Interactions Between Vascular Arbascular Mycorrhizal Fungi And *Streptomyces* As Biocontrol Agent For Tomato Damping-Off Disease Caused By *Rhizoctonia Solani*Kuhn

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**Abstract:** The most popular and important plant Lycopersicon esculentum Mill or Tomato was attacked by several fungal pathogens and several biocontrol agents can be used. Mycorrhizal fungi are mutuality associated with many plant root while actinomycete isolates of the genus Streptomyces enhanced plant growth and decreased plant pathogen development. Thus, interaction between Mycorrhiza and Streptomyces has been attracting attention due to the excellent benefit the plants by decreasing fungal infection and damping-off disease caused by *Rhizoctonia solani*. Out of 4 isolates of *R. solani*, the most pathogenic isolate was *R. solani* LB4 (% of plant survival, 30%), thus it was selected for more detail study. From rhizosphere soil of tomato, 15 actinomycetes isolates were obtained. The 15 isolates in addition to Streptomyces exfoliates LP10 were screened for inhibitory effect against *R. solani*. Out of the 15 isolates, 6 were inhibitory for *R. solani* LN4 in infected tomato plants. Plant fungal infection by *R. solani* LN4 decreased the numbers of the survived plants to 30%. Fungal infection decreased plant growth and mineral contents in 2 months old tomato plants. Proline was enhanced in diseased plants and decreased by the presence of biocontrol agents. Inoculation with biocontrol agents enhanced seedling growth, biomass of shoot and root after 2 months after infection with *R. solani* LB4 which may be attributed to hormone (IAA) and lytic enzymes (Chitinase and β-1,3 Glucanase) production by the Streptomyces isolate or the antagonistic activity and enhancing phosphorus uptake by mycorrhizal fungi. In conclusion it can be revealed that mycorrhizal fungi, *S. exfoliates* LP10 or their interactions effectively reduced *R. solani* LP4 infection on tomato roots under in vivo conditions and in whole fungal infection was significantly reduced by the used biocontrol treatments. The findings from this study clearly indicated the possibilities of using mycorrhizal fungi associated with actinomycete isolates as bio-inoculums for growth promotion and biocontrol of fungal infections of tomato.

**Keywords:** Streptomyces exfoliates, *Rhizoctonia solani*, mycorrhizae, Tomato, biocontrol, IAA

I. Introduction

*Lycopersicon esculentum* Mill (Tomato), originated in tropical America, is one of the most important and popular vegetables throughout the world. Tomatoes rich in vitamins A, B and C (Khosho, 1994). Every year many soil borne fungal pathogens are widely distributed throughout the world and are responsible for damaging this crop including that caused seedling damping-off by *Rhizoctonia, Fusarium, Pythium* and *Verticillium* (Kaprasilvi, 1996; Lucas et al., 1997). The soil born pathogenic fungus, *Rhizoctonia solani*, is worldwide distributed fungus that parasite plants by attacking the roots and lower stems of plants at seedling stages with a wide host range, causing serious plant losses. Since more than 100 years ago, this plant pathogen showed thread-like growth on plants causing various plant diseases such as root rot and, damping off or lived as saprophytic pathogen in the soil (Jiskani et al., 2007). *Rhizoctonia solani* mainly infected herbaceous plants and many studies was conducted for control of damping-off fungi associated with tomato roots using many different fungicides which usually associated with many side effects. Chemical control using pesticides polluted soil and caused harmful effects on human beings (Evangelista-Martinez, 2014). Accordingly, biological control of soil borne diseases using Gram-negative bacteria, especially *Pseudomonas* strains, have been intensively recorded due to production of antimicrobial agent and some metabolites while the Gram-positive bacteria belonging to genus *Bacillus* were less intensively used (Silosuhat et al., 1994,Tu et al., 2016). *Bacillus subtilis* is considered to be a safe biological agent (Chang and Kommedahl, 1968.), which may due to the antibiotic (ituran A) and surfactin productions (Hirakoa et al., 1992). The use of *B. subtilis* RB14 as biological control agent against the damping-off of tomato seedlings caused by *Rhizoctonia solani* was determined (Asaka and Shoda, 1996).

In recent years, the underground soil microbes have gained increased attention due to their interacting with plant roots and the interactions between pathogenic and beneficial isolates which are important.
implications for plant fitness. About 400 Mycorrhizal fungi (fungus root) form symbiotic relationships with plant roots. In terrestrial ecosystems, vascular arbuscular mycorrhizal (AM) fungi or endo-mycorrhizae are mutualistic with different plants and are important components with direct positive impacts on their host plants (Bergelson and Crawley, 1988, Callaway et al., 2004, Bardgett et al., 2006, Smith and Read, 2008), on contrast to the other parasitic fungi which influence negatively the host plants. Other reports (Atsatt, 1973; Brundrett, 2002) indicated that some plant roots have not any AM associations and direct interactions between mycorrhizal fungi and plants decreased fungal infection and significantly influenced growth of the plants (Lendzemo et al., 2005; Lopez-Raez et al., 2011). The obligate symbionts fungi of a majority of plants enhanced phosphorus uptake and plant protection from pathogens compared to non-mycorrhizal plants (Borowicz 2001). Azco’n-Aguilar et al. (2002) and (Smith and Read 2008) suggested several mechanisms to explain the protection process which may be due to improve nutrient content of the host plant. In this study, an attempt have been made to screen some actinomycetes for their biocontrol ability against the commonest fungal R. solani and the effect of the most potential strain and/or mycorrhizae as efficient biocontrol agents was studied.

II. Material and Methods

Media preparation
Each medium was solidified with 2.0% agar, when necessary and sterilized at 121°C for 15 min.

Microorganisms used
Tomato roots were collected and transported in ice tank to the laboratory and immersed in water to remove debris. After washing 3 times with sterile distilled water, the root tips were used for bacterial isolation. Rhizoctoni solani strains were isolated from infected tomato plants collected from different fields with seedling blight and root rot symptoms on potato dextrose agar medium. Based on morphological and physiological characters in addition to biochemical tests, identification of these isolates were determined at Biology Department, Faculty of Science, KAU, Saudi Arabia. The phytopathogenic fungal isolates were maintained on PDA medium containing (per liter) 200 g of potato infusion, 20 g of glucose, 1 g of peptone (pH 5.6) and 15 g of agar.

Tomato roots were used to isolate different actinomycete isolate on starch nitrate agar, which contained 5 μg/ml cycloheximide and 5 μg/ml nystatin. After 7 days at 45°C, isolates could be distinguished according to their morphological appearance (Hirsch and Christensen, 1983, Williams et al., 1989) and these were maintained on ISP2 agar (Shirling and Gottlieb, 1966) at 4°C until used. The isolated actinomycete isolates in addition to Streptomyces isolate obtained from contaminated soil previously identified as Streptomyces exfoliates LP10 (Aly et al., 2012) were tested for antifungal activity, indole acetic acid (IAA) and lytic enzymes productions.

The used mycorrhizal fungi
Inoculation with AMF included the application of the commercial AMF inoculum (Symbio - The environmental biotechnology company, Unit 8, Coopers Place, Combe Lane, Wombley, Surrey GU8 5SZ, UK) Themoilocums was composed of 5% organic material and 95% AM spores, including the following AM species: Scutellospora calospora, Acaulospora laevis, Gigaspora margarita, Glomus aggregatum, Rhizophagus irregularare (syn G. intraradices), Funneliformis mosseae (syn G. mosseae), G. fasciculatum, G. etunicatum, and G. deserticola. Total spore density in the inoculum was 25 spores of each species/g of mycorrhizalinoculum.

Antifungal activity
Agar disc of the selected actinomycetes on starch nitrate agar was prepared by using sterile cork borer with diameter of 8 mm, and placed at the centre of PDA plate, inoculated with fresh culture of R. solani. All the plates were inoculated at 25°C for 7 days and mean diameter of the inhibition was calculated. All experiments were carried out in triplicates.

Extraction of IAA from the actinomycete isolates
All actinomycete isolates were grown in production medium (Agwa et al., 2000) supplemented with 2 mg/ml L-tryptophan at a pH of 7.0 in 250 ml Erlenmeyer flask containing 50 ml of the broth medium. After 7 days of growth at 25°C, cells were collected and the supernatants were filtered using Milipore filter (0.45 mm) and the cell free filtrates were used for IAA detection and quantification. From the supernatants, IAA was extracted with ethyl acetate (Ahmad et al., 2005) and IAA detection on TLC plates (Silica gel, thickness 0.25 mm, Merck, Germany) was obtained after spraying Ehmann’s reagent (Ehmann, 1977). Quantity of IAA was determined according to the method of Bano and Musarrat (2003).

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Lytic enzyme assay

Chitinase assay was carried out in incubated mixture at 37°C for 2 h of 1 ml culture filtrate and 1 ml of 0.1% colloidal chitin in 0.05 M sodium acetate buffer (pH 5.2) and enzyme activity was detected by colorimetric method (Tsachowisan and Lumyong, 2003). One unit of chitinase was the amount of enzyme, produced 1 μ mole of N-acetylg glucosamine per ml of reaction mixture. Similarly, activity of β-1,3-Glucanase was detected using the method of Nelson (1955) and one unit of B-1,3-glucanase is the quantity of the enzyme giving 1μmol of reducing sugar (glucose) per min.

The effect culture filtrates of Streptomyceson seed germination

The supernatant of the six actinomycete isolates was used for soaking surface-sterilized tomato seeds or sterile distilled water incubated in the dark until the seedlings emerged (10 days) and percentage of germination and germination index were calculated(Dhamangaonkar and Pragati, 2009). Sterile dist. water was used as control

Germination Index = Total days x Total seeds

Soil treatments

The soil used in this study was taken from the garden of Faculty of Science, KAU, sieved through 2-mm-pore-sizescreen and air dried. The final concentrations of N, P₂O₅, and K₂O were 70 mg, 200 mg, and 70 mg/100 g dry soil, respectively. The prepared soil was sterilized by autoclaving for 60 min at 121°C two times. The soil was used to fill sterile pots (18 cm diameter), each was filled with 2.0 kg of air-dry sterile soil.

Inoculation of mycorrhiza into tomato seeds

The seed of tomato (Lycopersicon esculentum Mill. cv. ‘large cherry’) were obtained from American Seed Corp, USA. The seeds were soaked in 2% sodium hypochloride, NaOCl, for 30 min, washed several times and dried at room temperature. The mycorrhiza inoculum was mixed with tomato seeds at a rate of 2.0 g of the obtained inoculum/100 seeds.

Inoculation of soil with R. solani

Fungal mycelium was collected from the fungal growth on solid PDA medium from a Petri dish culture of R. solani in sterile saline water (5% NaCl, pH 7.0) and homogenized (5,000 rpm, 1 min, two successive times under cooling). About 15 ml of the obtained suspension was inoculated into the soil at the ratio of a Petri dish/pot. Five days before planting the tomato seedling. After inoculation, the pots were kept at 30°C and 60% moisture of the maximum waterholding capacity.

Application of Streptomycespores to soil

The selected actinomycete isolate, Streptomyces exfoliates LP10 was incubated in starch nitrate broth medium at 30°C and 100 rpm for 2 days and 5 ml of the culture was transferred to 100 ml of fresh medium. After 7 days of growth at 30°C and 100rpm, cells were collected after centrifugation at 5000 rpm for 5 min., washed and suspended in saline sterile water (5% NaCl, pH 7.0). Twenty ml of the cell suspension was used to inoculate soil of each a pot after transferring of the seedling to the soil.

Application of Mancozeb to the infected plants

After fungal infection, infected plants were sprayed with 20 ml Mancozeb (SHANGHAI) at 1000 ppm two times after 1 and 2 weeks of infection and plants in each pot were left to be air-dried.

Growth of tomato in sterilized soil

Sterile plastic pots containing 2 kg of sterile soil (autoclaved at 160°C for 60 min., two successive times) were used. Inoculated tomato seeds with mycorrhizal inoculum was germinated in the dark for 7 days at 30°C or stayed untreated. For each treatment, 10 pots were prepared. Soil inoculation with R. solani spore suspension was simultaneously carried out 5 days before planting of the germinated tomato seeds. Five groups of pots were prepared and studied. The pots were containing: 1. Normal plants (untreated or control plants) were grown in normal soil and uninoculated seeds were used. 2. Infected plants were grown in infected soil with R. solani and uninoculated seeds were used. 3. Plants treated with mycorrhizal fungi were grown in infected soil with R. solani and mycorrhizal inoculated seed were used. 4. Plants treated with Streptomyces were grown in infected soil with R. solani and soil was inoculated with Streptomyces spore suspension and uninoculated seeds were used. 5. Plants treated with mycorrhizal fungi and Streptomyces were grown in infected soil with R. solani and soil was inoculated with both Streptomyces spore suspension and mycorrhizal inoculated seeds were used. Infected plants with R. solanisprayed two times using the fungicide Mancozeb were also including.
Plant analysis

This work was carried out during the period 2015-2016. The germinated seeds were transferred to soil where each pot was receive 10 germinated seeds and all pots were labeled and incubated at 30°C with 60% relative humidity under 18 hr. of light. The diseased seedling percentages per pot were determined after two weeks. Each pot was irrigated with 200 ml distilled water two times per week.

After 2 months, shoots and roots were separated and rinsed in distilled water. Intensity of mycorrhizal infection (% M) in fresh roots of mycorrhizal infected plants were measured after clearing in 10% KOH and staining with trypan blue in lactophenol (Phillips and Hayman 1970). Shoot lengths (cm) and the root depth (cm) were measured, dried at 60°C until a constant weight. Shoot samples were powdered and total soluble sugars and total protein were determined in dry shoot samples of control, infected and treated plants according to Dubois et al. (1956) and Lowry et al. (1951) respectively. Subsamples of the dried shoots were digested in a mixture of H$_2$SO$_4$ and HClO$_4$, under heating on a hot plate for 5 hr. Phosphorus was measured using molybdenum phosphate method (Watanabe and Olsen, 1965) while nitrogen content were carried out after acid digestion using protocols found in Allen et al. (1974). Mineral contents (K$^+$ and Mg$^{++}$) were measured by Shimadzu Atomic Absorption Flame Spectrophotometer (Model 640/12) after acid digestion of the dried samples. The extracted proline was determined by the spectrophotometric method (Sadasivam and Manickam, 1996).

Statistical analysis

Each treatment was repeated at least 3 times and each pot (n=10) was receiving 5 seedlings. The dead plants were removed. The readings were taken and mean value±SD was represented. The data was analyzed using SPSS and Student t-test was applied.

III. Results

Four Rhizoctonia solani strains, LB1, LB2, LB3 and LB4 were isolated from infected roots plants. Identification was carried out and compared to reference isolate of Rhizoctonia solani. As shown in Table 1, the four fungal isolates were pathogenic and showed many symptoms including wilt, seed rot, stunting or pre/post-emergence damping off on the tomato plants. The most pathogenic isolate was LB4 (% of plant survival, 30%) while isolates LB 2 and LB 3 (% of plant survival, 40 and 48%) were the least pathogenic isolates. More than one symptom can be detected on the same plant. About 15 actinomycete isolates were recovered on starch nitrate with antibiotic addition. From rhizosphere of tomato plants, 15 isolates were obtained on starch nitrate medium containing antibiotics and the 15 isolates in addition to Streptomyces exfoliates LP10 were screened for the antifungal activities against Rhizoctonia solani LB4 in vitro. Out of them, 6 isolates belonging to the genus Streptomyces and named Streptomyces LN1, LN3, LN4, LN 9, LN11 and LN15 showed inhibitory activity against Rhizoctonia solani with inhibition zone diameter ranged from 10-21 mm. Inhibition zone diameter was 27 mm in case of Streptomyces exfoliates LP10. The most active isolate was S. exfoliates LP10 and the least active were isolates LN11 and LN15. It was found that S. exfoliates LP10 showed excellent production of both Chitinase, β-1,3 Glucanase, and indole acetic acid (IAA) in liquid media (Table 2). In liquid broth medium, the quantities of IAA produced the tested strains of actinomycetes were ranged from 4.5-22.0 μg/ml and maximum production was by S. exfoliates LP10 (22.0 μg/ml).

Tomato seeds were grown in sterile soil, previously infected with Rhizoctonia solani and some biocontrol agents including mycorrhizal fungi, Streptomyces or both were used to suppress the disease symptoms and development. Mancozeb was used as standard fungicide. It was found that % of root infection with mycorrhizae was 33% and increased to 43% in the presence of Streptomyces spores (Table 3). The % of recovered plants was about 100% in control plants and this percentage was decreased to 25% in the infected plants. These percentages were increased to 78, 89 and 86 % in case of infected and treated plants with mycorrhizae, Streptomyces or mycorrhizae+ Streptomyces, respectively. The percentage was 96% in case of using the fungicide Mancozeb. Root depth (cm), shoot length (cm) and root and shoot dry weight (g/plant) of tomato plants were decreased significantly by fungal infection and the decreases was completely or slightly removed by the used biocontrol agents. In case of fungal infected plants, root depth, shoot length and shoot dry weight were significantly lower in case of mycorrhizal inoculation and shoot dry weight was lower in case of Streptomyces inoculation compared to control plants (Table 3). The total soluble sugar, protein, proline, and mineral content (P$^{+++}$, N$^{+++}$, Mg$^{++}$ and K$^+$) of plants grown in sterile soil and either infected with Rhizoctonia solani or infected and treated with mycorrhizae, Streptomyces or both were determined and summarized in Table 4. From the previous Table, it was clear that fungal infection with R. solani LB4 increased TSS, protein and proline contents while the plant contents of phosphorus, nitrogen and the minerals Mg$^{++}$ and K$^+$ decreased compared to control plants. On the other hands, inoculation of the infected plant with mycorrhizal fungi, Streptomyces or mixed inoculums of both microbes enhanced growth and plant contents to levels nearly to that
of the healthy plants (Table 4) and treatment of fungal infection using bioagents (mycorrhizae, Streptomyces or both) was similar to that obtained using Mancozeb.

IV. Discussion

*Rhizoctonia solani* lives in the soil, at the upper layers about 2cm of the surface and under unfavorable conditions, this pathogen can live for many years as resting bodies, sclerotiora intertwined hyphae with dark colour (Sneh et al., 2006). Pathogenicity of *Rhizoctonia solani* on tomato seedlings and young plants was recorded. Twelve pathogenic isolates belonging to *Rhizoctonia solani* were recovered from *Vicia faba, Trifolium* and *Cotton* in Egypt. Many symptoms were caused by the pathogenic isolated on their hosts including seed rot, wilt, stunting, and pre and post emergence damping off (Mahmoud et al., 2007). Basu et al. (2016) reported that this pathogen showed more hyphal growth in the susceptible host than in the tolerant variety and more inter- and intracellular structures in the susceptible hosts. Many different microbes and fungicides were used to control damping-off disease and enhance plant growth (Durak, 2016). Molecular mechanisms can be used for studying the root colonization by AM fungi and root infection by root parasitic fungi. Enhancement of levels of antifungal compounds for biocontrol process may include the use of genetically modified microbes. Interactions between *Pseudomonas fluorescens* (biocontrol agents) and, an arbuscular mycorrhizal fungus, were used for enhancing *Tomato* (*Lycopersicum esculentum*) and *leek* (*Allium porrum*) plants. Mycorrhizal fungi help plants to grow healthier and better whereas they live inside the roots extending their hyphae outside to the soil to enhance phosphate, nutrients, and nitrogen addition to increasing water uptake to the host plant. The root area of the VAM plants effectively extended many hundreds of times which help plants to grow stronger, faster, and larger even with less fertilizer and/or water. Different species of VAM fungi infect many plants and increased plant growth, total soluble protein (Abdalla and Abdel-Fattah, 2000), uptake of phosphorus and nutrient fungal hyphae and total nitrogen content (Durga et al., 1995). Under laboratory conditions, inoculation of soil with *Trichoderma harzianum* or coating tomato fruits prevent *R. solani* infection and fruit rot by 43% and 85%, respectively but reduction was 86% in the field trial and fruit rot was reduced by 27–51%.

The trypan blue technique could demonstrate slight and rapid changes in cells infected with arbuscular mycorrhizal fungi during plant growth and there is a close positive link between mycorrhizal arbuscules, the main active site of P transfer to the host, and their host plants (Abdel-Fattah, 2001). It was found that inoculation of seeds *Pseudomonas or Bacillus* cells were antagonistic to *R. solani* (Georgakopoulos et al., 2002) and 28% fungal growth inhibition was recorded in agar plate while 80% disease was suppressed in vivo. Inoculation protect tomato seeds from infection (99% germination index) compared to control (untreated seeds). Growth and seed germination increased by bacterial inoculation due to production of phytohormone like indole-3-acetic acid improve (Bakonyi et al., 2013, Tu et al., 2016). When inoculated seeds were sown in the soil, the cells were released into the soil from the seed surface and produces lipopeptide antifungal antibiotic iturin A in the soil which could suppress the disease. The release of the antibiotic started approximately 24 h after seed planting (Szczzech and Shoda, 2006). Antibiotics and lytic enzymes such as glucanase, chitinase, cellulase can play crucial role to defend fungal pathogen *R. solani* K1 (Zohora et al., 2016).

The selection of effective antagonistic organisms is the basic step in biological control. On the basis of these studies, it is concluded that the *B. subtilis* RB14 coated seed has a direct inhibitory effect on *R. solani* K1 growth and development, thereby capable of suppressing damping-off diseases in tomato plants. Following the basic findings of applicability *B. subtilis* RB14 seed treatment against *R. solani* K1 in tomato plants, feasibility should be studied for other important plants and pathogens. In order to apply such seed treatment agents more successfully, a greater understanding of their ecology is required. The safety and efficacy of the inoculants will be determined by the ecological success of the applied strain in the environment into which they are introduced. Greater knowledge of diversity, distribution and activities of *Bacillus* spp. will be useful for identification of new inoculants strain for effective biocontrol. Further research is essential to elucidate the mechanisms underlying sensing of the rhizoplane environment and the production of antibiotics and enzymes for successful biocontrol. Natural auxin, indole acetic acid, produced by 80% of soil bacterial isolates (Bhavdhis et al., 2003) from organic nutrient and root exudates.

In this study, all *Streptomyces* species isolates produced IAA in liquid medium. Similar results were obtained for *Azotobacter* and *Streptomyces* with IAA production ranged from 4.9 - 11.4 μg/ml (Aly et al., 2012) while higher IAA production were recorded for bacteria and *Streptomyces* (Khamna et al., 2009). Furthermore, *Streptomyces* species from tomato rhizosphere have the ability to produce IAA and improve plant growth (Tokala et al., 2002, El-Tarabily, 2008). *Streptomyces* enhanced seed germination due to the presence of plant growth regulators (indole-3- acetic acid), vitamins, amino acids or secondary metabolites, P, Mg, N and total soluble sugars which enhanced root and shoot growth of wheat (El-Shanshoury, 1995, Ahmed et al. 2004). Many biologically active compounds such as antifungal and antibacterial compounds or plant growth promoting substances that have been developed for agricultural use were originated from *Streptomyces* (Illic et al., 2007, Passari et al., 2016). Alizadeh et al. (2012) reviewed that in China, many studies have been conducted on the

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yield increase after bacterial inoculation, wheat (8.5-16%), rice (8.1-16%), maize (6-11%), beans (7-16%), sugar beet (15-20%), sorghum (5-10%), sweet potato (15-19%), linen (6-13%), oily turnip (16-18%), peanut (10-15%) and vegetables (13-35%). Fungal infection decreased plant growth and increased proline concentration due to lipid peroxidation and increased production of superoxide radicals and, hydrogen peroxide. Sugars, sugar alcohols, polyols, inositol, quaternary amino compounds like glycine-betaine, proline and higher polyamines, serve as osmo-protectants under stress conditions and maintained membrane structure. Also, they act as free-radical scavengers preventing lipid per oxidation or as regulators of K+ channels in stomata (Hasegawa et al., 2000). The increase in the proline content under stress condition may be due to breakdown of proline rich protein or de novo synthesis of proline (Tewari and Singh, 1991). Finally, it was clear that, microbial inoculation with Streptomyces, mycorrhizal fungi or both decreased or prevent fungal infection or/and enhanced plant growth.

References


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Table 1. The symptoms detected by the four isolated Rhizoctonia solani strains, obtained from infected roots of germinated tomato seeds (N=25)

<table>
<thead>
<tr>
<th>Tested isolate</th>
<th>Seed rot</th>
<th>Wilt</th>
<th>Stunting</th>
<th>Pre-emergence damping off</th>
<th>Post-emergence damping off</th>
<th>No. of survival plant</th>
<th>% of survival plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizoctonia solani LB1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>10</td>
<td>9</td>
<td>36</td>
</tr>
<tr>
<td>Rhizoctonia solani LB2</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>8</td>
<td>12</td>
<td>48</td>
</tr>
<tr>
<td>Rhizoctonia solani LB3</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>Rhizoctonia solani LB4</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2. The most active Streptomyces isolates on inhibition of Rhizoctonia solani LB4 and production of lytic enzymes and indole acetic acid (IAA) in addition to enhancing tomato seeds germination

<table>
<thead>
<tr>
<th>Tested isolate</th>
<th>Inhibition of Rhizoctonia solani LB4</th>
<th>Lytic enzyme activity</th>
<th>IAA production</th>
<th>Effect on Tomato seed germination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diameter of inhibition zone (mm)</td>
<td>Chitinase</td>
<td>β-glucanase</td>
<td>Quantity (µg/ml)</td>
</tr>
<tr>
<td>Streptomyces LN 1</td>
<td>15±2.5*</td>
<td>1.20±0.13*</td>
<td>1.48±0.19*</td>
<td>++</td>
</tr>
<tr>
<td>Streptomyces LN 3</td>
<td>21±2.1*</td>
<td>0.78±0.19*</td>
<td>1.23±0.33*</td>
<td>+</td>
</tr>
<tr>
<td>Streptomyces LN 4</td>
<td>20±1.6*</td>
<td>0.98±0.15*</td>
<td>1.69±0.13*</td>
<td>+++</td>
</tr>
<tr>
<td>Streptomyces LN 9</td>
<td>14±2.0*</td>
<td>0.73±0.15*</td>
<td>1.33±0.19*</td>
<td>++</td>
</tr>
<tr>
<td>Streptomyces LN 11</td>
<td>11±1.4*</td>
<td>0.94±0.07*</td>
<td>1.1±0.30*</td>
<td>+</td>
</tr>
<tr>
<td>Streptomyces LN 15</td>
<td>2x±0.25*</td>
<td>1.40±0.25*</td>
<td>1.77±0.21*</td>
<td>++</td>
</tr>
<tr>
<td>Streptomyces exfoliates LP10</td>
<td>27±3.3</td>
<td>1.5±0.35</td>
<td>2.33±0.428</td>
<td>+++</td>
</tr>
</tbody>
</table>

*Significant results at P<0.05 compared to control Streptomyces exfoliates LP10

Table 3. Root depth (cm), shoot length (cm) and root and shoot dry weight (g/plant) of plants grown in sterile soil and either infected with Rhizoctonia solani or infected and treated with mycorrhizae, Streptomyces or both

<table>
<thead>
<tr>
<th>Treatment used</th>
<th>Measured parameters</th>
<th>Control plants</th>
<th>Infected plants with R. solani</th>
<th>Mycorrhizal fungi</th>
<th>Infected and treated plants</th>
<th>Streptomyces</th>
<th>Mycorrhizal fungi + Streptomyces</th>
<th>Fungicide (Mancozeb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% mycorrhizal infection</td>
<td>ND</td>
<td>ND</td>
<td>33%</td>
<td>ND</td>
<td>43%**</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>% of recovered plants</td>
<td>100±9%</td>
<td>25±9%</td>
<td>78±9%</td>
<td>86±9%</td>
<td>96±9%</td>
<td>74±9%</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>Shoot length (cm)</td>
<td>27.5±2.5</td>
<td>13.8±1.9*</td>
<td>18.7±1.9*</td>
<td>21.8±1.5</td>
<td>23.8±4.9</td>
<td>23.5±2.3</td>
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<td></td>
</tr>
<tr>
<td>Shoot dry weight (g/plant)</td>
<td>2.39±0.40</td>
<td>0.39±0.04*</td>
<td>1.98±0.17*</td>
<td>1.99±0.29*</td>
<td>2.33±0.43</td>
<td>2.11±0.31</td>
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<td></td>
</tr>
<tr>
<td>Root depth (cm)</td>
<td>15.2±2.0</td>
<td>9.6±1.4*</td>
<td>11.±3.3*</td>
<td>12.9±1.1</td>
<td>13.6±2.9</td>
<td>13.6±1.7</td>
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<td></td>
</tr>
<tr>
<td>Root dry weight (g/plant)</td>
<td>0.45±0.15</td>
<td>0.11±0.03*</td>
<td>0.55±0.09</td>
<td>0.43±0.07</td>
<td>0.49±0.11</td>
<td>0.49±0.13</td>
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<td></td>
</tr>
</tbody>
</table>

*Significant results at P<0.05 compared to control, **: Significant results at P<0.05 compared to inoculated plant, #: the values have the same letters were not significant

Table 4. The total soluble sugar, protein, proline, and mineral content (P, N, Mg and K) of plants grown in sterile soil and either infected with Rhizoctonia solani or infected and treated with mycorrhizae, Streptomyces or both.

<table>
<thead>
<tr>
<th>Treatment used</th>
<th>Measured parameters</th>
<th>Control plants</th>
<th>Infected plants with R. solani</th>
<th>Mycorrhizal fungi</th>
<th>Infected and treated plants</th>
<th>Streptomyces</th>
<th>Mycorrhizal fungi + Streptomyces</th>
<th>Fungicide (Mancozeb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS (mg/g, dry wt.)</td>
<td>133.6±49.11</td>
<td>191±11.4*</td>
<td>99±8.9*</td>
<td>108±8.64</td>
<td>112±5.9</td>
<td>104±7.7</td>
<td></td>
<td>130.5±4.0</td>
</tr>
<tr>
<td>Protein (mg/g, dry wt.)</td>
<td>131.7±34.1</td>
<td>147±56.16*</td>
<td>133±5.41</td>
<td>134±6.16</td>
<td>131±6.51</td>
<td>130.5±4.0</td>
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</tr>
<tr>
<td>Proline</td>
<td>1.34±0.03</td>
<td>1.7±0±3.3*</td>
<td>6.7±0.14*</td>
<td>4.9±0.92*</td>
<td>2.4±0.09</td>
<td>1.2±0.09</td>
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<tr>
<td>PO4**</td>
<td>12.4±2.2</td>
<td>7.2±1.21*</td>
<td>16.6±0.55*</td>
<td>13.7±1.22</td>
<td>12.3±3.10</td>
<td>13.8±1.17</td>
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</tr>
<tr>
<td>N†</td>
<td>22.3±0.11</td>
<td>16.4±1.51*</td>
<td>23.5±3.45</td>
<td>22.6±2.16</td>
<td>21.4±2.31</td>
<td>19.6±2.31</td>
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<td></td>
</tr>
<tr>
<td>Mg**</td>
<td>5.6±1.1</td>
<td>4.6±1.23*</td>
<td>5.4±0.7</td>
<td>5.3±0.4</td>
<td>4.9±0.6</td>
<td>4.8±0.2</td>
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</tr>
<tr>
<td>K†</td>
<td>13.4±1.3</td>
<td>9.4±0.091*</td>
<td>13.7±0.8</td>
<td>11.5±1.8</td>
<td>12.6±2.1</td>
<td>11.4±3.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant results at P<0.05 compared to control