IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 11, Issue 6 Ver. IV (Nov. - Dec.2016), PP 89-96
www.iosrjournals.org

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

Lubna S. Nawar
Biology Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.

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 rootswhile actinomycete isolates of the genus Streptomyces enhanced plant growth and decreased plant pathogen development. Thus, interaction between Mycorrhizae and Streptomyces has been attracting attention due to the excellentbenefitto the plantsby decreasing fungal infectionand damping-off disease caused by Rhizoctonia solani. Out of 4 isolates of R. solani, the most pathogenic isolatewas R. solani LB4 (% of plant survival, 30%), thus it was selected for more detail study. From rhizosphere soil of tomato,15 actinomyceteisolates 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 forR. solaniusing agar disc diffusion method but thehighest diameters of the inhibition zone (27 mm) was forStreptomyces exfoliates LP10. Production of varying levels of growth hormone (IAA) and lytic enzymes (Chitinase and β-1,3 Glucanase)by Streptomyces*isolates were also determined and *S. exfoliates LP10 was the most active isolate, thus it was selected in addition to mycorrhizal fungi for antifungal activity against R. solaniLN4 on 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 biochontrol agents. Inoculation with biochontrol 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 theStreptomyces* 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 infectionon 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. Tomatois rich in vitamins A, B and C (Khoso, 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* (Kaprashvili, 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 (Silosuh*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 (iturin A) and surfactin productions (Hiraoka *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 aremutualistic 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 theother 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 wasstudied.

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* strainswere 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 isolateswere determinedat Biology Department, Faculty of Science, KAU, Saudi Arabia.The phytopathogenic fungal isolates were maintained onPDA 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 andChristensen, 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, Wormley, Surrey GU8 5SZ, UK) Theinoculums was composed of 5% organic material and 95% AM spores, including the following AM species: *Scutellospora calospora, Acaulospora laevis, Gigaspora margarita, Glomus aggregatum, Rhizophagus irregulare* (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 filtrateswere used for IAA detection and quantification. From the supernatants, IAA was extracted with ethyl acetate (Ahmad *et al*.,2005) and IAA detectionon 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).

Lytic enzyme assay

Chitinase assay was carried out in incubated mixture at 37˚C for 2 h of1 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 (Taechowisan and Lumyong, 2003). One unit of chitinase was the amount of enzyme, produced 1 µ mole of N-acetylglucosamine 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 thequantity of the enzyme giving 1µmol of reducing sugar (glucose) per min.

The effect culture filtrates of *Streptomyces*on seed germination

The supernatant of the six actinomycete isolates was used for soakingsurface-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

$$\text{Germination Index} = \frac{\text{Sum of germinated seed for a certain period}}{\text{Total days x Total seeds}}$$

Soil treatments

The soil used in this study wastaken from the garden of Faculty of Science, KAU, sieved through 2-mm-pore-sizescreen and air dried. The final concentrations of N, P_2O_5, and K_2O were 70 mg, 200 mg, and 70 mg /100 g dry soil, respectively. The prepared soil wassterilized 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 mycorrhzae 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 time and dried at room temperature. The mycorrhizae inoculum was mixed with tomato seeds at a rate of 2.0 g of the obtained inoculums/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 15ml of the obtained suspension was inoculated into the soil at the ratio of a Petri dish/pot, 5 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 *Streptomyces*spores 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 transferredto 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 after1 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 2kg of sterile soil (autoclaved at 160°C for 60 min., two successive times) were used. Inoculated tomato seeds with mycorrhizal inoculumwas 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. solani*sprayed 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 receive10 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 dist.water two times per week.

After 2 months,shoots and roots were separated and rinsed in dist. 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 and weighted. Shoot samples were powdered and total soluble sugars and total protein were determined in dry shoot samples of control, infected and treated plantsaccording to Dubois *et al*. (1956) and Lowry *et al*. (1951) respectively.Subsamples of the dried shoots were digested in a mixture of H_2SO_4 and $HClO_4$ under heating on a hot plate for 5 hr. Phosphorus was measured using molybdophosphate method (Watanabe and Olsen, 1965) while nitrogen contentwere carried out after acid digestionusing 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 spectrophotometeric method (Sadasivam and Manickam, 1996).

Statistical analysis

Each treatment was repeated at least 3 times and each pot (n=10) was receiving 5seedlings. 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 isolatewas 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, LN11and 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* andsome 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 increase to78, 89 and 86 % in case of infected and treated plants with mycorrhizae, *Streptomyces* ormycorrhizae+ *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, S*treptomyces* 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, atthe upper layers about 2cm of the surface and under unfavorable conditions, this pathogen can live for manyyears as resting bodies, sclerotiaor intertwinedhyphae withdark colour (Sneh *et al*., 2006). Pathogenicity of *Rhizoctonia solani* on tomato seedlings and young plantswas 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 forbiocontrol 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 nitrogenin addition to increasingwater 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 manyplants and increased plant growth, total soluble protein (Abdalla and Abdel-Fattah 2000), uptake of phosphorusand nutrient fungalhyphae and total nitrogen content (Durga *et al*., 1995). Under laboratoryconditions,inoculation of soil with *Trichoderma harzianum* or coating tomato fruits prevent *R. solani* infection andfruit 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 *at el*., 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 (Szczech 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 seedhas a direct inhibitory effect on *R. solani* K1growth 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 (Bhavdish *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 rangedfrom 4.9 -11.4 µg/ml (Aly *et al*., 2012) while higherIAA 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* (Ilic *et al*., 2007, Passari *et al*., 2016). Alizadeh *et al.* (2012) reviewed that in China, many studies have been conducted on the

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, inositols, quarternary 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

[1]. Abdalla ME and Abdel-Fattah GM (2000). Influence of the endomycorrhizal fungus *Glomus mosseae* on the development of peanut pod rot disease in Egypt. Mycorrhiza, 10: 29–35.

[2]. Abdel-Fattah G M (2001). Measurement of the viability of arbuscular-mycorrhizal fungi using three different stains; relation to growth and metabolic activities of soybean plants. Microbiol. Res., 156, 359–367.

[3]. Agwa A, Aly MM, Bonaly R (2000). Isolation and characterization of two *Streptomyces* species produced non polyenic antifungal agents. J Union Arab Biol., 7: 62-84.

[4]. Ahmad F, Ahmad M, and Khan S (2005). Indole Acetic Acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan. Turk. J. Biol., 29: 29-34.

[5]. Ahmed W, Shahroona B, Zahir ZA and Arshad M (2004). Inoculation with ACC-deaminase containing rhizobacteria for improving growth and yield of wheat. Pak. J. Agric. Sci., 41: 119-124.

[6]. Alizadeh O, Sharafzadeh S and Firoozabadi AH (2012). The effect of plant growth promoting rhizobacteria in saline conditions. Asian Journal of Plant Sciences, 11: 1- 8.

[7]. Allen SE, Grimshaw HM, Parkinson JA, Quarmby C and Roerts JD (1974). Chemical Analysis of Ecological Materials. Blackwell Scientific Publications, Oxford, London, pp: 565.

[8]. Aly M M El-Sayed H A E Jastaniah S D. (2012). Synergistic effect between *Azotobacter vinelandii* and *Streptomyces* sp. Isolated From Saline Soil on Seed Germination and Growth of Wheat Plant. Journal of American Science, 8(5):667-676.

[9]. Ames RN (1989). Mycorrhiza formation in onion in response to inoculation with chitin-decomposing actinomycetes. New Phytol., 112:423–427. 25.

[10]. Asaka O and Shoda M (1996). Biocontrol of *Rhizoctonia solani* damping off of tomato with *Bacillus subtilis* RB14. Applied and Environmental Microbiology, 62, 4081-4085.

[11]. Atsatt PR (1973). Parasitic flowering plants, how did they evolve. American Naturalist., 107:502–510.

[12]. Azcon-Aguilar C, Jaizme-Vega MC and Calvet C (2002). The contribution of arbuscular mycorrhizal fungi for bioremediation. In: Mycorrhizal Technology in Agriculture. From Genes to Bioproducts, Gianinazzi, S., H. Schuepp, J.M. Barea and K. Haselwandter (Eds.). Birkhauser Verlag, Berlin, ISBN-10: 0-89054-245-71, pp: 187-197.

[13]. Bakonyi, N., Bott, S., Gajdos, E., Szabó, A., Jakab, A., Toth, B., Makleit, P. and Veres, S.Z. (2013). Using Biofertilizer to Improve Seed Germination and Early Development of Maize. Polish Journal of Environmental Studies, 22:1595-1599.

[14]. Bano N and Musarrat J (2003). Characterization of a new *Pseudomonas aeruginosa* strain NJ-15 as a potential biocontrol agent. Current Microbiology, 46: 324-328.

[15]. Bardgett RD, Smith RS, Shiel RS, Peacock S, Simkin JM, Quirk H, Hobbs PJ (2006). Parasitic plants indirectly regulate below-ground properties in grassland ecosystems. Nature 439:969–972.

[16]. Basu A., Chowdhury S., Ray Chaudhuri T. and Kundu S. (2016). Differential behavior of sheath blight pathogen *Rhizoctonia solani* in tolerant and susceptible rice varieties before and during infection. Plant Pathology, Vol. 65 (8):1333–1346.

[17]. Bergelson JM andCrawley MJ (1988). Mycorrhizal infection and plant-species diversity. Nature, 334: 200-202.

[18]. Bhavdish N, Johri A, Sharma J, Virdi S (2003). Rhizobacterial diversity in India and its influence on soil and plant health. Advances in Biochemical Engineering and Biotechnology, 84, 49-89.

[19]. Borowicz VA (2001). Do arbuscular mycorrhizal fungi alter plant-pathogen relations? Ecology, 82, 3057–3068

[20]. Brundrett MC (2002). Coevolution of roots and mycorrhizas of land plants. New Phytologist., 154:275–304

[21]. Callaway RM, Thelen GC, Rodriguez A and Holben WE (2004). Soil biota and exotic plant invasion. Nature, 427, 731–733.

[22]. Challis GL and Hopwood DA (2003). Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by Streptomyces species. Proc Natl Acad Sci USA, 100:14555–145561.

[23]. Chang I, and Kommedahl T (1968). Biological control of seedling blight of corn by coating kernels with antagonistic microorganisms. Phytopathology, 58:1395–1401.

[24]. Dhamangaonkar SN and Pragati M (2009). Effect of *Azotobacter chroococcum* (PGPR) on the growth of Bamboo (*Bambusa bamboo*) and Maize (*Zea mays*) plants. Biofrontiers Vol.1 (1): 24-31.

[25]. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem.; 28(3):350-356.

[26]. Durak E D (2016) Biological control of *Rhizoctonia solani* on potato by using indigenous *Trichoderma* spp. AIP Conf. Proc. 1726, : 21–23 April , Antalya, Turkey.

[27]. Durga V.K, Sanjay-Gupta S and Gupta S (1995). Effect of vesicular mycorrhizae on the growth and mineral nutrition of teak (*Tectona grandis* Linn.). Indian Forester, 121, pp. 518–527.

[28]. Ehmann A (1977). The van Urk Salkowski reagent a sensitive and specific chromogenic reagent for silica gel thin layer chromatographic detection and identification of indole derivatives. J Chromatogr, 132: 267-276.

[29]. El-Shanshoury A R (1995). Interactions of *Azotobacter chroococcum, Azospirillum brasilense* and *Streptomyces mutabilis*, in Relation to their effect on wheat development. Journal of Agronomy and Crop Science, Vol. 175(2): 119–127.

[30]. El-Tarabily KA (2008). Promotion of tomato (*Lycopersicon esculentum* Mill.) plant growth by rhizosphere competent 1-aminocyclopropane-1- carboxylic acid deaminase-producing streptomycete actinomycetes. Plant and Soil 308, 161-174.

[31]. Evangelista-Martinez Z (2014). Isolation and characterization of soil *Streptomyces* species as potential biological control agents against fungal plant Pathogens. World J. Microbiol. Biotechnol., 30: 1639–1647.

[32]. Georgakopoulos DG, Fiddaman P, Leifert C and Malathrakis NE (2002). Biological Control of Cucumber and Sugar Beet Damping-Off Caused by *Pythium ultimum* with Bacterial and Fungal Antagonists. Journal of Applied Microbiology, 92, 1078-1086.

[33]. Hasegawa T, Takizawa M, Tanida S (1983). A rapid analysis for chemical grouping of aerobic actinomycetes. J. Gen. Appl. Microbiol., 29: 319-322.

[34]. Hiraoka H, Asaka O, Ano T and Shoda M (1992). Characterization of *Bacillus subtilis* RB14, coproducer of peptide antibiotics Iturin A and Surfactin. The Journal of General and Applied Microbiology, 38, 635-640

[35]. Hirsch CF, Christensen DL (1983). Novel method for selective isolation of actinomycetes Appl Environ Microbiol., 46(4):925–929.

[36]. Homma Y and Suzui T (1989). Role of antibiotic production in suppression of radish damping-off by seed bacterization with *Pseudomonas cepacia*. Ann. Phytopathol. Soc. Jpn. 55:643–652.

[37]. Ilic SB, Konstantinovic SS, Todorovic ZB, Lazic ML, Veljkovic VB, Jokovic N, Radovanovic BC (2007). Characterization and antimicrobial activity of the bioactive metabolites in streptomycete isolates. Microbiology 76, 421-428.

[38]. Jiskani K MM , Pathan MA, Wagan KH, Imran M and Abro H (2007). Studies on the control of tomato damping-off disease caused by *Rhizoctonia solani.* Pak. J. Bot., 39(7): 2749-2754.

[39]. Khamna S, Yokota A and Lumyong S (2009). Actinomycetes isolated from medicinal plant rhizosphere soil: diversity and screening of antifungal compound, indole- 3-acetic acid and siderophore production. World J. Microbiol. Biotechnol., 25: 649 - 655.

[40]. Kuprashvili TD (1996). The use phytocides for seed treatment. Zashchita-I- Karantin- Rastenii, 55: 31-32.

[41]. Lendzemo VW, Kuyper TW, Kropff MJ, van Ast A (2005). Field inoculation with arbuscular mycorrhizal fungi reduces *Striga hermonthica* performance on cereal crops and has the potential to contribute to integrated *Striga* management. Field Crops Research., 91:51–61.

[42]. López-Ráez J A, Charnikhova T, Fernández I, Bouwmeester H, Pozo M J (2011). Arbuscular mycorrhizal symbiosis decreases strigolactone production in tomato. J. Plant Physiol., 168, 294–297.

[43]. Lowry OH, Rosebrough NJ, Farr AL et al (1951). Protein measurement with the Folin Phenol reagent. J Biol Chem., 193:265–275.

[44]. Lucas, G.B, Campbell C.L. and Lucas L.T (1997). Introduction to plant disease identification and management. CBS Pub. and Distributors, New Delhi. 364pp.

[45]. Mahmoud YAG, Gaafar RM., Mubarak HM (2007). Genetic diversity among Nile Delta isolates of *Rhizoctonia solani* Kühn based on pathogenicity, compatibility, isozyme analysis and total protein pattern. Turk J Bot., 31 19-29.

[46]. Montealegre J, Valderrama L, Sánchez S, Herrera R, Besoain X, Pérez L (2010). Biological control of *Rhizoctonia solani* in tomatoes with *Trichoderma harzianum* mutants. Electronic Journal of Biotechnology, North America, 1315 -1323.

[47]. Nelson NJ (1955). Colorimetric analysis of sugars. Methods Enzymol, 3, 85-86.

[48]. Passari A K, Mishra V K, Gupta V K,Saikia R and Singh B P (2016). Distribution and identification of endophytic *Streptomyces* species from *Schima wallichii* as potential biocontrol agents against fungal plant pathogens. Polish Journal of Microbiology, Vol. 65(3): 319–329.

[49]. Phillips JM and Hayman DS (1970). Improved procedures for clearing roots and staining parasitic and vesiculararbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc. 55, 158–161.

[50]. Sadasivam S and Manickam A (1996). Biochemical methods, 2nd ed. New International Limited Publishers, New Delhi p 291

[51]. Shirling EB and GottliebD(1966). Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol*., 16: 313-340.

[52]. Silosuh LA., Lethbridge B. J., Raffel S. J., He H., Clardy J. and Handelsman J. (1994). Biological activities of two fungistatic antibiotics produced by *Bacillus cereus* UW85. Appl. Environ. Microbiol., 60:2023–2030.

[53]. Smith SE and Read DJ. (2008). Mycorrhizal symbiosis. 3rd edn. Academic Press.

[54]. Sneh B, Jabaji-Hare S, Neate S and Dijst G (1996). *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Springer Netherlands.

[55]. Strashnov Y, Elad Y, Sivan A. Rudich Y., Chet I (1985).Control of *Rhizoctonia solani* fruit rot of tomatoes by *Trichoderma harzianum* Rifai. Crop Protection Vol. 4(3): 359-366.

[56]. Szczech M and Shoda M (2006). The effect of mode of application of *Bacillus subtilis* RB14-C on its efficacy as a biocontrol agent against *Rhizoctonia solani*. Journal of Phytopathology, 154, 370-377.

[57]. Taechowisan T and Lumyong S (2003). Activity of endophytic actinomycetes from roots of *Zingiber officinale* and *Alpinia galanga* against phytopathogenic fungi. Annals of Microbiology, 53, 291-298.

[58]. Tewari TN and Singh BB (1991). Stress studies in lentil (*Lens esculenta* Moench). II. Sodicity-induced changes in chlorophyll, nitrate, nitrite reductase, nucleic acids, proline, yield and yield components in lentil. Plant Soil, 135: 225-250.

[59]. Tokala RK, Strap JL, Jung CM, Crawford DL, Salove MH, Deobald LA, Bailey JF, Morra MJ (2002) Novel plant microbe rhizosphere interaction involving *Streptomyces lydicus* WYEC108 and the pea plant (*Pisum sativum*). Applied and Environmental Microbiology, 68, 2161-2171.

[60]. Tu, L., He, Y.H., Shan, C.H. and Wu, Z.S. (2016) Preparation of microencapsulated *Bacillus subtilis* SL-13 seed coating agents and their effects on the growth of cotton seedlings. BioMed Research International, Article ID: 3251357.

[61]. Vincent M. N, Harrison LA, Brackin J M, Kovacevich PA., Mukerji P, Weller DM and Pierson E A (1991). Genetic analysis of the antifungal activity of a soilborne Pseudomonas aureofaciens strain. Appl. Environ. Microbiol., 57:2928–2934.

[62]. Watanabe FS and Olsen SR (1965). Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Proceedings of the Soil Science Society of America, 29: 677–678.

[63]. Weller DM, Raaijmakers JM, Gardener BB, Thomashow LS (2002). Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu Rev Phytopathol., 40:309–348. 24.

[64]. Williams ST, Sharpe ME, Molt JC. (1989) Bergey's Mannual of Systematic Bacteriology. Williams and Wilkins (eds); 4: 2300-2648.

[65]. Zohora U, Ano T and Rahman M (2016). Biocontrol of *Rhizoctonia solani* K1 by Iturin A producer *Bacillus subtilis* RB14 seed treatment in tomato plants. Advances in Microbiology, 6, 424-431.

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

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

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

Tested isolate	Inhibition of *Rhizoctonia solani* LB4	Lytic enzyme activity		IAA production		Effect on Tomato seed germination	
	Diameter of inhibition zone (mm)	Chitinase	□-1,3 Glucanase	Dete-ction	Quantity (µg/ml)	%	Index
Streptomyces LN 1	15±2.5*	1.20 ±0.13*	1.48 ±0.19*	++	12.3 ±2.11*	79.4	0.56
Streptomyces LN 3	21±2.1*	0.78 ±0.19*	1.23 ±0.33*	+	4.5 ±5.21*	78.3	0.55
Streptomyces LN 4	20±1.6*	0.98 ±0.15*	1.69 ±0.13*	+++	13.0 ±3.23*	71.9	0.52
Streptomyces LN 9	14±2.0*	0.73 ±0.15*	1.33 ±0.19*	++	14.8 ±1.34*	70.0	0.50
Streptomyces LN 11	11±1.4*	0.94 ±0.07*	1.11 ±0.30*	+	7.9 ±1.15*	78.9	0.55
Streptomyces LN 15	10±1.1*	1.4 0 ±0.25*	1.77 ±0.21*	++	11.7 ±1.42*	79.1	0.56
Streptomyces exfoliates LP10	27±3.3	1.5 ±0.35	2.33 ±0.428	+++	22.0 ±5.39	79.3	0.56
Control (dist. water)	0.0	ND	ND	ND	ND	74.9	0.53

*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

Treatment used / Measured parameters	Control plants	Infected plants with *R. solani*	Infected and treated plants			
			Mycorrhizal fungi	*Streptomyces*	Mycorrhizal fungi + *Streptomyces*	Fungicide (Mancozeb)
% mycorrhizal infection	ND	ND	33%	ND	43%**	ND
% of recovered plants	$100^{bc\#}$	25^{d}	78^{a}	89^{ab}	86^{ab}	96^{b}
Shoot length (cm)	27.2±2.5	13.8±1.7*	18.7±1.9*	21.8±1. 5	23.8±4.9	23.5±2.3
Shoot dry weight (g/plant)	2.39±0.40	0.39±0.04*	1.98±0.17 *	1.99±0.29*	2.33±0.43	2.11±0.31
Root depth (cm)	15.2±2.0	9.6±1.4*	11.1±3.3*	12.9±1. 1	13.6±2.9	13.6±1.7
Root dry weight (g/plant)	0.45±0.15	0.11±0.03*	0.55±0.09	0.43±0.07	0.49±0.11	0.49±0.13

*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.

Treatment used / Measured parameters	Control plants	Infected plants with *R. solani*	Infected and treated plants			
			Mycorrhizal fungi	*Streptomyces*	Mycorrhizal fungi + *Streptomyces*	Fungicide (Mancozeb)
TSS (mg/g, dry wt.)	133.6±9.11	191±11.4*	99±8.9*	108.8±6.4	112±5.9	104±7.7
Protein (mg/g, dry wt.)	131.7±3.41	147.5±6.16*	133.3±5.41	134.4±6.16	131.6±5.61	130.5±4.0
Proline	1.54±0.01	17.4±2.33*	6.7±0.14*	4.9±0.92*	2.2±0.09	1.2±0.09
PO_4^{+++}	12.4±2.2	7.2±1.21 *	16.6±0.55*	13.7±1.22	13.2±3.10	13.8±1.17
N^{+++}	22.5±0.11	16.4±1.51 *	23.5±3.45	22.6±2.16	21.4±2.31	19.6±2.31
Mg^{++}	5.6±1.1	4.6±1.23*	5.4±0.7	5.3±0.4	4.9±0.6	4.8±0.2
K^{+}	13.4±1.3	9.4±0.91 *	13.7±0.8	11.5±1.8	12.6± 2. 1	11.4±3.5

*Significant results at P<0.05 compared to control