Zagazig J. Agric. Res., Vol. 46 No. (5) 2019 1395-1418

Plant Protection and Pathology Research

http:/www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master

ANTAGONISTIC POTENTIAL OF RHIZOSPHERIC BIOCONTROL AGENTS AGAINST SOYBEAN ROOT ROT- WILT DISEASE COMPLEX SYNDROME

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Received: 01/07/2019 ; Accepted: 14/07/2019

ABSTRACT: Biological control is an environmentally safe alternative technique for management of plant pathogenic fungi. This work aims to identify and characterize potential bioagents prevalent in soybean soil. A total of 27 fungal isolates were recovered from soybean plants exhibiting typical wilt and root rot symptoms. Pathogenicity of each isolate was examined on soybean seedlings, and the most pathogenic isolates, were selected. Those isolates were identified based on their microscopic features as *Fusarium oxysporum* Schlecht, *F. solani* Mart and *Rhizoctonia solani* Kuhn. The identity of each isolate was confirmed by molecular phylogeny based on internal transcribed spacer (ITS) sequence. Additional 31 microorganisms of the population within soybean rhizosphere were isolated and their potential antagonistic ability was characterized *in vitro* and *in vivo*. A collection of three fungi, four bacteria, one actinomycete and one yeast isolate showed significant reduction of mycelial growth of the tested fungal pathogens. The same potential bioagents allocate significant decrease in disease severity percentage of wilt-root rot complex disease under greenhouse and open field conditions compared with mycorrhizae and Rhizolex-T. Potential mechanisms by which the bioagents suppressed soybean wilt-root rot complex disease, were addressed. Application with bioagents induced expression of peroxidase, polyphenol oxidase, chitinase, β-1,3-gluctronase, total carbohydrates and total phenols in treated soybeans, which significantly contribute to plant disease resistance. Quality parameters of soybean seeds from bioagent-treated plants showed favorable elevation in total proteins and oils within the seeds.

Key words: Soybean, root rot, wilt, disease complex, antagonism, biological control, rhizosphere.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is one of the most valuable legume crops cultivated worldwide **(Patkowska and Konopinski, 2013).** Diseases have been a major problem for soybean production **(Wrather and Koenning, 2006 and 2009)**. The majority of losses in soybean yield are attributed to microbial diseases. The total loss value differs from one year to another based on production area and environmental conditions (**Hartman *et al.*, 1999)**. The most common soil-borne disease affecting soybean is root rot caused by *Phytophthora sojae*, *Rhizoctonia solani, Pythium* spp. and *Fusarium* spp., and charcoal rot disease caused by *Macrophomina phaseolina* (**Naito *et al.*, 1993; Nelson *et al.*, 1997; Hartman *et al.*, 1999**). Several members of the genus *Fusarium* cause deleterious diseases on soybean such as Fusarium wilt, which caused by *F. oxysporum* (**Armstrong and Armstrong, 1950; El-Kazzaz *et al.*, 2008**), sudden death syndrome (SDS), caused by *Fusarium virguliforme* (**Aoki *et al.*, 2003; Rodríguez and Meneses, 2005**), seed and seedling diseases, and root rot (**Yang and Feng, 2001; Broders *et al.*, 2007)**. At least 18 *Fusarium* species have been isolated from soybean roots on different soybean production areas (**Martinelli *et al.*, 2004; Pioli *et al.*, 2004; Navi and Yang, 2008; Bienapfl *et al.*, 2010; Arias *et al.*, 2011; Bienapfl, 2011**).

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Microscopic methods **(Roy, 1997; Li *et al*., 1998)** are used to identify *Fusarium* spp. but it is often difficult to detect the fungus species in roots and soil that contain other morphologically similar fungal pathogens. A molecular approach using random amplified polymorphic DNA (RAPD) markers as a diagnostic tool for the identification of *Fusarium* spp. cultures, has been reported **(Achenbach *et al*., 1996)**. **O'Donnell and Gray (1995)** designed *F. solani* f. sp. *phaseoli*-specific PCR primers based on rDNA sequences and used to detect the fungus in inoculated soybean roots.

Biological control of plant pathogens is one of the most promising methods for the management of soil-borne diseases. However, the use of fungicides in the management of plant diseases not only pollutes the environment, but also has hazardous effect on human health **(Bürger *et al*., 2012)**. Therefore, there is a need to explore alternative methods for controlling the soybean root rot diseases **(Li and Ma, 2012)**. Microbial communities are abundantly present in rhizosphere or areas under the influence of the root and its close vicinity. Rhizosphere gives support to many active microbial populations capable of exerting beneficial, neutral or detrimental effects on plant growth **(Whipps, 2001).** Microorganisms inhabiting rhizosphere, which beneficially enhance the growth of plants, are specifically known as plant growth promoting rhizobacteria (PGPR). Some genera of bacteria have been determined as PGPR including *Bacillus, Pseudomonas, Azospirillum, Azotobacter, Bradyrhizobium* and *Rhizobium*. Effective PGPR should have at least three characters of promoting plant activities, which are root colonization competency, phytostimulator and biocontrol agent against plant pathogens **(Bloemberg and Lugtenberg, 2001)**. Members of the genus *Trichoderma* are among the most prevalent biocontrol and growth promoting agents for many plants **(Verma *et al*., 2007; Bai *et al*., 2008; Savazzini *et al*., 2009)**. The competition with pathogens, parasitism and the production of antifungal compounds are the most important mechanisms in biocontrol activity **(Verma *et al*., 2007; Savazzini *et al*., 2009)**. Obtained bacteria from soybean rhizosphere, reduced soybean root rot in pot experiment (**Huang *et al*., 2017**). *Trichoderma harzianum* isolate T-soybean, showed growth-promoting activity for soybean seedlings and induced resistance to *Fusarium oxysporum* under greenhouse conditions (**Zhang *et al*., 2009)**. Biotic agents, *i.e., B. subtilis* and *Saccharomyces cerevisiae* were effective in decreasing pre and post damping-off, increasing plant height and branch number. Seed quality was increased as a result (**Abd El-Hai *et al*., 2016)**. Application of *Trichoderma harzianum*, *T. hamatum* and *Bacillus subtilis* significantly decreased soybean root rot disease in naturally pathogen-infested fields (**El-Sayed *et al*., 2009)**.

Thus, the present investigation has been carried out to determine the antagonistic potentiality of some rhizospheric micro-organisms inhabiting soybean root system to the causal agents of wilt/root rot disease complex and in consequence their contribution with plant resistance and vigor.

MATERIALS AND METHODS

Survey of Soybean Wilt-Root Rot Complex Disease

Wilt and root rot diseases were surveyed at three different soybean production areas in Dakahlia, Beni-Suef and Minia Governorates. Samples were collected from three districts of each governorate and two villages per district, during 2014 growing season using the method described by **Horricks (1980)**.

Disease incidence (DI) and disease severity (DS) percentages for each individual location were calculated as described by **Cooke *et al.* (2006)** using the following formula:

Disease incidence (DI) = (number of infected plants/total number of inspected plants) x 100

Disease severity (DS) = [E (n.v) / X.N] x 100

Where:

N = total number of all inspected plants.
n = number of plants at the rate v.
v = the rate of the disease on the following scale.
X = the highest disease severity rate determined from the scale.

Plant diseases severity was calculated according to the scale described by **Abdou *et al.***

(2001). Symptomatic plants were rated on the basis of gradual progression of root discoloration and leaf collapse and yellowing as follows, 0 = neither root discoloration nor leaf yellowing were observed, 1= 1-25% of the total root mass exhibited discoloration and one leaf started to collapse, 2= 26-50% root discoloration and more leaves decline, 3= 51-75% root discoloration plus and leaf wilted, 4= up to 76% root discoloration or more than one leaf wilted, and 5= the entire plant is dead.

Isolation, Identification and Frequency of Fungi Associated with Symptomatic Soybean Plants

Soybean plants showing root rot -wilt disease symptoms were collected from the previously mentioned survey locations for isolation. The root samples were thoroughly washed under running tap water, cut into small pieces (0.5 cm), and surface sterilized by dipping in 0.1% sodium hypochlorite for 2 minutes, then washed three times with sterile distilled water. The surface disinfected pieces were dried under laminar flow hood, then transferred individually to Petri plates containing potato dextrose agar (PDA) medium which were incubated at 25°C for 5 days.

Once growth occurred, the developed fungal colonies were purified using the hyphal tip **(Brown, 1924)** and/or single spore isolation techniques **(Hansen, 1926)**. The purified fungi were identified to their species according to the fungal morphological and microscopical characteristics as described by **Barnett and Hunter (1986)**, **Booth (1977) and Leslie and Summerell (2006)** Fungal Taxonomy Res., Dept., Plant Pathology Res. Inst., Agricultural Res. Center. The obtained fungal isolates were maintained on PDA slant and kept in refrigerator at 4°C for further studies. The frequency of the isolated fungi was calculated separately for each collected sample per district according to **Cooke *et al.* (2006)**.

Pathogenicity of Isolated Fungi from Soybean Root Rot-Wilted Infected Plants

The pathogenicity tests for previously isolated and identified pathogens that showed the most significant isolation frequencies were carried out using soybean cv. Giza 22 in pots infested soil using the homogenized culture technique according to the method devised by **Muthomi *et al.* (2007)**, under greenhouse conditions at the experimental greenhouse of Legumes and Forage Diseases Res. Dept., Plant Pathol. Res. Inst. ARC, Giza, Egypt. Soybean seeds (cv Giza22) were sown at the rate of 10 seeds/pot (30 cm in diameter). A set of five replicates were used for each fungus. Five pots containing non-infested soil were used as control. Percentage of damping-off was recorded 30 days after sowing. Percentage of healthy survival plants show no visual evidence of disease were calculated 3 months after sowing. Severity of wilt diseases was determined after 90 days according to **Abdou *et al.* (2001)** using a previously rating scale of 0 to 5 on the basis of root discoloration or leaf yellowing.

Molecular Identification of the Fungal Pathogens

Extraction of DNA

Identification was carried out based on rDNA-ITS sequence for each potential pathogen. Fungi were cultured on PDA medium for seven days at 28^{o}C, and aerial mycelium was scraped gently under aseptic conditions. Two hundred mg of freshly harvested mycelium were used for DNA extraction using Qiagen DNA extraction kit (QIAamp® DNA Mini Kit 50) according to the manufacturer's protocol. Aliquots of DNA were treated with RNase enzyme type A at 37°C for one hour to remove any RNA contaminants. Purified DNA extracts were stored in White Point upright freezer at -20°C till further use.

PCR Amplification

A 1:100 dilution of DNA was used as a template for PCR amplification with the aid of ITS1 + ITS4 primers designed by **White *et al.* (1990)** targeting the ribosomal DNA- Internal transcribed spacer (rDNA-ITS) region.

Polymerase chain reaction was conducted using Vent DNA Polymerase (NEB Biolabs, UK). The PCR reaction mixture was built for each pathogen and subjected to the thermal cycler protocol recommended by the DNA polymerase manufacturer. The PCR products were analyzed by gel electrophoresis on 1%

agarose gel. DNA bands were visualized and photographed using Gel Doc EZ Imager, Bio-Rad at Genetics DEPT., Faculty of Agriculture, Zagazig University. For sequencing purpose, PCR products were purified using Qiagen gel extraction kit (QIAquick Gel Extraction Kit) and sent for sequencing at AuGCT facility, China. Bidirectional sequencing was performed using the same primers based on Sanger's sequencing method **(Sanger *et al*., 1977)**.

Phylogenetic analysis

Phylogenetic reconstruction was performed using bidirectional sequences of ITS-5.8S rDNA. Consensus sequences were determined and assembled using MEGA X **(Kumar *et al*., 2018)** and compared to the database in NCBI GenBank using BLASTn search tool. Reference strains were selected and used for multiple sequence alignment using MUSCLE algorithm in MEGA X. GenBank accession number for each reference strain is indicated in the resulted phylogenetic tree. Sequence alignments were manually edited and trimmed when needed. The tree-making algorithms, namely Neighbor-Joining method **(Saitou and Nei, 1987)** from the MEGA X was used to infer the phylogenetic evolutionary trees. To assess branch support, data sets were sampled with 500 bootstrap replicates. *Gibberella zeae* strain CZ545-1 and *Athelia rolfsii* were used as outgroups for Fusarium and Rhizoctonia, respectively.

Biological Control

Selection and Evaluation of Potential Bioagents, *in vitro*

Isolation of microorganisms

Soil samples collected from soybean plots located at Agricultural Research Center in Giza, were used as source of microorganisms. Serial dilutions from the rhizospheric soil were prepared in a sterilized distilled water and used for isolation using the dilution plate method described by **Aneja (2003)**. The dilutions 10^{-2} to10^{-4} were used for fungal isolation on Peptone Dextrose Rose Bengal Agar medium **(Johnson *et al.,* 1960)**, 10^{-5} to10^{-6} were used for bacterial isolation on Soil Extract Agar medium **(Lochhead, 1940)**, and 10^{-6} to10^{-7} were used for actinomycete isolation on Jensen's agar medium **(Jensen, 1930)**. Petri dishes were incubated at 28^{o}C±2. The developed microbial colonies were purified and characterized based on their morphological features and microscopical characteristics based on **Schaad *et al.* (2001)** for bacteria, **Domsch *et al.* (2007)** for fungi, **Sakure *et al.* (2015)** for actinomycetes, and **Botha (2006)** for yeast.

Selection of potential bioagents

A total of 17 bacterial isolates, 10 yeast isolates, 3 fungal isolates and one actinomycete isolate were obtained after isolation process. The inhibitory effect of each microbial isolate was evaluated *in vitro* against the pathogenic fungal isolates using the dual culture assay for fungal isolates and the streaking plate method 2 days after pathogen inoculation for bacteria and actinomycete isolates **(Henis and Inbar, 1968)**. Reduction percent of the pathogen's growth were calculated after full growth of the fungal pathogen in the control treatments.

Identification of bioagents

The identification processes of antagonistic bacterial isolates were carried out in the Department of Bacterial Diseases, Plant Pathology Research Institute (PPRI), Agricultural Research Center (ARC), Egypt based on their morphological and microscopical characteristics. Fungal isolates with potential antagonistic capability were identified in the Department of Fungal Taxonomy Res. Dept, PPRI, ARC.

Evaluation of beneficial traits of selected bioagents

To analyze the inhibition mechanism and growth promotion of soybean seedings, the following metabolic activities were measured within the growth cultures of each potential bioagent.

a. Measurement of IAA: the endogenous plant auxin (indole acetic acid IAA) was quantified by the method of **Patten and Glick (2002)**, and expressed as g/l.

b. Phosphate solubilization: The ability of microbial bioagents to solubilize tricalcium phosphate was evaluated using Pikovskaya's Agar **(Wahyudi *et al.,* 2011)**.

c. Siderophore production: Qualitative measurement of siderophore production in the microbial

cultures was carried out using chrome azurol S (CAS-medium) according to **Husen (2003)**.

Biocontrol Efficiency of Bioagents on Soybean Root Rot-wilt, *In vivo*

Effect of bioagents on artificially infected soybeans under greenhouse conditions

The biocontrol capability of the isolated microorganisms was examined in pot experiment under greenhouse conditions. Sterilized pots (25cm in diameter) containing sterilized soil were used. The soil was infested with each examined pathogen individually and combined at equal ratios. Healthy looking soybean seeds cv. Giza 22 were sorted out into 11 groups and coated with different bioagents, chemical and mycorrhizae. Biocontrol agents were grown on 200 ml potato dextrose broth medium, filtered and air-dried. Fungal growth was mixed with talc powder at the rate 1:1 (*W:W*) and used for seed dressing with the aid of the arabic gum. The chemical pesticide Rizolex-T was used as a positive control as seed coating at the rate of 3g/kg. A commercial formulation containing the mycorrhizal fungus *Glomus* sp. was purchased from Microbiology Res. Dept., ARC, and used for seed dressing as well. Additional group of seeds was coated with the arabic gum and used as a negative control. Percentage of damping-off and survived healthy or infected plants was calculated as mentioned earlier.

Biochemical analysis of soybean yield

Five soybean plants were randomly selected from each treatment for biochemical evaluation of enzymatic activities, phenol and carbohydrate contents at reproductive stages two and four (R2 and R4). All measurements were evaluated using the Milton Roy Spectronic 601 spectrophotometer.

Analysis of enzymatic activity

Colorimetric analysis was performed for the following enzymes to measure their activity at the growth stage R2-R4. Leaves of soybean seedlings treated with bioagents and grown in pathogen-infested soil were collected and homogenized in liquid nitrogen. One gram of powdered sample was extracted with 2 ml of sodium phosphate buffer 0.1 M (pH 7.0). The homogenate was centrifuged for 20 min at 10,000 rpm and the protein extract were used for estimation of defense enzymes. Wavelength was set to the required value for each enzyme. The Enzyme Commission (EC number), which indicates the numerical class of each enzyme based on the chemical reactions it catalyzes, is shown next to each enzyme below.

a- Peroxidase PO (EC 1.11.1.7) activity was recorded as the change in absorbance per minute immediately after the addition of substrate according to **Kochba *et al.* (1977)**. The activity was expressed as the increase in absorbance at 430 nm min^{-1} mg^{-1} of protein.

b- Polyphenol oxidase PPO (EC 1.14.18.1) activity was measured according to the method described by **Maxwell and Bateman (1967)** and the activity was expressed as change in absorbance min^{-1} mg^{-1} of protein.

c- Chitinase (EC 3.2.1.14) was extracted from vegetative parts according to the method described by **Reid and Ogrydziak (1981)**. Enzyme activity was determined at wavelength 544 nm. according to **Reissig *et al.* (1955)**. The enzyme activity was expressed as *n* moles O-linked N-acetylglucosamine (GlcNAc) equivalents min^{-1} g^{-1} fresh weight.

d- B-1,3-glucanase (E.C. 3.2.1.39) activity was assayed by the laminarin-dinitrosalicylic acid method described by **Dann and Deverall (2000)** at wavelength 610 nm. The enzyme activity was expressed as mg glucose released min^{-1} mg^{-1} of sample.

Analysis of phenolic content

Total phenols were determined using the Folin-Ciocalteau reagent as mentioned by **Snell and Snell (1953)**. The absorbance was measured at 520 nm and the phenol amount was expressed as mg/g.

Analysis of carbohydrates

Carbohydrates were extracted as described by **Snell and Snell (1953)** and color optical density of the reacted mixture was measured on absorbance at 540 nm using the picric acid technique described by **Thomas and Dutcher (1924)**, and results were expressed as mg/g fresh weight.

Effect of bioagents on naturally infected soybeans under field conditions

The experiments were conducted in naturally infected field at Itay El-Baroud Agriculture Research Station, Behaira Governorate, Egypt (GPS coordination: 30°53'09.8"N 30°38'55.8"E) during two successive growing seasons, 2015 and 2016. The experimental design was completely randomized block. The plot was divided to equal subplots each one consisting of 4 ridges (3.5 m long and 60 cm in width). Three replicates were used for each treatment. Soybean seeds cv. Giza 22 were individually treated with fungicide, bioagents and mycorrhizae, as previously mentioned. All treatments were sown in hills 20 cm apart on both sides of the ridge and two seeds per hill (plant population = 140,000 plants/faddan). All recommended agricultural practices were adopted.

Percentages of damping–off and healthy survived and infected plants were calculated as mentioned earlier. At harvest stage, plant growth parameters including plant height, number of branches and pods per plant and seed yield per faddan were recorded.

Biochemical analysis of total protein and oils in seed yield

Seed samples were collected and ground to a fine powder using a mechanical grinder.

a. Total protein content within seed powder was quantified by Bradford method **(Bradford, 1976)** at wavelength 610 nm.

b. Total oils were determined in seed powder as described by **Adnan (2016)** using Electrothermal Sexhulet System EME6 9599/CE MK1.

Statistical Analysis

Data were statistically analyzed using one- and two-way ANOVA in SPSS software **(Snedecor and Cochran, 1980)**. Means were compared by the least significant difference value "LSD" at 5% level of probability.

RESULTS AND DISCUSSION

Survey of Soybean Root Rot-Wilt Disease Complex

Results in Table 1 reveal that the disease incidence percentage (DI%) ranged from 15.4% to 32.2% in different locations, while disease severity percentage (DS%) ranged from 9.5 % to 18.4%.

Frequency (%) of the Isolated Fungi from Root Rot-Wilt Infected Soybeans

Results in Table 3 shows that 27 fungal pathogens were isolated from infected soybean roots. Soybean root rot-wilt disease, caused by a complex infection of many soil inhabitants, has become an increasing problem for soybeans **(Roy *et al*., 2000; Wang *et al*., 2004)**.

Fusarium solani, *Fusarium oxysporum* and *R solani* were the most frequently isolated fungi (Table 2). *F. solani* presented only in Minia Governorate (40% in Minia, 70% in Samalot, and 66.6% in Matai). The highest frequency 76.6% of *F. oxysporum* obtained from El-Fashn, Beni-Suef Governorate followed by 66.7% of Nasser city of the same governorate. *F. oxysporum* presented also in Dakahlia Governorate but did not presented in Minia. Possible reasons for these patterns include differences in climate and topography, crop management practices and environmental conditions possible related to soil types **(Arias, 2012)**. The highest frequency 70% of *Rhizoctonia solani* was obtained from Talkha, Dakahlia Governorate followed by 63.3% from Meet-Ghamr of the same governorate.

Many investigators have reported that *Fusarium oxysporum*, *F. solani*, *Rhizoctonia solani*, *Macrophomina phaseolina* and *Sclerotium rolfsii* are the causal agents of root rot/wilt disease of soybean plants in Egypt **(Fayzalla *et al.,* 2009; Abd El-Hai *et al*., 2010 and 2016; Ragab *et al.*, 2017)**.

Pathogenicity Test

Significant variations in aggressiveness was observed among pathogenic isolates. When plants infected by *F. solani*[2], *F. oxysporum*[4] and *R. solani*[4], there were no plants to be survived, however, the highest percentage of survived plants was recorded when they infected by *Sclerotium rolfesii* [3] and *M. phaseolina*[2] (66.7%) as shown in Table 3. These observations would indicate that *F. oxysporum* may be more important as a pathogen on cotyledons, and *F. solani* more important as a root pathogen. The

explain of the high percentage of seedling and plants mortality could be due to isolate aggressiveness. Morphology and genetic diversity might provide important information on the biology and epidemiology of *Fusarium* and *Rhizoctonia* species on soybean **(Arias, 2012)**.

Many investigators noted that *F. solani*, *R. solani*, *M. phaseolina*, *F. oxysporum* and *S. rolfsii* are considered among the main pathogens causing root rot diseases of soybean plants **(Abd El-Hai *et al.*, 2010; Mishra *et al.*, 2011; Dalal and Kulkarni, 2014; Abd El-Hai *et al.*, 2016; Ragab *et al.*, 2017)**. In Egypt, **Abd El-Hai *et al.*, (2010)** reported that *F. oxysporum*, *F. solani*, *Macrophomina phaseolina* and *R. solani* were the main pathogens for root diseases affecting soybean plants.

Table 1. Survey of soybean root rot-wilt disease complex during 2014 growing season in three governorates. Percentages of disease incidence (DI) and disease severity index (DS) were calculated

Gov.	Minia			Beni-Suef			Dakahlia		
City	Minia	Samalot	Matai	Beni- suef	Nasser city	El-Fashn	Mit Ghamr	Belqas	Talkha
DI %	32.2	32.2	27.9	27.5	22.7	29.7	15.6	17.5	15.4
DS %	17.8	18.4	12.2	16.1	13.3	10.2	11.1	10.4	9.5
F. sol	+	+	+	-	-	-	-	-	-
F. oxy	-	-	-	+	+	+	+	+	+
R. sol	+	-	-	+	+	-	+	-	+

Gov.= Governorate, F. sol= *Fusarium solani*, F. oxy= *Fusarium oxysporum*, R. sol=*Rhizoctonia solani*

Table 2. Frequency (%) of the isolated fungi from diseased soybeans collected from Minia, Beni-Suef and Dakahlia governorates during the growing seasons 2014

Governorate		*F. solani*	*F. oxysporum*	*F. avenaceum*	*F. subglutinans*	*F. proliferatum*	*F. semitectum*	*A. alternata*	*R. solani*	*S. rolfsii*	*M. phaseolina*	*P. gregata*	Unidentified fungal genera
Minia	**Minia**	40	0	0	20	20	0	0	16.7	0	3.3	0	0
	Samalot	70	0	0	0	0	0	0	0	13.3	13.3	0	3.4
	Matai	66.6	0	0	0	0	0	0	0	16.7	16.7	0	0
Beni-Suef	**Beni-Suef**	0	23.3	20	0	0	23.3	10	13.3	0	0	10	0
	Nasser city	0	66.7	0	0	0	0	0	30	0	0	0	3.3
	El- Fashn	0	76.6	0	0	0	0	0	0	0	16.7	0	6.7
Dakahlia	**Mit-Ghamr**	0	30	0	0	0	0	0	63.3	0	0	0	6.7
	Belqas	0	70	0	0	0	0	0	0	20	0	0	10
	Talkha	0	23.3	0	0	0	0	0	70	0	0	0	6.7
Total		19.6	32.2	2.2	2.2	2.2	2.6	1.1	21.5	5.6	5.6	1.1	4.1

Table 3. Pathogenicity test of isolated fungi from soybean root rot-wilt infected plants under greenhouse conditions

Fungi isolate	Location	Pre- (%)	Post- (%)	Dead plants (%)	Survivals (%)
F. solani [1]	El-Minia	26.7	26.7	30.0	16.7
F. solani [2]	Samalot	56.7	26.7	16.7	0.0
F. solani [3]	Matai	23.3	26.7	23.3	26.7
F. oxysporum [4]	Beni-Suef	0.0	33.3	66.7	0.0
F. oxysporum [5]	Nasser City	0.0	33.3	36.7	30.0
F. oxysporum [6]	El-Fashn	0.0	30.0	40.0	33.0
F. oxysporum [7]	Mit-Ghamr	0.0	23.3	36.7	40.0
F. oxysporum [8]	Belqas	0.0	26.7	33.3	40.0
F. oxysporum [9]	Talkha	0.0	36.7	23.3	40.0
F. avenaceum	Beni-Suef	20.0	13.3	20.0	46.7
F. subglutinans	El-Minia	23.3	26.7	23.3	26.7
F. proliferatum	El-Minia	23.3	40.0	13.3	33.3
F. semitectum	Beni-Suef	23.3	26.7	23.3	26.7
A. alternata	Beni-Suef	20.0	26.7	0.0	3.3
R. solani [1]	El-Minia	13.3	20.0	16.7	50.0
R. solani [4]	Beni-Suef	56.7	33.3	13.3	0.0
R. solani [5]	Nasser City	10.0	23.3	30.0	36.7
R. solani [7]	Mit-Ghamr	3.3	30.0	30.0	40.0
R. solani[9]	Talkha	13.3	16.7	13.3	36.7
S. rolfsii [2]	Samalot	16.7	10.0	20.0	53.3
S. rolfsii [3]	Matai	10.0	6.7	16.7	66.7
S. rolfsii [8]	Belqas	16.7	20.0	3.3	60.0
M. phaseolina [1]	El-Minia	6.7	16.7	13.3	63.3
M. phaseolina [2]	Samalot	6.7	23.3	13.3	66.7
M. phaseolina[3]	Matai	3.3	13.3	20.0	63.3
M. phaseolina [6]	El-Fashn	3.3	16.7	16.7	63.3
P. gregata	Beni-Suef	13.3	13.3	23.3	53.3
Control		0.0	0.0	0.0	100.0
LSD (5 %)		1.367	1.810	3.884	4.699

Pre- = Pre-emergence damping-off, Post- = Post-emergence damping-off.

Molecular Identification of Most Virulent Isolated Fungi

Three potential pathogens were isolated from symptomatic soybean plants and characterized to the genus level based on morphological and microscopical features as *Fusarium* sp.1, *Fusarium* sp.2 and *Rhizoctonia* sp. Phylogenetic analyses based on rDNA-ITS molecular marker inferred two clades and resolved Fusarium isolate 1 into *Fusarium solani* clade with 100% nucleotide sequence identity to the tested reference strains, while Fusarium isolate 2 was associated to *Fusarium oxysporum* clade with highest level of identity to *F. oxysporum* strain GG2F93 (Fig. 1A). *Rhizoctonia* sp. on the other hand, showed the highest levels of similarity to *Rhizoctonia solani* clade and its teleomorph stage, *Ceratobasidium* (Fig. 1B). Thus, these three potential pathogens were identified as *F. solani*, *F. oxysporum* and *R. solani*.

Biological Control

Many researchers used microorganisms as antagonistic to plant pathogens as an alternative control method to fungicides as seed or soil treatments **(Raspor *et al*., 2010).**

Selection and Evaluation of Potential Bioagents, *In vitro*

Isolation of microorganisms

In the present investigation, rhizospheric isolation yielded several kinds of microorganisms including different three fungal isolates, seventeen bacterial ones, ten isolates of yeast and only one isolate of actinomycete. Rhizospheric microorganisms provide primary defense line against pathogen attacks **(Weller, 1988)**. Interaction of beneficial microbes with plant root in the rhizospheric region help in plant growth promotion resulted in high yield **(Jain *et al*., 2016)**.

Selection of potential bioagents

Results in Table 4 reveals that the bacterial isolates 10, 13, 16 and 17 had the highest effect on the mycelial growth reduction percent of each of the identified three fungi (*F. oxysporum*, *F. solani* and *Rhizoctonia solani*). Actinomycetes isolate had a moderate effect on *F. oxysporum*, *F. solani* and *R. solani* (46.3, 52.8 and 54.6%, respectively).

Results in Table 5 denote that the fungal isolate F.3 imply the highest growth mycelial reduction percentage of all tested pathogens. When fungus F.2 had been tested, *F. oxysporum* was the most suppressed pathogen (80.6%) followed by *F. solani* (72.9%), while the least suppressed one was *R. solani* (59.2%). The possible agent fungus F.1 reduced the growth of *F. oxysporum*, *F. solani* and *R. solani* by 73.2, 58.9 and 65.2%, respectively. The possible agent yeast Y6 had a similar effect on pathogens growth reduction. It reduced the growth of *F. oxysporum*, *F. solani* and *R. solani* by 68.5, 57.2 and 70.9%, respectively.

Identification of bioagents

Microorganisms of high biocontrol potential were identified to the species level based on their morphological, microscopic and physiological characters. The identification processes resulted in four bacterial agents namely as *Bacillus subtilis* 2, *Pseudomonas* sp.1, *Bacillus subtilis1* and *Pseudomonas* sp.2; three fungal isolates, *Trichoderma viride*, *Trichoderma harzianum* and *Chaetomium globosum*; an actinomycete isolate (*Streptomyces* sp.) and a yeast agent identified as *Debaryomyces* sp. (Table 6).

Several mechanisms are responsible for the suppression of fungal pathogens by bacteria, including competition, antibiotic and metabolite production. The inhibition of radial growth by the forming inhibition zone is considered as antibiosis, whereby the antibiotic metabolites may penetrate the pathogen cell and inhibit its activity by chemical toxicity. *B. subtilis* produced several kinds of antimicrobial peptide substances such as subtilin, bacilysin, mycobacillisyn, and iturin **(Yoshida *et al*., 2001)**. This also confirmed by **Carvalho *et al*. (2014)**, who indicated that selected bioagents might have several modes of action in inhibiting colonial growth of pathogens, such as competition for nutrients and space, mycoparasitism and antibiosis.

Evaluation of beneficial traits of selected bioagents

a. Measurement of IAA: All tested bioagents showed IAA production in culture supplemented with tryptophan in the range of 0.132 to 0.318 g/l (Table 7). The ability of isolates to use tryptophan supplemented in the cultivation

Table 4. Effect of rhizospheric bacteria (B.) and actinomycete (A) on growth of the pathogenic fungi, *in vitro.* Results are presented as growth reduction (%)

Microbial isolate	*F. oxysporum*	*F. solani*	*R. solani*
B. 1	38.3	44.4	36.5
B. 2	25.2	24.8	27.0
B. 3	49.4	21.3	22.4
B. 4	29.6	26.3	27.9
B. 5	29.6	21.8	12.6
B. 6	45.4	23.1	20.4
B. 7	28.2	37.0	25.5
B. 8	25.4	45.2	20.0
B. 9	20.9	20.4	21.3
B. 10	70.0	68.3	62.8
B. 11	31.5	29.7	22.8
B. 12	32.0	25.9	29.0
B. 13	69.4	69.5	85.7
B. 14	26.5	27.8	29.6
B. 15	30.6	25.0	39.9
B. 16	67.1	58.3	67.6
B. 17	60.2	55.6	59.8
A. 1	46.3	52.8	54.6
Control	0.0	0.0	0.0
LSD 5%	0.938	1.244	1.206

Table 5. Effect of rhizospheric yeast (Y.) and fungi (F.) on growth of the pathogenic fungi, *in vitro.* Results are presented as growth reduction (%)

Microbial isolate	*F. oxysporum*	*F. solani*	*R. solani*
Y. 1	32.4	27.6	29.6
Y. 2	33.3	23.5	25.2
Y. 3	27.8	28.2	11.1
Y. 4	37.0	28.0	29.4
Y. 5	31.5	30.4	26.9
Y. 6	68.5	57.2	70.9
Y. 7	34.2	28.0	11.1
Y. 8	32.4	35.2	31.5
Y. 9	34.1	27.4	27.2
Y. 10	32.4	29.3	26.7
F. 1	73.2	58.9	65.2
F. 2	80.6	72.9	59.2
F. 3	94.4	75.4	77.2
Control	0.0	0.0	0.0
LSD 5%	2.302	1.061	1.417

Table 6. Identification of the bioagents isolated from soybean rhizosphere

Isolate code	Scientific name
B. 10	*Bacillus subtilis*2 Randy
B. 13	*Pseudomonas* sp.1
B. 16	*Bacillus subtilis*1 Randy
B. 17	*Pseudomonas* sp.2
A.1	*Streptomyces* sp.
Y. 6	*Debaryomyces* sp. Meyen
F. 1	*Chaetomium globosum* Kunze
F. 2	*Trichoderma viride* Pers
F. 3	*Trichoderma harzianum* Rifai

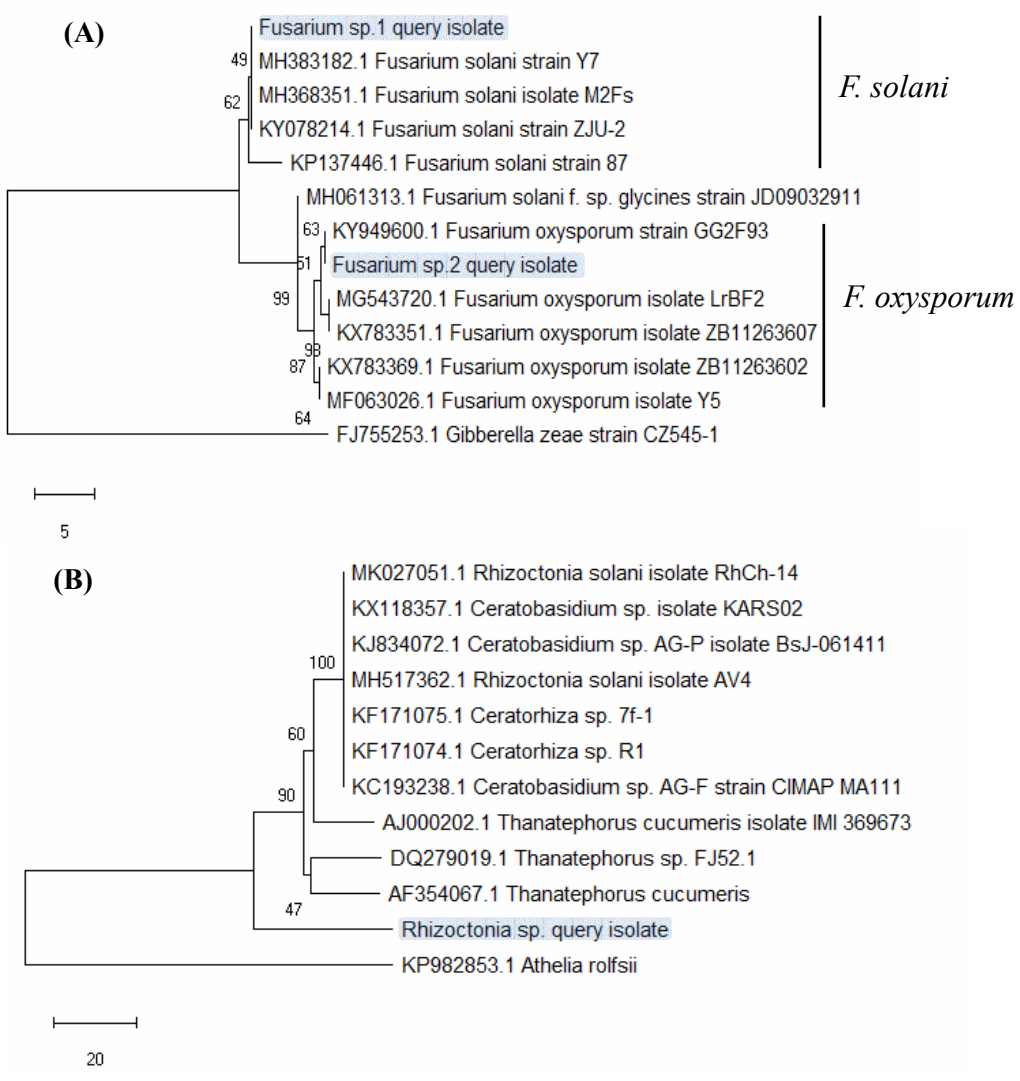

Fig. 1. Phylogenetic tree showing the relationships of three fungal isolates to closer species within the genera *Fusarium* (A) and *Rhizoctonia* (B). The tree was based on an alignment of ITS-5.8S rDNA marker and constructed using the Neighbour-Joining analysis. The numbers at the nodes indicate the percentages of bootstrap support of 500 resampled datasets. The scale bar indicated 5 and 20 substitutions per nucleotide position for panel A and B, respectively. Evolutionary analyses were conducted in MEGA X

Table 7. Beneficial traits of selected bioagents for plant growth and development

Bioagent	IAA (g/l)	Siderophores (+/–)	P-solubilization (+/–)
***B. subtilis*1**	0.286	+	+
***Pseudomonas* sp.1**	0.318	+	+
***B. subtilis*2**	0.227	+	+
***Pseudomonas* sp.2**	0.183	+	–
***Debaryomyces* sp.**	0.287	+	+
***Streptomyces* sp.**	0.177	+	+
T. viride	0.174	+	+
T. harzianum	0.167	+	+
C. globosum	0.132	+	–

medium is one of the important points to determine IAA producing activity. Tryptophan is the main precursor of IAA biosynthesis in bacteria *via* indole pyruvic acid (IPA) pathway. The ability of the isolates to increase plant growth in germinating seed bioassay is highly related to the IAA production, which was produced by bioagents isolates **(Wahyudi *et al*., 2011)**.

b. Phosphate solubilization: *Chaetomium globosum* and *Pseudomonas* sp.1 did not show phosphate solubilization, while the other seven bioagents showed significant phosphate solubilization activity. *Bacillus* spp. that significantly promoted soybean seedling, were able to solubilize phosphate **(Wahyudi *et al*., 2011)**. The ability of several isolates to solubilize tricalcium phosphate *in vitro* shows the possible application of the isolates in crop cultivation fields. The obtained results were in accordance with **Rodriguez and Fraga (1999)** who demonstrated that, *Pseudomonas* sp. and *Bacillus* sp. were able to increase the availability of phosphorus in soil. Such bacterial isolates could be used as phosphate solubilizers in alkaline soil, based on the ability to release phosphate bounded by calcium which mostly exists in alkaline soils, whereas in the acidic soil, phosphate is mostly fixed by Fe or Al **(Glodstein, 1995)**.

b. Siderophore production: All nine bioagents were able to produce siderophore. All isolates which produced the bioactive compound siderophore were able to inhibit the phytopathogenic fungi (**Wahyudi *et al*., 2011).** Siderophore is one of the biocontrol mechanisms belonging to PGPR groups, under iron limiting condition. PGPR produces a range of siderophore which have a very high affinity for iron. Therefore, the low availability of iron in the environment would suppress the growth of pathogenic organisms including plant pathogenic fungi **(Whipps, 2001)**.

Biocontrol Efficiency of Bioagents on Soybean Dease, *In vivo*

Effect of bioagents on artificially infected soybeans under greenhouse conditions

Results in Table 8 indicate variable significant effects of seed treatment with each bioagent on the artificial infection under greenhouse conditions. Seed coating with biocontrol agents was the most effective treatment for controlling root rot diseases as shown by **Loeffez *et al*. (1986), Callan *et al*. (1990 and 1991), Jahn and Puls (1998), Abdel-Kader and Ashour (1999) and Warren and Bennett (1999)**.

a. Pre- and post-emergence damping-off: There was no pre-emergence damping-off caused by *F. oxysporum* in all treatments. The nine tested bioagents and treatment with mycorrhizae significantly reduced pre and post-emergence damping-off of soybean seedlings compared with untreated control.

Table 8. Effect of seed treatment with bioagents on the artificially infected soybean plants by different pathogens and their mixture, under greenhouse condition (season 2014)

Treatment	*Fuasrium oxysporum*					*Fuasrium solani*					*Rhizoctonia solani*					Fungal mixture				
				Survivals					Survivals					Survivals					Survivals	
	Pre emergence damping-off (%)	Post emergence damping-off (%)	Dead Plants (%)	Infected (%)	Healthy (%)	Pre emergence damping-off (%)	Post emergence damping-off (%)	Dead Plants (%)	Infected (%)	Healthy (%)	Pre emergence damping-off (%)	Post emergence damping-off (%)	Dead Plants (%)	Infected (%)	Healthy (%)	Pre emergence damping-off (%)	Post emergence damping-off (%)	Dead Plants (%)	Infected (%)	Healthy (%)
***B. subtilis*1**	0.0	15.0	8.3	3.3	73.4	6.7	6.7	10.0	3.3	73.3	6.7	13.3	10.0	0.0	70.0	20.0	10.0	16.7	3.3	50.0
***Pseudomonas* sp.1**	0.0	15.0	11.7	6.7	66.6	13.3	10.0	10.0	0.0	66.7	6.7	13.3	3.3	10.0	66.7	16.7	6.7	13.3	6.7	56.6
***B. subtilis* 2**	0.0	20.0	6.7	3.3	70.0	10.0	10.0	5.0	5.0	70.0	6.7	16.7	3.3	3.3	70.0	20.0	13.3	3.3	10.0	53.4
***Pseudomonas* sp.2**	0.0	20.0	6.7	3.3	70.0	0.0	13.3	13.3	0.0	73.4	13.3	10.0	0.0	3.3	73.4	23.3	10.0	10.0	3.3	53.4
***Debaryomyces* sp.**	0.0	20.0	10.0	6.7	63.3	8.3	8.3	11.4	8.3	63.7	6.7	10.0	8.3	8.3	66.7	20.0	11.7	8.3	6.7	53.3
***Streptomyces* sp.**	0.0	13.3	6.7	10.0	70.0	10.0	10.0	6.7	3.3	70.0	3.3	13.3	10.0	0.0	73.4	23.3	11.7	5.0	3.3	56.7
T. viride	0.0	3.3	10.0	16.7	70.0	0.0	13.3	13.3	0.0	73.4	8.3	15.0	0.0	6.7	70.0	13.3	11.7	6.7	10.0	58.3
T. harzianum	0.0	15.0	8.3	6.7	70.0	13.3	13.3	0.0	3.4	70.0	8.3	10.0	8.3	3.4	70.0	20.0	11.7	8.3	6.7	53.3
C. globosum	0.0	16.7	6.7	16.7	59.9	16.7	11.7	.3	3.3	60.0	8.3	13.3	8.3	6.7	63.4	23.3	6.7	16.7	0.0	53.3
Mycorrhizae	0.0	13.3	10.0	6.7	60.0	16.7	13.3	10.0	0.0	60.0	13.3	13.3	8.3	8.3	56.8	23.3	13.3	10.0	3.3	50.1
Rizolex-T	0.0	13.3	0.0	0.0	86.7	3.3	3.3	6.7	0.0	86.7	13.3	0.0	0.0	0.0	86.7	13.3	11.7	5.0	0.0	70.0
Control	0.0	36.7	16.7	26.8	20.0	23.3	30.1	23.3	0.0	23.3	20.0	26.7	23.3	0.0	30.0	46.7	43.3	6.7	3.3	0.0
LSD (5%)	0.0	1.783	0.833	1.958	5.558	1.215	1.675	1.083	0.738	3.392	0.975	1.117	0.589	1.337	4.725	1.391	1.891	0.771	0.315	4.883

b. Dead plants: Results revealed that bioagent treatments reduced dead plants compared with untreated control.

c. Healthy survived plants: Bioagent treatments reduced the infection and induced the survived plants percentage. The highest percentage of healthy survived plants was achieved in case of *T. harzianum* treatment, when soil was infested with *F. oxysporum*, *F. solani* and *R. solani*. The highest percentage of survived plants was in case of *P. fluorescens* and *T. viride*, when soil was infested with a mixture of the three fungi. This result confirmed by **Ragab *et al.* (2017)**, who indicated that dressed soybean seeds with *T. harzianum* or Rizolex-T gave the highest increase in survived plants when compared with other treatments. The species of *Trichoderma* are known to produce antibiotics, such as trichodermin, trichodermol, trichotoxin, harzianum A and harzianolide **(Živković *et al.*, 2010)**. These compounds were responsible for inhibiting pathogen isolates.

Wahyudi *et al.* (2011) suggested that, siderophore produced by bacteria functions as suppressor of the phytopathogenic fungi such as *F. oxysporum* or *R. solani*. In addition to IAA production, phosphate solubilization and siderophore, other mechanisms of biocontrol including antibiotic compounds, elicitation of induced systemic resistance (ISR) of plant, and lytic enzyme secretion **(Haas and Defago, 2005)**.

Biochemical Analysis of Soybean Yield

Analysis of enzymatic activity

Results in Table 9 reveal variable effects of bioagents upon artificial infection on the activity of certain enzymes *e.g.* Peroxidase, Polyphenol oxidase, chitinase and/or β-1,3-gluctronase enzyme in soybean leaf samples. Enzymatic activities were increased in soybean plant tissues grown from seeds pre-treated with the different bioagents and mycorrhizae if compared with fungicide and untreated control.

Peroxidase and polyphenol oxidase enzymes are involved in the oxidation of specific host metabolites which in turn act as growth inhibitors of the phytopathogen **(Chen *et al.*, 2000)**. Meanwhile, **Majdah and Al-Tuwaijri (2009)** predicate that treatment with the biocontrol agents (*T. viride* and *B. subtilis*) resulted in a significant decrease in the activity of all the oxidative enzymes tested in the tissues of infected cucumber plants as compared to infected untreated plants. The rates of activity became comparable to that of the healthy plants. This might be due to that chitinase hydrolyzes the N-acetylglucosamine polymer chitin, and occur in diverse plant tissues over a broad range of crop and non-crop species (**Zamir and Zhang, 1993)**. The production of cell wall-degrading enzymes is considered as an action involved in biocontrol of pathogen **(Adams, 2004; Zeilinger and Omann, 2007; Vinale *et al*., 2008).** According to **Zeilinger and Omann (2007)** both enzymes production and infection structure formation are induced responses activated by the diffusible factor.

Total phenols and carbohydrate contents

Phenol contents as shown from Table 10 were increased as a result of application of the bioagents in soybean plant tissues in all the investigated treatments compared with the control and even of Rhizolex-T treatment. The same trend was also detected for most of the applied bioagents in increasing the total carbohydrates. Seeds pre-treated with *Streptomyces* sp. denote great increase of both of total phenols and total carbohydrate contents. Increasing the phenol contents might be due to that biocontrol agents lead to an increase of the control ability. Biocontrol agents might interact with the plant for exchange metabolites and that could cause significant changes in plant metabolism **(Vinale *et al*., 2008)**.

Effect of bioagents on naturally infected soybeans under field conditions

Results in Table 11 indicate that soybean seeds treated with either bioagents, mycorrhizae and/ or fungicide, under field conditions of two successive seasons 2015 and 2016, significantly reduced pre and post-emergence damping-off compared with untreated control and/or Rhizolex-T. *Trichoderma harzianum* and Rizolex-T were the best treatments in reducing dead plants and consequently increased survived plants in the first growing season. In the second growing one most of bioagents denote best results side by side with Rhizolex-T.

Table 9. Effect of bioagents on the enzymatic activity in soybean *cv.* Giza 22 tissues grown in soil infested with different pathogens and their mixture, under greenhouse conditions

Treatment	Peroxidase activity min^{-1} mg^{-1} of protein				Polyphenol oxidase activity min^{-1} mg^{-1} of protein				Chitinase activity min^{-1} g^{-1} fresh weight				B-1,3-gluctronase activity min^{-1} mg^{-1} of sample			
	F. oxysporum	*F. solani*	*R.. solani*	Fungal mixture	*F. oxysporum*	*F. solani*	*R. solani*	Fungal mixture	*F. oxysporum*	*F. solani*	*R. solani*	Fungal mixture	*F. oxysporum*	*F. solani*	*R. solani*	Fungal mixture
***B. subtilis*1**	0.919	0.216	0.853	0.185	0.179	0.155	0.178	0.098	1.414	3.317	1.563	3.643	17.143	15.794	18.111	16.095
***Pseudomonas* sp.1**	0.826	0.219	0.687	0.176	0.177	0.126	0.148	0.092	1.786	2.563	1.167	2.333	17.865	15.024	17.00	16.183
***B. subtilis* 2**	0.708	0.211	0.684	0.257	0.169	0.127	0.135	0.086	1.468	5.508	3.151	2.294	18.365	12.429	18.841	17.008
***Pseudomonas* sp.2**	0.550	0.271	0.628	0.181	0.166	0.121	0.199	0.086	1.333	2.706	1.159	2.421	18.500	11.425	17.746	15.937
***Debaryomyces* sp.**	0.576	0.311	0.707	0.192	0.188	0.513	0.177	0.095	1.690	4.714	1.611	2.817	21.452	16.032	18.00	17.627
***Streptomyces* sp.**	0.740	0.299	0.831	0.248	0.136	0.234	0.178	0.097	1.624	4.786	1.695	3.984	18.405	13.016	17.333	18.405
T. viride	0.456	0.230	0.744	0.193	0.166	0.189	0.134	0.086	2.167	7.183	1.468	5.00	17.349	17.619	16.024	16.365
T. harzianum	0.788	0.223	0.743	0.193	0.144	0.138	0.145	0.089	2.048	7.865	1.294	5.542	23.34	17.28	15.66	13.93
C. globosum	0.382	0.193	0.771	0.257	0.136	0.125	0.108	0.082	1.357	4.548	1.548	4.603	17.51	14.45	15.71	17.94
Mycorrhizae	0.469	0.200	0.783	0.267	0.135	0.144	0.130	0.088	1.825	11.62	1.865	5.595	18.31	8.032	16.10	17.59
Rizolex-T	0.190	0.104	0.427	0.139	0.091	0.085	0.076	0.049	0.919	1.643	0.675	1.183	9.778	7.849	8.07	7.595
Control	0.298	0.101	0.313	0.097	0.108	0.087	0.072	0.041	0.905	1.627	0.397	1.083	9.413	7.167	8.00	6.095

Table 10. Effect of bioagents on total phenols and total carbohydrates within soybean leaves, grown in soil infested with different pathogens and their mixture, under greenhouse conditions

Microbial bioagents	Total phenols (mg/g)				Total carbohydrates (mg/g)			
	F. oxysporum	*F. solani*	*R. solani*	Fungal mix.	*F. oxysporum*	*F. solani*	*R. solani*	fungal mix.
***B. subtilis*1**	2.322	2.243	2.481	2.169	5.754	1.302	5.778	4.960
***Pseudomonas* sp.1**	2.480	2.329	2.790	2.265	4.190	1.357	3.595	4.238
***B. subtilis* 2**	2.286	2.322	2.579	2.308	3.111	3.214	3.738	3.638
***Pseudomonas* sp.2**	2.703	2.344	2.675	2.140	3.825	1.722	3.667	3.976
***Debaryomyces* sp.**	2.380	2.401	2.699	2.625	5.548	1.969	4.976	3.937
***Streptomyces* sp.**	2.761	2.416	2.822	3.121	5.111	5.651	5 .698	5.279
T. viride	2.999	2.207	2.565	2.106	2.810	1.690	4.067	4.786
T. harzianum	2.840	2.279	2.717	2.308	3.397	1.849	4.024	5.103
C. globosum	2.329	2.277	2.551	2.009	2.429	4.341	3.167	4.071
Mycorrhizae	2.624	2.552	2.365	2.344	4.587	2.437	3.857	4.817
Rizolex-T	1.927	2.207	2.351	1.997	1.556	1.198	2.994	1.584
Control	1.639	1.712	2.022	1.661	1.246	1.035	2.881	1.571

Table 11. Effect of bioagents on soybean root rot-wilt disease grown under field conditions in two successive seasons. Results are represented as percentage of each parameter

Bioagent	Season 2015					Season 2016				
	Pre-	Post-	Dead plants	Infected survivals	Healthy survivals	Pre-	Post-	Dead plants	Infected survivals	Healthy survivals
***B. subtilis*1**	8.2	5.7	2.4	3.8	79.9	8.7	8.5	4.4	4.4	74.0
***Pseudomonas* sp.1**	7.9	6.5	2.5	3.8	79.3	8.2	8.2	4.2	4.4	75.0
***B. subtilis* 2**	8.7	4.0	4.7	2.7	79.9	7.6	7.9	5.4	3.1	76.0
***Pseudomonas* sp.2**	6.7	6.7	4.6	3.0	79.0	9.3	8.0	3.9	3.7	75.1
***Debaryomyces* sp.**	9.1	4.8	5.3	2.8	78.0	8.7	6.8	6.8	2.9	74.8
***Streptomyces* sp.**	8.0	5.3	4.5	3.5	78.7	8.5	2.0	5.9	5.8	77.8
T. viride	8.2	8.8	5.8	2.6	74.6	11.8	6.8	3.2	7.1	71.1
T. harzianum	7.3	5.2	4.7	2.4	80.4	9.3	4.9	5.4	7.4	73.0
C. globosum	8.7	7.7	4.2	5.7	73.7	10.0	7.8	4.8	5.8	71.6
Mycorrhizae	8.8	8.6	4.7	6.1	71.8	9.0	10.3	7.8	7.8	65.1
Rizolex-T	0.9	4.2	4.0	2.7	88.2	4.5	5.5	2.4	1.0	86.6
Control	13.3	13.1	4.4	1.6	67.6	21.3	5.3	12.6	5.8	55.0
LSD (5%)	0.684	0.284	0.979	0.358	0.951	0.922	0.376	1.304	0.276	1.902

The ability of the different bioagents to increase plant growth highly related to the IAA production which was produced by *Bacillus* spp. isolates (**Wahyudi *et al*., 2011)**. Yet, there is stimulation of bacterial IAA to the development of the host plant root system **(Patten and Glick, 2002)**. They also reported that low levels of IAA can stimulate root elongation, while high levels of bacterial IAA stimulate the formation of lateral and adventitious roots. **Rodriguez and Fraga (1999)** demonstrated that, *Pseudomonas* and other phosphate solubilizing bacteria (PSB) like *Bacillus* spp. were able to increase the availability of phosphorus in soil.

When *Trichoderma* spp. colonize plant roots, they invade only in the surface layers of the root, further penetration can be controlled by the plant defense reactions. Therefore, *Trichoderma* spp. are usually non-virulent irrespective of their intrinsic ability to attack plants **(Harman *et al*., 2004)**. Even though some *Trichoderma* spp. grow only on roots, the plant defense reactions can become systemic and protect the entire plant from a range of pathogens and diseases **(Harman *et al*., 2004)**.

The effect of bioagents on plant growth parameters and yield was studied. Results presented in Table 12 reveal the various responses of the tested soybean cultivar of the growth parameters (plant height, number of branches and number of pods/plant) and yield component such as seed weight (g/plant), 100 seed weight (g), seed yield (kg/plot/faddan) as affected by different bioagents, mycorrhizae and Rhizolex-T. Results indicated that all bioagent treatments significantly improved plant height, number of branches and number of pods. Treatments increased seed weight (g/plant), 100 seed weight (g), seed yield (kg/plot).

Many investigators noted that coated seeds by bioagents and seed bio-priming cause significant increase of vegetative growth of many crops **(El-Mohamedy *et al.*, 2006).**

Vinale *et al*. (2008) suggested that the secondary metabolites such as auxin like compounds or auxin inducing substances by Trichoderma-plant interaction might be a reason for the improved growth. Also, this might be due to effectiveness of bioagents such as *Bacillus* spp. and streptomyces, which promoted soybean seedling, able to solubilize phosphate and produce a range of siderophores which have a very high affinity for iron chelating **(Wahyudi *et al*., 2011).**

The root colonization of *Trichoderma* spp. increases the growth of the entire plant and thus results in an increase of plant productivity and the yields. Symbiotic association with rhizosphere of the plant helps to surmount abiotic stresses and improve nutrient uptake **(Harman *et al*., 2004)**.

Biochemical analysis of total protein and oil in seed yield

Application of bioagents, Rizolex-T and mycorrhizae increased the percentage of oil and total protein contents within soybean seeds comparing to untreated plants, as shown in Table 13. The mechanism of such increase could be attributed to the ability of *Trichoderma* spp. to colonize the root system of soybean and increase the absorption surface, which in turn will increased nutrient uptake. Biocontrol agents could also build a symbiotic relationship with plant roots for the exchange of metabolites and carbohydrates which could enhance plant metabolism and increase photosynthesis **(Vinale *et al*., 2008)**. Upregulation of plant metabolism and photosynthesis will increase the oil and protein content within seeds.

In conclusion, rhizospheric bioagents inhabiting the soybean soil can provide an efficient alternative solution for management of root rot-wilt diseases complex. Selected bioagents could directly eliminate the fungal pathogens and suppress diseases incidence using their antagonistic secretions, and trigger plant resistance and growth which will indirectly help in suppressing the disease.

Table 12. Effect of bioagents on soybean plant growth parameters and yield component under field conditions in two successive seasons

	Season 2015						Season 2016					
	Plant height (cm)	No. of branches	No. of pods	Seed weight (g)/ plant	100seed weight (g)	Seed yield (kg) /plot	Plant height (cm)	No. of branches	No. of pods	Seed weight (g)/ plant	100seed weight (g)	Seed yield (kg) /plot
***B. subtilis*1**	72.4	3.5	25.4	47.9	66.5	13.025	69.7	3.0	26.7	48.9	61.8	13.300
***Pseudomonas* sp.1**	78.6	4.3	23.2	47.7	64.2	12.974	71.5	4.0	22.3	47.9	67.2	13.028
***B. subtilis* 2**	68.7	3.1	22.2	47.3	59.2	12.865	66.3	3.0	22.2	47.8	63.0	13.001
***Pseudomonas* sp.2**	69.5	3.8	22.7	47.4	61.8	12.892	66.5	3.3	23.3	46.9	59.8	12.756
***Debaryomyces* sp.**	71.8	3.9	23.1	47.7	64.6	12.89	67.4	3.3	25.0	47.7	66.4	12.892
***Streptomyces* sp.**	74.3	4.0	23.8	48.1	66.0	13.001	72.1	4.0	25.0	48.3	66.0	13.137
T. viride	72.3	3.4	19.8	36.9	66.4	10.036	69.7	3.4	21.6	48.0	53.2	13.056
T. harzianum	69.5	3.5	23.3	38.2	67.7	10.390	68.7	3.0	26.6	47.8	61.7	13.001
C. globosum	73.9	3.2	21.3	37.2	57.2	10.118	68.7	3.2	23.0	47.7	55.5	12.890
Mycorrhizae	79.7	4.1	21.7	37.4	57.2	10.172	76.9	4.0	23.0	47.0	55.5	12.784
Rizolex-T	61.8	3.5	22.0	36.9	58.0	10.036	64.4	3.0	22.0	47.0	55.8	12.784
Control	61.3	2.9	19.2	36.3	52.0	9.873	60.6	2.4	17.0	46.4	33.0	1.9

Table 13. Effect of bioagents on total proteins and total oils contents in soybean seeds on naturally infected soybean under field conditions

Treatments	Total protein (mg/g)	Total oils (mg/g)
***B. subtilis*1**	0.472	0.227
***Pseudomonas* sp.1**	0.435	0.201
***B. subtilis* 2**	0.426	0.198
***Pseudomonas* sp.2**	0.456	0.227
***Debaryomyces* sp.**	0.417	0.221
***Streptomyces* sp.**	0.485	0.199
T. viride	0.441	0.222
T. harzianum	0.420	0.210
C. globosum	0.400	0.200
Mycorrhizae	0.312	0.212
Rizolex-T	0.300	0.189
Control	0.305	0.188

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القدرة التنافســـية لميكروبات المكافحة الحيوية ضد مرض متلازمة ذبول وعفن جـذور فـول الصـويا

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تعتبر المكافحة الحيوية بديل آمن لمكافحة الفطريات الممرضة للنبات، تهدف تلك الدراسة إلي عزل وتعريف وتشخيص قدرة الميكروبات النافعة التي تقطن التربة الزراعية لنبات فول الصويا، تم الحصول علي ٢٧ عزلة من الفطريات المصاحبة لجذور نباتات فول الصويا تظهر عليها أعراض متلازمة الذبول وعفن الجذور، وبدراسة القدرة المرضية لكل منها علي نباتات سليمة تحت ظروف الصوبة تبين أنها متفاوته في قدرتها علي إحداث المرض لذا تم اختيار العزلات الأكثر شراسة، أوضحت دراسات التعريف الميكروسكوبي أن عزلات الفطريات الممرضة هي فيوزاريم أوكسيسبورم و فيوزاريم سولاني وريزوكتونيا سولاني، وتم التأكد من هوية تلك الفطريات بإستخدام تقنية تفاعل البلمرة المتسلسل المعتمدة علي تتابع الحمض النووي أي تي اس (ITS) بكل فطر، تم عزل مجموعة أخري من الميكروبات المصاحبة لمنطقة الريزوسفير بهدف اختبار قدرتها على مكافحة مرض متلازمة ذبول وعفن جذور فول الصويا بالمعمل والحقل، أظهرت الدراسات المعملية قدرة ثلاثة أجناس فطرية وأربعة أجناس بكتيرية وجنس من الخمائر وآخر من الأكتينوميسيتات على تثبيط نمو الفطريات الممرضة معملياً بشكل معنوي، كما أظهرت تلك الميكروبات قدرة ملحوظة علي كبح المرض محل الدراسة تحت ظروف الصوبة والحقل المفتوح مقارنة بالميكورهيزا المتوفرة تجارياً والمبيد الكيماوي ريزوليكس تي. درست النظريات التي تفسر ميكانيكيات التأثير الإيجابي لتلك الميكروبات علي النبات سواء بخفض شدة المرض أو برفع كفاءة النبات. أوضحت تلك الدراسات أن عوامل المكافحة الحيوية المستخدمة كان لها دور في تنشيط بعض الإنزيمات والفينولات التي تؤثر سلباً علي نشاط الفطريات الممرضة سواء بشكل مباشر أو غير مباشر للنبات، كما لوحظ قدرة تلك الميكروبات علي تشجيع نمو النبات لقدرتها علي إفراز هرمون النمو وتيسير الفوسفات والحديد بالتربة، وكذا رفع جودة المحصول كماً ونوعاً والذي انعكس على محتوى البذور من الزيوت والبروتينات.

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