Effect of *Trichoderma* species on damping off diseases incidence, some plant enzymes activity and nutritional status of bean plants

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ABSTRACTS: *Fusarium solani* and *Rhizoctonia solani* are the common causal pathogens causes the damping off disease of beans (*Phaseolus vulgaris* L.) in Egypt. The antagonistic effect of four *Trichoderma* species, i.e. *Trichoderma album, Triechoderma hamatum, Trichoderma harzianum* and *Trichoderma viride*, was tested against *F. solani* and *R. solani in vitro*, in greenhouse and in field. *In vitro* tests, all *Trichoderma* spp. significantly reduced the mycelial growth of two pathogenic fungi. In greenhouse experiment, *T. album, T. hamatum, T. harizianum* and *T. viride*, as soil treatments, significantly reduced the pre- and post-emergence damping off disease incidence under artificial infection with *F. solani* and *R. solan*i. Soil treatments with four *Trichoderma* species significantly reduced the incidence of damping off disease where the percentages disease incidence were in the range of 7.0 -20.0% and 2.4 – 6.5%, compared to 25.7 and 13.5% in control plants, at pre- and post-emergence stages ,respectively. The best protection to damping off disease was obtained by *T. hamatum*, followed by *T. viride*, *T. album* and *T. harzianum*, respectively. The treatments gave the highest plant survival (%) and improved the growth and yield parameters. Results showed that the levels of chitinase, peroxidase and polyphenol oxidase activities highly increased in treated bean plant compared in untreated plants. The macro- and micro-elements content in treated bean plants was affected by *Trichoderma* species treatments compared to elements content in untreated plants. The relationship between plant nutrient content and some plant enzymes activity was studied. [Journal of American Science. 2011;7(1):156-167]. (ISSN: 1545-1003).

Key words: *Fusarium solani, Rhizoctonia solani, Phaseolus vulgaris, Trichoderma* spp., biological control, nutritional atatus.

INTRODUCTION

Beans (*Phaseolus vulgaris* L.) are considered one of the most important economic legumes in Egypt. Bean is very important as a human food, animal feed and its beneficial effects in improving the soil fertility **(Anonymous, 2005 and Broughton *et al*., 2003).** *Fusarium* root rot on beans is caused by the fungus *Fusarium solani* f. sp. *phaseoli*. The fungus can attack older seedlings, and is most severe on plants growing under stressful conditions. The pathogen usually survives as thick-walled chlamydospores in soil. *Rhizoctonia* root rot, caused by *Rhizoctonia solani*, is common throughout the world. It is one of the most economically important root diseases of beans. It has a broad host range that includes most annual and many perennial plants. Generally, *Rhizoctonia* survives between crops as sclerotia or as fungal mycelia in the soil. Young plants are more susceptible to infection than older plants. Application of the fungicides is not economical in the long time because they pollute the environment, leave harmful residues and can lead to the development of resistant strains of the pathogen with repeated use **(Vinale *et al.*, 2008).** Replacement of fungicides with bio-control agents is an alternative mean to; manage the plant pathogens, produce safety food and reduce the environment pollution **(Barakat and Al-Masri, 2005).** One of the most important bio-control agents is *Trichoderma* spp. that the most frequently isolated soil fungi and present in plant root ecosystems **(Harman *et al*., 2004)**.*Trichoderma* spp. also are commercially marketed as biopesticides, bio-fertilizers and soil amendments. The use of *Trichoderma* fungi in agriculture can provide numerous advantages ; 1) colonization of the root and rhizosphere of plant, 2) control of plant pathogens by different mechanisms such as parasitism, antibiosis production and induce systemic résistance , 3) improvement of the plant health by promote plant growth , and 4) stimulation of root growth **(Harman *et al*.,2004)**.

The antagonistic activity of the genus *Trichoderma* to *F. solani* and *R. solani* has been widely demonstrated **(Lewis *et al*., 1998).** *Trichoderma harzianum* protected the bean seedlings against pre-emergence damping off infection, reduced the disease severity and increased the plant growth in the presence of *R. solani* pathogen **(Paula *et al*., 2001). El-Kafrawy (2002)** reported that the *T. harzianum*, *Trichoderma hamatum*, *Trichoderma pseudoknonningii* and *Trichoderma polysporum* inhibited the radial mycelial growth of *R. solani in vitro* test from 59.6 to 78.4 %. Soil treatment with *T. hamatum*, *T. harzianum* and *T. viride* gave the maximum protection against pre- and post-emergence

damping off and reduced the disease incidence from 50 to 6.6, 10 and 10%, respectively, compared to fungicide Rizolex (tolclofos-methyl) at 10%. The bio-control agent treatments improved the plant heights, fresh and dry weight and increased dry seeds yield comparing with the control **(El-Kafrawy, 2002).** The seed treatment with the bio-control agents was less than soil treatment. **Gonzalez *et al*. (2005)** showed that the field application with *T. viride* and/or *T. harzianum* as soil application gave the same effectiveness (99%) against *R. solani* pathogen, comparing with the seed immersion. Soil amendments with *T. harzianum* significantly increased the heights and weight of plants and significantly reduced the *R. solani* infection **(Malik *et al.*, 2005).** Application of *T. harzianum* as seed treatment significantly reduced the incidence of damping-off diseases some leguminous crops, i.e. faba bean, lentil, and chickpea, when planted in a soil naturally infested with *Fusarium* spp. and *R. solani* **(Abou-Zeid *et al*., 2003).***T. harzianum*, *Trichoderma koningii* and *T. viride*, as seed dressing, improved the seedling emergence and health of runner bean (*Phseous coccineus* cv. Eureka) **[Pieta *et al*., 2003**]. Seeds of common bean were dressed, prior to sowing; with conidia of *T. harzianum* protected the germinating seedlings and plants against infection by soil borne pathogenic fungi, i.e. *Fusarium* spp. and *R. solani* **(Pieta and Pastucha, 2004).**

Soil provides the medium for root development and with the exception of carbon, hydrogen, oxygen and some nitrogen, plants depend on soil for all other nutrients and water. The soil microbes that include bacteria, fungi, actinomycetes, protzoa and algae play a significant role in the nutrient cycling **(Nannipieri *et al.*, 2003)**. Interactions between plant root systems and bio-control agents such as rhizobacteria are able to generate a wide array of secondary metabolites which can have a positive influence on plant growth, enhancing the availability of minerals and nutrients, improving nitrogen fixation ability and improving plant health through the bio-control of phytopathogens **(Sturz and Christie, 2003).** Applying biological control agents to infected plants increase mineral levels [(nitrogen (N), phosphorous (P), potassium (K) and magnesium (Mg)], and both chlorophyll biosynthesis and photosynthetic activity, which in turn led to the accumulation of metabolites i. e., carbohydrates and proteins (**Mahmoud *et al*.,2004).** *Bacillus subtilis* and *T. viride* only or combined were significantly increased the values of NPK concentration on tomato plants compared to control treatments **(Henry *et al*., 2009 and Morsy *et al*., 2009).**

The objective of this search was to evaluate the antagonistic potential effect of *Trichoderma* species (spp.), i.e. *T. album, T. hamatum, T. harzianum* and *T. viride* as bio-control agents against *F. solani* and/or *R. solani* the causal organisms of damping off disease and nutritional atatus in bean plants. The antagonistic activity of *Trichoderma* spp. was tested *in vitro,* in pot and in field. Role of bio-agents in enhance of some enzymes (chitinase, peroxidase and polyphrnol oxidase) related to disease control in plant was detected. The relationship between nutritional status of bean plants and *Trichoderma* spp. application was tested.

MATERIALS AND METHODS

1- Plant material:

Bean seeds (*Phaseolus vulgaris* L.) cv. Pulista was obtained from Vegetable Crops Research Depart., Agricultural Research Centre, Giza, Egypt, to produce the host plants in this search. Health test of bean seeds was made. Seeds were placed on sterile cotton and filter paper moistened with sterile distilled water in Petri dishes and incubated at 25 ºC. Ten replicates of 20 seeds were used for each dish .No infected bean seeds were recorded through the test **(Coskuntuna and Özer, 2008).**

2- Pathogens:

Fusarium solani and *Rhizoctonia solani* were isolated from naturally infected bean plants, showing damping off and root rot symptoms, cultivated in Qalyubiya Governorate, Egypt. The isolated fungi were identified on the basis of cultural and microscopic morphological characters according to the key given by **Barnett & Hunter (1972) and Booth (1985).** Pathogencity of isolated fungi toward bean plants (cv. Pulista) was estimated **(Sallam *et al*., 2008).** Artificial inoculum of pathogenic fungi was prepared by growing each fungus on sorghum - sand medium as described by **Abd El- Khair and El-Mougy (2003).** The most aggressive isolate of each pathogenic fungus was used *in vitro* and in pot experiments.

3- Isolation of *Trichoderma* spp.:

Four fungi of *Trichoderma* species, i.e. *T. album, T. hamatum, T. harzianum* and *T. viride* were isolated from healthy bean plants rhizosphere collected from Qalyubiya Governorate , using dilution plate technique . All *Trichoderma* spp. fungi were purified by hyphal tip technique and identified on the basis of cultural and microscopic morphological characters (**Barnett & Hunter, 1972 and Bissctt, 1991)** in Plant Pathology Department, National Research Centre. The fungi of *Trichoderma* spp. were used *in vitro*, in pots and in field experiments.

4- Preparation of *Trichoderma* spp. inoculums:

The propgules (colony forming unit, cfu) suspension of each *T. album, T. hamatum, T. harzianum* and *T. viride* fungus was prepared in sterile distilled water from 7-days-old-culture on potato dextrose agar [PDA] **(Rojo *et al*., 2007).** The fungal inoculum was harvested by flooding the culture with SDW and then rubbing the culture surface with a sterile glass rod. The fungal propgules concentration in each suspension was determined by counting using a haemocytometer slide (Adjusted at 10^8 cfu / ml). A mixture of milted soybean and talc powder (1:1, w: w) was used as a carrier mixture for antagonistic fungal propgules. A carrier mixture was added at rate of 50% to fungal suspension and mixed to even distribution of fungal propgules **(Abd El- Khair and El -Mougy, 2003).**

5- Evaluation of antagonistic activity of *Trichoderma* species:

5.1- In dual culture technique (*in vitro*):

The antagonistic effect of *T. album, T. hamatum, T. harzianum* and *T. viride* against *F. solani* and *R. solani* pathogens *in vitro* was evaluate using the dual culture technique **(Coskuntuna and Özer, 2008)**. Each *Trichoderma* spp., *F. solani* and *R. solani* were cultured, separately, on PDA medium for 7 days at 25ºC. Disc (5mm- diameter) from each bio-control fungus was inoculated on surface of PDA medium in side of Petri dish. A disc (5 mm - diameter) of *F. solani* and *R. solani*, separately, was inoculated at equal distance of the opposite side of Petri dish. Petri dishes were inoculated with each pathogenic fungus only as control. Four Petri dishes for each bio-control - pathogenic fungus treatment, as well as the control, were used as replicates. The inoculated Petri dishes were incubated at 25 ºC at 7 days. Antagonistic effect of *Trichoderma* spp., as decrease of the mycelial growth of pathogenic fungi, was determined using the following formula.

Antagonistic effect =A-B/A x100

Where, **A**: The diameter of mycelial growth of pathogenic fungus in control and

B: The diameter of mycelial growth of pathogenic fungus with *Trichoderma* fungus.

5.2- In pot experiment:

Antifungal activity of *T. album, T. hamatum, T. harzianum* and *T. viride* against *F. solani* and *R. solani* pathogens was evaluated in pots under artificially infestation conditions. The experiment were designed under greenhouse conditions in Pest Rearing Department, Central Agricultural Pesticides Laboratory, using pots (40 cm – diameter) containing 4 kg of sterilized loamy clay soil. First, soil was infested with each pathogenic fungus grown on sorghum-sand medium at rate of 5 g/ kg soil in different pots and then the pots were irrigated for 7 days before bio-control agent inoculation. Next, soil was inoculated with each *Trichoderma* fungus at 5 g/ kg soil, and then pots were watered for 7 days before sowing. Ten of bean seeds (cv. Pulista) were sown in each pot. Five pots as replicates were used for each treatment as well as the control. The experiment included the following treatments; 1) non-infested soil (control), 2) soil treated with *F. solani* only, 3) *F. solani* + each *Trichoderma* fungus, separately, 4) soil treated with *R. solani* only and 5) *R. solani* + each *Trichoderma* fungus , separately. Pots were kept under greenhouse conditions till the end of the experiment. Disease incidence of pre- and post-emergence of damping off disease incidence and survival (%) of bean plants were recorded after 15, 30 and 45 days, respectively.

5.3- In field experiment:

The efficacy of soil treatment with *T. album, T. hamatum, T. harzianum* and *T. viride*, in separated block, against the incidence of damping off disease, were evaluated in a commercial bean field with a previous history of the disease in Qalyubiya Governorate. The field trial (20 plots) was designed in complete randomized block with four replicates. Each plot was 3x3 m in area and had four rows of 3m in length and 75 cm in width. First, each bio-control agent inoculum was applied at rate of 50g/1 m^2 of soil was incorporated with the 20 cm of the soil surface. Next, the soil was irrigated before 7 days before sowing. Bean seeds (cv. Pulista) were planted at rate of 3 seeds / hole at 20cm space. The field treatments were as follows; 1) soil naturally infected (control), 2) soil treated with *T. album*, 3) soil treated with *T. hamatum*, 4) soil treated with *T. harzianum* and 5) soil treated with *T. viride*.

Effect of the tested *Trichoderma* spp on:

5.3.1- Disease assessment:

Effect of the tested *Trichoderma* spp. in reducing the damping off disease incidence at pre- and post-emergence stages as well as the percentages of the survival of healthy plants were recorded after 15 , 45 and 60 days.

5.3.2- Plant growth and yield parameters.

Random samples of ten bean plants were collected at 60 days of sowing for each bio-control agent treatment as well as the control plants. The plant growth parameters as number of branches per plant, plant height, and number of leaves per plant and fresh weight of plant were determined. The yield parameters

also recorded as number of pods per plant and the average pod weight were recorded.

5.3.3- Some plant enzymes activity:

Effect of *Tricoderma* spp. application on the activity of chitinase, peroxidase and polyphenol oxidase enzymes related to plant defense against pathogens infection were determined in leaves of bean plants.

Extraction of enzymes:

Plant tissue (g) was homogenized with 0.2 Tris Hcl buffer (pH 7.8) containing 14mM B-Mercaptoethanol at the rate of 1/3 w/v. The homogenate was centrifuged at 3000 rpm for 15 minutes. The supernatant was used to determine enzyme activities **(Tuzun *et al*., 1989).**

5.3.3.1-Chitinase assay:

Colloidal chitin was prepared from chitin powder (Sigma Co.) according to the method described by **Ried and Ogryd –Ziak (1981).**Twenty five grams of chitin powder suspended in 250 ml of 85 % phosphoric acid (H_3PO_4) and stored at 4 ^{O}C for 24 h., then blended in 2 liter of distilled water using blender. The suspension was centrifuged. This washing procedure was repeated twice. The colloidal chitin suspension was adjusted at PH 7 with (1N) NaOH and re-centrifuged. The pelleted colloidal chitin was stored at 4 c until used. Determination of enzyme activity was carried out according to the method of **Monreal and Reese (1969).**One ml of colloidal chitin in 0.05 M citrate phosphate buffer (pH 6.6) in test tubes. One ml of enzyme extract was added and mixed by shaking. Tubes were incubated in a water bath at 37 ^{O}C for 60 min., then cooled and centrifuged before assaying. Reducing sugars were determined in 1 ml of the supernatant by dinitro-salysilic acid (DNS). Optical density was determined at 540 nm.

5.3.3.2 - Peroxidase assay:

Proxidase activity was measured by incubation 0.1 of enzyme extract with 4 ml of guaiacol for 15 minutes at 25 ^{O}C and absorbance at 470 nm was determined. The guaiacol solution consisted of 3 ml of 0.05 M potassium phosphate. PH 7, 0.5 ml of 2 % guaiacol and 0.5 ml of 0.3 % H_2O_2 (**Abeles *et al*., 1971)**. Peroxidase activity was expressed as the increase in absorbance at 470 nm/gram fresh weight/15 minutes.

5.3.3.3 - Polyphenol oxidase assay:

Activity of polyphenol oxidase was determined using the colorimetric method described by **Matta and Dimond (1963).** The reaction mixture contained 1.0 ml of crude enzyme extract, 1.0 ml 0.2 M sodium phosphate at pH 7.0 and 1.0 ml of 10 M catchall brought to final volume of 6.0 ml with distilled water **(Morsy, 2005).** The activity of polyphenol oxidase was expressed as the optical density at 475 nm.

5.3.4 - Macro- and micro-nutrient elements content:

Representative soil sample (0-30 cm) was taken before cultivation to determine some physico-chemical characteristic (Table, 1) according to standard methods described by **Jackson (1973)** and **Lindsay & Norvel (1978).** At early F1 (pods <10 cm long) growth stage, young mature blade from terminal and green pods were taken for determine their macro- and micro-elements content. The dry aching technique as described by Chapman and Pratt (1978) was used to extract macro- and micronutrients from dried leaves and pods.Total nitrogen (N) in leaves was determined by Buechi-320 apparatus, while potassium (K) and calcium (Ca) were measured using flame photometer, Genway instrument and phosphorus (P) was measured by using Spectrophotometer, Perkin Elemer instrument. The leaves content of iron (Fe), manganese (Mn), zinc (Zn) and magnesium (Mg) were measured by using Atomic Absorption Spectrophotometer, Perkin, Elemer, and Model 1100B.

5.4- Statistical analysis:

Data obtained were subjected to Computer Statistical Package (CO-STATE) originated by **Anonymous (1989).**

RESULTS

1 - *In vitro* tests:

The inhibitory effect of *T. album*, *T. hamatum*, *T. harizianum* and *T. viride* against the mycelial growth of *F. solani* and *R. solani in vitro* test are shown in Table (2). The antagonistic effects of *Trichoderma* spp. against *F. solani* were in the range of 38.9 – 57.5 %. *T. hamatum* gave the highest effect about 57.5 %, followed by *T. album* (49.1%), *T. harzianum* (44.4%) and *T. viride* (38.9 %), respectively. Results showed that the growth inhibition of *R. solani* by *Trichoderma* spp were in the range of 41.7 – 70.3 %. *T. hamatum* also highly inhibited the mycelial growth of *R. solani*, where the growth inhibition was 70.3 %, followed by *T. harzianum* (61.1%), *T. album* (53.7%) and *T. viride* (41.7%), respectively. Results showed that the best growth inhibition against two pathogenic fungi was obtained by *T. hamatum*, while the lowest one was obtained by *T. viride* (Table 2).

2 - In greenhouse experiment:

Soil treatments with *T. album, T. hamatum, T. harizianum* and *T. viride* significantly reduced the pre- and post-emergence damping off disease incidence under artificial infection with *F. solani* and *R. solan*i in greenhouse conditions (Table, 3). The damping off disease incidence caused by *F. solani* under application of *Trichoderma* spp. were in the range of 9.5 – 19.0 % and 2.5 - 7.5% ,compared to 48.5 and 55.8 % at pre- and post-emergence stages ,respectively**.** At pre-emergence**,** *T. hamatum* gave the highest reduction to disease incidence about 81%, followed by *T. viride* (71 %), *T. album* (69%) and *T. harzianum* (61%). At post-emergence stage, *T. album* gave the best growth reduction to disease incidence about 96%, followed by *T. harzianum* (90%), *T. viride* (90%) and *T. hamatum* (87 %), respectively. The percentages of survival bean plants were in the range of 92.5 – 97.5 % compared to 44.2 % healthy bean plants in the control treatment. *T. album* gave 97.5 % healthy plants, followed by *T. harzianum* (94.5%), *T. viride* (94.3%) and *T. hamatum* (92.5%) where the percentages of the increase of healthy plants were 121 , 114, 113 and 109% ,respectively (Table 3).

The damping off disease incidence caused by *R. solani* under application of *Trichoderma* spp. were in the range of 4.0 – 16.0 % and 2.1 – 5.9% , compared to 35.0 and 52.5 % in the control plants , at pre- and post-emergence stages (Table,3). *T. hamatum* gave the highest reduction (80%) of disease incidence, followed by *T. viride* (63%), *T. album* (62%), and *T. harzianum* (54%) at pre-emergence stage. While at post-emergence, *T. album* gave the highest reduction (96%) against damping off disease, followed by *T. harzianum* (90%), *T. hamatum* (90%) and *T. viride* (89%), respectively (Table,3). The percentages of healthy bean plants were in the range of 94.1 – 97.9 %. *T. album* produced the highest percentage of healthy bean plants (97.9%), followed by *T. harzianum* (94.8%), *T. viride* (98%) and *T. hamatum* (95%), compared to 47.5 % in the control plants, where the percentages of the increase were 106, 100, 98 and 95, respectively.

3 - In field experiment: Effect of *Trichoderma* spp. on

3.1- Damping off disease incidence:

Soil treatments with four *Trichoderma* species, i..e., *T. album, T. hamatum, T. haezianum* and *T. viride*, significantly reduced the incidence of damping off disease in the field comparing with the control plants (Table, 4). Results showed that the percentages of damping off disease incidence were in the range of 7.0 -20.0% and 2.4 – 6.5%, compared to 25.7 and 13.5%, at pre- and post-emergence stages, respectivily. The best protection against the disease obtained by *T. hamatum*, where the disease incidence reduction was 73 %, followed by *T. viride* (53%), *T. album* (38%) and *T. harzianum* (22%) at pre-emergence, respectively (Table 3). At post-emergence, *T. album* gave the highly reduction (82%) to damping off incidence, followed by *T. viride* (65%), *T. harzianum* (63%) and *T. hamtum* (52%), respectively. Application of *Trichoderma* spp. also reduced the root rot disease of bean plants where the disease incidence were in the range of 0.0 – 2.2 % compared to 10.7 % in control bean plants.

Trichoderma spp. also significantly increased the percentages of healthy plants compared to control plants (Table, 4). Results showed that the survival bean plants were in the range of 91.3 – 97.6%, while in control bean plants was 75.5%. Application of *T. album* produced the highest percentage of healthy plants (97.6%), followed by *T. viride* (95.3%), *T. harzianum* (92.5%) and *T. hamatum* (91.3%), respectively.

3.2- Growth and yield parameters:

Results revealed that the average of bean plant height with *Trichoderma* application were in the range of 46.0-49.8 cm compared to 37.3 cm in the control plants (Table,5).*T. hamatum* gave the highest increase of plant height (34%) , followed by *T. harzianum* (26%) , *T. viride* (26%) and *T. album* (23%). No significant differences were recorded among *Trichoderma* treatments, while significant once were recorded between *Trichoderma* treatments and the control plants. The branches number average per plant as result for application of *Trichoderma* spp. were in the range of 5.0 – 6.3 branch/plant, compared to 3.7 branch /plant in control treatment. *T. harzianum* significantly increased the branches number average (70%), followed by *T. viride* (49%), *T. hamatum* (41%) and *T. album* (35). The leaves number average in treated bean plants were in the range of 11.5 – 15.2 leaves/plant; while in untreated plants were 9.5. *T. harzianum* significantly increased number of leaves (60%) , followed by *T. hamatum* (56%) , *T. viride* (50%) and *T. album* (21%).Results also showed that the fresh weight average without pods were in the range of 43.1 – 77.4% g , compared to 42.5g in the control plants (Table,5). *T. hamatum* significantly increased the fresh weight of bean plant (82%), followed by *T. harzianum* (36%), *T. viride* (21%) and *T. album* (2%).

Application of *Trichoderma* spp. significantly increased the pods number average per plant where the number were in the range of 15.2 – 20.0 pods/plant ,compared to 10.8 pods/plant in the control plants (Table,5). No significant differences were recorded among *Trichoderma* treatments. *T. hamatum* significantly increased the pods number average (85%), followed by *T. harzianum* (62%), *T. album*

(45%) and *T. viride* (41%).Results indicated that *T. hamatum* and *T. album* significantly increased the pod weight average about 48 and 15 % , respectively, comparing with other *Trichoderma* treatments as well as the control plants(Table,5).

3.3 - Some plant enzymes activity:

All *Trichoderma* spp. treatments stimulated the activity of chitinase, Perxidase and polyphenol oxidase enzymes, comparing with the control treatment (Table, 6). The optical density of chitinase enzyme activity in treated bean plants were in the range of 0.271 – 0.620, compared to 0.117 in control plants. The increases of chitinase enzyme activity were in the range of 132-430 %. *T. viride* gave the highest enzymatic activity of chitinase (430%), followed by *T. album* (174%), *T. harzianum* (150%) and *T. hamatum* (132%). The optical density of peroxidase enzyme activity was in the range of 0.437-0.775 in bean plants under *Trichoderma* application, compared to 0.346 in untreated bean plants. The peroxidase enzymatic activity was in the range of 26-124 % with *Trichoderma* treatments application. *T. harizanum* significantly increased the activity of peroxidase activity about 124 %, followed by *T. hamatum* (100%), *T. viride* (34%) and *T. album* (26%).Results showed that the optical density of polyphenol oxidase were in the range of 0.231 – 0.518 in treated bean plants , compared to 0.146 in control plants (Table,6). *Trichoderma* application enhanced the activity of polyphenol oxidase enzyme in bean plants from 58 to 255 %. *T. harizanum* significantly increased the enzyme activity (255%), followed by *T. viride* (108%), *T. hamatum* (103%) and *T. album* (58%), respectively (Table, 6).

3.4 - Macro- and micro-nutrient elements content:

3.4.1 – In bean leaves:

Results showed that the *Trichoderma* spp. treatments affected on the level of nutrients leave content from macro- and micro-elements (Table, 7). The *Trichoderma* spp. treatments significantly decreased the level of nitrogen and phosphorus content in bean leaves in treated plants .The level of nitrogen content was in the range of 2.19 – 2.60 %, compared to level of 2.73 % in untreated leaves. *T. harzianum* highly decreased the level of nitrogen content, where the level was 2.19 %, followed by *T. album* (2.21%), *T. hamatum* ((2.36 %) and *T. viride* (2.60 %). No significant differences were found between *T. album* and *T. harizanium* treatments. The level of phosphorus in leaves of treated plants was in the range of 15 – 17 %, compared to 0.21% in untreated control. The level of phosphorus was 15, 16, 16 and 17% in leave plants treated with *T. hamatum*, *T. album*, *T. viride* and *T. hariznum*, respectively. No significant differences were noticed among *T. hamatum*, *T. album* and *T. viride* (Table, 6).Results also revealed that the treatments increased the levels of potassium, magnesium, calcium and sodium in treated bean leaves than untreated one (Table,7). The level of potassium was in the range of 1.73 – 2.88% in treated leaves, compared to 1.39 % in untreated plants. *T. harzianum* significantly increased the level of potassium (2.88%) in treated leaves, followed by *T. viride* (20.16%), *T. album* (1.87%) and *T. hamatum* (1.73%). Treatment with *T. album*, *T. hamatum* and *T. viride* increased the level of magnesium in bean leave plants to 0.91, 0.88 and 0.84 %, except *T. harzianum* treatment decreased the level to 0.68 %, compared to level of 0.80% in untreated control. Results also showed that the level of calcium was in the range of 2.80 – 3.25 % in treated plants, compared to level of 2.75 % in controls. *T. viride* significantly increased the level of calcium in leaves (3.25%), followed by *T. album* (3.15%), *T. hamatum* (3.10%) and *T. harzianum* (2.80%). Treatment with both *T. hamatum* and *T. harzianum* significantly increased the level of sodium to 0.088 and 0.039 % in treated leaves, respectively, compared to the control (0.023%).

Effect of *Trichoderma* spp. treatments on the content of bean leaves in treated plants from micro-elements i.e. iron, manganese, zinc and cupper as shown in Table (7). The treatments significantly increased the levels of both iron and manganese in leave of treated plants. On the other hand, the same decreased the levels of zinc and cupper (Table, 7). The level of iron was in the range of 1250 – 2150 ppm .*T. hamatum* significantly increased the level of iron element (2150 ppm), followed by *T. album* (1550 ppm), *T. viride* (1425 ppm) , while *T. harzianum* significantly decreased the level of iron to 1250 ppm , compared to level of 1350 ppm in control . The level of manganese was in the range of 75 – 84 ppm, compared to level of 71 ppm in control plants. *T. viride* treatment only significantly increased the level of cupper in treated leaves. It is clear that *Trichoderma* spp. significantly increased the levels of potassium, calcium, iron and manganese in leaves in treated bean plants. *T. hamatum* gave the highest values of magnesium, iron and manganese in bean leaves.

3.4.2 – In fresh bean pods:

Results showed that *Trichoderma* spp. treatments significantly decreased the levels of both nitrogen and phosphorous in bean pods of treated plants, compared to control treatments (Table, 8).The level of nitrogen was in the range of 1.79 – 3.09 % , while the level of phosphorous was in the range of 0.42 – 0.50% , compared to 3.37 and 0.56 % in untreated pods. *T. harzianum* significantly increased

the level of potassium to level of 4.42 %, while other *Trichoderma* spp. significantly decreased it as compared to level of 3.65 % in treated pods. The level of magnesium was in the range of 0.32 – 0.38 % in treated pods, compared to level of 0.43 % in untreated pods. *T. harzianum* only significantly increased the level of calcium and sodium to 0.35 and 0.022 % in treated pods, compared to levels of 0.25 and 0.014 %, respectively.

Effect of *Trichoderma* spp. on the levels of micro-elements in bean leaves as shown in Table (8). The treatments of *Trichoderma* significantly increased the content of pods from zinc*;* the level of element was in the range of 49 – 58 ppm, compared to level of 44 ppm in control. *T. harzianum* only significantly increased the levels of iron (550) and cupper (10 ppm) in pods of treated bean plants, compared to level of 475 and 6 ppm in untreated pods (Table, 8).

3.4.3 – Correlation coefficient:

The correlation coefficient between macro- and micro-element nutrients concentration and some plant enzymes i.e. chitinase, peroxidase and polyphenol oxidase which related to plant disease resistance in bean leaves as average of two seasons are shown in Table (9). Results indicated that the significantly correlation was found between chitinase activity and the leaves content from phosphorous, potassium, calcium, manganese and cupper). The positive correlations were found between potassium, calcium, manganese and cupper, while the highly significant negatively one was found with phosphorous (Table, 9). Results showed that negatively correlation was noticed between the activity of peroxidase enzyme and macro-elements of nitrogen, phosphorus, magnesium, zinc and copper while positive correlation was detected with potassium and sodium. The activity of polyphenol enzyme in bean plants was negatively correlated with nitrogen, phosphorus and magnesium, while the significantly positive correlation was found with potassium. It is clear that the activity of peroxidase was highly correlated with nutrients elements, followed by chitinase and polyphenol oxidase. The highly significant positive correlation was recorded between the potassium and the activity of studied plant enzymes which may reflect emphatic role of potassium in plant resistance. The activity of polyphenol oxidase was correlated with micro-elements.

Table (1): Some physico-chemical properties of soil sample from the experimental site.

Properties	**Items**
Sand %	29.2
Silt %	25.0
Clay %	25.2
Texture	Clay loam
pH (1:2.5 soil : water)	8.36
E. C. (1:2.5 soil : water)dS/m	0.30
$CaCO_3$	1.20
Organic matter %	1.50
Available macronutrients (mg/100 g soil)	
P	3.24
K	55.86
Mg	289
Co	693
Na	36.40
Available micronutrients (mg/Kg soil)	
Fe	7.40
Mn	3.30
Zn	1.80
Cu	0.90

Table (2): Effect of *Trichoderma* species treatments aganist the leaner mycelial growth of *Fusarium solani* and *Rhizoctonia solani* in *vitro* tests .

Trichoderma species	Antagonistic effect against			
	Fusarium solani		*Rhizoctonia. solani*	
	Mycelial diameter (cm)	**Reduction %**	Mycelial diameter (cm)	**Reduction %**
Trichoderma album	4.9 c[(1)]	**49**	4.2 c	**54**
Trichoderma hamatum	3.8 d	**58**	2.7 e	**70**
Trichoderma harzianum	5.0 c	**44**	3.5 d	**61**
hoderma viride	5.5 b	**39**	5.3 b	**42**
Control	9.0 a	-	9.0 a	-

(1) Means in each column followed by the same letter are not significantly different according to LSD test (P=0.05).

Table (3): Effect of *Trichoderma* species treatments on the percentage of damping-off disease of bean plants under greenhouse condition (artificially infection) .

Trichoderma species	Disease assessment					
	Damping-off				Survival %	
	pre-emergence		Post-emergence			
	Incidence %	**Reduction %**	Incidence %	**Reduction %**	Healthy plants %	**Increase %**
Fusarium solani						
Trichoderma album	15.0 c[(1)]	**69**	2.5 e	**96**	97.5 a	**121**
Trichoderma hamatum	9.0 e	**81**	7.5 b	**87**	92.5 d	**109**
Trichoderma harzianum	19.0 b	**61**	5.5 d	**90**	94.5 b	**114**
Trichoderma viride	14.0 d	**71**	5.7 c	**90**	94.3 c	**113**
Control	48.5 a	**-**	55.8 e	**-**	44.2 e	**-**
Rhizoctonia solani						
Trichoderma album	14.0 bc	**62**	2.1 c	**96**	97.9 a	**106**
Trichoderma hamatum	7.0 d	**80**	5.5 b	**90**	92.5 d	**95**
Trichoderma harzianum	16.0 b	**54**	5.2 b	**90**	94.8 b	**100**
Trichoderma viride	13.0 c	**63**	5.9 b	**89**	94.1 c	**98**
Control	35.0 a	**-**	52.5 a	**-**	47.5 a	**-**

(1) Means in each column (for each pathogenic fungus) followed by the same letter are not significantly different according to LSD test (P = 0.05).

Table (4): Effect of *Trichoderma* species treatments on the percentage of damping-off disease of bean plants under field applications (natural infection).

Trichoderma species	Disease assessment						
	Damping-off %				Root-rot %	Survival %	
	Pre-emergence		Post-emergence				
	Inc. %	**Red. %**	Inc. %	**Red. %**		Healthy plants %	**increase %**
Trichoderma album	16.0 c [(1)]	**38**	2.4 e	**82**	0.0	97.6 a	**29**
Trichoderma hamatum	7.0 e	**73**	6.5 b	**52**	2.2 b	91.3 d	**21**
Trichoderma harzianum	20.0 b	**22**	5.0 c	**53**	2.2 b	82.5 c	**22**
Trichoderma viride	12.0 d	**53**	4.7 d	**65**	0.0	95.3 b	**26**
Control	25.7 a	**-**	13.5 a	**-**	10.7 a	75.8 e	**-**

(1) Means in each column followed by the same letter are not significantly different according to LSD test (P=0.05).

Table (5): Effect of *Trichoderma* species treatments on some growth and yield parameters of bean plants under field applications (natural infection).

Growth and yield prameters		*Trichoderma* spp.				
		Trichoderma album	*Trichoderma hamatum*	*Trichoderma harzianum*	*Trichoderma virde*	control
Growth parameters						
Plant height (cm)	Average	46.0 a [(1)]	49.8 a	47.0 a	46.8 a	37.3 b
	Increase %	**23**	**34**	**26**	**26**	**-**
Branches no./	Average	5.0 b	5.2 b	6.3 a	5.5 ab	3.7 c

plant	**Increase %**	**35**	**41**	**70**	**49**	**-**
Fresh weight / plant	Average	43.1 c	77.4 a	57.9 b	51.2 bc	42.5 c
	Increase %	**2**	**82**	**36**	**36**	**21**
Leaves no./ plant	Average	11.5 b	14.8 ab	15.2 a	14.2 b	9.5 c
	Increase %	**21**	**56**	**60**	**50**	**-**
Yield parameters						
Pods no./ plant	Average	15.7 a	20.0 a	17.5 a	15.2 a	10.8 b
	Increase %	**45**	**85**	**62**	**41**	**-**
Pods weight **(g)**	Average	3.1 b	4.0 a	2.7 c	2.7 c	2.7 c
	Increase %	**15**	**48**	**0**	**0**	**-**
Pods fresh yield	Average	3.3 b	5.1 a	3.2 b	3.0 b	1.6 c
	Increase %	**106**	**219**	**100**	**99**	**-**

(1) Means in each row followed by the same letter are not significantly different according to LSD test (P = 0.05).

Table (6): Enzymatic activity of chitinase, peroxidase and polyphenol oxidae, in bean plants treated with *Trichoderma* species in field applications.

Trichoderma species	Enzymatic activities					
	Chitinase		Peroxidase		Polyphenol oxidase	
	Activity	**Increase %**	Activity	**Increase %**	Activity	**Increase %**
Trichoderma album	0.321 b[(1)]	**174**	0.437 c	**26**	0.231 d	**58**
Trichoderma hamatum	0.271 e	**132**	0.693 b	**100**	0.296 c	**103**
Trichoderma harzianum	0.293 c	**150**	0.775 a	**124**	0.518 a	**255**
Trichoderma viride	0.620 a	**430**	0.465 c	**34**	0.303 b	**108**
Control	0.117 d	**-**	0.346 d	**-**	0.146 e	**-**

(1) Means in each column followed by the same letter are not significantly different according to LSD test (P = 0.05).

Table (7) : Effect of *Trichoderma* species on the nutrient elements content in bean leaves in field application (Average of two season).

Nutrient elements	Nutrient content of leaves with *Trichoderma* species				
	Trichoderma album	*Trichoderma hamatum*	*Trichoderma harzianum*	*Trichoderma viride*	Control
Macro-elements (%)					
Nitrogen	2.21 d[(1)]	2.36 c	2.19 d	2.60 b	2.73 a
Phosphorus	0.16 bc	0.15 c	0.17 b	0.16 bc	0.21 a
Potassium	1.87 c	1.73 d	2.88 a	2.16 b	1.39 e
Magnesium	0..91 a	0.88 ab	0.68 d	0.84 bc	0.80 c
Calcium	3.15 b	3.10 c	2.80 d	3.25 a	2.75 e
Sodium	0.023 c	0.088 a	0.039 b	0.022 c	0.023 c
Micro-elements (ppm)					
Iron	1550 b	2150 a	1250 e	1425 c	1350 d
Manganese	75 b	84 a	74 b	82 a	71 c
Zinc	29 bc	28 c	31 b	31 b	34 a
Cupper	9 cd	9 cd	8 d	13 a	10 b

(1) Means in each row followed by the same letter are not significantly different according to LSD test (P = 0.05).

Table (8) : Effect of *Trichoderma* species on nutrient elements content in bean green pods in field application (Average of two season).

Nutrient elements	Nutrient content of bean pods with *Trichoderma* species				
	Trichoderma album	*Trichoderma hamatum*	*Trichoderma harzianum*	*Trichoderma viride*	Control
Macro-elements (%)					
Nitrogen	2.03 d (1)	2.13 c	3.09 b	1.79 e	3.37 a
Phosphorus	0.45 d	0.42 e	0.48 c	050 b	0.56 a
Potassium	3.60 c	2.26 e	4.42 a	3.55 d	3.65 b
Magnesium	0.38 b	0.32 c	0.38 b	0.37 b	0.43 a
Calcium	0.25 c	0.28 b	0.35 a	0.26 c	0.25 c
Sodium	0.017 b	0.015 c	0.022 a	0.016 b	0.014 c
Micro-elements (ppm)					
Iron	245 e	390 d	550 a	407 c	475 b
Manganese	19 d	20 c	22 b	26 a	26 a
Zinc	50 b	49 b	51 b	58 a	44 c
Cupper	6 c	6 c	10 a	7 b	6 c

(1) Means in each row followed by the same letter are not significantly different according to LSD test (P = 0.05).

Table (9): Correlation coefficient between nutrient elements content in bean leaves and enzymatic activity in leaves (Average of two seasons).

Nutrients content In bean leaves	Enzymatic activity in leaves		
	Chitinase	Peroxidiase	Polyphenol oxidase
Macro-elements (%)			
Nitrogen	0.0010(NS)	– 0.6489**	– 0.6148 **
Phosphorus	– 0.5174 **	– 0.4641 **	– 0.3671 *
Potassium	0.3904 *	0.6958 **	0.9547 **
Magnesium	0.1568 (NS)	– 0.4415 *	– 0.6449 **
Calcium	0.7629 **	– 0.1073 (NS)	– 0.1210 (NS)
Sodium	– 0.2055 (NS)	0.6556 **	0.2125 (NS)
Micro- elements (ppm)			
Iron	– 0.0696 (NS)	0.2685 (NS)	– 0.1905 (NS)
Manganese	0.5566 **	0.3103 (NS)	0.1205 (NS)
Zinc	– 0.2094 (NS)	– 0.3840 *	– 0.1934 (NS)
Cupper	0.6092 **	–.4507 *	– 0.2837 (NS)

(NS) = Non significant * , ** = Significant at the probability levels of 0.05 and 0.01 ,respectively r 0.05 = 0.361 r 0.01 = 0.463

DISCUSSION

Our results revealed that the *T. album*, *T. hamatum*, *T. harzianum* and *T. viride*, which obtained from the rhizosphere of healthy bean plants, have bean reported as the best antagonists for damping off disease caused by *F. solani* and *R. solani* under laboratory, pot and field conditions. All *Trichoderma* spp. treatments reduced the mycelial growth of two pathogenic fungi. It is very important, especially the chemical methods are not economical in the long run, because the pollution the atmosphere, damage the environment, leave harmful residues, and can lead to the development of resistant strains among the target organisms with repeated use. Results indicated that all *Trichoderma* spp. significantly reduced the disease incidence at pre- and post-emergence stages in pot and field experiments. These results agree with those recorded by **Abou-Zeid *et al.* (2003)** and **Pieta and Pastucha (2004).** They reported that *T. harzianum*, *T. koningii* and *T. viride* protected the germinating bean seedlings against *Fusarium* spp. and *R. solani* infection. Our results, based on soil treatments with the tested *Trichoderma* spp. demonstrated a significant reduction to incidence of damping off disease in bean under pot and field infection (**El-Kafrawy, 2002, Gonzalez *et al.*, 2005 and Malik *et al.*, 2005).**

Our results showed that the use of *Trichoderma* spp. as bio-control agents induced the accumulation of some enzymes such as chitinase, peroxidase and

polyphenol oxidase which play an important role in plant defense mechanisms against pathogens infection. Results cleared that the enzymatic activity in treated bean plants increased than in untreated one. **Nawar and Kuti (2003**) reported that there are positive relationships between peroxidase and resistance development in plants. **Caruso *et al*. (2001)** also experimentally supported the idea that peroxidase play a defense role against invading pathogens. **Hassan *et al*. (2007)** recorded the lowest percentages of chocolate spot disease severity and the highest levels of peroxidase activities. Treatments with *Trichoderma* spp. gave the highly protection o bean seedlings against damping off disease at post-emergences stage comparison with per-emergence one. It is may be related to the ability of *Trichoderma* spp. to stimulate the enzymes in bean plants associated with increased the protection against disease. Our results revealed that *Trchoderma* spp. treatments increased some macro- and micro-elements content in leave and pods of bean and decreased the content of nitrogen, phosphorus and magnesium. These results agree with those reported by **Snoeijers *et al* (2000**). They reported that the successful colonization of plants by pathogens requires efficient utilization of nutrient resources available in host tissue. Our results revealed that the *Trichoderma* treatments highly increased the activity in bean leaves than pods. The treatments significantly increased the macro-elements of potassium, magnesium, and calcium and micro-element of iron which play an important role in defense plant tissues against plant pathogen infection. Results indicated that the activity of plant enzymes (chitinase, peroxidase and polyphenol oxidase) was correlated with level of macro- and micro-elements in bean plants.

Trichoderma is listed both in Europe and USA as an active principal ingredient permitted for use in organic farming for plant disease control. *Trichoderma* spp utilize various mechanisms including nutrient competition, antibiosis, antagonism, inhibition of pathogen or plant enzymes; processes of biodegradation, carbon and nitrogen cycling; complex interactions with plants in the root zone of the rhizosphere, which involve various processes such as colonization, plant growth stimulation, bio-control of diverse plant pathogens, decomposition of organic matter, symbiosis, and nutrient exchange **(Howell,, 2003 and Harman, 2006).** Recent studies indicate that these fungi can induce systemic resistance in plants, thus increasing the plant defense response to diverse pathogen attack **(Harman *et al.*, 2004).**

CONCLUSION

The previous results concluded that the soil treatments with *Trichoderma* spp significantly reduced the incidence of damping off disease .Results showed iit was positive correlation between the activity of chitinase , peroxidase and polyphenol oxidase and the level of nutrient elements especially potassium. Results recommended that the determine the nutritional status of plant under field treated with bio-control agents helps to select the suitable fertilization programs as well as diagnose nutritional deficiencies

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