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ORIGINAL PAPER

Efficiency Assessment of Combinations Between *Rhizobium leguminosarum* and *Trichoderma* spp. for Controlling of Pea (*Pisum sativum* L.) Damping-off Disease

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ABSTRACT

Pea (*Pisum sativum* L.) is subjected to attack by certain soil-borne phylogenetic of fungus-like eukaryotic microorganisms such as *Pythium debaryanum* and soil-borne fungi *Rhizoctonia solani* and *Fusarium solani* which cause damping-off diseases. Isolation of associated fungi and *P. debaryanum* and three species of *Trichoderma* was carried out from soil rhizosphere of pea plants. Antagonistic effect of *Rhizobium leguminosarum* combined with *Trichoderma lignorum*, *T. longibrachiatum* and *T. koningii* against pathogenic fungi was investigated *in vitro* and *in vivo* under greenhouse conditions. The effect of combinations between the tested *Rhizobium* sp. and the other tested *Trichoderma* spp. on the disease incidence caused by the pathogenic microorganisms was evaluated when used as seed and soil treatments. Disease assessments, nitrogen fixation and yield parameters after 50 and 90 days from sowing in comparison with un-treated and fungicide treatments were recorded. Mycoparasitic activity of the tested *Trichoderma* spp. against each of the pathogenic fungi and *Pythium debaryanum* was studied using scanning electron microscope. Results showed that the soil treatments were more effective in controlling damping-off disease than seed treatments in overall experiments. Combination of *R. leguminosarum* with *T. longibrachiatum* gave the best results in reducing percentage of post-emergence damping-off (13.33 and 6.67%) and root rot (14.72 and 9.58%) caused by *P. debaryanum* and *R. solani*, respectively. Survived plants, nitrogen fixation and yield parameters were also increased. Treatment of *R. leguminosarum* combined with *T. koningii* against infection by *F. solani* gave the best results in reducing percentages of post-emergence damping-off (6.67%) and root rot (13.06%) with increment of survived plants, nitrogen fixation and yield parameters. In conclusion, combinations of *R. leguminosarum* with the *Trichoderma* species were effective than application of each one alone against pea damping-off disease.

Keywords: Damping-off, pea, root rot, *Rhizobium leguminosarum*, *Trichoderma* spp.

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INTRODUCTION

Pea (*Pisum sativum* L.) is considered to be an important leguminous crop in Egypt for both local consumption and exportation. Although, highly yield cultivars have been produced, the average of seed yield per unit is still not satisfied (Hamid *et al*., 2012). Several biotic and abiotic factors are responsible for low yield which affecting pea production. El-Mohamedy and Abd El-Baky (2008) reported that if biotic factors *e.g*., pea diseases are not managed well, the substantial yield losses must be occurred. Pea damping-off and root rot diseases caused by certain soil-borne pathogenic fungi are considered among the most serious seedling diseases which causing substantial losses either in seed quality or in yield (Persson *et al*., 1997). Unfortunately, commonly approved control strategy used against such kind of plant diseases still mainly depends on application of hazardous chemical fungicides. Chemical fungicides are considered serious problems to health and environment, for that reason there is an increase demand to find alternative eco-friendly ways to chemical fungicides in control processes. So, biological control strategy could be considered as an alternative way in management of many diseases to reduce the amount of used chemical

fungicides. Lifshitz *et al*. (1986) reported that application of conidia produced by *Trichoderma harzianum* or *T. koningii* as seed treatment reduced the pea damping-off disease incidence induced by *Pythium* sp. Application of *Trichoderma* spp. as seed or soil treatments against damping-off and root rot diseases of leguminous crops was done by many investigators (Abou-Zeid *et al*., 2003; Abd El-Khair *et al*., 2010 and El-Khateeb 2014). Certain isolates of *Rhizobium* sp. were reported to reduce disease incidence of *Pythium* damping-off of pea and protect a lot of leguminous crops to be infected by soil-borne fungi such as *Fusarium* spp., *Rhizoctonia* sp., *Macrophomina phaseolina* and *Sclerotium rolfsii* (Arfaoui *et al*., 2005 and Baraka *et al*., 2009). Combinations between *Rhizobium* sp. and *Trichoderma* spp. were reported not only for suppressing the soil-borne fungi, but also for improving the yield of chickpea through enhancing the growth parameters and nutrient uptake (Rudresh *et al*., 2005). Damping-off and root rot diseases of *Lupines terms*, *Cicer arietinum* and *Vicia fabae* were controlled biologically using *Rhizobium* and *Trichoderma*. Results obtained proved that previous microorganisms could be used in biological control against soil-borne fungi responsible for huge yield losses in fields of legume crops (Shaban and El-Bramawy, 2011). Recently, combinations between *Rhizobium* sp. and *Trichoderma* spp. were applied in faba bean and chickpea against damping-off disease caused by *Fusarium solani* (El-Khateeb 2014). The antagonistic potential effect of *Rhizobium* sp. combined with *Bacillus subtilis* (Rhizo-N) and *T. harzianum* (Plantgard) against *F. solani*, the causal agent of faba bean root rot was studied by Abd El-Khair *et al*. (2018) and a reasonable reducing of the disease was found. Additionally, faba bean growth parameters were improved and yield components were also increased as a result of combination application. Schmidt *et al*. (2019) found that there was indirect effect in plant growth caused by rhizobia inoculation in common bean plants against the fungal pathogens *Rhizoctonia solani*, *Fusarium oxysporum*, *Fusarium solani* and *Macrophomina phaseolina*.

The present work was established to study the efficiency of *R. leguminosarum* and *Trichoderma* spp., separately and in combinations against *P. debaryanum*, *R. solani* and *F. solani*, the causal agents of pea damping-off and root rot diseases in addition to improving of pea growth, nitrogen fixation parameters and yield components.

MATERIALS AND METHODS

Plant materials:

Pea (*Pisum sativum* L.) seeds of cv. Master B were obtained from Horticulture Research Institute, Agricultural Research Center (ARC), Dokki, Giza, Egypt.

Isolation and identification of pathogenic agents:

Infected pea plants showing typical symptoms of damping-off and root rot were collected from different locations of cultivated area at Kafr El-Sheikh governorate, Egypt. Infected plant parts were washed under running tap water, then surface sterilized using sodium hypochlorite (5%) for 3 min. Then all samples were passed through several replacements of sterilized distilled water, and finally dried between two folds of sterilized filter papers (Whatman, 1). Potato dextrose agar (PDA) medium amended with penicillin as antibiotic (20 Iu/ml) in Petri dishes (9cm) was used for growing the pathogenic microorganisms with incubation at 25±2ºC. All incubated dishes were observed daily for fungal growth, then the isolated fungi and phylogenetic of fungus-like eukaryotic microorganisms were purified using hyphal tip or single spore techniques according to Dhingra and Sinclair (1985). Identification of fungal isolates and phylogenetic of fungus-like eukaryotic microorganism according to Plaats-Niterick (1981); Sneh *et al*. (1991) and Leslie and Summerell (2006) was done in the Myco. Rese. and Dis. Surv Dept, Plant Pathology Res. Inst., A.R.C., Giza, Egypt.

Pathogenicity test:

Seeds of pea cultivar Master B were obtained from Horticulture Research Institute, Agricultural Research Center (ARC), Dokki, Giza, Egypt. Inocula of the three pathogens (*P. debaryanum*, two isolates of *R. solani* and three isolates of *F. solani*) were prepared by growing isolates in autoclaved bottles contained (95 g clean moistened sand: 5 g corn meal) incubated at 25±2ºC (Ghoneim and Belal, 2013). The sandy loam soil was autoclaved at 121ºC for 2 hr. Plastic pots 30 cm diameter were sterilized using 5% formalin and left for 2 days to ensure complete formalin evaporation. Soil infestation was carried out by adding the previous inoculum to each pot at the rate of 3% of the soil weight. After seven days from soil infestation, five surface sterilized pea seeds were sown for each pot in controlled greenhouse conditions (24ºC, 60% humidity and 11 hr day light). Three replicates were used for each pathogen and pots

had pathogen-free sandy loam soil were used for planting a check treatment. According to Shaban and El-Bramawy (2011) percentage of damping-off 15, 30 and 45 days after sowing was recorded.

Isolation and identification of *Trichoderma* spp.:

Using dilution plate technique, three *Trichoderma* species, were isolated from the rhizosphere of healthy pea plants grown at Kafr El-Shiekh governorate, Egypt. All *Trichoderma* species were purified by hyphal tip technique and the identification was done based on the cultural and morphological characteristics according to Bissett (1991) by the specialists of the Mycological Research and Disease Survey Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

***In vitro* experiments:**

Efficiency assessment of antagonistic effects of *Trichoderma* species against *P. debaryanum* (1 isolate), *R. solani* (2 isolates) and *F. solani* (3 isolates) (causal agents of pea damping-off diseases) were evaluated using dual culture technique according to Coskuntuna and Özer (2008). Reduction percentage and decrease of mycelial growth of the causals as a result of antagonistic effect of *Trichoderma* species was estimated according to Abd El-Khair *et al*. (2010) using the following formula:

$$\text{Antagonistic effect} = A\text{-}B/A \times 100$$

Where, A = mycelial growth diameter of pathogenic fungus or *P. debaryanum* in control, B = mycelial growth diameter of the pathogenic fungus or *P. debaryanum* in dual cultured with *Trichoderma* species.

Scanning electron microscope examination:

To study the interaction between the causals of damping-off and root rot of pea and *Trichoderma* spp., small pieces of agar were cut at the margin of the causals and antagonistic fungi growth and transferred for dehydrating and subsequently sputter coated with gold according to methods of Harley and Ferguson (1990). Examination and photographing were done using scanning electron microscope (SEM) JEOL JSM 6510 Iv, Faculty of Agriculture, Mansoura University, to observe the antagonistic effect through parasitism action.

Culturing of *Rhizobium leguminosarum*:

One loopful of the purified isolate of *R. leguminosarum* bv. *vicieae* obtained from Agricultural Botany Department, Faculty of Agriculture, Kafrelsheikh University was cultured on yeast extract mannitol broth (YMB) medium and incubated in a shaken incubator (150 rpm) at 30ºC for 3-5 days (Belal *et al*., 2013).

***In vivo* (greenhouse) experiments:**

Pots 30 cm diameter filled with sandy loam soil and pea seeds were sown each with 5 seed/pot. Three pots were used for each treatment. Infested soil with the pathogenic agents, each alone was prepared one week before sowing of seeds. Preparation of the pathogenic agents inocula were done as mentioned before. All pots were irrigated 3 times through the seven days. Antagonistic treatments were applied as seed and soil treatments at the time of sowing in controlled greenhouse conditions (24ºC, 60% humidity and 11 hr day light) as follows:

Seed treatment:

Pea cv. Master B seeds were immersed for 20 min in spore suspension of *Trichoderma* species (each one alone) in a concentration of 10^6 spore/ml. For collecting the spores of *Trichoderma* species (each alone), medium consists of wheat bran, sawdust and tap water in ration 3:1:4 v/v, media were inoculated separately with any of the tested species of *Trichoderma* and incubated at 28 ºC for 10 days according to Elad *et al.* (1980), then sterilized water was added to wheat grain medium and filtered through cheesecloth. Spore suspensions were adjusted to 10^6 spore/ml. Application of *R. leguminosarum* to be combined with *Trichoderma* spp., was done according to methods of Vincent (1970). Seeds covered with spores of *Trichoderma* species (each one alone) were sprayed with 10% Arabic gum solution and mixed immediately with the prepared peat-based inoculum [cultures of *R. leguminosarum* were adjusted to 10^8 CFU/ml and mixed well at room temperature (antagon. 30 ºC) with sterilized peat moss at the rate of 1 ml/g peat moss and left for 48 hr]. Seeds were dried at room temperature for 30 min in shade and then sown according to Goniem and Belal (2013). Seed treatments were applied against *P. debaryanum*, *R. solani* and *F. solani*. For comparison, the fungicide Moncut® 25% WP (Flutolanil) was used as seed dressing (3 g/kg seeds).

Soil treatment:

Spore suspensions of *Trichoderma* species (each one alone) were added at the rate of 100 ml/pot in a concentration of 10^6 spore/ml + 100 ml/pot of bacterial suspension of *R. leguminosarum* in a concentration of 10^8 CFU/ml at the sowing time as soil drench. Soil treatments were applied against *P. debaryanum*, *R. solani* and *F. solani*. Fertilization, irrigation and insect control were applied as recommended.

Disease assessment:

Percentages of pre- and post-emergence damping-off and survived plants were calculated according to Shaban and El-Bramawy (2011), while for root rot assessments, the scale 0-4 of Hwang and Chang (1989) with minor modification was used as follow: 0 = healthy roots, 1 = 1-9%, 2 >= 9-39%, 3 >= 39-69% and 4 >= 69% and above of root discoloration. Root discoloration was recorded at the end of experiment and calculated according to the following formula:

Root rot index = [total of all ratings / (total number of plants × 4)] × 100

Measurement of studied characters:

After 50 days from sowing, samples of pea plants belonging to each treatment were removed from the soil and washed thoroughly with tap water. Then, nitrogen fixation parameters *viz*., No. of nodules/ plant, dry weight of nodules (g)/ plant, shoot length (cm), dry weight of shoots (g), N% and total N (mg)/plant were taken. Yield parameters after 90 days from sowing *viz*., dry weight of pods (g)/plant, dry weight of seeds (g)/plant, N% and total N (mg)/plant were also determined.

Determination of nitrogen percentage

Plant samples were air dried, grinded and a known weight (0.2 gm) was digested according to method of Chapman and Parker (1963) in 5 ml H_2SO_4 and 1 ml of perchloric acid, to determine the nitrogen percentage and total content. For distillation, digested materials were treated with 40% NaOH and the evaporated ammonia was received in 4% boric acid solution. According to methods of Black *et al*. (1965), the distillates were titrated with 0.02 M H_2SO_4, using a mixture of methyl red and bromocrystal green as an indicator. Nitrogen percentage was calculated based on dry weight, while total nitrogen content was determined as follows:

Total nitrogen content =

N% × dry weight of plants (Black *et al.* 1965).

Effect of fungicide on *Rhizobium* sp. and *Trichoderma* spp.:

To study the effect of Moncut®25% WP (Flutolanil) fungicide on *R. leguminosarum, T. lignorum, T. longibrachiatum* and *T. koningii*, three concentrations 0.75, 1.5 and 3g/l were used in *in vitro* experiments. Prepared Petri dishes with PDA medium containing any of the three concentrations of Moncut fungicide tested were inoculated at the center with discs (5 mm diameter) obtained from 5 days old cultures of any tested *Trichoderma* spp. and incubated at 28ºC. Three replicates were used for each treatment and culture media free of fungicide were used as a control treatment. All treatments were observed daily for growth. At the time of full growth of control treatment (10 days), the measurements of growth diameter (cm) for each isolate were taken. Spores were collected by gently rubbing the agar surface with a sterilized brush and rinsed with 10 ml of sterilized water. The suspension was filtered through cheesecloth to remove all mycelial fragments. Spores were counted by hemocytometer in complete volume of 100 ml.

With *R. leguminosarum*, the inhibitory effect of fungicide was evaluated by the disc diffusion method described by Thornberry (1950). Yeast mannitol agar medium was inoculated by *Rhizobium* before pouring in Petri dishes, then filter paper discs (1 cm diameter) were immersed in 0.05 ml of any of the previously mentioned fungicide concentrations and placed onto the surface of culture media. Three dishes were used as replicates for each treatment as well as control treatment (discs immersed in sterilized water). The diameter of inhibition zone as effect of fungicide on the growth, was measured after incubation at 30ºC for 48 hr.

Statistical analysis:

All experiments were designed as randomized complete with factorial arrangement. One-way analysis of variance (ANOVA) was carried out by Costat software. Means were compared using L.S.D. (Stell and Torrie, 1980) and Duncan multiple range test DMRT (Duncan, 1955).

RESULTS

Isolation and identification of pathogenic agents:

Five fungal isolates and a fungus-like eukaryotic microorganism were isolated from diseased pea cv. Master B plants showing typical symptoms of damping-off and root rot. These fungi were purified and identified as *Rhizoctonia solani* Kühn (isolates No. R1 and R2) and *Fusarium solani* (Mart) Sacc. (isolates No. F1, F2 and F3) and a fungus-like eukaryotic microorganism was identified as *Pythium debaryanum* (R. Hesse).

Pathogenicity test:

Data summarized in Table (1) show that only one isolate of *P. debaryanum* evaluated for its pathogenicity test gave 53.33% % pre-emergence damping-off, 20.00% post-emergence damping–off, 26.67% survived plants and 75.00% root rot (Table 1) in comparison with control treatment (un-infested soil). It is clear from the obtained data presented in Table (1) that between

Fusarium isolates tested *F. solani*, isolate No. F1 gave the highest pre-emergence damping-off (46.67% %), 20.00% post-emergence damping-off, 33.33% survived plants and 66.67% root rot in comparison with control treatment (pea grown in un-infested soil). Whereas *R. solani*, isolate No. R1 gave 40.00% % pre-emergence damping-off, 26.67% post-emergence damping-off, 33.33% survived plants and 58.33% root rot (Table 1) in comparison with control treatment (un-infested soil).

Table (1): Pathogenicity evaluation of five fungal isolates and a fungus-like eukaryotic microorganism

Treatment	Disease assessment			
	% Pre-emergence damping-off	% Post-emergence damping-off	% Survived plants	% Root rot
Control (un–infested soil)	0.00 g	0.00 a	100.00 f	0.00 g
Pythium debaryanum	53.33 a	20.00 d	26.67 e	75.00 a
Rhizoctonia solani (R1)	40.00 c	26.67 c	33.33 d	58.33 c
Rhizoctonia solani (R2)	26.67 e	20.00 d	53.33 a	48.61 e
Fusarium solani (F1)	46.67 b	20.00 d	33.33 d	66.67 b
Fusarium solani (F2)	33.33 d	26.67 c	40.00 c	51.39 d
Fusarium solani (F3)	20.00 f	33.33 b	46.67 b	44.45 f

The numbers in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

Isolation and identification of *Trichoderma* spp.:

Three *Trichoderma* species were isolated from the rhizosphere of healthy pea plants grown at Kafr El-Shiekh governorate, Egypt. All *Trichoderma* species were purified by hyphal tip technique and identified as *Trichoderma lignorum*, *T. longibrachiatum* and *T. koningii* based on the cultural and morphological characteristics according to Bissett (1991).

Antagonistic activity of *Trichoderma* spp. *in vitro*:

Antagonistic effect of *Trichoderma* spp. against *P. debaryanum*, *F. solani* and *R. solani* was studied *in vitro* and the results of reduction percentage and mycelial diameter of causals were tabulated in Table (2). Results presented in Table (2) show that the antagonism of *T. longibrachiatum* against *P. debaryanum* was significantly effective than other used *Trichoderma* species representative in reduction percentage in mycelial growth (59.74%). Similarly, application of *T. longibrachiatum* against *R. solani* (R1 isolate) was significantly effective than other used *Trichoderma* species representative in Table (2) as a reduction percentage in mycelial growth (71.48%). While, application of *T. koningii* against *F. solani* (F1 isolate) was significantly effective than other used *Trichoderma* species representative in Table (2) as a reduction percentage in mycelial growth (68.14%).

Table (2): *In vitro* effect of *Trichoderma* species treatment on the radial growth of *P. debaryanum*, *R. solani* (R1 isolate) and *F. solani* (F1 isolate)., incubated at 27°C.

Interaction between pathogenic fungi and *Trichoderma* spp.	Antagonistic effect	
	Mycelial diameter of pathogenic fungi (cm)	Reduction (%)
*P. debaryanum**	9.00 a	0.00 d
T. lignorum	4.76 b	47.04 c
T. koningii	4.26 c	52.59 b
T. longibrachiatum	3.60 d	59.74 a
R. solani (R1), control plates	9.00 a	0.00 d
T. lignorum	2.93 c	67.40 b
T. longibrachiatum	2.56 d	71.48 a
T. koningii	3.33 b	62.96 c
F. solani (F1), control plates	9.00 a	0.00 d
T. lignorum	3.93 b	56.30 c
T. koningii	2.87 d	68.14 a
T. longibrachiatum	3.36 c	62.59 b

* control plates.; The numbers in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

Scanning electron microscope observations:

Mycoparasitic activity of *Trichoderma* spp. against *P. debaryanum, R. solani* (R1 isolate) and *F. solani* (F1 isolate) was examined using SEM. *Trichoderma* spp. parasitized hayphae of *P. debaryanum* when grown in dual culture on agar medium. Several abnormalities were noted when *P. debaryanum* was parasitized by *T. longibrachiatum*, which gave the best inhibitory effect. Coiling, swelling, lysis and empty hyphae of *P. debaryanum* were observed and hyphae of *T. longibrachiatum* advanced over the colony of pathogenic fungus-like eukaryotic micro-organism (Fig. 1A). *Trichoderma* spp. were also observed to be sporulated abundantly in areas of hyphal interactions (Fig. 1A).

Results show that *R. solani* (R1 isolate) was also parasitized by *T. longibrachiatum*, which gave the best inhibitory effect as a result of interaction between them (Fig. 1B). Empty hyphae as an interaction of *T. koningii* growing with *F. solani* (F1 isolate) was also observed (Fig. 1 C).

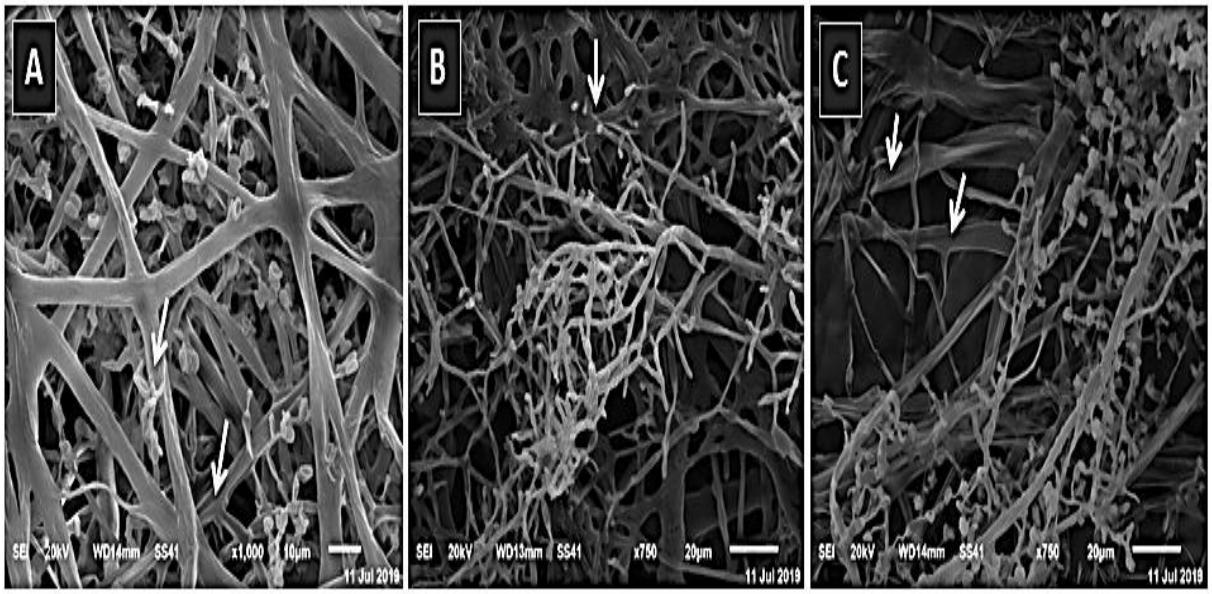

Figure (1): Interaction between pathogenic agents and *Trichoderma* spp. in dual culture: A) coiling and empty hyphae as interaction of *T. longibrachiatum* sporulation and growing with *P. debaryanum* (white arrows), B) coiling hyphae as interaction of *T. longibrachiatum* growing with *R. solani* (white arrows) and C) empty hyphae as interaction of *T. koningii* growing with *F. solani* (white arrows).

Combinations between *Rhizobium* sp. and *Trichoderma* spp.:

***In vivo* effect against *P. debaryanum*:**

Obtained results summarized in Table (3) show that soil treatments were significantly effective than seed treatments in controlling booth damping-off and root rot of pea. The most effective treatment against *P. debaryanum* under the study was Moncut 25% WP fungicide treatment. Interestingly, combination of *R. leguminosarum* with *T. longibrachiatum* gave the best reduction percentage with pre-emergence damping-off recorded 6.67%, post-emergence damping-off (13.33%), meanwhile survived plants were 80% and root rot recorded 14.72 % against *P. debaryanum* (Table 3) when applied as soil treatments. Application of *R. leguminosarum* alone against *P. debaryanum* resulted in significant reduction percentage with pre-emergence damping-off (26.67%), post-emergence damping-off (26.67%), but survived plants recorded 46.67% and root rot (25.00%) compared with the inoculated control infested with *P. debaryanum* alone (Table 3).

Plant growth and N-fixing parameters were also studied. Results presented in Table (4) show that applied treatments significantly increased the plant growth and N-fixing parameters of pea plants measured after 50 days from sowing compared to infected control (Table 4). Combinations of *R. leguminosarum* with *T. longibrachiatum* gave the best increasing of all N-fixing parameters of pea plants such as No. of nodules/plant (52.03), dry weight of nodules/plant (0.42 g), shoot length (24.56 cm), dry weight of shoots/plant (5.45 g), percentage of N/plant (2.55 %) and total N/plant (128.98 mg) when applied as soil treatments compared to other used treatments (Table 4).

Table (3): *In vivo* effect of combinations between *Rhizobium* sp. and *Trichoderma* spp. against *P. debaryanum*

Treatment	Disease assessment			
	% Pre-emergence damping–off	% Post-emergence damping–off	% Survived plants	% Root rot
Control healthy	0.00 f	0.00 d	100.00 a	0.00 h
Control healthy + *R. leguminosarum*	0.00f	0.00d	100.00 a	0.00 h
Control infested	60.00 a	13.33 c	26.67 h	73.83 a
Control infested + *R. leguminosarum*	26.67 b	26.67 a	46.67 g	25.00 b
Seed treatment				
R. leguminosarum + *T. lignorum*	26.67 b	20.00 b	53.33 f	20.83 c
R. leguminosarum. + *T. longibrachiatum*	13.33 d	20.00 b	66.67 d	18.76 cd
R. leguminosarum. + *T. koningii*	13.33 d	26.67 a	60.00 e	20.14 c
R. leguminosarum + Moncut 25%WP	20.00 c	0.00 d	80.00 b	10.42 g
Soil treatment				
R. leguminosarum + *T. lignorum*	20.00 c	13.33 c	66.67 d	17.28 de
R. leguminosarum + *T. longibrachiatum*	6.67 e	13.33 c	80.00 b	14.72 f
R. leguminosarum + *T. koningii*	13.33 d	13.33 c	73.33 c	15.89 ef

The numbers in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

Table (4): *In vivo* effect of combinations between *Rhizobium* sp. and *Trichoderma* spp. on N-fixation parameters after 50 days from sowing, in artificially infested soil by *P. debaryanum*

Treatment	N- fixation parameters after 50 days from sowing					
	No. of nodules/ plant	Dry weight of nodules (g)/ plant	Shoot length (cm)	Dry weight of shoots (g)/plant	% N	Total N (mg)/plant
Control healthy	19.05 i	0.18 e	27.24 a	7.54 b	1.67 d	125.88 b
Control healthy + *R. leguminosarum*	82.08 a	0.52 a	27.58 a	8.27 a	2.69 a	222.19 a
Control infested	9.80 k	0.09 f	17.56 f	1.54 g	1.31 e	20.28 i
Control infested + *R. leguminosarum*	23.56 h	0.16 e	21.67 e	2.70 f	1.64 d	44.52 h
Seed treatment						
R. leguminosarum + *T. lignorum*	38.14 g	0.25 d	23.55 d	3.92 e	1.97 c	77.57 g
R. leguminosarum. + *T. longibrachiatum*	42.67 e	0.32 c	24.98 bc	4.25 e	2.17 bc	92.37 e
R. leguminosarum. + *T. koningii*	40.63 f	0.28 cd	24.34 cd	4.19 e	2.06 bc	86.53 f
R. leguminosarum + Moncut 25%WP	12.99 j	0.12 ef	25.65 b	5.61 c	1.53 d	86.31 f
Soil treatment						
R. leguminosarum + *T. lignorum*	46.03 d	0.39 b	23.39 d	4.83 d	2.16 bc	104.58 d
R. leguminosarum + *T. longibrachiatum*	52.03 b	0.42 b	24.56bcd	5.45 c	2.55 a	128.98 b
R. leguminosarum + *T. koningii*	49.37 c	0.40 b	24.16 cd	5.15 cd	2.26 b	116.30 c

The numbers in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

Data in Table (5) show that combinations of *R. leguminosarum* with *T. longibrachiatum* gave the best increasing of all yield parameters of pea plants measured after 90 days from sowing, expressed as dry weight of pods/plant (13.88 g), dry weight of seeds/plant (7.27 g), percentage of N/plant (3.14 %) and total N/plant (228.66 mg) when applied as soil treatments (Table 5) compared to other used treatments. Although, application of Moncut fungicide significantly decreased disease incidence and increased the growth parameters and yield parameters, unfortunately harmful effect in the plants was observed on N-fixing parameters (Table 5).

Table (5): *In vivo* effect of combinations between *Rhizobium* sp. and *Trichoderma* spp. on yield parameters after 90 days from sowing, in artificially infested soil by *P. debaryanum*

Treatment	N-fixation and yield parameters after 90 days from sowing			
	Dry weight of pods (g)/ plant	Dry weight of seeds (g)/plant	% N	Total N (mg)/plant
Control healthy	16.25 ab	9.59 b	2.18 cd	207.54 d
Control healthy + *R. leguminosarum*	18.68 a	10.58 a	3.68 a	385.29 a
Control infested	7.54 d	3.15 g	1.74 d	54.85 j
Control infested + *R. leguminosarum*	11.36 c	4.16 f	2.26 cd	93.88 i
Seed treatment				
R. leguminosarum + *T. lignorum*	12.82 bc	5.33 e	2.65 bc	147.59 h
R. leguminosarum. + *T. longibrachiatum*	13.27 bc	6.28 d	2.97 b	186.40 e
R. leguminosarum. + *T. koningii*	9.77 cd	5.77 d	2.76 bc	159.15 g
R. leguminosarum + Moncut 25%WP	14.27 bc	7.48 c	1.97 d	147.11 h
Soil treatment				
R. leguminosarum + *T. lignorum*	13.15 bc	6.13 d	2.87 b	180.54 f
R. leguminosarum + *T. longibrachiatum*	13.88 bc	7.27 c	3.14 b	228.66 b
R. leguminosarum + *T. koningii*	13.44 bc	6.89 c	3.08 b	215.77 c

The numbers in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

***In vivo* effect against *R. solani*:**

Results show that the soil treatments were significantly more effective than seed treatments in overall experiments (Table 6). The most effective treatment against *R. solani* was Moncut 25% WP fungicide treatment. Within the soil treatment experiments, combination of *R. leguminosarum* with *T. longibrachiatum* gave the best reduction percentage with pre-emergence damping-off (6.67%), post-emergence damping-off (6.67%), while survived plants recorded 86.67% and root rot (9.58%) caused by *R. solani* (Table 6). Application of *R. leguminosarum* alone against *R. solani* resulted in significant reduction percentage with pre-emergence damping-off (20.00%), post-emergence damping-off (20.00%), but survived plants resulted 60.00% and root rot (19.45%) compared with the control of plants grown in infested soil only with *R. solani* alone (Table 6).

Table (6): *In vivo* effect of combinations between *Rhizobium* sp. and *Trichoderma* spp. against *R. solani*

Treatment	Disease assessment			
	% Pre-emergence damping–off	% Post-emergence damping–off	% Survived plants	% Root rot
Control healthy	0.00 e	0.00 a	100.00 a	0.00 g
Control healthy + *R. leguminosarum*	0.00 e	0.00 d	100.00 a	0.00 g
Control infested	46.67 a	20.00 a	33.33 h	62.50 a
Control infested + *R. leguminosarum*	20.00 b	20.00 a	60.00 g	19.45 d
Seed treatment				
R. leguminosarum + *T. lignorum*	6.67 d	20.00 a	73.33 e	13.89 d
R. leguminosarum. + *T. longibrachiatum*	6.67 d	13.33 b	80.00 d	13.06 d
R. leguminosarum. + *T. koningii*	20.00 b	13.33 b	66.67 f	15.28 c
R. leguminosarum + Moncut 25%WP	6.67 d	0.00 d	93.33 b	5.42 f
Soil treatment				
R. leguminosarum + *T. lignorum*	13.33 c	6.67 c	80.00 d	10.42 e
R. leguminosarum + *T. longibrachiatum*	6.67 d	6.67 c	86.67 c	9.58 e
R. leguminosarum + *T. koningii*	13.33 c	13.33 b	80.00 d	13.06 d

The numbers in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

Plant growth and N-fixing parameters were also studied. Results show that applied treatments significantly increased the plant growth and N-fixing parameters of pea plants measured after 50 days from sowing compared to infected control (Table 7). Combinations of *R. leguminosarum* with *T. longibrachiatum* gave the best increasing of all N-fixing parameters of pea plants such as No. of nodules/plant (62.99), dry weight of nodules/plant (0.47 g), shoot length (26.46 cm), dry weight of shoots/plant (6.84 g), percentage of N/plant (2.46 %) and total N/plant (168.49 mg) when applied as soil treatments (Table 7) compared to other used treatments. Results of yield parameters were similar to those obtained with growth parameters.

Data in Table (8) indicate that combinations of *R. leguminosarum* with *T. longibrachiatum* gave the best increasing of all yield parameters of pea plants measured after 90 days from sowing, such as dry weight of pods/plant (15.65 g), dry weight of seeds/plant (8.86 g), percentage of N/plant (3.16 %) and total N/plant (280.38 mg) when applied as soil treatments (Table 8) compared to other used treatments.

Table (7): *In vivo* effect of combinations between *Rhizobium* sp. and *Trichoderma* spp. on N-fixation parameters after 50 days from sowing, in artificially infested soil by *R. solani*

Treatment	N-Fixation parameters after 50 days from sowing					
	No. of nodules/ plant	Dry weight of nodules (g)/ plant	Shoot length (cm)	Dry weight of shoots (g)/plant	% N	Total N (mg)/plant
Control healthy	19.05 g	0.18 g	27.24 ab	7.54 ab	1.67 g	126.22 e
Control healthy + *R. leguminosarum*	82.08 a	0.52 a	28.56 a	8.27 a	2.69 a	222.19 a
Control infested	13.20 h	0.14 h	20.66 f	1.94 f	1.49 h	28.97 i
Control infested + *R. leguminosarum*	29.06 f	0.25 f	23.87 e	4.31 e	1.78 f	77.01 h
Seed treatment						
R. leguminosarum + *T. lignorum*	41.18 e	0.35 e	24.82 de	5.40 d	2.18 de	117.72 f
R. leguminosarum. + *T. longibrachiatum*	45.85 d	0.39 d	25.06 cde	6.34 cd	2.29 cd	144.90 c
R. leguminosarum. + *T. koningii*	39.27 e	0.33 e	24.15 e	5.19 de	2.08 e	108.37 g
R. leguminosarum + Moncut 25%WP	17.85 g	0.15 h	27.93 a	7.16 bc	1.62 g	116.32 f
Soil treatment						
R. leguminosarum + *T. lignorum*	56.38 c	0.44 c	25.97 bcd	6.25 cd	2.34 c	146.54 c
R. leguminosarum + *T. longibrachiatum*	62.99 b	0.47 a	26.46 bc	6.84 bc	2.46 b	168.49 b
R. leguminosarum + *T. koningii*	54.32 c	0.41 d	25.27 cde	6.14 cd	2.27 cd	139.86 d

The numbers in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

Table (8): *In vivo* effect of combinations between *Rhizobium* sp. and *Trichoderma* spp. on yield parameters after 90 days from sowing, in artificially infested soil by *R. solani*

Treatment	N-fixation and yield parameters after 90 days from sowing			
	Dry weight of pods (g)/ plant	Dry weight of seeds (g)/plant	% N	Total N (mg)/plant
Control healthy	16.25 b	9.59 ab	2.18 f	209.21 f
Control healthy + *R. leguminosarum*	18.68 a	10.58 a	3.68 a	389.35 a
Control infested	8.94 e	3.84 g	1.96 g	75.46 i
Control infested + *R. leguminosarum*	12.51 d	6.28 f	2.47 e	154.82 h
Seed treatment				
R. leguminosarum + *T. lignorum*	14.38 c	7,54 de	2.96 cd	223.22 e
R. leguminosarum. + *T. longibrachiatum*	14.83 bc	8.15 cde	3.06 bc	249.66 d
R. leguminosarum. + *T. koningii*	14.18 c	7.33 e	2.86 d	209.64 f
R. leguminosarum + Moncut 25%WP	15.88 bc	9.03 bc	2.16 f	196.33 g
Soil treatment				
R. leguminosarum + *T. lignorum*	15.33 bc	8.54 bcd	3.09 bc	261.81 c
R. leguminosarum + *T. longibrachiatum*	15.65 bc	8.86 bc	3.16 b	280.38 b
R. leguminosarum + *T. koningii*	14.83 bc	8.29 cde	3.07 bc	254.78 cd

The numbers in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

In vivo* effect against *F. solani

Evaluation the effect of combinations between *Rhizobium* sp. and *Trichoderma* spp. against *F. solani*, indecated that the soil treatments were significantly effective than seed treatments in overall experiments. The most effective treatment against *F. solani* under study was Moncut 25% WP fungicide treatment. Within soil treatment experiments, combination of *R. leguminosarum* with *T. koningii* gave the best reduction percentage in pre-emergence damping-off (13.33%), post-emergence damping-off (6.67%), meanwhile survived plants recorded 80.00% and root rot (13.06%) against infection by *F. solani* (Table 9). Application of *R. leguminosarum* alone against *F. solani* resulted in significant reduction percentage with pre-emergence damping-off (20.00%), post-emergence damping-off (26.67%), increased survived plants 53.33% and minimized root rot (22.22%) compared with the control of pea plants grown in infested soil with *F. solani* alone (Table 9).

Table (9): *In vivo* effect of combinations between *Rhizobium* sp. and *Trichoderma* spp. against *F. solani*

Treatment	Disease assessment			
	% Pre-emergence damping–off	% Post-emergence damping–off	% Survived plants	% Root rot
Control healthy	0.00 e	0.00 e	100.00 a	0.00 g
Control healthy + *R. leguminosarum*	0.00 e	0.00 e	100.00 a	0.00 h
Control infested	46.67 a	26.67 a	26.67 h	70.92 a
Control infested + *R. leguminosarum*	20.00 b	26.67 a	53.33 9	22.22 b
Seed treatment				
R. leguminosarum + *T. lignorum*	20.00 b	20.00 b	60.00 f	19.45 c
R. leguminosarum. + *T. longibrachiatum*	13.33 c	26.67 a	66.67 e	17.36 d
R. leguminosarum. + *T. koningii*	20.00 b	13.33 c	66.67 e	15.28 e
R. leguminosarum + Moncut 25%WP	6.67 d	6.67 d	86.67 b	7.50 g
Soil treatment				
R. leguminosarum + *T. lignorum*	20.00 b	6.67 d	73.33 d	15.98 e
R. leguminosarum + *T. longibrachiatum*	6.67 d	13.33 c	80.00 c	14.72 e
R. leguminosarum + *T. koningii*	13.33 c	6.67 d	80.00 c	13.06 f

The numbers in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

Results of plant growth and N-fixing parameters show that applied treatments significantly increased the plant growth and N-fixing parameters of pea plants measured after 50 days from sowing compared to infected control (Table 10). Combinations of *R. leguminosarum* with *T. koningii* gave the best increasing of all N-fixing parameters of pea plants such as No. of nodules/plant (55.95), dry weight of nodules/plant (0.46 g), shoot length (25.36 cm), dry weight of shoots/plant (5.94 g), percentage of N/plant (2.32 %) and total N/plant (137.53 mg) when applied as soil treatments (Table 10) compared to other used treatments. Results of yield parameters showed the same trend those obtained with growth parameters. Data in Table (11) report that combinations of *R. leguminosarum* with *T. koningii* gave the best results in increasing all yield parameters of pea plants measured after 90 days from sowing, such as dry weight of pods/plant (15.11 g), dry weight of seeds/plant (7.69 g), percentage of N/plant (3.13 %) and total N/plant (240.54 mg) when applied as soil treatments (Table 11) compared to other used treatments.

Effect of fungicide on *Rhizobium* sp. and *Trichoderma* spp.

To achieve a real plant protection, integrated pest management must be effective and sufficient. For that purpose, Moncut 25% WP fungicide was tested for controlling pre- and post-emergence damping-off diseases in pea plants and its effect on *Rhizobium* sp. and *Trichoderma* spp. Results summarized in Table (12) indicate that the used fungicide Moncut was toxic to both *Rhizobium* sp. and *Trichoderma* spp. at the recommended doses. Moncut at the concentration of 3 g/l inhibited the growth of *R. leguminosarum* (4.43 cm) compared to untreated control. Consequently, the adverse effect of used fungicide was very clear on the three *Trichoderma* species either on growth diameter or sporulation (Table 12). Low concentrations had less inhibitory effect against *R. leguminosarum*, *T. lignorum*, *T. longibrachiatum* and *T. koningii.*

Table (10): *In vivo* effect of combinations between *Rhizobium* sp. and *Trichoderma* spp. on N-fixation parameters after 50 days from sowing, in artificially infested soil by *F. solani*

Treatment	N-fixation parameters after 50 days from sowing					
	No. of nodules/ plant	Dry weight of nodules (g)/ plant	Shoot length (cm)	Dry weight of shoots (g)/plant	% N	Total N (mg)/plant
Control healthy	19.05 h	0.18 f	27.24 b	7.54 b	1.67 d	126.11 c
Control healthy + *R. leguminosarum*	81.97 a	0.52 a	28.56 a	8.27 a	2.68 a	222.26 a
Control infested	13.03 i	0.09 g	18.36 e	1.64 i	1.43 d	23.56 h
Control infested + *R. leguminosarum*	28.00 g	0.19 f	22.14 d	3.05 h	1.78 c	54.14 g
Seed treatment						
R. leguminosarum + *T. lignorum*	41.63 f	0.31 e	23.84 c	4.35 g	2.09 bc	91.13 f
R. leguminosarum. + *T. longibrachiatum*	42.81 f	0.34 de	24.45 c	4.62 fg	2.15 bc	99.25 e
R. leguminosarum. + *T. koningii*	46.16 e	0.37 cde	24.64 c	4.85 ef	2.20 bc	106.93 d
R. leguminosarum + Moncut 25%WP	13.25 i	0.15 f	25.77 c	6.24 c	1.58 d	98.49 e
Soil treatment						
R. leguminosarum + *T. lignorum*	50.84 d	o.40 bcd	24.68 c	5.16 e	2.16 bc	111.70 d
R. leguminosarum + *T. longibrachiatum*	54.27 c	0.43 bc	24.86 c	5.67 d	2.24 bc	126.27 c
R. leguminosarum + *T. koningii*	55.95 b	0.46 b	25.36 c	5.94 cd	2.32 b	137.53 b

The numbers in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

Table (11): *In vivo* effect of combinations between *Rhizobium* sp. and *Trichoderma* spp. on yield parameters after 90 days from sowing, in artificially infested soil by *F. solani*

Treatment	N-fixation and yield parameters after 90 days from sowing			
	Dry weight of pods (g)/ plant	Dry weight of seeds (g)/plant	% N	Total N (mg)/plant
Control healthy	16.24 b	9.59 b	2.18 e	209.21 d
Control healthy + *R. leguminosarum*	18.69 a	10.58 a	3.69 a	389.35 a
Control infested	7.93 f	3.56 f	1.84 f	71.99 h
Control infested + *R. leguminosarum*	11.92 e	5.19 e	2.39 d	123.79 g
Seed treatment				
R. leguminosarum + *T. lignorum*	13.86 cd	6.36 d	2.74 c	174.05 f
R. leguminosarum. + *T. longibrachiatum*	13.33 d	6.68 cd	2.81 c	193.23 e
R. leguminosarum. + *T. koningii*	14.21 cd	7.14 cd	3.03 b	216.21 c
R. leguminosarum + Moncut 25%WP	15.22 bc	8.14 c	2.13 e	173.18 f
Soil treatment				
R. leguminosarum + *T. lignorum*	14.24 cd	6.93 cd	2.98 b	206.95 d
R. leguminosarum + *T. longibrachiatum*	14.74 cd	7.28 cd	3.05 b	221.27 c
R. leguminosarum + *T. koningii*	15.11 bc	7.69 cd	3.13 b	240.54 b

The numbers in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

Table (12): *In vitro* effect of Moncut 25%WP fungicide on *Rhizobium* sp. and *Trichoderma* spp.

Treatment	Conc. (g/l)	*R. leguminosarum* Growth diameter (cm)	*Trichoderma* spp.					
			T. lignorum		*T. longibrachiatum*		*T. koningii*	
			Growth diameter (cm)	Sporulation (spores/ml wash)	Growth diameter (cm)	Sporulation (spores/ml wash)	Growth diameter (cm)	Sporulation (spores/ml wash)
Control	0	0.00 d	9.00 a	7.50 x10^7a	9.00 a	7.77 x 10^7 a	9.00 a	7.17 x 10^7a
Moncut 25%WP	3	4.43 a	3.30 d	0.00 d	3.57 d	0.00 d	2.50 d	0.00 c
	1.5	3.50 b	4.33 c	4.40 x 10^6c	4.43 c	6.10 x 10^6 c	4.17 c	0.00 c
	0.75	2.57 c	6.77 b	2.33 x 10^7 b	7.13 b	3.17 x 10^7 b	6.07 b	9.01 x 10^6 b

The numbers in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

DISCUSSION

Previous reports of Hamid *et al*. (2012) and El-Abd *et al*. (2013) confirmed that *P. debaryanum*, *R. solani* and *F. solani* (which were isolated from diseased pea cv. Master B plants showing typical symptoms of damping-off and root rot) are related to pea damping-off and root rot diseases. So, the need to find an eco-friendly way for avoidance of hazardous effect produced by chemical fungicides has become very important. *Trichoderma* species as bio-agents have been used effectively against certain soil-borne pathogenic fungi. Mycoparasitic activity of *Trichoderma* spp. against each of the pathogenic fungi and fungus-like eukaryotic organism *P. debaryanum* of pea was studied *in vitro* using scanning electron microscope. Coiling, swelling, lysis and empty hyphae of the pathogenic fungi were observed, and hyphae of *Trichoderma* advanced over the colony of pathogenic causals were also detected. Hyphal coiling as an early stage of *Trichoderma* parasitism was observed as previously results reported by Lifshitz *et al*. (1986) and Almeida *et al*. (2007). Coiling of *T. hamatum* around hyphae of *R. solani* and *Pythium* spp. give chance to produce the hydrolytic enzymes such as cellulase and β-1,-3-glucanase, which hydrolysis β-glucans and cellulose content and make hyphae appear empty (Chet *et al*., 1981). Toxic volatile metabolites excreted by *Trichoderma* species were also reported by Qualhato *et al*. (2013) to have a significant effect on growth and development of many plant pathogenic fungi. Interestingly, combinations between *Rhizobium* sp. and *Trichoderma* spp. have been used effectively against soil-borne pathogenic fungi. Combinations between *Rhizobium* sp. and *Trichoderma* spp. were reported not only for suppressing the soil-borne fungi, but also for improving the yield of chickpea through enhancing the growth parameters and nutrient uptake (Rudresh *et al*., 2005). Damping-off and root rot diseases of *Lupines terms*, *Cicer arietinum* and *Vicia fabae* were controlled biologically using *Rhizobium* and *Trichoderma*. In addition, biological control of certain fungal diseases through using of *R. leguminosarum* have been reported and its mechanisms in biological control were explained. Obtained results with *R. leguminosarum* alone against *P. debaryanum* are in agreement with previous findings of Bardin *et al*. (2004) who reported that the application of *R. leguminosarum* against Pythium damping-off of pea and sugar beet was very effective in reducing the causal pathogen of this disease. These mechanisms were reported mainly through iron or nutrient competition, antibiotic production, and promotion of the plant growth through enhancement of host resistance (Arfaoui *et al*., 2005). So, combinations between *Rhizobium* sp. and *Trichoderma* spp. in biological control processes has many advantages against plant pathogens under the present study. Generally, these results are in agreement with work done by Goniem and Belal (2013) who reported that cowpea soil-borne pathogens which cause pre- and post-emergence damping-off diseases were effectively controlled through using combinations between *T. longibrachiatum* and *Bradyrhizobium* sp. Combinations between *Rhizobium* sp. and *Trichoderma* spp. were reported not only for suppressing the diseases but also for enhancing the survived plants, nitrogen fixation and yield parameters. Similar results were obtained by Saleh *et al*. (2000) who reported that, significant increases of faba bean cv. Giza 674 components of nodules number, dry weight, and N-content of shoots, when infested seeds with *R. leguminosarum* bv. *viceae*, were found. The obtained results about growth parameters and yield components are in harmony with those of Khalequzzaman and Hossain (2007) who reported that when seeds of faba bean were treated with *Rhizobium* strains, the length of green pods, weight of green pods, number of green pods, weight of seeds and discolored seeds were significantly influenced positively. Similar results were also obtained by Ghazi (2006) who established that inoculation of broad bean seeds with *R. leguminosarum* strain 317 enhanced the number and dry weight of shoots, pods, seeds and nitrogen content of treated plants. Antagonistic activity of *R. leguminosarum* against *F. oxysporium* f. sp. *lentis* has been studied by Essalmani and Lahlou (2002) who reported that the antagonistic effects were due to antibiotic substances and microbial activity which have protein nature act as fungicidal substances on conidia of *Fusarium* sp. Data reported by Baraka *et al*. (2009) showed that inoculation of faba bean plants by *R. leguminosarum* bv. *viceae* against *F. solani*, significantly increased the N-fixing and yield parameters compared to infected control by pathogen alone. The previous findings of Shaban and El-Bramawy (2011) confirmed that application of *Trichoderma* spp. in combination with *Rhizobium* sp. significantly developed and increased the growth and yield parameters of faba bean plants. Although, application of Moncut fungicide significantly decreased the disease

incidence and increased the growth parameters and yield components, unfortunately harmful effect in the plants was observed on N-fixing parameters. The obtained results are in agreement with those obtained by Singh and Wright (2002) who reported adverse effect of herbicides on the nodulation, nitrogen fixation of pea plants grown in soil infested with *R. leguminosarum* bv. *viceae*. Similar results were also obtained by El-Khateeb (2014) who tested these pesticides against *Rhizobium* cultures. The effect of pesticides was also reported with *Tichoderma* species by Sarkar *et al*. (2010) who stated that systemic fungicides such as propinconazole, triflumizole and hexaconazole were toxic to the antagonistic species of the fungus.

It could be concluded that the used combinations in the present work between *R. leguminosarum* and *Trichoderma* species were effective against soil-borne pathogens, *P. debaryanum*, *R. solani* and *F. solani* (causal agents of pea damping-off and root rot diseases). Obtained results show that the soil treatments were effective in controlling damping-off and root rot diseases than seed treatments in overall experiments. Additionally, nitrogen fixation parameters after 50 days and yield parameters after 90 days from sowing were improved when these combinations were applied.

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