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Efficiency of some Antioxidants and Bioagents in Controlling Rhizoctonia Damping-off of Snap Bean

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ABSTRACT

Four antioxidants, *i.e.* ascorbic, citric , oxalic and salicylic acid and eight bioagents, *i.e. Bacillus megaterium, B. subtilis, Pseudomonas fluorescens*, *Serratia marcescens, Trichoderma album, T. harzianum, T. lignorum* and *T. viride* were tested *in vitro*, under greenhouse and *in vivo* against *Rhizoctonia* damping-off of snap bean plants. *In vitro*, all the tested bioagents caused significant reduction to the linear growth of *R. solani. T. viride* caused the highest reduction followed by *T. lignorum* then *T. harzianum*, respectively. Under greenhouse conditions, sowing soaked bean seeds in the tested antioxidants or coated with the tested bioagents in soil artificially inoculation with *R. solani* significantly reduced the incidence of pre- and post-emergence damping- off with significant increase in the fresh and dry weight of roots and shoots compared with control treatment. Salicylic acid and *S. marcescens* gave the highest percentages of survived plants. *S. marcescens* induced the highest increase of fresh and dry weight of shoots followed by *B. megaterium* then salicylic acid and *T. viride*. *S. marcescens* followed by *B. megaterium* and *T. viride* resulted in the highest increase in the fresh and dry weight of roots. In general, all the tested antioxidants and bioagents significantly reduced the incidence of damping-off and root-rot under field conditions. The obtained results showed that, the use of antioxidants and bioagents caused considerable increase in the activity of peroxidase, polyphenol oxidase, chitinase and β-1,3-glucanase enzymes that play an important role in plant defence mechanisms against pathogens infection.

Key words: Snap Bean, damping-off, bioagents, antioxidants and enzymatic activity.

Introduction

Bean plants *(Phaseolus vulgaris* L.) is one of the most important leguminous crops grown in Egypt. Bean plants cultivated in Egypt for local consumption and exportation. Damping-off and root rot diseases are serious and persistent problem for bean plants during growing season (Filion *et al.,* 2003; Harveson *et al.,* 2005; Wen *et al.,* 2005 and Ragab *et al.,* 2015). *Rhizoctonia solani* is considered one of the most important causal of damping- off and root rot diseases. These diseases reduced seedling emergence and final plant stand and cause great loss in the yield of the affected crops (Mahmoud, 1985 and Rizvi & Yang, 1996). The fungus can persist for many years in previously infected bean and other hosts debris and infested the soil by producing small dark sclerotia. Fungal disease control is achieved through the use of fungicides which are hazardous and toxic to both people and domestic animals and lead to environmental pollution. Therefore, a more balanced, cost effective and eco-friendly approach must be implemented and adopted farmers (El-Mougy *et al.,* 2013). Options available for controlling root rots after planting are very limited and of questionable effectiveness (Abawi and Pastor-corrales 1990). Reduction of the pathogenic fungi *Fusarium oxysporum* growth by the antagonist *in vitro* and suppression of the bean damping- off disease caused by the pathogen under greenhouse condition demonstrated the ability of *Trichoderma* species to control diseases caused by the pathogen (Muriungi *et al.,* 2013). Control of root rot disease in many crops can be achieved by several means, among them antioxidants and the biological control methods being nowadays the most accepted. Benzoic, salicylic and ascorbic acid significantly reduced linear growth of *Fusarium oxysporum*, *F. solani* and *Rhizoctonia solani* and reduced spore germination of *Fusarium* spp. The 3 antioxidants significantly reduced damping- off of tomatoes (Shahda, 2000). More recently, several bioagents such as *Trichoderma* spp,, *Bacillus* spp., *Pseudomonas* spp. and *Serratia marcensens* are recommended to control several root pathogens (Elad *et al.,* 2003; Fayez *et al.,* 2004 and Ahmed, 2011). *In vitro* inhibition of many root rot pathogens and control of the disease that they cause in the greenhouse and field by the various species of the antagonistic fungi *Trichoderma* has been reported by many researchers (Luban 2005, Ebtsum *et al.,* 2009 ; Zahoor *et al.,* 2012 and Ragab *et al.,*

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2015). *T. harzianum* protected 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). *Trichoderma* spp. are widely used as antagonistic fungal agents against several pathogens as well as plant growth enhancers. Faster metabolic rates, anti-microbial metabolites, and physiological conformation are key factors, which chiefly contribute to antagonism of these fungi. Mycoparasitism, spatial and nutrient competition, antibiosis by enzymes and secondary metabolites and induction of plant defence system are typical bio-control actions of these fungi (Verma *et al.,* 2007). The use of *Trichoderma* spp., *Bacillus* spp. *Pseudomonas* spp. and *Serratia marcensens* as bioagents induced the accumulation of some enzymes such as chitinase, peroxidase and polyphenol oxidase, which play an important role in plant defence mechanisms against pathogens infection in treated bean plants, which increased in them more than in untreated one (Abd-El-Khair *et al.,* 2011 and Ahmed, 2011).

The present work was designed to evaluate the efficiency of some antioxidants and some bioagents against snap bean damping-off and root rot diseases caused by *R. solani* and the role of them in enhancing enzymes activity related to disease control.

Materials and Methods

1- Sources of the used materials:

Bean seeds *(Phaseolus vulgaris* L.) cv. Bronco used in this study were obtained kindly from Legume Crop Res. Dept., Agric. Res. Cent., Giza, Egypt.

Rhizoctonia solani was isolated from naturally infected snap bean plants, showing damping-off and root- rot symptoms, cultivated in the experimental farm of the Fac. of Agric. at Moshtohor, Benha Univ., Egypt. The isolated fungus was identified on the basis of cultural and microscopic morphological characters according to the key given by Gilman (1957).

The tested bioagents, i.e. *Bacillus megaterium, B. subtilis, Pseudomonas fluorescens*, *Serratia marcesens, Trichoderma album, T. harzianum, T. lignorum* and *T. viride* used in this study were kindly obtained from the fungal collections bank of Plant Pathol. Dept., Fac. of Agric. at Moshtohor, Benha Univ. Egypt.

Four antioxidants, *i.e.* ascorbic, citric, oxalic and salicylic acid were tested at concentration of 5.0 mM as seed soaking.

2- Preparation of Rhizoctonia solani inocula:

R. solani was grown in 500 mL glass bottles contained autoclaved sand-barley medium (1:3 w:w and 40% water). Autoclaved bottles, containing the medium, were inoculated with *R. solani* and incubated for 15 days at 28 ± 2°C.

3- Effect of some bioagents on the growth of R. solani in vitro.

The antagonistic effect of *Bacillus megaterium, Bacillus subtilis, Pseudomonas fluorescens*, *Serratia marcescens, Trichoderma album, Trichoderma harzianum, Trichoderma lignorum* and *Trichoderma viride* against *R. solani* pathogens *in vitro* was evaluated using the dual culture technique (Coskuntuna and Ozer, 2008). *Bacillus megaterium, B. subtilis, Pseudomonas fluorescens* and *Serratia marcescens* were grown on nutrient broth medium for 2 days at 28 ± 2°C. Meanwhile, *Trichoderma* spp. and *R. solani* were cultured, separately, on PDA medium for 7 days at 27 ± 1°C. In this respect, loop growth of each antagonistic bacterium was streaked individually in the opposite side of inoculated *R. solani* isolate (disc, 5mm ϕ) on PDA plates.

Meanwhile, 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 *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. Three Petri dishes for each bio-agent, as well as the control, were used as replicates. The inoculated Petri dishes were incubated at 25 °C for 7 days.

The inoculated plates were examined daily and then the linear growth area of *R. solani* was measured to determine the most effective antagonistic isolate among the tested bio-agents (Abou-Zeid and Hassanien, 2000). Percentages of the fungal growth reductions (X) were calculated using the following formula:

$X = G_1 - G_2 / G_1 \times 100$

Where:
X= fungal growth reduction.
G_1= linear growth of the pathogen grown alone.
G_2= linear growth of the pathogen in presence of tested bio-agent.

4- Effect of treating bean seeds with some antioxidants bioagents on damping off diseases incidence:

In this experiment, surface sterilized bean seeds were soaked for 2.5 hours (Shalaby, 1997) in 5.0 mM concentration of ascorbic acid, citric acid, oxalic acid and salicylic acid. The wetted seeds were spread out in a thin layer and left to 2 hours then they were sown in pathogen-infested potted soils at the rate of five seeds/pot. Surface sterilized bean seeds coated with suspension of any of the following antagonistic microorganisms (prepared as described below) to evaluate their efficiency in controlling damping-off disease incidence. The tested microorganisms including *Bacillus megaterium, Bacillus subtilis, Pseudomonas fluorescens*, *Serratia marcesens, Trichoderma album, Trichoderma harzianum, Trichoderma lignorum* and *Trichoderma viride.* Each of an antagonistic fungus was grown on PDA plates for 10 days at 27 ± 1°C then its growth was flooded with sterile-distilled water, scraped with a camel brush then filtered thorough sterilized filter papers. The resulted spore suspensions were found to be contained approx. 5 X 10^8 condia/mL in case of all *Trichoderma* spp. A known amount of surface sterilized bean seeds placed in plastic bags was thoroughly mixed and shacked slowly for 5 minutes with mixture consisted of 2 mL spore suspension plus 1 mL of 1% Arabic gum solution as sticker (modified from Harman *et al*., 1980). Bean seeds were treated with antagonistic bacteria according to Park *et al.,* (1991). Any of the tested antagonistic bacterial isolates was grown for 48 h at 28 ± 2°C on nutrient broth medium and then their cell suspensions were adjusted at rate 2.8 x10^8 cfu/mL for each one of them. Agar slants of surface sterilized bean seeds were thoroughly mixed with 2 mL of bacterial suspension plus 1 mL of 1% Arabic gum solution as sticker for 5 minutes then left for 2 h to air dried in a laminar-flow before planting Callan *et al*., (1990).

Bean seeds whether treated or non-treated with the antagonistic microorganisms were sown in plastic pots (25 cm diameter), uniformly filled with sterilized air-dried soil artificially infested with the causal fungus at the rate of 3.0% (w/w). Five seeds of common bean (cv. Bronco) were sown in each pot. Four replicates were used for each particular treatment. After 15, 30 and 45 days after planting, the percentages of pre- and post-emergence damping-off and the survived plants were recorded, respectively. Whole plants were removed gently to avoid root damage and washed under current of tap water. Plants were then separated into roots and shoots and oven dried at 70 °C for 48 h.

The damping off disease assessment was carried out as:

$$\% \text{ Pre-emergence} = \frac{\text{Number of non germinated seeds}}{\text{Number of sown seeds}} \text{ X } 100$$

$$\% \text{ Post-emergence} = \frac{\text{Number of dead seedlings}}{\text{Number of germinated seeds}} \text{ X } 100$$

5- *Determination the activity* of oxidative and catalyzed enzymes:

Leaves of treated snap bean plants were taken 30 days after sowing. Leaf samples were ground with 0.2 M Tris HCl buffer (pH 7.8) containing 14 mM β-mercaptoethanol at the rate 1/3 w/v. The extracts were centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used to determine enzyme activities (Tuzun *et al.,* 1989).

5.1. Determination of peroxidase (PO):

Peroxidase activity was determined according to the method described by Allam and Hollis (1972), Peroxidase activity was expressed as the increase in absorbance at 425 nm/g fresh weight/minutes.

5.2. Determination of polyphenoloxidase (PPO):

The polyphenoloxidase activity was determined according to the method described by Matta and Dimond (1963). Polyphenoloxidase activity was expressed as the increase in absorbance at 420nm/g fresh weigh/min.

5.3. Determination of chitinase:

Determination the activity of chitinase was carried out according to the method of Boller and Mauch, (1988). Chitinase activity was expressed as mM N-acetylglucose amine equivalent released/g fresh weight tissue/60 minutes.

5.4. Determination of *β-1,3-Glucanase:*

Determination the activity of the β-1,3-glucanase was carried out according to the method of (Sun *et al.,* 2006.). β-1,3-glucanase was expressed as mM glucose equivalent released /g fresh weight tissue /60 minutes.

6- *Field experiments:*

The efficacy of soaking bean seeds in the tested antioxidants or coating with the suspension of any of the tested antagonistic microorganisms, *i.e. B. megaterium, B. subtilis, P. fluorescens*, *S. marcescens, T. album, T. harzianum, T. lignorum* and *T. viride,* against the incidence of damping off was evaluated in the experimental farm of the Fac. of Agric. at Moshtohor, Benha Univ., during two successive seasons of 2013 and 2014. Bean seeds were coated with suspension of the antagonistic microorganisms as described before. The experimental design was a randomized complete block in three replicates. The area of the plot was 10.5 m^2. Next, the soil was irrigated 7 days before sowing. Bean seeds (cv. Bronco) were planted at rate of 2 seeds / hole at 20 cm space. The usual agricultural practices were followed as recommended. After 15, 30 and 45 days after planting, the percentages of pre- and post-emergence damping-off and the survived plants were recorded, respectively.

7-Statistical analyses:

Statistical analyses of the obtained data have been carried out according to the procedures (ANOVA) reported by Snedecor and Cochran (1989). Treatment means were compared by the least significant difference test "L.S.D" at 5% level of probability.

Results

1- Effect of some bioagents on the linear growth of *R. solani in vitro*

Data in Table 1 indicate that all the tested antagonists caused significant reduction to the linear growth of *R. solani. Trichoderma viride* caused the highest reduction (80.37%) followed by *T. lignorum* (78.52 %) then *T. harzianum* (74.08%) and *T. album* (71.86 %). Whereas, *Pseudomonas fluorescens,* and *Bacillus megaterium* were the most efficient antagonistic bacteria, which reduced the growth of *R. solani* to 62.59 and 61.48%, respectively.

Table 1: Effect of some bioagents on the linear growth of *R. solani,* after incubation at 25 °C for 7 days.

Bioagent	Linear growth (mm)	Efficiency (%)
B. megaterium	34.67	61.48
B. subtilis	37.33	58.52
P. fluorescens	33.67	62.59
S. marcescens	40.67	54.81
T. album	25.33	71.86
T. lignorum	19.33	78.52
T. harzianum	23.33	74.08
T. viride	17.67	80.37
Control	90.00	0.00
L.S.D. at 5%	6.14	------

2. Effect of the tested antioxidants and bioagents on the incidence of damping-off under greenhouse conditions

Results in Table 2 reveal that seeds treated with the tested antioxidants or bioagents significantly reduced pre- and post-emergence damping-off incidence under artificial inoculation with *R. solani* under greenhouse conditions.

The incidence of damping-off caused by *R. solani* under application of the tested bioagents at pre- and post-emergence stages were in the range of 5.0 - 25.0 % and 5 - 16.25%, compared to 30.0 and 28.75 % in the control plants. Hence, the percentages of the survived plants were increased due to the application of antioxidants and /or the bioagents compared with the control. Both salicylic acid and *S. marcescens* gave the highest percentage of survived plants (90%) followed by both oxalic acid and *B. megaterium* (88.75%) then both *B. subtilis* and *T. viride* (85%) and ascorbic acid, *T. harzianum* and *T. lignorum* (83.75 %). On the other hand, *T. album* gave the lowest percentage of survived plants (53.75%).

Table 2: Effect of the tested antioxidants and bioagents on incidence of damping-off under greenhouse conditions

Treatment	%, Damping- off		Survived plant %	%, Reduction		%, Increase in survived plants
	Pre-	Post-		Pre-	Post-	
Salicylic acid	5.00	5.00	90.00	83.33	82.61	118.18
Citric acid	10.00	10.00	80.00	66.67	65.22	93.94
Oxalic acid	5.00	6.25	88.75	83.33	78.26	115.15
Ascorbic acid	10.00	6.25	83.75	66.67	78.26	103.03
T. harzianum	10.00	6.25	83.75	66.67	78.26	103.03
T. viride	10.00	5.00	85.00	66.67	82.61	106.06
T. album	25.00	16.25	53.75	16.67	43.48	30.30
T. lignorum	10.00	6.25	83.75	66.67	78.26	103.03
B. subtilis	10.00	5.00	85.00	66.67	82.61	106.06
B. megaterium	5.00	6.25	88.75	83.33	78.26	115.15
P. fluorescens	15.00	5.00	80.00	50.00	82.61	93.94
S. marcescens	5.00	5.00	90.00	83.33	82.61	118.18
Control	30.00	28.75	41.25	0.00	0.00	0.00
L.S.D. at 5%	9.65	8.34	10.23	----	----	----

3- Effect of the tested antioxidants and bioagents on shoot and root weight of snap bean plants under greenhouse conditions.

Results presented in Table 3 reveal that the treated bean seeds with the tested antioxidants and bioagents significantly increased fresh and dry weight of roots and shoots under artificial inoculation with *R. solani* under greenhouse conditions. The highest increases in shoot fresh and dry weight were recorded by *S. marcescens* (124.76 and 138.79%, respectively) followed by *B. megaterium* (123.79 and 132.01%, respectively), salicylic acid (116.52 and 89.72 %, respectively) and *T. viride* (110.29 and 91.36%, respectively).

As for root fresh and dry weight also *S. marcescens* induced the highest increase, being 130.39 and 123.76% respectively followed by *B. megaterium* being 118.14 and 109.9%, respectively, salicylic acid , being100.98 and 98.02 %,, respectively and *T. viride* being 83.58 and 93.56%, respectively. On the other hand, *T. album* recorded the lowest increase in shoot fresh and dry weight being 34.21 and 34.38%, respectively. Whereas, *T. lignorum* recorded the lowest increase in root fresh and dry weight, being 29.90 and 53.47%, respectively.

4- Effect of the tested antioxidants and bioagents on the activity of peroxidase and polyphenoloxidase enzymes in snap bean plants.

Results presented in Table 4 indicate that treating seeds of bean with antioxidants or bioagents increased the activity of peroxidase and polyphenol oxidase enzymes compared with untreated control. Generally, *B. subtilis, T. lignorum* and *T. album* were the superior for increasing the activity of peroxidase enzyme and increasing the activity of peroxidase by 219.03, 195.52 and 172.01%, respectively. Meanwhile, citric acid was the lowset effective one, which increased the activity of peroxidase to 72.20%.

On the other hand, *B. subtilis* followed by *B. megaterium* and *T. lignorum* were the most effective bioagents and increased the activity of polyphenoloxidase by 238.46, 236.92 and 153.85% respectively. Whereas, citric acid increased the activity of peroxidase by 47.26% and was the lowest effective one.

Table 3: Effect of the tested antioxidants and bioagents on shoot and root weight of snap bean plants under greenhouse conditions.

Treatment	Shoot weight (g/ plant)		Root weight (g/ plant)		%, Increase			
					Shoot weight (g/ plant)		Root weight (g/ plant)	
	Fresh weigh	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
Salicylic acid	33.67	8.10	8.20	4.00	116.53	89.25	100.98	98.02
Citric acid	21.45	6.53	5.22	3.02	37.94	52.57	27.94	49.50
Oxalic acid	26.16	7.02	6.14	3.38	68.23	64.02	50.49	67.33
Ascorbic acid	23.20	6.84	5.65	3.27	49.20	59.81	38.48	61.88
T. harzianum	22.64	5.93	6.52	3.53	45.59	38.55	59.80	74.75
T. viride	32.70	8.19	7.49	3.91	110.29	91.36	83.58	93.56
T. album	20.87	5.76	5.78	3.36	34.21	34.58	41.67	66.34
T. lignorum	21.95	5.53	5.30	3.10	41.16	29.21	29.90	53.47
B. subtilis	31.05	7.78	6.99	3.85	99.68	81.78	71.32	90.59
B. megaterium	34.80	9.93	8.90	4.24	123.79	132.01	118.14	109.90
P. fluorescens	22.79	6.03	6.70	3.65	46.56	40.89	64.22	80.69
S. marcescens	34.95	10.22	9.40	4.52	124.76	138.79	130.39	123.76
Control	15.55	4.28	4.08	2.02	0.00	0.00	0.00	0.00
L.S.D. at 5%	1.48	0.74	0.76	0.41	----	----	----	----

Table 4: Effect of the tested antioxidants and bioagents on the activity of peroxidase and polyphenol oxidase enzymes in snap bean plants.

Treatment	Peroxidase	Polyphenol-oxidase	%, Increase to the control	
			Peroxidase	Polyphenol- oxidase
Salicylic acid	10.26	28.71	91.42	145.38
Citric acid	9.23	17.23	72.20	47.26
Oxalic acid	11.88	28.62	121.64	144.62
Ascorbic acid	11.43	25.26	113.25	115.90
T. harzianum	10.17	23.76	89.74	103.08
T. viride	13.50	23.31	151.87	99.23
T. album	14.58	22.14	172.01	89.23
T. lignorum	15.84	29.7	195.52	153.85
B. subtilis	17.10	39.6	219.03	238.46
B. megaterium	13.14	39.42	145.15	236.92
P. fluorescens	13.86	21.15	158.58	80.77
S. marcescens	14.40	18.45	168.66	57.69
Control	5.36	11.7	0.00	0.00

5- Effect of the tested antioxidants and bioagents on the activity of chitinase and β-1, 3-glucanase enzymes in snap bean plants.

The effect of treating bean seeds with antioxidants or bioagents on the activity of chitinase and β-1,3-glucanase is presented in Table 5. All the tested antioxidants and bioagents increased chitinase activity. The highest activity of chitinase was induced by *B. megaterium* and *S. marcescens* (331.05 and 325.58% increase. respectively), followed by salicylic acid, *P. fluorescens* and *T. viride* (309.03, 303.42 and 292.34% increase. respectively). Citric acid was the lowest effective one, which increased the activity by 74.69%.

As for β-1,3-glucanase, all tested antioxidants and bioagents increased β-1,3-glucanase activity. The highest increase was recorded by *S. marcescens* (271.15%) followed by *B. megaterium* and *P. fluorescens* (210.75 and 175.00%. respectively). Meanwhile *T. harzianum* induced the lowest increase (19.87%).

Table 5: Effect of the tested antioxidants and bioagents on the activity of chitinase and β-1,3-glucanase enzymes in snap bean plants

Treatment	Chitinase	β-1,3-glucanase	%, Increase to the control	
			Chitinase	β-1,3-glucanase
Salicylic acid	29.9	5.01	309.03	60.58
Citric acid	12.77	4.00	74.69	28.21
Oxalic acid	21.61	5.77	195.62	84.94
Ascorbic acid	17.07	4.25	133.52	36.22
T. harzianum	27.07	3.74	270.31	19.87
T. viride	28.68	7.23	292.34	131.73
T. album	13.13	5.07	79.62	62.50
T. lignorum	23.63	5.38	223.26	72.44
B. subtilis	14.14	6.30	93.43	101.92
B. megaterium	31.51	9.69	331.05	210.58
P. fluorescens	29.49	8.58	303.42	175.00
S. marcescens	31.11	11.58	325.58	271.15
Control	7.31	3.12	0.00	0.00

6. Effect of the tested antioxidants and bioagents on the incidence of damping-off under field conditions

Data in Table 6 show that, all tested antioxidants and bioagents were significantly effective in controlling disease incidence and reducing the pre- and post-emergence damping-off under field conditions. As for, the disease control at seedling stage in term of % survived seedlings, it could be noticed that the maximum percentage of survived seedlings was produced by *Serratia marcescens* (82.23 and 78.07%) and *B. megaterium* (80.29 and 76.44%) followed by salicylic acid (81.10 and 77.75%), *T. viride* (78.10 and 73.80%) and *B. subtilis* (76.88 and 72.35%) at the two seasons respectively.

Table 6: Effect of the tested antioxidants and bioagents on incidence of damping-off under field conditions

Treatment	Season, 2013			Season, 2014		
	Damping off		Survival plant%	Damping off		Survival plant %
	Pre%	Post%		Pre%	Post%	
Salicylic acid	10.5	8.40	81.10	12.00	10.25	77.75
Citric acid	14.20	10.75	75.05	16.25	13.00	70.75
Oxalic acid	11.50	9.65	78.85	12.80	11.40	75.8
Ascorbic acid	13.75	10.30	75.95	15.25	12.40	72.35
T. harzianum	14.24	11.44	74.32	16.50	13.75	69.75
T. viride	12.75	9.15	78.10	14.25	11.95	73.80
T. album	18.50	16.50	65.00	19.50	16.91	63.59
T. lignorum	14..50	13.41	72.63	17.00	14.27	68.73
B. subtilis	13.00	10.12	76.88	15.00	12.65	72.35
B. megaterium	10.75	8.96	80.29	12.75	10.81	76.44
P. fluorescens	15.25	15.04	69.71	18.25	15.42	66.33
S. marcescens	9.75	8.02	82.23	11.50	10.43	78.07
Control	23.50	20.30	56.20	25.75	22.57	51.68
L.S.D. at 5%	1.41	1.64	1.81	1.17	1.73	2.03

Discussion

Common bean (*Phaseolus vulgaris* L.) is one of the most important legume in human diets. Damping-off and root rot diseases are serious and persistent problem for snap bean plants during growing season (Filion *et al.*, 2003; Harveson *et al.*, 2005 and Ragab *et al.,* 2015). *In vitro,* all tested antagonists caused significant reduction to the linear growth of *R. solani. T. viride* caused the highest reduction followed by *T. Lignorum* the *T. harzianum*. This results are in harmony with those obtained by Pieta and Pastucha (2004) and (Abd-El-Khair *et al.,* 2011 and Ragab *et al.,* 2015**).** They reported that, *T. album, T. hamatum, T. harizianum* and *T. viride* significantly reduced the mycelial growth of *R. solani*. El-Mougy *et al.,* (2013**)** reported that, *P. fluorescens* and *T. harzianum* reduced the growth area more than 90.6 and 87.4 % for *F. solani* and *R solani*, respectively. Several mechanisms could be suggested to interpret antagonistic potentiality of the tested antagonists. For example, ability to produce lytic enzymes (Fridlender *et al.,* 1993), antibiotics (Bender *et al.,* 1999), volatile compounds (Claydon *et al.,* 1987; Bakker and Schippers, 1987), and phytotoxic substances (Hoagland and Cutler, 2000).

Greenhouse experiment revealed that soaking bean seeds in the tested antioxidants or coating the seeds with the tested bioagents significantly reduced pre- and post-emergence damping- off under artificial inoculation with *R. solani* as well as significantly increased fresh and dry weight of roots and shoots. Salicylic acid and *S. marcescens* gave the highest percentage of survived plants followed by oxalic acid, *B. megaterium*, *B. subtilis* and *T. viride.* Moreover, *S. marcescens* followed by *B. megaterium* then salicylic acid and *T. viride* increased fresh and dry weight of roots and shoots.

All the tested antioxidants and bioagents significantly reduced the incidence of pre- and post-emergence damping-off under field conditions. The obtained results are in agree with the obtained data by Abdel- Kader & Ashour 1999 and El-Mougy *et al.,* 2013. They reported an announced reduction in root rot incidence of bean and cowpea, caused by the pathogens *R. solani* and *F. solani*, was achieved using the antagonists *T. harzianum*. Similar results were also reported by El-Mougy (2001); Pieta and Pastucha (2004) **,** (Abd-El-Khair *et al.,* 2011) and Ragab *et al.* (2015) .They reported that *T. harzianum, T. koningii* and *T. viride* protected the germinating bean seedlings against *Fusarium* spp. and *R. solani* infection. In this regard, *S. marcescens* significantly reduced Fusarium wilt of cucumber when applied as root treatments (Liu *et al.,* 1995). Bio-control of damping-off diseases has been successfully applied using *B. subtilis* (Harris and Adkins 1999 and Schmidt *et al.* 2004). Application of antioxidants, e.g. ascorbic, salicylic, coumaric, benzoic acids and propylgalate as either seed soaking or soil drench proved sufficient protection against cumin diseases caused by *Fusarium oxysporum* f. sp. *cumini* and *Acremonium egyptiacum* (Mostafa, 2006).

The obtained results showed that the use of antioxidants or bioagents caused considerable increases in the activity of peroxidase, polyphenol oxidase, chitinase and β-1,3-glucanase enzymes that play an important role in plant defence mechanisms against pathogens infection. Results cleared that the enzymatic activity in treated snap bean plants increased than that in untreated one. This results are in harmony with those recorded by Abd-El-Khair *et al.* (2011) they reported that, the use of *Trichoderma* spp. as bioagents induced the accumulation of some enzymes such as chitinase, peroxidase and polyphenol oxidase in treated snap bean plants. Many plant enzymes are involved in defence reactions against plant pathogens. Oxidative enzymes such as peroxidase and polyphenol oxidase enhance formation of lignin, while other oxidative phenols contribute in formation of defence barriers for reinforcing the cell structure (Avdiushko *et al.,* 1993). Enzyme activity plays an important role in plant disease resistance through increasing plant defence mechanisms that are considered the main tool of varietals resistance (Takuo *et al.,* 1993). Caruso *et al.* (2001**),** also, experimentally supported the idea that peroxidase play a defence role against invading pathogens. Chitinase and β-1, 3 glucanase enzymes play an important role in plant defence against fungi by hydrolyse their cell wall (Tian *et al.,* 2006; Imran *et al.,* 2007 and Barilli *et al.,* 2010). Many oxidative enzymes such as peroxidase, catalase, ascorbate oxidase and polyphenol oxidase were detected as a result of treatments with various antioxidants (Takahama and Oniki, 1994, El-Khallal, 2007 and Abdel-Monaim, 2008).

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