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Promoting Resistance of Snap Bean against Damping-off Disease Caused by *Rhizoctonia solani* Using the Integration between Some Antioxidants and Bioagents

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ABSTRACT

In both greenhouse and field experiments, the effect of three antioxidants, *i.e.*, ascorbic acid, oxalic acid and salicylic acid (at 5.0 mM) as a seed soaking, followed by the bioagents, *i.e*., *Bacillus megaterium, B. subtilis, Serratia marcesens, Trichoderma harzianum, T. lignorum* and *T. viride* as a seed coating were appliedagainst damping-off disease of bean plants, in addition to study the biochemical response of treated plants. Results revealed that all tested treatments significantly reduced pre- and post-emergence damping-off in addition to inducing an increase in survived plants under both conditions. Complete protection of damping-off incidence was obtained in the case of using salicylic acid as a seed soaking and *Serratia marcesens* as a seed coating compared to other treatments and control. All tested treatments significantly increased either fresh or dry weight of shoots and roots per plant. Moreover, under field conditions, the best integration treatments were ascorbic acid, oxalic acid and salicylic acid as a seed soaking and with *S. marcesens* as a seed coating where the lowest pre and post emergence damping-off % ranged between (3.88 and 7.4%); in addition to increasing the survival plants more than (88.5%), but other treatments showed moderate effect. As for, biochemical response of snap bean plants under greenhouse condition results indicated that all tested treatments increased the flavonoids content and affected positively the activities peroxidase, polyphenoloxidase and phenylalanine ammonia lyase, chitinase and β-1,3-glucanase enzymes of the plants. As for chitinase activity, the most effective treatment (with efficacy being 315.21%) was ascorbic acid with *S. Marcescens.* On the other hand, the highest increase of β-1,3-glucanase activity was recorded with the integration of oxalic acid and each of *S. marcescens* and *B. megateriu* (382.50 and 316.88%), respectively were recorded.

Key words: Bean plants, damping-off disease, ascorbic acid, bioagents, oxalic acid, salicylic acid.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a major grain legume consumed worldwide for its edible seeds and pods. Rhizoctonia root rot caused by *Rhizoctonia solani* is a widely distributed disease of common bean in the world (Paula Júnior *et al.*, 2007). Fungal disease control depends mainly on using the fungicides but risks to human health and the environment are expected. Therefore, alternatives eco-friendly approaches for the control of the disease were emphasized (Mandal *et al.,* 2009). Such strategy is use of bio-control agents to control fungal plant diseases as well as other alternatives to fungicides (Punja and Utkhede, 2004). The integration of biological agents with additional strategies is increasingly recommended to enhance Rhizoctonia root rot control (Sweetingham, 1996).

Trichoderma spp. are effective bio-control agents for a number of soil-borne plant pathogens where known for their ability to enhance plant growth (Sharon *et al.,* 2001 and Rose *et al*., 2003). Moreover, it was found that bioagents stimulated the plant resistance reaction. In this respect, Jain *et al.,* (2012) reported that *Pseudomonas aeruginosa, Trichoderma harzianum* and *Bacillus subtilis* from rhizosphaeric soils were used as consortia to assess their ability to trigger the phenylpropanoid and antioxidant activities and accumulation of proline, total phenol and pathogenesis-related (PR) proteins in pea under the challenge of the soft-rot pathogen *Sclerotinia sclerotiorum*. Recently, it was suggested that *Trichoderma* affects the induced systemic resistance (ISR) mechanism in plants (Shoresh *et al*., 2005 and Hoitink *et al*., 2006). Jaiganesh, *et al*., (2007) reported that out of the six-bio protectants tested, *S. marcescens* was found very effective against *Pyricularia. oryzae* under *in vitro* and *in vivo* conditions. On the other hand, antioxidants which save to human and environment had been used successfully to control root and pod rot diseases in peanut (Mahmoud *et al.*, 2006), Fusarium wilt in chickpea (Nighat- Sarwar *et al.*, 2005), faba bean chocolate spot (Hassan *et al.*, 2006), Fusarium wilt in tomato (El- Khallal, 2007), root rot and leaf blight in lupine (Abdel-Monaim, 2008), damping-off in pepper (Rajkumar, 2008) and faba bean damping-off (Khalifa *et al.*, 2016).

The objective of the present work was to evaluate the effect of integrated treatments between antioxidants and bioagents for controlling damping-off disease of bean plants under greenhouse and field conditions in addition to biochemical response of treated plants.

MATERIALS AND METHODS

Source of bean seeds, pathogenic fungus and bi-agents

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* (Kühn) was isolated from naturally infected bean plants; showing damping-off and root-rot symptoms, cultivated in the experimental farm of the Faculty of Agriculture, Benha University, Egypt. The isolated fungi were identified based on cultural and microscopic morphological characters according to the key given by Gilman (1957). The tested bioagents, *i.e., Bacillus megaterium, B. subtilis, Serratia marcesens, Trichoderma harzianum, T. lignorum* and *T. viride,* were kindly obtained from the fungal collections, bank of Plant Pathology Dept., Fac. of Agric., Benha Univ. Egypt. Three antioxidants, i.e., ascorbic acid, oxalic acid and salicylic acid were tested at a concentration of 5.0 mM.

Preparation of *Rhizoctonia solani* inoculum

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. Soil was artificially infected with *R. solani* at a rate of 3.0% (w/w).

Greenhouse experiment

Effect of integration between antioxidant and bioagent on damping-off incidence of bean plants

In this experiment, surface sterilized bean seeds were soaked for 2.5 hrs in 5.0 mM of ascorbic acid, oxalic acid and salicylic acid. The wetted seeds were spread out in a thin layer and left to 2 hrs then coated individually with suspension of any of the following antagonistic microorganisms to evaluate their efficiency in controlling damping-off disease incidence. The tested microorganisms included; *B. megaterium, B. subtilis, S. marcesens, T. harzianum, T. lignorum* and *T. viride.* Each antagonistic fungus was grown on PDA plate for 10 days at 27±1°C, then its growth was flooded with sterile-distilled water, scraped with a camel brush, then filtered through sterilized filter papers. The resulted spore suspensions were found to be contained approximately a $5x10^8$ 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 min with mixture consisted of 2 ml spore suspension plus 1 ml of 1% Arabic gum solution as a sticker. However, bean seeds were treated by antagonistic bacteria, according to Parke *et al.* (1991). Antagonistic bacterial isolates was each grown for 48 h at 28±2°C on nutrient broth medium and then their cell suspensions were adjusted at rate of 2.8 $x10^8$ cuf/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. Bacterial population determined per seed was $1x10^8$ c.f.u/seed according to dilution plate assay described by Callan *et al*., (1990).

Bean seeds (cv. Bronco) treated or non-treated with antagonistic microorganisms were sown in plastic pots (25 cm diameter) were uniformly packed with sterilized air-dried soil infested artificially with *R. solani* at a rate of 3.0% (w/w). Five seeds of common bean were sown in each pot. Five replicates were used for each particular treatment. After 15, 30 and 45 days after planting, the percentages of pre-, post-emergence damping-off and the survived healthy plants were recorded, respectively. Whole plants were removed gently to avoid root damage and washed under gentle 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:

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

$$\% \text{ Post-emergence} = \frac{\text{No. of diseased and dead seedlings}}{\text{No. of germinated seeds}} X\,100$$

Effect of antioxidant and bioagent treatments on biochemical changes in leaves:

1- Determination of total flavonoids content

Half gram of fresh plant tissue was ground using a pestle and mortar with 10 ml of 80% ethanol then, filtered and centrifuged at 10,000 rpm for 20 min. The supernatant was evaporated till dryness. The residue was dissolved in 5 ml of 80% ethanol and used as the extract. The flavonoids content was determined according to the method described by (Peixoto Sobrinho *et al.,* 2008). The flavonoids content was expressed as milligrams of rutin equivalents per gram of sample (mg RE/g), at wavelength of 420 nm.

2- Determination the activities of enzymes

Leaf samples of treated 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).

3- Determination of phenylalanine ammonia lyase (PAL)

Activity of PAL was determined at the rate of conversion of L-phenylalanine to trans-cinnamic acid as described by Dickerson *et al.* (1984). The optical density (O.D.) value was recorded at 290 nm and enzyme activity was expressed as µmol transcinnamic acid min^{-1} g^{-1} protein.

4- 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- 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.

6- Determination of Chitinase

Determination of chitinase activity 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.

7- 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-,3-glucanase was expressed as mM glucose equivalent released /g fresh weight tissue /60 min.

Field experiment

Effect of antioxidants and bioagents on damping-off incidence of snap bean plants

The efficacy of soaking bean seeds in testing antioxidants and coated with suspension of any of the following antagonistic microorganisms *Bacillus megaterium, B. subtilis, Serratia marcescens, T. harzianum, T. lignorum* and *T. viride,* against the incidence of damping-off disease, was evaluated at the experimental farm of the Faculty of Agriculture, Benha University, Egypt during the period (March and April) of the two successive seasons 2014 and 2015. Bean seeds were treated as described above. The experimental design was a randomized complete block in three replicates. The area of the plot was 10.5 m^2. The soil was irrigated 7 days before sowing. Bean seeds (cv. Bronco) were planted at a rate of 3 seeds / hole at 20cm space. The usual agricultural practices were practiced as the recommendation. After 15, 30 and 45 days post planting, the percentages of pre- and post-emergence damping-off and the survived healthy plants were recorded.

Statistical analyses

Statistical analyses of all the previously designed experiments were carried out using (ANOVA) as reported by Snedecor and Cochran (1989). Treatment means were compared by the least significant difference test "L.S.D" at the 5% level of probability.

RESULTS AND DISCUSSION

Greenhouse experiments

Effect of antioxidants and bioagents treatments on damping-off incidence

Three antioxidants, *i.e.,* Ascorbic acid, oxalic acid and salicylic acid at 5.0 mM (as seed soaking) were integrated with 6 bioagents, *i.e., B. megaterium, B. subtilis, S. marcesens, T. harzianum, T. lignorum* and *T. viride* (as seed coating) to study their effects against damping-off disease on snap bean plants caused by *R. solani* under greenhouse conditions. Results in table (1) indicated that integration between the tested antioxidants and bioagents had a great comfortable impact in reducing damping-off and increasing the survived plants of snap bean compared to untreated control treatment. In this respect, complete protection of damping-off incidence was obtained in the case of using salicylic acid as seed soaking and *Serratia marcesens* as seed coating

Table (1): Effect of integration between antioxidants and bioagents treatments on controlling damping-off incidence in snap bean plants under greenhouse conditions

Antioxidants	Bioagents	Damping-off		Survived plants	Reduction		% Increase
		Post%	Pre%	%	Post%	Pre%	Survived plants %
Salicylic acid	*T.harzianum*	8.00	5.00	87.00	66.67	78.26	64.15
	T.viride	8.00	5.00	87.00	66.67	78.26	64.15
	T.lignorum	4.00	0.00	96.00	83.33	100.00	81.13
	B. subtilis	12.00	9.00	79.00	50.00	60.87	49.06
	B.megaterium	4.00	4.00	92.00	83.33	82.61	73.58
	S. marcescens	0.00	0.00	100	100.00	100.00	88.68
Oxalic acid	*T.harzianum*	8.00	9.00	83.00	66.67	60.87	56.60
	T.viride	12.00	8.00	80.00	50.00	65.22	50.94
	T. lignorum	4.00	4.00	92.00	83.33	82.61	73.58
	B. subtilis	12	10.00	78.00	50.00	56.52	47.17
	B. megaterium	4.00	5.00	91.00	83.33	78.26	71.70
	S. marcescens	4.00	4.00	92.00	83.33	82.61	73.58
Ascorbic acid	*T.harzianum*	8.00	9.00	83.00	66.67	60.87	56.60
	T.viride	8.00	9.00	83.00	66.67	60.87	56.60
	T. lignorum	4.00	4.00	92.00	83.33	82.61	73.58
	B. subtilis	12.00	9.00	79.00	50.00	60.87	49.06
	B. megaterium	4.00	5.00	91.00	83.33	78.26	71.70
	S. marcescens	4.00	0.00	96.00	83.33	100.00	81.13
Control		24.00	23.00	53.00	0.00	0.00	0.00
LSD 0.05		8.14	8.43	9.70	------	------	------

Table (2):Effect of integration between antioxidants and bioagents treatments on some vegetative characteristics of snap bean plants under greenhouse conditions

Antioxidants	Bioagents	Shoot wt. (g/ plant)		Root wt. (g/ plant)		Increase			
						Shoot wt. (g/ plant)		Root wt. (g plant)	
		Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
Salicylic acid	*T.harzianum*	33.49	9.70	7.76	3.72	81.71	97.15	63.37	48.21
	T.viride	35.47	9.92	8.74	3.84	92.46	101.63	84.00	52.99
	T. lignorum	39.98	10.72	9.23	4.32	116.93	117.89	94.32	72.11
	B. subtilis	28.54	7.03	6.55	3.53	54.86	42.89	37.89	40.64
	B. megaterium	37.65	10.53	8.33	3.92	104.29	114.02	75.37	56.18
	S. marcescens	40.86	10.80	9.40	4.45	121.70	119.51	97.89	77.29
Oxalic acid	*T.harzianum*	29.28	6.74	6.63	3.35	58.87	36.99	39.58	33.47
	T.viride	34.69	9.78	7.85	4.15	88.23	98.78	65.26	65.34
	T. lignorum	33.61	9.38	7.25	3.80	82.37	90.65	52.63	51.39
	B. subtilis	28.33	7.34	6.50	3.45	53.72	49.19	36.84	37.45
	B. megaterium	36.01	10.32	8.36	4.20	95.39	109.76	76.00	67.33
	S. marcescens	35.16	9.85	7.60	3.65	90.78	100.20	60.00	45.42
Ascorbic acid	*T.harzianum*	31.20	8.02	7.20	3.53	69.29	63.01	51.58	40.64
	T.viride	32.08	7.97	6.86	3.39	74.06	61.99	40.21	35.06
	T. lignorum	37.60	10.45	8.25	4.10	104.02	112.40	73.68	63.35
	B. subtilis	29.13	7.55	6.96	3.78	58.06	53.46	46.53	50.60
	B. megaterium	34.70	10.20	7.43	4.25	88.28	107.32	56.42	69.32
	S. marcescens	43.97	11.62	9.64	4.55	138.58	136.18	102.95	81.27
Control		18.43	4.92	4.75	2.51	0.00	0.00	0.00	0.00
LSD 0.05		1.60	0.53	0.46	0.28	------	------	------	------

compared to other treatments and control. The highest % reduction of damping-off disease incidence was obtained in the case of using oxalic acid and ascorbic acid as seed soaking with *Serratia marcesens* as a seed coating where they reduced the disease incidence to more than 82.61%, meanwhile, the survived plants were 92 and 96.0 % respectively. On the other hand, the other treatments were moderately effective in this respect. These results could be interpreted in light the findings of Khalifa *et al.* (2016) who found that soaking faba bean seeds in both of the biotic inducers *(T. hamatum* and *B. subtilis*) and abiotic inducer (salicylic acid) reduced damping-off diseases more than using any of them individually. Also, these results are in harmony with those obtained by El-Mohamedy *et al.,* 2014) who verified the success of using salicylic acid and bio-agents in controlling root rot diseases of tomato plants caused by *F. solani, R. solani* and *Sclerotium rolfsii.*

Effect of antioxidant and bioagent treatments on some vegetative characteristics

Some vegetative characters, *i.e.,* fresh and dry weight of shoots and roots per plant were determined 45 days post sowing. Results in table (2) revealed that integration between the tested treatments significantly increased both fresh and dry weights of shoots and roots per plant. The highest increase % of both fresh and dry weights of shoots (138.58, 136.18%, respectively) and those of roots (102.95 and 81.27%, respectively) was obtained in the case of using ascorbic acid as seed soaking with *S. marcesens* as a seed coating. These results are in agreement with those obtained by Eid, (2014) who found that soil drenching with some bioagents significantly reduced damping-off disease caused by *Sclerotium rolfsii* and increased the fresh and dry weights of shoot and root of snap bean plants compared to untreated control.

Effect of integration between antioxidants and bioagents treatments on biochemical changes in leaves

Three antioxidants, *i.e.,* ascorbic acid, oxalic acid and salicylic acid at 5.0 mM (as seed soaking) were integrated with 6 bioagents, *i.e., B. megaterium, B. subtilis, S. marcesens, T. harzianum, T. lignorum* and *T. viride* (as seed coating) to study their effects on biochemical changes of snap bean plants under greenhouse conditions.

1. Effect on total flavonoids content

Results in table (3) revealed that all tested treatments increased the flavonoids content of snap bean plants. The highest effective treatment was in case of the integration between salicylic acid (as seed soaking) and *B. megaterium* (as a seed coating) where

Table (3): Effect of integration between antioxidants and bioagents treatments on flavonoids content of bean plants under greenhouse conditions

Antioxidants	Bioagents	Flavonoids content (mg/g)	% Efficacy
Salicylic acid	*T.harzianum*	4.21	159.88
	T.viride	5.40	233.33
	T. lignorum	2.70	66.67
	B. subtilis	5.78	256.79
	B. megaterium	9.94	513.58
	S. marcescens	6.59	306.79
Oxalic acid	*T.harzianum*	2.97	83.33
	T.viride	5.29	226.54
	T. lignorum	2.75	69.75
	B. subtilis	4.21	159.88
	B. megaterium	6.64	309.88
	S. marcescens	5.72	253.09
Ascorbic acid	*T.harzianum*	3.24	100.00
	T.viride	5.39	232.72
	T. lignorum	2.81	73.46
	B. subtilis	4.70	190.12
	B. megaterium	6.05	273.46
	S. marcescens	5.24	223.46
Control		1.62	0.00
LSD 0.05		0.14	------

it recorded the highest flavonoids content (9.94 mg) with efficacy (513.6%) comparing to other treatments and control. On the other hand, the integration between oxalic acid and *B. smegaterium* or salicylic acid and *S. marcescens* came in the second rank where the flavonoids content were 6.64 and 6.59mg with efficacy of 306.8 and 309.9%, respectively. However, the other integrated treatments were less effective. Obtained results could be interpreted in light of the findings of Fekadu and Tesfaye (2013) who found that applying the *Pseudomonas fluorescens* 9 and 10 by bio-primed seed of faba bean treatment enhanced the accumulation of total phenols and flavonoids. In addition, Kessler *et al*, (2003) mentioned that the anti-oxidant activity of flavonoids is due to their ability to reduce free radical formation and to scavenge free radicals or chelating process.

2- Effect on some oxidative enzyme activities

Results in table (4) revealed that all tested treatments of integration between some antioxidants and bioagents affected positively the activities of Peroxidase (PO), Polyphenoloxidase (PPO) and Phenylalanine Ammonia Lyase (PAL) enzymes in leaves of snap bean plants compared to control treatment. In this respect, the highest effective treatment of PPO enzyme was that expressed on the integration between salicylic acid (as a seed soaking) and *T. hamatum* (as a seed coating) where the recorded efficacy was (355.12%), while the treatment of oxalic acid and *B. subtilis* was the highest effective one on PO where the recoded efficacy was (221.47%). On the other hand, the highest increase of PAL activity was recorded by the integration between oxalic acid and *S. Marcescens*, where the recorded efficacy was (1122.45%). In general, most of the other treatments of integration between some antioxidants and bioagents were moderately effective in this respect, but all of them were more effective than the control treatment. These results are in agreement with those obtained by Abdel-Monaim *et al.* (2011), El-Mohamedy *et al*, (2013) and Khalifa *et al.* (2016) who found that a combination among SA, *T. hamatum* and *B. subtilis* at the 15th day recorded the highest value of peroxidase activity in infected plants with *F. solani* and *R. solani*. As for polyphenoloxidase activity results, it could be interpreted in light the findings of Chranowski *et al.* (2003) who mentioned that polyphenoloxidase is a widespread enzyme found in plant cells in the chloroplast membranes, where hydroxylation of monophenols to diphenols, dehydrogenating O-diphenol to produce O-quinone and metabolization of these phenolic compounds transforming them into toxic forms. Also, Khalifa, *et al.* (2016) indicated that combination treatment among SA, *T. hamatum* and *B. subtilis* recorded a maximum increase in polyphenoloxidase activity in leaves and roots at the 30th day of infected faba bean plants with *F. solani* and *R. solani*. Also, the role of oxidative enzymes may be explained as an oxidation process of phenol compounds to oxidized products (quinines), which may limit the fungal growth (Ragab *et al.*, 2015).

***3- Effect on* some hydrolysed enzyme activities**

Results in table (5) indicate that all tested treatments of integration between some antioxidants and bioagents affected positively the activities of hydrolysed enzymes like chitinase and β-1,3-glucanase in leaves of snap bean plants. As for chitinase activity, the most effective treatment was ascorbic acid with *Serratia marcescens* where recorded the highest activity with efficacy being (315.21%). On the other hand, the highest increase of β-1,3-glucanase activity was recorded with the integration between oxalic acid and any of *S.*

Table (4): Effect of integration between antioxidant and bioagent treatments on some oxidative enzyme activities in leaves of snap bean plants

Antioxidants	Bioagents	PO	PPO	PAL	%Efficacy		
					PO	PPO	PAL
Salicylic acid	*T.harzianum*	11.28	55.98	129.78	106.97	355.12	86.73
	T.viride	10.49	25.79	472.86	92.48	109.67	580.37
	T. lignorum	15.10	18.15	310.32	177.06	47.56	346.50
	B. subtilis	13.56	31.14	220.86	148.81	153.17	217.78
	B. megaterium	10.38	34.20	99.36	90.46	178.05	42.96
	S. marcescens	12.66	46.62	810.00	132.29	279.02	1065.47
Oxalic acid	*T.harzianum*	13.74	32.22	130.14	152.11	161.95	87.25
	T.viride	11.27	50.58	718.00	106.79	311.22	933.09
	T. lignorum	14.37	26.91	364.5	163.67	118.78	424.46
	B. subtilis	17.52	29.25	190.62	221.47	137.80	174.27
	B. megaterium	13.21	27.41	120.24	142.39	122.85	73.01
	S. marcescens	15.18	20.57	849.6	178.53	67.24	1122.45
Ascorbic acid	*T.harzianum*	13.38	27.09	180.72	145.50	120.24	160.03
	T.viride	13.11	47.52	347.04	140.55	286.34	399.34
	T. lignorum	16.44	22.89	301.14	201.65	86.10	333.29
	B. subtilis	13.56	19.05	188.64	148.81	54.88	171.42
	B. megaterium	14.19	21.72	106.92	160.37	76.59	53.84
	S. marcescens	13.02	34.92	720.00	138.90	183.90	935.97
Control		5.45	12.30	69.5	0.0	0.00	0.00
LSD 0.05		0.19	0.52	1.27	------	------	------

Table (5): Effect of integration between antioxidantsand bioagents treatments on some hydrolysed enzyme activities in leaves of snap bean plants

Antioxidants	Bioagents	Chitinase	β-1,3-glucanase	%Efficacy	
				Chitinase	β-1,3-glucanase
Salicylic acid	*T.harzianum*	27.70	7.22	283.13	125.63
	T.viride	18.99	3.53	139.83	10.31
	T. lignorum	18.99	10.84	162.66	238.75
	B. subtilis	19.08	10.53	163.90	229.06
	B. megaterium	21.78	9.28	201.24	190.00
	S. marcescens	27.30	10.88	277.59	240.00
Oxalic acid	*T.harzianum*	22.07	5.64	205.26	76.25
	T.viride	18.55	3.92	156.57	22.50
	T. lignorum	19.96	9.67	176.07	202.19
	B. subtilis	21.30	10.80	194.61	237.50
	B. megaterium	27.76	13.34	283.96	316.88
	S. marcescens	21.54	15.44	197.93	382.50
Ascorbic acid	*T.harzianum*	26.89	8.03	271.92	150.94
	T.viride	20.33	4.29	181.19	34.06
	T. lignorum	19.96	7.76	176.07	142.50
	B. subtilis	20.15	6.69	178.70	109.06
	B. megaterium	27.50	10.49	280.36	227.81
	S. marcescens	30.02	10.45	315.21	226.56
Control		7.23	3.20	0.00	0.00
LSD 0.05		0.36	0.21	------	------

Table (6): Effect of integration between some antioxidants and bioagents treatments on damping-off incidence in snap bean plants under field conditions

Antioxidants	Bioagents	Season, 2014			Season, 2015		
		Damping-off		Survival plant %	Damping-off		Survival plant %
		Pre%	Post%		Pre%	Post%	
Salicylic acid	*T.harzianum*	10.40	8.89	80.71	10.20	7.26	82.54
	T.viride	10.60	9.95	79.45	10.00	8.17	81.83
	T. lignorum	7.20	5.50	87.30	6.40	5.62	87.98
	B. subtilis	12.60	12.78	74.62	12.20	9.63	78.17
	B. megaterium	9.00	7.06	83.94	8.20	6.10	85.70
	S. marcescens	6.00	4.41	89.59	4.80	3.88	91.32
Oxalic acid	*T.harzianum*	11.00	10.26	78.74	11.20	8.46	80.34
	T.viride	11.80	10.42	77.78	11.80	9.12	79.08
	T. lignorum	8.00	6.07	85.93	7.40	5.83	86.77
	B. subtilis	13.00	13.32	73.68	13.40	10.85	75.75
	B. megaterium	9.60	8.47	81.93	8.80	7.05	84.15
	S. marcescens	7.40	5.89	86.71	6.00	5.50	88.50
Ascorbic acid	*T.harzianum*	11.40	9.24	79.36	10.60	8.30	81.10
	T.viride	12.20	11.23	76.57	11.20	7.43	81.37
	T. lignorum	8.60	6.89	84.51	7.00	4.33	88.67
	B. subtilis	13.20	12.17	74.63	12.60	10.17	77.23
	B. megaterium	8.40	7.42	84.18	9.20	6.73	84.07
	S. marcescens	6.60	4.92	88.48	5.40	5.15	89.45
Control		25.40	22.22	52.38	22.80	20.26	56.94
LSD at 5%		1.32	1.45	2.11	1.20	1.37	2.15

marcescens and *B. megaterium*, where the recorded efficacy were (382.50 and 316.88%), respectively. Additionally, the other treatments of integration between some antioxidants and bioagents were moderately effective, but they were more effective than control treatment. Generally, the present study showed that all tested antioxidants reduced damping-off incidence and increased the flavonoids, PO, PPO and PAL, chitinase and β-1,3-glucanase enzymes of bean plants. In this respect, antioxidants might be right regulating plant growth by increasing enzyme activity as α-amylase and nitrate reductase, which accelerate the sugar translocation from the leaves to developing fruit (Sharma *et al.*, 1986). Also, the obtained results could be interpreted in light of the findings of Avdiushko *et al.* (1993) who reported that many plant enzymes are involved in defense reactions against plant pathogens. Enzyme activity plays an important role in plant disease resistance through increasing plant defense mechanisms that were considered the main tool of varietal resistance (Takuo *et al.*, 1993).

Field experiment

Effect of antioxidant and bioagent treatments on damping-off incidence

The effect of integration between three antioxidants, *i.e.*, ascorbic acid, oxalic acid and salicylic acid at 5.0 mM (as a seed soaking) and 6 bioagents *i.e. B. megaterium, B. subtilis, S. marcesens, T. harzianum, T. lignorum* and *T. viride* (as a seed coating) on damping-off disease of snap bean plants were studied under field conditions. Results in table (6) indicated that all tested integration treatments

reduced the pre- and post-emergence damping-off as well as increased the survival plants. In this respect, the best integration treatments were ascorbic acid, oxalic acid and salicylic acid as seed soaking and with *S. marcesens* as a seed coating, where the lowest pre and post emergence damping-off % were recorded with those treatments. In addition, the previously mentioned integration treatments increased the survived plants (88.5–91.32%) compared to other treatments and control. On the other hand, the rest of integration treatments were moderately effective in this respect, but they were more effective than control treatment. These results were in line with that obtained by Abdel-Monaim (2008). Jaiganesh *et al.* (2007) who reported that out of the 6-bio protectants tested, *S. marcescens* was found very effective against *P. oryzae* under *in vitro* conditions. The results revealed that rice blast control was achieved by spraying *S. marcescens*. Also, these obtained results could be supported with the findings of El-Mohamedy *et al.* (2014) and Khalifa *et al.* (2016).

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