Biological Control of Bean Damping- off Caused by *Sclerotium rolfsii*

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The role of four bioagents, *i.e. Bacillus subtilis Pseudomonas fluorescens Saccharomyces cerevisiae* and *Trichoderma viride* in controlling damping-off disease of bean (*Phaseolus vulgaris* L.) caused by *Sclerotium rolfsii* was evaluated under greenhouse and field conditions. Greenhouse experiment indicated that all the tested bioagents significantly reduced the incidence of the disease compared with control treatment. In addition, the most effective treatments were *B. subtilis, T. viride* and *P. fluorescence*, which reduced disease incidence more than 83.7 and 74.5% for pre- and post-emergence damping-off, respectively and increased the survived plants to 90.3, 86.1 and 87.6%,respectively compared with 26.3% in untreated plants. Also, they significantly increased dry and fresh weight of bean shoot & root as well as resulted in considerable increase to the activity of peroxidase, polyphenol oxidase and chitinase activity more than 260.0, 109.0 and 218.3%, respectively. Under field conditions during 2009 and 2010 growing seasons, data revealed, also, that all the tested bioagents significantly reduced disease incidence with considerable increase to the survived plants and the produced seed yield compared with control treatment. As for the first season, the most effective treatments were *B. subtilis T. viride, S. cerevisiae* and *P. fluorescens*, which reduced disease incidence more than 61.3 and 41.3 % than the control for pre- and post-emergence damping-off, respectively. The corresponding percentages of survived plants were 78.2 , 79.0 , 75.2 and 76.8 %, respectively viz. 38.5 % for the control. On the other hand, the most effective treatments for increasing seed yield was *S. serivisae* followed by *P. fluorescens*, being 894.95 and 748.1 kg/feddan viz. 269.2 kg/feddan for the control. The other two bioagents showed moderate effect. The same trend was obtained during the second season. It could be suggested that such bioagents might be promising as alternatives to control bean damping-off caused by *S. rolfsii*.

Keyword: Bean, bioagents, enzymes, fresh and dry weight, *Sclerotium rolfsii*, seed yield .

Bean plants (*Phaseolus vulgaris* L.) is one of the most important leguminous crops in Egypt. Damping-off disease is a serious and persistent problem of bean plants during growing season (Filion *et al.,* 2003; Harveson *et al.,* 2005 and Wen *et al.,* 2005). *Sclerotium rolfsii* Sacc. [*Athelia rolfsii* (Curzi) Tu & Kimbrough] causes a disease known as southern blight or white mold in a wide variety of crops all over the world. Sclerotium root-rot is also a difficult disease to manage since the fungal sclerotia can survive in the soil and crop residues for several years (Punja, 1985).

Nowadays, the world is suffering from great pollution from many pollutants agrochemicals such as pesticides. Therefore, the current strategy of management plant pests, especially of vegetables and fruits depends on using alternative methods rather than pesticides and/or using these chemicals at the first periods of plant growth prior to fruit maturity. Hence, this work aimed to use bioagents for controlling bean damping-off. Biological control through the use of antagonistic microorganisms is a potential, non chemical means of controlling plant disease by reducing inoculums level of the pathogens. Such management could be help in preventing pollution and also health hazards (Kumar, 2007). *Trichoderma* spp. are now the

most common fungal bioagents that have been extensively researched and deployed throughout the world (Khalifa *et al.,* 2013). *Bacillus* spp, *Pseudomonas* spp. and yeast (*Saccharomyces cerevisiae*) also are among the most important genera of antagonistic microorganisms for controlling fungal diseases (Meena *et al*., 2001 Ibrahim *et al.,* 2008 and Abdel-Kader *et al.*, 2012). Application of *B. subtilis* under greenhouse and field conditions, reduced damping–off and root rot diseases of many crops (El Fiki *et al.,* 2004, Mahmoud *et al.,* 2006 and Khalifa *et al.,* 2007). *Pseudomonas fluorescens* is, also, considered as an important group of the antagonistic bacteria, where it was effective against several soil borne pathogens in field and greenhouse trails (Jayashree *et al.,* 2000 and Karunanithi *et al.,* 2000).

The present study aimed to evaluate the effect of different bioagents, *i.e. S. cerevisiae, T. viride*, *B. subtilis* and *P. fluorescens* on management of bean damping-off compared to the fungicide Vitavax-200 under greenhouse and field conditions.

M a r e r i a l s a n d M e t h o d s

1- Source of the materials:

A white moldy layer with small, smooth and brown sclerotia was detected in the parts of common beans in contact with the soil, which was initially identified as *Sclorotium rolfsii* infection according to Schwartz *et al.,* (2005) and FAO (2007). Further confirmation of *S. rolfsii* was obtained through the morphological characteristics identified under the microscope by the Dept. of Fungal Taxonomy, Plant Pathol. Res. Instit., Agric. Res. Cent. (ARC), Giza - Egypt.

The tested bioagents *i.e. Trichoderma veredi, Bacillus subtilis* and *Pseudomonas fluorescence* were kindly obtained from Botany Dept., Fac. of Agric., Benha Univ. Meanwhile, yeast *Saccharomyces cerevisiae* was obtained from Microbiol. Res. Cent., Cairo MIRCEN, Ain Shams Univ., Egypt.

Bean seeds cv. Bronco were obtained from Veg. Crops Res. Dept., Agric. Res. Cent., Giza - Egypt.

2-Greenhouse experiments:

2.1. Effect of the tested bioagents on incidence of damping-off :

The antagonistic bacteria, *i.e. P. flourescens* and *B. subtilis* were grown in nutrient broth medium, while *S. cerevisiae* was grown on nutrient yeast dextrose broth medium NYDB (Abd-Alla *et al.*, 2007). All tested bacteria and yeast were incubated in a rotary shaker at 200 rpm for 48 h at 28 ± 2°C. The bacterial and yeast cells were harvested by centrifugation at 6,000 rpm for 10 min, washed twice in 0.05 M. phosphate buffer pH 7.0, and re-suspended in sterilized distilled water. The concentration of both yeast and bacterial cells in the suspensions was adjusted to $3X10^6$ cells per milliliter (cfu/ml) by a haemocytometer slide for yeast cells (Abdel-Kader *et al.* 2012), while bacterial concentration was determined according to its turbidity using spectrophotometer at 400nm. Both *T. viride* and *S. rolfsii* were 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 any of *S. rolfsii* and *T. viride* and incubated for 15 days at 28 ± 2°C.

Plastic pots (25 cm in diameter) were sterilized by dipping in 5% formalin solution for 5 minutes, then thoroughly washed with tap water and left to get rid of the remained formalin, then filled with sandy loam soil sterilized with 5% formalin solution and left to aerate. Pots were infested with *S. rolfsii* inoculum at the rate of 3.0% (w/w)**.** After 14 days of soil infestation, *T. viride* was applied at a rate of 5% (w/w), meanwhile, either antagonistic bacteria or yeast were used at a rate of 50 ml/pot (each 1 ml contains about 3×10^6 cells (Abdel-Kader *et al.* 2012). Seeds moisten with super film as sticker were dressed with Vitavax-200 at a rate of 3g/kg seed were used for comparison. Five surface sterilized bean seeds with 2% sodium hypochlorite (cv. Bronco) were sown in each pot. Five replicates were used for each treatment.

Pots infested with the pathogenic fungus and sown with untreated sterilized seeds were used as control.

Percentages of pre- and post-emergence damping-off as well as healthy survived plants were recorded 15, 30 and 60 days after planting, respectively.

Fresh and dry weight of shoot and root systems were determinate at the end of the experiment (60 days after planting).

2.2. Determination of enzymes activity:

The four tested bioagents as well as the fungicide Vitavax-200 were evaluated for their effect on the activity of peroxidase, polyphenoloxidase and chitinase in bean plants.

2-2.a.Extraction of enzymes:

Five g. of bean leaves were taken 6 weeks after sowing and ground in a mortar in presence of purified sand plus 4ml of 0.1 M sodium phosphate buffer (pH 7.1) (Tuzun *et al.*, 1989). The homogenate of each sample was filtered through four layers of cheesecloth then filtrates were centrifuged at 3000 rpm for 20 min. at 6°C. The obtained supernatant fluids (crude enzyme extracts) were used for assaying activities of peroxidase, polyphenoloxidase (PPO) and chitinase enzymes at 425, 420 and 540 nm, respectively using Spectrophotometer (Spectronic 20-D). Enzyme extract was replaced" by distilled water in control blank cuvette. Changes in absorbency for all previous enzymes were recorded. In this regard, the activity of peroxidase enzyme (Allam and Hollis, 1972), polyphenoloxidase enzyme (Matta and Dimond, 1963) and Chitinase enzyme (Boller and Mauch, 1988) were determined.

3- Field experiments:

A field has a back history of natural infestation with *S. rolfsii* (located at vegetables Farm of Hort., Fac. Agric. Moshtohor, Benha Univ., Egypt) was chosen to carry out to evaluate role of the tested bioagents, *i.e. B. subtilis, P. flourescense, S. cerevisiae* and *T. viride* in reducing damping-off incidence and the produced seed yield during 2009 and 2010 growing seasons . The field was prepared for sowing bean as usual. Mechanical and chemical analyses of the field soil are presented in Table (1). A field experiment, consisted of plots area of 10.5m^2 (3x3.5) each comprised of 3 rows and 16 hill/row, was conducted in Complete Randomized Block Design with three replicates (plots) for each treatment as well as control. Bean seeds cv. Bronco were used in all treatments at rate of 2 seeds / hill.

Table 1. Mechanical and chemical analyses of field soil during two growing seasons 2009 and 2010.

Soil characteristics	2009	2010
Coarse sand (%)	2.00	2.2
Fine sand (%)	23.41	24.71
Silt (%)	33.45	36.0
Clay (%)	41.14	46.4
Textural class	Clay loam	Clay loam
$CaCO_3$ (g kg^{-1})	25.10	22.10
Organic matter (g kg^{-1})	1.51	2.35
pH	7.83	7.67
EC (dS m^{-1})	2.43	2.17
Total N (mg kg^{-1})	1154.00	3139.00
Available P (mg kg^{-1})	43.12	41.1

Soil infestation with inocula of the tested bioagents was carried out by using 360g of *T. viride* inoculum/row and 500ml ($3X10^6$) of *B. subtilis, P. flourescense* and *S. cerevisiae* inoculum /row by incorporating the inoculum with the top 20cm of soil surface of the rows just

before sowing (El-Mougy, 2001). Seeds dressed with Vitavax-200 at a rate of 3g/kg seed were used for comparison.

Disease assessment of pre- and post-emergence damping-off as well as survived plants were recorded 15, 30 and 60 days after planting, respectively.

Fresh and dry weights of the plants were determined at flowering stage by using five randomly selected plants from each plot. Meanwhile, Beans pods of each plot were harvested at proper maturity stage, then weighed then total seed yield/fadden was estimated.

Statistical analysis

Data collected were analyzed with the statistical analysis system (CoStat Pro., 2005). All multiple comparisons were first subjected to analysis of variance (ANOVA). The differences between the mean values of various treatments were compared by Duncan s multiple range test (Duncan, 1955).

RESULTS

1-Effect of four bioagents compared to the fungicide Vitavax-200 on incidence of damping-off under greenhouse conditions:

Data presented in Table (2) indicate that all the tested four bioagents significantly reduced pre- and post-emergence damping-off caused by *S. rolfsii* compared to untreated control. In addition, The most effective bioagent in this regard was *B. subtilis* followed by *P. fluorescence* then *T. viride*, which reduced the disease more than 83.71 and 74.53% for pre- and post-emergence damping-off. The respective averages of survived plants for these bioagents were 90.3, 86.1 and 87.6%, respectively compared with 26.3 % for untreated plants. Meanwhile, *S. cerivisae* reduced pre- and post-emergence damping-off by 79.7 and 43.96 %, respectively with 77.53% survived plants.

Table 2. Effect of four bioagents compared to the fungicide Vitavax-200 on incidence of bean damping-off (Pronco cv.) under greenhouse conditions.

Treatments	% Pre-emergence damping off	% Reduction	% Post-emergence damping off	% Reduction	% Survived plants
S. cerivisae	10.73 b	79.70	11.73 bc	43.96	77.53 b
T. viride	8.60 b	83.71	5.33 cd	74.53	86.07 ab
B. subtilis	5.93 b	88.77	3.80 d	81.84	90.27 a
P. fluorescens	7.20 b	86.36	5.20 cd	75.16	87.60 ab
Vitavax -200	12.80 b	75.76	12.07 b	42.33	78.60 b
Control	52.80a	00.00	20.93 a	00.00	26.27 c

2. Effect of four bioagents compared to the fungicide Vitavax-200 on some crop parameters of bean plants under greenhouse conditions:

Data shown in Table (3) show that all the tested bioagents significantly increased shoot and root fresh and dry weight compared to untreated control. Yeast and fungicide treatments were the most effective treatments for enhancement the vegetative characters of bean plants, followed by *P. fluorescence* then *B. subtilis* and *T. viride* in most cases.

Table 3. Effect of some bioagents compared to the fungicide Vitavax-200 on some vegetative characters of bean plants under greenhouse conditions.

Treatments	Shoot system weight (g plant^{-1})		Root system weight (g plant^{-1})	
	Fresh	Dry	Fresh	Dry
S. cerivisae	51.96 a	16.10 a	10.84 a	3.78 a
T. viride	39.76 b	9.73 c	6.60 c	3.20 b
B. subtilis	38.70 b	11.46 c	8.49 b	3.23 b
P. fluorescens	40.42 b	13.51 b	8.35 b	3.14 b
Vitavax -200	50.91 a	13.83 b	9.09 b	3.08 b
Control	24.06 c	7.73 d	4.93 d	2.50 c

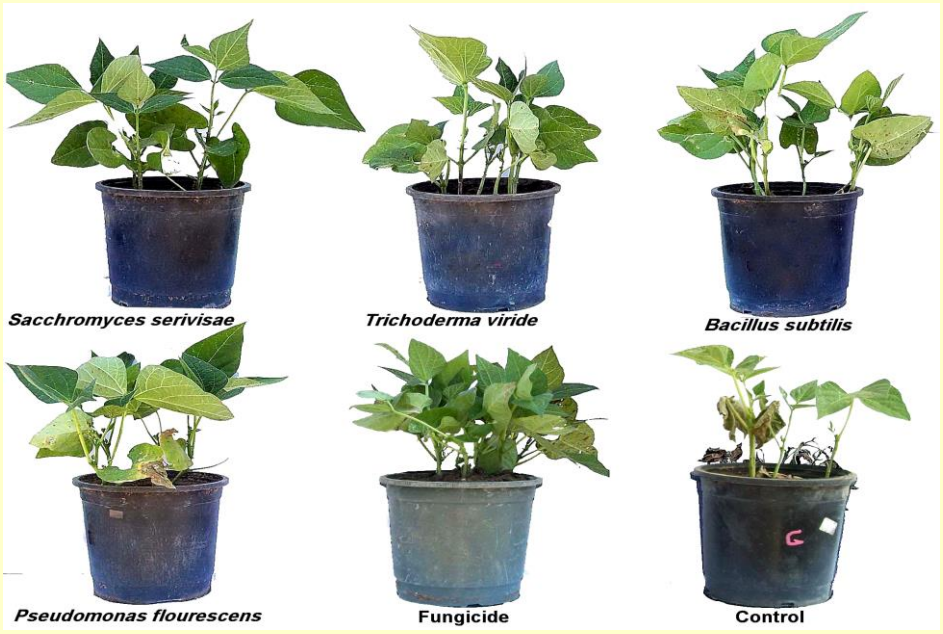

Fig 1. Effected of four bioagents, *i.e. S. cerevisiae, T. veredi, B. subtilis* and *P. fluorescens* on growth of bean plants infested with *S. rolfsii* under greenhouse conditions.

3. Effect of four bioagents compared to the fungicide Vitavax-200 on the enzymatic activity of bean plants .

Data presented in Table (4) reveal that all treatments increased the activity of all the assessed enzymes compared to untreated control. Generally, all the tested bioagents were superior for increasing the activity of all the tested enzymes compared to the tested fungicide. *T. virde* resulted in the highest increase in the activity of chitinase and polyphenoloxidase, whereas *B. subtilis* caused highest increase in the activity of peroxidase.

Table 4. Effect of four bioagents on the enzymatic activity of bean plants .

Treatments	Chitenase		Peroxidase		Polyphenoloxidase	
	Activity	Increase %	Activity	Increase %	Activity	Increase %
S. cerivisae	19.1	218.3	22.3	346.0	45.4	167.0
T. viride	22.8	280.0	18.0	260.0	62.1	265.3
B. subtilis	19.1	218.3	24.9	398.0	43.7	157.0
P. fluorescens	20.0	233.3	23.1	362.0	35.6	109.4
Vitavax -200	9.3	55.0	13.0	140.0	29.7	74.7
Control	6.0	--------	5.0	--------	17.0	-------

4-Effect of four bioagents compared to the fungicide Vitavax-200 on incidence of bean damping off under field conditions:

The four bioagents, *i.e. T. viride, B. subtilis*, *P. fluorescens* and *S. serivisae* were tested for their effect on incidence of damping-off under field conditions. Data shown in Table (5) indicate that all bioagents significantly reduced the disease. AS for first season, the most effective treatments were *B. subtilis T. viride, S. serivisae* and *P. fluorescens*, which reduced the disease more than 61.3 and 41.3% for pre and post emergence respectively. The corresponding percentages of survived plants for these bioagents were 78.2, 79.0, 75.2 and 76.8%, respectively viz. 38.5% for the control. On the other hand, the most effective treatments for increasing seed yield was *S. serivisae* followed by *P. fluorescens*, being 894.95 and 748.1 kg/feddan viz. 269.2 kg./ feddan for the control. The other two bioagents showed moderate effect. The same trend was obtained during the second season.

Table 5. Effect of four bioagents on incidence of damping-off of bean under field conditions during 2009 and 2010 growing seasons.

	Treatments	% Pre-emergence Damping-off	% Reduction	% Post-emergence Damping-off	% Reduction	% Survived plants
First season, 2009	*S. cerivisae*	11.5bc	71.67	10.3bc	50.48	78.2 a
	T. viride	15.7 b	61.33	7.6 c	63.46	76.8 a
	B. subtilis	13.9bc	65.76	10.9bc	47.60	75.2 a
	P. fluorescens	8.9 c	78.08	12.2 b	41.35	79.0 a
	Vitavax-200	11.5bc	71.67	8.0 c	61.54	80.5 a
	Control	40.6 a	0.00	20.8a	0.00	38.5 b
Second season, 2010	*S. cerivisae*	24.0 b	47.60	5.2 b	63.38	70.8 b
	T. viride	18.1 b	60.48	2.8 b	80.28	79.2 a
	B. subtilis	25.1 b	45.20	4.2 b	70.42	70.8 b
	P. fluorescens	25.0 b	45.41	4.9 b	65.49	70.1 b
	Vitavax-200	25.0 b	45.41	4.2 b	70.42	70.8 b
	Control	45.8 a	0.00	14.2 a	0.00	39.9 c

5. Effect of four bioagents compared to the fungicide Vitavax-200 on some crop parameters of bean plants under field conditions:

The tested four bioagents were tested for their effect on some crop parameters of bean plants under field conditions. Data shown in Table (6) indicate that all bioagents significantly increased the assessed crop parameters of bean plants under field conditions as compared to untreated plants (control) in the two seasons. As for, in the two seasons, the highest increase was obtained with *S. serivisae* ,which increased the shoot system fresh weight; shoot system dry weight; the root system fresh weight; root system dry weight and seed yield (105.26, 29.38, 18.64, 6.92 g plant^{-1} and 894.95 kg feddan^{-1} respectively, in the first season. While, 100.26, 37.88, 23.34, 8.63 g plant^{-1} and 1142.24 kg feddan^{-1} respectively, in the second season).

Table 6. Effect of different bioagents on some crop parameters of bean (Pronco cv.) under filed conditions during 2009 and 2010 growing seasons.

Treatments		Shoot system weight (g/plant)		Root system weight (g/plant)		Seed yield (kg/feddan)
		Fresh	Dry	Fresh	Dry	
First season, 2009	*S. cerivisae*	105.26a	29.38 a	18.64 a	6.92 a	894.95 ab
	T. viride	61.34 c	19.89 b	10.61 d	4.44 c	605.32 c
	B. subtilis	61.49 c	20.60 b	15.25 b	5.79 b	650.03 c
	P. fluorescens	81.53 b	24.55 ab	14.58 bc	6.14 ab	748.18 bc
	Vitavax-200	96.79 a	21.48 b	12.28 cd	4.03 c	1048.3 a
	Control	32.35 d	9.63 c	6.12 e	3.08 d	269.2 d
Second season, 2010	*S. cerivisae*	100.26 ab	37.88 a	23.34 a	8.63 a	1142.24 a
	T. viride	97.70 ab	28.38 b	19.42 c	7.78 b	1060.72 a
	B. subtilis	92.46 b	26.97 b	19.02 c	7.12 c	974.40 a
	P. fluorescens	97.62 ab	28.76 b	18.82 c	7.41 bc	902.21 a
	Vitavax-200	106.37 a	33.35 ab	21.88 b	7.75 b	1091.30 a
	Control	62.24 c	19.49 c	13.58 d	5.91d	468.58 b

Discussion

Bean plants (*Phaseolus vulgaris* L.) is one of the most important leguminous crops 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) and Wen *et al.* (2005). *Sclerotium rolfsii* causes the disease known as southern blight in a wide variety of crops. Due to the pollution to the human food by agrochemicals, especially pesticides, therefore there is a growing need to develop alternative approaches for controlling plants diseases rather than pesticides. So, bioagents are risk free both for environment and non-target organisms, and could reduce the use of chemical products for controlling plant diseases. Most bioagents (BAs) have varied performance in different environmental conditions. Some of this variability has been attributed to differences in physical and chemical properties found in natural environments where bioagents are applied Thomashow and Weller (1996); Duffy *et al*. (1997).

In the present study results indicated that all the tested bioagents significantly reduced the incidence of bean damping-off caused by *S. rolfsii* with significant increase to shoot and root dry and fresh weight. In addition, all the tested bioagents caused considerable increased in the activity of peroxides, polyphenol oxidase and chitinase. Furthermore, under field conditions results of two successive seasons showed that all bioagents have significantly reduced the disease and increased the produced seed yield. The most effective treatments were *S. serivisae* and *B. subtilis*, which increased the seed yield per feddan. Application of *S. cerevisiae* resulted in the highest reduction to pre- and post-emergence damping-off and increased the Survived plants in comparison with the control. Hassan and Abd El-Rehim (2002) observed that increasing yeast concentration (0.05 to 0.1%) resulted in gradual reduction to onion neck rot. Lokesh *et al*. (2007) mentioned that using several taxa included yeast genera as plant growth promoters and/or as bioagents significantly reduced the infection of watermelon by *Fusarium* spp. and increased seed germination**.** In addition, bacterial species like *Bacillus*, *Pseudomonas*, have been proved in controlling the fungal diseases. Bacteria identified as plant growth promoting rhizobacteria and biocontrol strains often belong to the genera of *Bacillus* (Nair *et al.*, 2002) and *Pseudomonas* (Mark *et al.*, 2006). Moreover, *Pseudomonas* spp. received great attention as bioagents because of their catabolic versatility, excellent root-colonizing abilities and production of broad range antifungal metabolites such as 2,4-diacetylphloroglucinoal (DAPG), pyoluteorin, pyrrolnitrin and phenazines Chin-A-Woeng *et al*. (2001) and Raaijmaker *et al*. (2002). The mechanisms through which *Pseudomonas* spp.

control plant diseases involve (i) competition for niches and nutrients, (ii) antibiosis, (iii) predation, and (iv) induction of plant defense responses. Biocontrol of damping-off diseases has been successfully applied using *B. subtilis* Berger *et al*. (1996); Harris and Adkins (1999); Georgakopoulos *et al*. (2002) and Schmidt *et al.* (2004). Fernando, *et al.* (2007) found that in field studies over a period of two years indicated that disease control with *Pseudomonas chlororaphis* (PA-23), *Bacillus amyloliquefaciens* (BS6) was comparable to that achieved with the fungicide Rovral (iprodione). They added that here was no significant difference between single- and double-spray application of PA-23 and BS6 in the management of canola stem rot.

Mukherjee and Raghu (1997) observed that *Trichoderma* spp. were highly effective in suppressing *S. rolfsii* on ginger rhizomes and on several vegetables in storage. Also Rekha *et al*. (2012) found that isolates Tri-13 (*T. viride*) and Tri-29 (*T. viride*) reduced the growth of *S. rolfsii* through volatile metabolites compare to other tested isolates and control. Similarly, Chakrabortys and Bhawmik (1985) found that *T. harzianum* and *T. viride* highly effective in the controlling of sunflower collar rot caused by *S. rolfsii*. It could be suggested that bioagents as safety method could be commercially used for controlling bean damping-off disease under field conditions.

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المقاومة الحيوية لمرض سقوط البادرات في الفاصوليا الناتج عن الاصابة بالفطر *Sclerotium rolfsii* .

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فرع أمراض النبات - قسم النبات الزراعي - كلية الزراعة- جامعة بنها.

تم دراسة تأثير أربعة من كائنات المكافحة الحيوية وهي الخميرة *Saccharomyces cerevisiae* وفطر *Trichoderma viride* وبكتريا *Bacillus subtilis* و *Pseudomonas fluorescens* لمكافحة مرض سقط البادرات في نباتات الفاصوليا الناتج عن الإصابة بالفطر *Sclerotium rolfsii* . أشارت النتائج المتحصل عليها تحت ظروف الصوبة أن جميع كائنات المكافحة الحيوية محل الدراسة أدت إلي خفض معدل الإصابة بالفطر *S. rolfsii* . وكانت أكثر كائنات المكافحة الحيوية المستخدمة خفضا لمعدل الاصابة هي بكتريا *B. subtilis* و فطر *T. viride* وبكتريا *P. fluorescens* حيث حققت أعلى إنخفاض لمعدل الإصابة والتي تراوحت بين ٨٣,٧ و ٧٤,٥% لنسبة سقوط البادرات قبل وبعد الظهور فوق سطح التربة ، وبالتالي أعلى نسبة نباتات نامية (باقية) حيث سجلت ٩٠,٣ و ٨٦,١ و٨٧,٦% ، علي التوالي بالمقارنة بالنباتات الغير معاملة حيث سجلت ٢٦,٣% للنباتات المتبقية . سجلت كل كائنات المكافحة الحيوية المستخدمة زيادة ملحوظة في الوزن الطازج والجاف للمجموع الخضري والجذري لنباتات الفاصوليا ، كما أدت إلي حدوث زيادة ملحوظة في نشاط إنزيمات البيروكسيديز والبولي فينول أكسيديز والشيتنيز بمعدل أكثر من ٢٦٠ و ١٠٩ و ٢١٨,٣% ، علي التوالي بالمقارنة بالنباتات الغير معاملة. علاوة على ذلك ، أوضحت نتائج تجارب الحقل خلال موسمى ٢٠٠٩ ، ٢٠١٠ ، أن كل عوامل المكافحة الحيوية المستخدمة أدت إلى خفض نسبة الإصابة بمرض سقوط البادرات. ففي الموسم الأول كانت أكثر كائنات المقاومة الحيوية فاعلية هي بكتريا *B. subtilis* وفطر *T. viride* و الخميرة *S. cerevisiae* وبكتريا *P. fluorescens* حيث خفضت نسبة الإصابة بمرض سقوط البادرات قبل وبعد الظهور فوق سطح التربة بمعدل أكثر من ٦١,٣ و ٤١,٣% ،علي التوالي. وسجلت هذه الكائنات نسب ٧٥,٢ و ٧٦,٨ و ٧٨,٢ و ٧٩,٠% نباتات قائمة (متبقية) ،علي التوالي بالمقارنة للنباتات الغير المعاملة حيث سجلت ٣٨,٥%. أما بالنسبة إلي المحصول فقد كانت اكثر المعاملات فاعلية هي الخميرة *S. cerevisiae* ثم بكتريا *B. subtilis* حيث أدت إلي زيادة محصول بذور الفاصوليا للفدانبدرجة *كبيرة* ، وكان تأثير باقي عوامل المكافحة الحيوية متوسط الفاعلية علي زيادة محصول بذور الفاصوليا للفدان. وتم الحصول على نتائج مشابة في موسم التمو الثاني. وبهذا يمكن الاقتراح باستخدام عوامل المكافحة الحيوية السابقة كطريقة واعدة و آمنة لمكافحة مرض سقوط البادرات في نباتات الفاصوليا المتسبب عن الإصابة بالفطر *S. rolfsii* .