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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,* yeast (*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. The greenhouse experiment indicated that all the tested bioagents significantly reduced the incidence of the disease compared to control treatment. The most effective treatments were *B. subtilis, T. viride* and *P. fluorescens* which reduced disease incidence by more than 83.7 and 74.5% for pre- and post-emergence damping-off, respectively, while increased the plant survival by 90.3, 86.1 and 87.6%, respectively, compared to 26.3% in untreated plants. These treatments also increased the dry and fresh weights of bean shoot and root as well as resulted in considerable increases in the activities of peroxidase, polyphenoloxidase and chitinase by 260.0, 109.0 and 218.3%, respectively. Under field conditions, all the tested bioagents significantly reduced disease incidence with considerable increases in plant survival and higher seed yield during the two seasons (February of 2009 and 2010) of study. During the first season, *B. subtilis T. viride, S. cerevisiae* and *P. fluorescens* were found to be the most effective bioagents which reduced the disease incidence with 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 were *S. cerevisiae* 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 synthetic fungicides for controlling bean damping-off caused by *S. rolfsii*.

Keywords: Bean, bioagents, enzymes, *Sclerotium rolfsii* and seed yield.

Bean plants (*Phaseolus vulgaris* L.) are 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 mould in a wide variety of crops all over the world. Sclerotium root-rot disease is difficult to manage since the fungal sclerotia can survive for several years in soil and crop residues (Punja, 1985).

Nowadays, the world is suffering from pollution caused by the extensive uses of the agrochemicals in agriculture such as pesticides. Therefore, the plant pests, especially of vegetables and fruits, could be more preferably controlled using more safe methods than pesticides. This work aimed at using bioagents for controlling bean damping-off. The biological control of plant pests using antagonistic microorganisms is a potentially, non-chemical means of controlling plant disease by reducing inoculums level of the pathogens. Such management could help in preventing pollution and no health hazards (Kumar, 2007). *Trichoderma* spp. are the most popular bioagents that have been extensively researched and deployed throughout the world (Khalifa *et al.*, 2013). *Bacillus* spp., *Pseudomonas* spp. and yeast (*Saccharomyces cerevisiae*) are also among the most important genera of the antagonistic microorganisms for controlling fungal diseases (Meena *et al*., 2001, Ibrahim *et al.*, 2008 and Abdel-Kader *et al.*, 2012). *Bacillus subtilis* reduced damping-off and root-rot diseases of many crops under greenhouse and field conditions (El-Fiki *et al.*, 2004; Mahmoud *et al.*, 2006 and Khalifa *et al.*, 2007). *Pseudomonas fluorescens* is also an important antagonistic bacterium against several soil borne pathogens (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 controlling *S. rolfsii*, in beans compared to the fungicide Vitavax-200 under greenhouse and field conditions.

Materials and Methods

Source of the materials:

A white mouldy 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 *Sclerotium rolfsii* infection according to Schwartz *et al.* (2005) and Anonymous (2007). Further confirmation of *S. rolfsii* was performed through the morphological characteristics identified under the microscope by the Dept of Mycol. Res. and Dis. survey, Plant Pathol. Res. Inst., ARC. The tested bioagents, *i.e. Trichoderma viride, Bacillus subtilis* and *Pseudomonas fluorescens,* were kindly obtained from Botany Dept., Fac. of Agric., Benha Univ. Meanwhile, yeast (*Saccharomyces cerevisiae*) was obtained from Microbiol. Res. Centre, Cairo MIRCEN, Ain Shams Univ., Egypt. Bean seeds (cv. Bronco) were obtained from Veg. Crops Res. Dept., ARC, Giza, Egypt.

Greenhouse experiments:

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

The antagonistic bacteria, *i.e. P. fluorescens* and *B. subtilis*, were grown in nutrient broth medium, while yeast (*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 suspensions of the bacterial and yeast cells were adjusted to $3x10^6$ cfu/ml (Omar *et al.*, 2011). 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 at 28±2°C. for 15 days.

Plastic pots (25-cm-diam.) 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 m contains about $3x10^6$ 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).

Determination of enzymes activity:

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

Extraction of enzymes:

Five grams 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 the 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 540nm, respectively, using spectrophotometer (Spectronic 20-D). Enzyme extract was replaced by distilled water in controlling 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 Diamond, 1963) and Chitinase enzyme (Boller and Mauch, 1988) were determined.

Field experiments:

A field originally contaminated with *S. rolfsii*, (located at the vegetable farm of Horticulture Dept., Fac. of Agric. Moshtohor, Benha Univ., Egypt) was chosen to evaluate the effectiveness of the tested bioagents, *i.e. B. subtilis*, *P. fluorescens*, *S. cerevisiae* and *T. viride*, in reducing the damping-off incidence and their further effects on the seed yield during the two seasons (February of 2009 and 2010) of study. Physical and chemical properties of the soil of study are presented in Table (1). A field experiment was conducted in Complete Randomized Block Design with three replicates (plots) for each treatment as well as control. The plots area were $10.5m^2$ (3x3.5), each comprised of 3 rows and 16 hill/row. Bean seeds (cv. Bronco) were used in all treatments at rates of 2 seeds/hill.

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

Soil characteristic	2009	2010
Coarse sand (%)	2.0	2.2
Fine sand (%)	23.4	24.7
Silt (%)	33.4	36.0
Clay (%)	41.1	46.4
Textural class	Clay loam	Clay loam
$CaCO_3$ (g kg^{-1})	25.1	22.1
Organic matter (g kg^{-1})	1.5	1.3
pH	7.8	7.7
EC (dS m^{-1})	2.4	2.2
Total N (mg kg^{-1})	1154.0	2139.0
Available P (mg kg^{-1})	43.1	41.1
Available K (mg kg^{-1})	976.0	688.0

Soil infestation with inocula of the tested bioagents was carried out by using 360g of *T. viride* inoculum/row and 500ml ($3x10^6$ cfu/ml) of *B. subtilis*, *P. fluorescens* and *S. cerevisiae* inoculum /row by incorporating the inoculum on the top 20cm of soil surface of the rows before sowing (El-Mougy, 2001). Seeds were dressed by 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 was recorded 15, 30 and 60 days after planting, respectively. Fresh and dry weights of the plants were determined at flowering stage by selecting five plants randomly from each plot. Beans pods of each plot were harvested at proper maturity stage, and then weighed then total seed yield/feddan was estimated.

Statistical analysis:

Data collected were analysed with the statistical analysis system (Anonymous, 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

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

Data presented in Table (2) and Fig. (1) indicate that all the tested 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. fluorescens* then *T. viride*, which reduced the disease more than 83.7 and 74.5% 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 to 26.3% for untreated plants. Meanwhile, *S. cerevisiae* reduced pre- and post-emergence damping-off by 79.7 and 44.0%, respectively, with 77.5% survived plants.

Table 2. Effect of bioagents and the fungicide Vitavax-200 on incidence of bean (cv. Bronco) damping-off under the greenhouse conditions

Treatment	Pre-emergence Damping-off (%)	Reduction (%)	Post-emergence Damping-off (%)	Reduction (%)	Survived plants (%)
S. cerevisiae	10.7 b	79.7	11.7 bc	44.0	77.5 b
T. viride	8.6 b	83.7	5.3 cd	74.5	86.1 ab
B. subtilis	5.9 b	88.8	3.8 d	81.8	90.3 a
P. fluorescens	7.2 b	86.4	5.2 cd	75.2	87.6 ab
Vitavax-200	12.8 b	75.8	12.1 b	42.3	78.6 b
Control	52.8 a	00.0	20.9 a	00.0	26.3 c

Values with the same letter are not significantly different (P = 0.05).

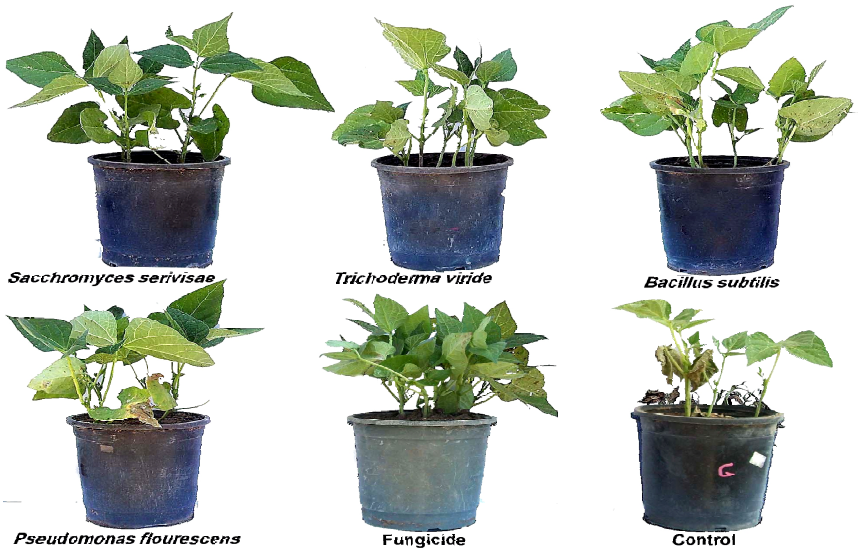

Fig. 1. Effect of four bioagents, *i.e.* yeast (*S. cerevisiae*), *T. viride, B. subtilis* and *P. fluorescens,* on growth of bean plants infested with *S. rolfsii* under greenhouse conditions.

Effect of four bioagents compared to the fungicide Vitavax-200 on fresh and dry weight of shoot and root system of bean plants under greenhouse conditions:

Data shown in Table (3) reveal that all the tested bioagents significantly increased shoot and root fresh and dry weight compared to untreated control. The highest increases in root and shoot dry weights were recorded for yeast (*S. cerevisiae*) treatment. *Pseudomonas fluorescens* recorded statistically similar effects to those of the fungicide treatment on root and shoot dry weights. Although there were no statistical differences between *T. viride* and *B. subtilis* and that of the fungicide treatment on the root dry weight, while their effects were significantly lower than that of the fungicide treatment on shoot dry weight.

Table 3. Effect of some bioagents compared to the fungicide Vitavax-200 on fresh and dry weight for shoot and root system of bean plants (cv. Bronco) under greenhouse conditions

Treatment	Shoot system weight (g plant^{-1})		Root system weight (g plant^{-1})	
	Fresh	Dry	Fresh	Dry
S. cerevisiae	52.0 a	16.1 a	10.8 a	3.8 a
T. viride	39.8 b	9.7 c	6.6 c	3.2 b
B. subtilis	38.7 b	11.5 c	8.5 b	3.2 b
P. fluorescens	40.4 b	13.5 b	8.3 b	3.1 b
Vitavax-200	50.9 a	13.8 b	9.1 b	3.1 b
Control	24.1 c	7.7 d	4.9 d	2.5 c

Values with the same letter are not significantly different (P = 0.05).

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. viride* 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 (cv. Bronco) plants

Treatment	Chitinase		Peroxidase		Polyphenoloxidase	
	Activity(1)	Increase (%)	Activity(2)	Increase (%)	Activity(3)	Increase (%)
S. cerevisiae	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	-------

1- Chitinase activity was expresses as mM N-acetyl glucose amine equivalent released / gram fresh weight tissue / 60 minutes.
2- Peroxidase activity was expressed as the change in absorbance (O.D) / minute/gram fresh weight.
3- The polyphenoloxidase activity was assayed as the change in absorbency (O.D)/ minute/gram fresh weight.

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

The four bioagents, *i.e.* yeast (*S. cerevisiae*), *T. viride*, *B. subtilis* and *P. fluorescens*, were tested for their effect on incidence of damping-off under field conditions in two successive seasons 2009 and 2010. Data shown in Table (5) indicate that Vitavax-200 fungicide and all bioagents significantly reduced the

Table 5. Effect of four bioagents on incidence of damping-off of bean plants (Bronco cv.) under field conditions during 2009 and 2010 seasons

Treatment		Pre-emergence damping-off (%)	Reduction (%)	Post-emergence damping-off (%)	Reduction (%)	Survived plants (%)
First season	*S. cerevisiae*	11.5 bc	71.7	10.3 bc	50.5	78.2 a
	T. viride	15.7 b	61.3	7.6 c	63.5	76.8 a
	B. subtilis	13.9 bc	65.7	10.9 bc	47.6	75.2 a
	P. fluorescens	8.9 c	78.1	12.2 b	41.3	79.0 a
	Vitavax-200	11.5 bc	71.7	8.0 c	61.5	80.5 a
	Control	40.6 a	0.0	20.8 a	0.0	38.5 b
Second season	*S. cerevisiae*	24.0 b	47.6	5.2 b	63.4	70.8 b
	T. viride	18.1 b	60.5	2.8 b	80.3	79.2 a
	B. subtilis	25.1 b	45.2	4.2 b	70.4	70.8 b
	P. fluorescens	25.0 b	45.4	4.9 b	65.5	70.1 b
	Vitavax-200	25.0 b	45.4	4.2 b	70.4	70.8 b
	Control	45.8 a	0.00	14.2 a	0.00	39.9 c

Values with the same letter are not significantly different (P = 0.05).

disease. As for first season 2009, the most effective treatments were *B. subtilis, T. viride, S. cerevisiae, P. fluorescens* and Vitavax-200 fungicide, which reduced the disease more than 61.3 and 41.4% for pre- and post-emergence, respectively. The corresponding percentages of survived plants for these bioagents were 78.2, 79.0, 75.2, 76.8 and 80.5%, respectively, with no significant between them viz. 38.5% for the control. On the other hand, *T. viride* was the most significant effective treatment for decreasing pre- and post-emergence damping-off and increasing survived plants compared with the others, which gave 79.2% of survived plants followed by the other treatment with no significant in between, viz. 39.9% for the control in the second season 2010**.**

Effect of four bioagents compared to the fungicide Vitavax-200 on fresh and dry weight for shoot and root system and seed yield of bean plants under field conditions:

The four bioagents were evaluated for their effect on some crop parameters of bean plants under field conditions in two growing seasons 2009 and 2010. Data shown in Table (6) indicate that all bioagents and Vitavax-200 significantly increased the assessed crop parameters of bean plants under field conditions as compared to untreated plants (control) in the two seasons. The highest increase of all assessed crop parameters except for seed yield was obtained with *S. cerevisiae*, which increased the shoot system fresh weight, shoot system dry weight, the root system fresh weight and root system dry weight, (105.3, 29.4, 18.7, and 6.9 g plant^{-1}, respectively), however, Vitavax-200 fungicide was the most effective treatment for increasing seed yield production (1048.3 kg/feddan), in the first season; while, 100.3, 37.9, 23.3, 8.6 g plant^{-1} and 1142.2 kg feddan^{-1}, respectively, in the second season. The same trend was obtained during the second season 2010 with few exceptions.

Table 6. Effect of different bioagents on fresh and dry weight for shoot and root system and seed yield of bean plants (cv. Bronco) under field conditions during 2009 and 2010 growing seasons

Treatment		Shoot system weight (g/plant)		Root system weight (g/plant)		Seed yield (kg/feddan)
		Fresh	Dry	Fresh	Dry	
First season	*S. cerevisiae*	105.3a[(1)]	29.4 a	18.6 a	6.9 a	894.9 ab
	T. viride	61.3 c	19.9 b	10.6 d	4.4 c	605.3 c
	B. subtilis	61.5 c	20.6 b	15.2 b	5.8 b	650.0 c
	P. fluorescens	81.5 b	24.5 ab	14.6 bc	6.1 ab	748.2 bc
	Vitavax-200	96.8 a	21.5 b	12.3 cd	4.0 c	1048.3 a
	Control	32.3 d	9.6 c	6.1 e	3.1 d	269.2 d
Second season	*S. cerevisiae*	100.3 ab	37.9 a	23.3 a	8.6 a	1142.2 a
	T. viride	97.7 ab	28.4 b	19.4 c	7.8 b	1060.7 a
	B. subtilis	92.5 b	27.0 b	19.0 c	7.1 c	974.4 a
	P. fluorescens	97.6 ab	28.8 b	18.8 c	7.4 bc	902.2 a
	Vitavax-200	106.4 a	33.3 ab	21.9 b	7.7 b	1091.3 a
	Control	62.2 c	19.5 c	13.6 d	5.9 d	468.6 b

Values with the same letter are not significantly different (P = 0.05).

D i s c u s s i o n

Bean (*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 of 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 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 and Duffy *et al*., 1997).

In the present study, under greenhouse conditions 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, polyphenoloxidase 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. cerevisiae* 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). 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 defence 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), 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*. In a conclusion, 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 موت البادرات في الفاصوليا الناتج عن الإصابة بالفطر *Sclerotium rolfsii*

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تم دراسة تأثير أربعة من كائنات المكافحة الحيوية وهي الخميرة *Saccharomyces cerevisiae* وفطر *Trichoderma viride* وبكتريا *Bacillus subtilis* و *Pseudomonas fluorescens* لمكافحة مرض سقوط البادرات في نباتات الفاصوليا الناتج عن الإصابة بالفطر *Sclerotium rolfsii*. أشارت النتائج المتحصل عليها تحت ظروف الصوبة أن جميع عوامل المكافحة الحيوية محل الدراسة أدت إلى 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 انخفاض 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% ، على التوالي بالمقارنة بالنباتات غير المعاملة 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 إلى حدوث زيادة ملحوظة في نشاط إنزيمات البيروكسيديز والبولي فينول أكسيديز والشيتنيز بمعدل أكثر من ٢٦٠ و ١٠٩ و ٢١٨.٣% ، على التوالي بالمقارنة بالنباتات غير المعاملة. 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* والخميرة *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% ،على التوالي. وسجلت هذه الكائنات نسب ٧٥.٢ و ٧٦.٨ و ٧٨.٢ و ٧٩.٠% نباتات قائمة (متبقية) ،على التوالي بالمقارنة للنباتات غير المعاملة حيث سجلت ٣٨.٥%.

أما بالنسبة إلي المحصول فقد كانت أكثر المعاملات فاعلية هي الخميرة *S. cerevisiae* ثم بكتريا *B. subtilis* حيث أدت إلى زيادة محصول بذور الفاصوليا للفدان بدرجة كبيرة ، وكان تأثير باقي عوامل المكافحة الحيوية متوسط الفاعلية على زيادة محصول بذور الفاصوليا للفدان. وتم الحصول على نتائج مشابه في موسم النمو الثاني. وبهذا يمكن الاقتراح باستخدام عوامل المكافحة الحيوية السابقة كطريقة واعدة وآمنة لمكافحة مرض سقوط البادرات في نباتات الفاصوليا المتسبب عن الإصابة بالفطر *S. rolfsii*.