Journal of Phytopathology and Pest Management 3(2): 1-14, 2016
pISSN:2356-8577 eISSN: 2356-6507
Journal homepage: http://ppmj.net/

Evaluation of certain antagonistic fungal species for biological control of faba bean wilt disease incited by *Fusarium oxysporum*

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Abstract

Fusarium wilt incited by *Fusarium oxysporum* Schlecht. causes a remarkable economic losses in faba bean (*Vicia faba* L.) growing areas. In this study, fourteen isolates of *F. oxysporum* were isolated from the diseased faba bean plants, showing wilt symptoms, obtained from different localities in Assiut governorate. The isolates proved to be pathogenic on Masr-1 faba bean cultivar under greenhouse conditions. The effectiveness of five antagonistic fungal species (*Trichoderma harzianum* Rifai., *Trichoderma viride* Pers., *Gliocladium roseum* Bainier, *Gliocladium catenulatum* Gilman & Abbott and *Saccharomyces cerevisiae*) in growth inhibition of the pathogen *in vitro* as well as controlling the disease in greenhouse were evaluated. All tested antagonistic fungi were able to inhibit the growth of *F. oxysporum* in dual culture, significantly, as compared with control. Results also indicate that *F. oxysporum* suppressed strongly by coating seeds of faba bean by *T. harzianum*, *T. viride*, *G. roseum*, *G. catenulatum* and *S. cerevisiae* before sowing in the soil. Furthermore, the lowest percentages of Fusarium wilt severity were recorded by *T. viride* and *G. roseum* which reduced disease incidence to 22.25 % and 25.25% respectively, compared with 75.50 % in untreated seeds. It could be suggested that such antagonistic fungal species might be promising as alternatives for controlling faba bean wilt caused by *Fusarium oxysporum*.

Key words: antagonistic fungi, biological control, faba bean, Fusarium wilt, *Fusarium oxysporum*.

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Introduction

Faba bean (*Vicia faba* L.) is one of the most common fast food items in the Egyptian diet. It is an excellent source of protein (20-25%), calcium (0.15%), phosphorus (0.50%), lysine (1.5%) and methionine-cystine (0.5%), dry weight, as well as an excellent source of complex carbohydrates, dietary fiber, choline, lecithin, minerals and secondary metabolites (Rabey et al., 1992). It is susceptible to a number of soil-borne pathogenic fungi including *Fusarium oxysporum* that causes wilt disease, which decrease the crop productivity and lower the quality of seeds (Elwakil et al., 2009; Mazen et al., 2008; Abou-Zeid et al., 1997). Moreover, Faba bean Fusarium wilt caused by *Fusarium oxysporum* was reported by Dong et al. (2014). *F. oxysporum* was found to be the causal pathogen of faba bean plants wilt collected from different fields in New Valley governorate, Egypt. All the obtained isolates were able to attack faba bean plants causing damping-off and wilt diseases (Abdel-Monaim, 2013). *Rhizoctonia solani, Fusarium solani* and *Macrophomina phaseolina* are reported to attack faba bean roots and stem base causing serious losses in seed germination and plant stand (Abdel-Kader et al., 2011). Biological control of *Fusarium species* has shown promise in previous studies due to their low environmental impact, and their ability to help reduce growers' dependency on chemicals, thereby slowing the development of fungicide resistance in pathogen populations (Crane et al., 2013; Jochum et al., 2006). In the previous studies, a great attention has been focused on the possibility to use the antagonistic fungi as a bio-control agent for Fusarium wilt. Several antagonistic microorganisms (*Trichoderma, Gliocladium, Streptomyces, Pseudomonas* and *Bacillus*) have been identified as bio-control agents of plant pathogenic fungi. The common mode of action was antibioses and mycoparasitism (Mahmoud & Abo-Elyousr, 2014; Aryantha & Guest, 2006). *Trichoderma species* are biological control agents that control ascomycetous and basidiomycetous fungi, which are mainly soil-borne but also airborne pathogens. Antagonists of phytopathogenic fungi have been used to control plant diseases, and 90% of such applications have been carried out with different strains of the fungus Trichoderma (Monte, 2001). The genus Trichoderma comprises a great number of fungal strains that act as biological control agents, the antagonistic properties of which are based on the activation of multiple mechanisms. Trichoderma can indirectly biocontrol phytopathogens by competing for nutrients and space nutrients, through the secretion of antibiotic volatiles and/or diffusible metabolites, which modify soil conditions promoting growth and plant defense mechanisms. Moreover, mycoparasitism is considered a direct biocontrol mechanism (Benítez et al., 2004; Howell, 2003). The addition of Trichoderma metabolites that may act as elicitors of plant resistance, or the expression in transgenic plants of genes whose products act as elicitors, also results in the synthesis of phytoalexins, PR proteins and other compounds, and in an increase in resistance against several plant pathogens, including fungi and bacteria (Dana et al., 2001; Elad et al.,

2000). Successful reductions of Fusarium wilt in many crops by application of different species of Trichoderma have been reported by Bell et al. (1982) and Ramezani (2009). Application of bioagents *Trichoderma viride* and *Trichoderma harzianum* were significantly reduced the disease severity of *Fusarium* wilt of chickpea caused by *Fusarium oxysporum* (Rehman et al., 2013). *Clonostachys rosea* (syn. *Gliocladium roseum*) was identified as a mycoparasite for against the causal pathogens of pea root rot, including *Fusarium oxysporum* f. sp. *pisi* and *F. solani* f. sp. *pisi* (Xue, 2003). The most successful antagonists against seedborne *Fusarium culmorum* were isolates of *Chaetomium* sp., *Idriella bolleyi* and *Gliocladium roseum* (Knudsen et al., 1995). Furthermore, the biocontrol fungus *Gliocladium catenulatum* is an effective antagonist against several root and foliar greenhouse pathogens. The biocontrol agent forms dense networks of hyphae on plant roots, grows internally in root epidermal cells, and produces hydrolytic enzymes, all of which lead to a reduction in pathogen propagules (Chatterton & Punja, 2010). Antibiotic production by biocontrol fungi has most commonly been reported for isolates of *Trichoderma* and *Gliocladium* (Whipps, 2001). An endophytic *Gliocladium sp*. has also been found to produce volatile organic compounds (VOC) that have strong antimicrobial activity (Strobel, 2006). The volatile organic compound annulene was produced in the greatest amount by *Gliocladium spp*. (Stinson et al., 2003). *G. catenulatum* have shown mycoparasitic activity against several plant pathogenic fungi *in vitro* (McQuilken et al., 2001). Microscopic observations showed that the biocontrol agent destroyed hyphal cells of *S. sclerotiorum* and *Fusarium spp*. through direct contact, resulting in collapse and disintegration of the host cells without visible penetration (Huang, 1978). Common seed-borne fungi of faba bean can be controlled by the seed treatment with bread yeast before sowing in the soil (Elwakil et al., 2009; Zhang et al., 2003). The soil-borne pathogenic fungus *Fusarium oxysporum* was suppressed by using *S. cerevisiae* as biocontrol agent of sugar beet plants (Shalaby & El-Nady, 2008). Yeasts possess many features which make them eligible as biocontrol agents. They have simple nutritional requirements; the capacity to grow in fermenters on inexpensive media; the ability to survive in a wide range of environmental conditions and no production of anthropotoxic compounds (Wilson & Wisniewski, 1989). They have been recorded to occupy the rhizosphere region and to show a rhizosphere effect (Babeva & Belyanin, 1966). The aim of this study was planned to identify the causal pathogen of wilt disease of faba bean plants in Assiut governorate and to investigate the ability of *T. harzianum, T. viride, G. roseum, G. catenulatum* and *S. cerevisiae* to parasitize on *F. oxysporum* to reduce the disease incidence of faba bean Fusarium wilt under Egyptian climate.

Materials and methods

Isolation and identification of the causal pathogen: Naturally infected faba bean plants showing wilt symptoms were collected from several fields in Assiut governorate, Egypt from November 2011

to February 2012 growing season. The infected parts were cut into small pieces of 0.5 cm and surface disinfected with 2% sodium hypochlorite solution for 2 min, rinsed twice in sterile distilled water, dried between sterile filter paper, then placed into Petri dishes containing Potato Dextrose Agar medium (PDA) amended with streptomycin sulphate (120 mg L^{-1}) and incubated for three days at 25±2°C in the dark. Pure cultures of the developing fungi were obtained using single hyphal-tip isolation technique (Dhingra & Sinclair, 1995; Booth, 1985; Domsch et al., 1980). Hyphae were stained with lacto-phenol cotton blue and examined to determine hyphae morphology. The Leica-ICC50-HD, with a high definition digital microscope camera, was used to observe the specimen. The pure cultures were kept at 5°C in the refrigerator for further studies.

Pathogenicity tests: Pathogenicity tests were carried out at greenhouse of Plant Pathology Department, Faculty of Agriculture, Assiut University, Egypt. Pathogenic capabilities of fourteen isolates of *Fusarium oxysporum* were tested on Masr-1 faba bean cultivar. Inocula of the tested isolates were prepared by growing in sterilized conical flasks (500 ml) containing barley medium (100g barley supplemented with 2g glucose + 1g yeast extract + 100 ml distilled water) and incubated at 25±2°C for 15 days. Sterilized pots (30 cm in diameter) were filled with sterilized sandy-loam soil which mixed thoroughly with equal amounts of *F. oxysporum* inoculum at the ratio of 2% soil weight, mixed well and thoroughly irrigated. Soil infestation was carried out five day before sowing seeds. Seeds of Masr-1 faba bean cultivar were surface sterilized by dipping in 2% sodium hypochlorite solution for 3 min, followed by washing with sterilized water. Each pot was sown with ten faba bean seeds. Pots containing 1% non-infested barley medium were used as control. Four pots were used as replicates for each tested isolate. Experiment was carried out under greenhouse conditions, the prevailing temperatures during pathogenicity tests were 18±2°C (minimum) and 24±2°C (maximum). The plants were irrigated when necessary and daily observed for infection.

Disease severity assessment: Disease severity for each tested isolate was estimated after 10 weeks from planting date, as a wilting percentage, on the basis of root discoloration or leaf yellowing. Plants with typical Fusarium wilt symptoms were assessed according to the type of symptoms that was observed using a numerical grades ranging from 0 to 5 as follows: (0)= No visible symptoms; (1)= 1-<20 % of plant leaves are yellow and of the vascular systems are light brown (discoloration); (2)= 20-<40 % of plant leaves are yellow and of the vascular systems are brown (discoloration); (3)= 40-<60 % of plant leaves are yellow and of the vascular systems are dark brown (discoloration); (4)= 60-<80 % of plant leaves are yellow and of the vascular systems are dark brown (discoloration); (5)= 80-≤100 % of plant leaves are yellow and of the vascular systems are dark brown (discoloration) or completely dead plants. Disease severity (%) =

$$\frac{\sum[(N\times 0)+(N\times 1)+.....(N\times 5)]}{5T}\times 100$$

Where: (N) = the number of plants corresponding to the numerical grade, 0, 1, 2, 3, 4 and 5. (5T) = the total number of plants (T) multiplied by maximum numerical grade (5).

Isolation of antagonistic fungal species: *Trichoderma harzianum*, *Trichoderma viride, Gliocladium roseum* and *Gliocladium catenulatum* were isolated from soil rhizosphere of healthy faba bean plants on Potato Dextrose Agar *(*PDA) amended with streptomycin sulphate (120mg L^{-1}). PDA culture plates were incubated at 25±2ºC. Obtained isolates, which belong to *Trichoderma spp*. and *Gliocladium spp*. were purified by single spore technique according to Brown (1924) and kept at 5°C for further studies. Whereas, for isolation of *Saccharomyces cerevisiae;* about 1g of soil was added to 50 ml of Yeast Extract Peptone Dextrose (YPD) broth medium (1% yeast extract, 2% peptone, 2% dextrose, pH 4), and incubated at 28±2ºC with rapid shaking at 160 rpm for 24 hours. Then 25 µl was spread onto YPD Agar plates supplemented with 500µg/ml streptomycin sulfate salt to inhibit bacterial growth. After incubation for 3 days at 28±2ºC, the colonies belonging to *S. cerevisiae* were selected according to their colors and surface features.

Efficacy of antagonistic fungi against *Fusarium oxysporum in vitro*: Antagonistic capability of five isolates of *T. harzianum*, three isolates of *T. viride*, four isolates of each of *G. roseum* and *G. catenulatum* and three isolates of *S. cerevisiae* were tested against the highly pathogenic isolate (*F. ox*. 10) *in vitro.* Dual culture technique was followed; mycelial disks (5 mm in diameter) were cut from the edges of actively growing colonies of *F. oxysporum* and Gliocladium or Trichoderma isolates, and were placed opposite each other, 1cm from the edge of 9 cm Petri dishes containing PDA medium, amended with streptomycin sulphate (120mg L^{-1}). Petri dishes inoculated with *F. oxysporum* alone served as controls. Each pair was replicated four times and incubated for five days at 25±2°C in darkness. Colony diameter of *F. oxysporum* was measured using ruler. For test the antagonistic capability of the three isolates of *S. cerevisiae* against *F. oxysporum* the pathogen agar disc was inoculated at the middle of PDA plate. Single streaks of yeast drawn across the plate at two equidistant points located 1cm from plate edge. Plates were incubated at 28±2ºC. Four replicates were used for each treatment. Antagonism was determined by measuring the pathogen colony diameters and percentage of inhibition in mycelial growth of *F. oxysporum* was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{A - B}{A} \times 100$$

Where: (A) is the colony diameter of pathogen alone (control); (B) is the colony diameter of pathogen after antagonist effect.

Efficacy of antagonistic fungi on controlling Fusarium wilt of faba bean under greenhouse conditions: Isolate (3) of *T. harzianum*, isolate (1) of *T. viride*, isolate (2) of *G. roseum*, isolate (1) of *G. catenulatum* and isolate (2) of *S. cerevisiae*, which produced the highest inhibition percentage against *F. oxysporum in vitro*, were selected to investigate their ability to reduce the

incidence of Fusarium wilt in faba bean plants. Inoculum of the highly pathogenic isolate of *F. oxysporum* (no. 10) was prepared as previously mentioned in pathogenicity tests. Inoculums of the antagonistic fungi (*T. harzianum, T. viride, G. roseum* and *G. catenulatum*) were prepared using Potato Dextrose Broth (PDB) amended with streptomycin sulphate (120 mg L^{-1}) and incubated for 15 days at 25±2°C in the dark. Inoculums were harvested by passing the liquid culture through double layer cheesecloth, and adjusted to $5x10^5$ conidia mL^{-1}. Surface sterilized seeds of Masr-1 faba bean cultivar (by dipping for 2 min in 2% hypochlorite solution) were coated with 1% water solution of acacia gum as adhesive material. Seeds were coated with each antagonistic fungus separately at a rate of $5x10^5$ conidia mL^{-1} on seed surface for 5 hours and then air-dried before sowing in greenhouse pots. Whereas, *S. cerevisiae* was grown on YPD broth medium for 2 days at 28±2ºC with shaking. Cultures were centrifuged and pellets were washed twice with sterile dH_2O, and the concentration of the yeasts was adjusted to 10^9 cfu ml^{-1}, then seeds of faba bean were coated with a water suspension (10^9 cfu ml^{-1}) of *S. cerevisiae*. Sterilized pots (30 cm in diameter) filled with sterilized sandy-loam soil were infested by mixing inoculum of *F. oxysporum* with the soil at the rate of 2% w/w. Infested pots were thoroughly irrigated (after infestation) and sown 5 days later, to insure establishment and distribution of the pathogen inoculums in soil. Each pot was sown by ten treated seeds of Masr-1 faba bean cultivar and arranged in complete randomized design. Untreated seeds were used for controls; pots of positive controls were inoculated similarly with *F. oxysporum* only, while negative controls were treated with distilled water. Four replicate pots per treatment were used. The experiment was repeated twice. Experiment was carried out under greenhouse conditions at Department of Plant Pathology, Faculty of Agriculture, Assiut University. The prevailing temperatures during the experiment were 18±2°C (minimum) and 24±2°C (maximum). The plants were irrigated when necessary and daily observed for infection. Disease severity percentages were estimated after 10 weeks post-sowing, as mentioned in pathogenicity tests. The percentage of reduction in the incidence of Fusarium wilt was calculated as follow:

$$\text{Reduction (\%)} = \frac{A - B}{A} \times 100$$

Where: A= disease incidence of positive control; B= disease incidence of treatment.

Statistical analysis: The results were analyzed using ANOVA test and the means differences were regarded as significant using LSD test at 5% level of probability according to Gomez and Gomez, 1984.

Results

Identification of the causal pathogen: Fourteen isolates were obtained from naturally infected plants of faba bean. The identification of the isolates was carried out based on the morphological features of mycelia and spores as well as culture characteristics, the obtained isolates were identified as *Fusarium*

oxysporum Schlecht. emend. Snyder & Hansen. (Armstrong & Armstrong, 1978; Both, 1971; Gerlach & Nirenberg, 1982; Kleczewski and Egel, 2011; Nelson et al., 1983; Snyder & Hansen, 1940).

Pathogenicity tests: Data in Table (1) indicate that, all the tested isolates able to infect faba bean plants causing wilt disease with different degrees of severities. *F. oxysporum* isolates varied significantly in their virulence; isolates no. 10, 9 and 11 proved to be the most aggressive isolates and showed the highest percentage of disease severity, followed by isolate no. 12. Whereas, *F. oxysporum* isolates no. 2, 3 and 1 exhibited the lowest percentage of disease severity. No symptoms were observed in the control plants. According to these results isolate (*F. ox*. 10) was selected for biological control experiment *in vitro* and in greenhouse.

Table 1: Pathogenicity tests of *F. oxysporum* isolates on Masr-1 faba bean cultivar under greenhouse conditions.

Isolates No	Disease severity (%)*
F. ox.1	49.25 HI
F. ox.2	46.50 I
F. ox.3	48.25 I
F. ox.4	54.00 GH
F. ox.5	55.75 EF
F. ox.6	55.00 FG
F. ox.7	60.00 DE
F. ox.8	59.50 EF
F. ox.9	72.75 AB
F. ox.10	73.75 A
F. ox.11	69.25 ABC
F. ox.12	65.00 CD
F. ox.13	54.75 FG
F. ox.14	60.50 DE
Control	0.0 J

*Means within the same column followed by different letters are significantly different (P≤0.05) based on LSD.

Identification of antagonistic fungal species: Five isolates were identified as *Trichoderma harzianum* Rifai., three isolates were identified as *Trichoderma viride* Pers., four isolates were identified as *Gliocladium roseum* Bainier (syn. *Clonostachys rosea* f. *rosea* (Link) Schroers) and four isolates were identified as *Gliocladium catenulatum* Gilman & Abbott (syn. *Clonostachys rosea* f. *catenulata* Gilman & Abbott). Isolates were identified based on morphological characteristics of mycelia and conidiophores as described by Domsch et al. (1980); Dhingra and Sinclair (1995); Schroers et al. (1999) and Schroers (2001). Whereas, three isolates of yeast were identified as *Saccharomyces cerevisiae* Meyen ex E.C. Hansen based on their sporulation, fermentation, morphological and physiological characteristics as described by Barnett et al. (1990); Lodder (1970); Vaughan-Martini and Martini (1998); Yarrow (1984).

Efficacy of antagonistic fungi against *Fusarium oxysporum in vitro*: Antagonistic capability of five isolates of *T. harzianum*, three isolates of *T. viride*, four isolates of each of *G. roseum* and *G. catenulatum* and three isolates of *S. cerevisiae* were tested against the highly pathogenic isolate (*F. ox*.10) *in vitro.* Data presented in Table (2) indicated that, all tested isolates of antagonistic fungi were able to inhibit the growth of *F. oxysporum*, and they were varied in their antifungal effect on the mycelial growth of the pathogen. Among the *Trichoderma species*, *T. viride* isolate (1) and *T. harzianum* isolate (3) showed the best performance *in vitro* as a biological control agent of *F. oxysporum* resulting

in 65.83 and 63.33 % reduction in colony growth of the pathogen, respectively. *G. roseum* isolates significantly reduced the growth of *F. oxysporum* in dual culture with the inhibition rate ranging between 62.5 to 59.72%. Whereas, *G. catenulatum* isolate (1) is produced inhibition rate of 56.94%. As a biological control agent, *S. cerevisiae* was tested *in vitro* against *F. oxysporum* and the obtained results show various inhibitory effects against *F. oxysporum.* Isolate (2) of *S. cerevisiae* proved to be the most effective isolate against *F. oxysporum.*

Table 2: Effect of antagonistic fungi on colony diameter of *F. oxysporum* in dual culture.

Treatments	Colony diameter of *F. oxysporum* (cm)*	Inhibition of *F. oxysporum* growth (%)
F. ox.+ T. harzianum -1	3.97 FG	55.83
F. ox.+ T. harzianum -2	4.60 BC	48.88
F. ox.+ T. harzianum -3	3.30 LM	63.33
F. ox.+ T. harzianum -4	3.80 GHI	57.77
F. ox.+ T. harzianum -5	4.32 DE	51.94
F. ox.+ T. viride -1	3.07 M	65.83
F. ox.+ T. viride -2	3.85 FGH	57.22
F. ox.+ T. viride -3	4.10 EF	54.44
F. ox.+ G. roseum -1	3.62 HIJK	59.72
F. ox.+ G. roseum -2	3.37 KL	62.50
F. ox.+ G. roseum -3	3.45 JKL	61.66
F. ox.+ G. roseum -4	3.55 IJKL	60.55
F. ox.+ G. catenulatum -1	3.87 FGH	56.94
F. ox.+ G. catenulatum -2	4.45 CD	50.55
F. ox.+ G. catenulatum -3	4.70 BC	47.77
F. ox.+ G. catenulatum -4	4.67 BC	48.05
F. ox.+ S. cerevisiae -1	3.77 GHI	58.05
F. ox.+ S. cerevisiae -2	3.55 IJKL	60.55
F. ox.+ S. cerevisiae -3	4.57 CD	49.16
Control	9.0 A	0.0

*Means within the same column followed by different letters are significantly different (P≤0.05) based on LSD.

Efficacy of antagonistic fungi on controlling Fusarium wilt of faba bean under greenhouse conditions: Isolate (3) of *T. harzianum*, isolate (1) of *T. viride*, isolate (2) of *G. roseum*, isolate (1) of *G. catenulatum* and isolate (2) of *S. cerevisiae* were investigated for their ability to reduce the incidence of Fusarium wilt of faba bean plants under greenhouse conditions. Obtained results are summarized in Table (3) indicated that, application of tested microorganisms as seed coat were reduced significantly the incidence of Fusarium wilt compared to untreated seeds. *T. viride* and *G. roseum* showed the highest reduction in Fusarium wilt severity (70.52 and 66.55%, respectively). While *T. harzianum* reduced severity by (65.23%) compared with the untreated controls. Disease incidence of treated seeds by *S. cerevisiae* decreased to 30.25 % in

comparison with 75.50 % for the pots containing untreated seeds. Results of the current study indicate that *T. harzianum*, *T. viride*, *G. roseum*, *G. catenulatum* and *S. cerevisiae* have strong potential as biocontrol agents of the soil borne fungal plant pathogen *F. oxysporum* causing wilt symptoms in faba bean plants.

Table 3: Efficacy of antagonistic fungi on controlling Fusarium wilt of faba bean under greenhouse conditions.

Treatments	Fusarium wilt severity (%)*	Reduction (%)
F. ox.+ *T. harzianum* -3	26.25 C	65.23
F. ox.+ *T. viride* -1	22.25 D	70.52
F. ox.+ *G. roseum* -2	25.25 CD	66.55
F. ox.+ *G. catenulatum* -1	27.75 BC	63.24
F. ox.+ *S. cerevisiae* -2	30.25 B	59.93
F. ox. alone (positive control)	75.50 A	0.0
Uninfected control	0.0 E	100

*Means within the same column followed by different letters are significantly different ($P \leq 0.05$) based on LSD.

Discussion

Biological control is an efficient and environmentally friendly way to reduce the disease severity of Fusarium wilt. The aim of this study was to evaluate the effect of certain bio-control agents on the control of Fusarium wilt of faba bean. The results of the present study declared that, all the antagonistic fungi inhibited the growth of *F. oxysporum* in dual culture. The tested microorganisms reduced the wilt incidence under greenhouse conditions. *Trichoderma viride* and *T. harzianum* were the most effective species, inhibiting the growth of the pathogen *in vitro*. In greenhouse, *T. viride* and *T. harzianum* reduced the severity of Fusarium wilt by 70.52% and 65.23%, respectively. Furthermore, *T. viride* is able to produce the highest level of disease protection when used for seed coating. Previous studies reported that production of antifungal secondary metabolites by Trichoderma can induce resistance of plants against infection by pathogenic microorganisms. Trichoderma strains exert biocontrol against fungal phytopathogens either indirectly, by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and plant defensive mechanisms and antibiosis, or directly, by mechanisms such as mycoparasitism (Müllenborn et al., 2007; Benítez et al., 2004). Trichoderma strains grow rapidly and they are naturally resistant to many toxic compounds, including herbicides, fungicides and pesticides such as DDT, and phenolic compounds (Chet et al., 1997) and because the strains recover very rapidly after the addition of sublethal doses of some of these compounds. Resistance to toxic compounds may be associated with the presence in Trichoderma strains of ABC transport systems (Harman et al., 2004). *Gliocladium roseum* and *G. catenulatum* showed remarkable protection to faba bean plants against Fusarium wilt. Result indicated that seed treatment with *G. roseum* and *G. catenulatum* reduced significantly the wilt incidence as compared to control. In

greenhouse, application of *G. roseum* reduced significantly the incidence of Fusarium wilt (66.55%), compared to untreated seeds. Previous studies reported that, the mechanisms of action of *Gliocladium spp.* involved in disease suppression may be due to the ability of the bioagent to be rhizosphere competent and can endophytically colonize roots as well as stems of plants. Chatterton et al. (2008) found that colonization of *G. catenulatum* in the root zone reduced pathogen development and disease incidence. An endophytic *Gliocladium spp*. has also been found to produce volatile organic compounds (VOC) that have strong antimicrobial activity (Strobel, 2006) and that are inhibitory against soilborne fungi. The volatile organic compound annulene was produced in the greatest amount by *Gliocladium spp.* (Stinson et al., 2003). Microscopic observations showed that the biocontrol agent destroyed hyphal cells of *Fusarium spp*. through direct contact, resulting in collapse and disintegration of the host cells without visible penetration (Huang, 1978). The present study shows that, *Saccharomyces cerevisiae* isolates are antagonistic to *F. oxysporum* in dual culture. The percentage of inhibition reached 60.55% compared with control. Moreover, the use of *S. cerevisiae* as seed treatment indicated that seed coating with an antagonistic isolate of *S. cerevisiae* suppressed Fusarium wilt and decreased the incidence of the disease under greenhouse conditions. These results are supported by the previous finding of Elwakil et al. (2009) who used bread yeast, *S. cervisia*, as seed treatment for controlling seed-borne fungi of faba bean. Moreover, results are in agreement with Hassanein et al. (2002), who reported that yeasts were effective producers of antifungal metabolites. The mechanism of yeast as biocontrol agent involves in nutrient competition, direct parasitism, and perhaps induced resistance (Wisniewski, 1991). Moreover, Arras (1996) stated that antibioses, competition for colonization sites and nutrients and degradation of the pathogen toxins and enzymes by yeasts, may be involved in the suppression of growth of pathogenic fungi *in vitro* and *in vivo*. Antagonistic activity of the ascomycetous yeast strain *Pichia anomala* against *Fusarium spp* contaminated barley grains was also reported by Laitila et al. (2007). Furthermore, Gianluca et al. (2006); Lee et al. (1991) and Weller (1988) reported that a microorganism that colonizes roots is ideal for use as a biocontrol agent against soil-borne diseases and, consequently, improving plant growth. Yeasts applied for the control of plant pathogens were found to produce proteinaceous killer toxins lethal to susceptible yeast and fungi strains (Santos et al., 2004; Hodgson et al., 1995). In conclusion, the control of Fusarium wilt of faba bean can be achieved by seed coating using antagonistic fungi before sowing in the soil.

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