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Effect of some Fungicides and Bioagents on Controlling Seed-Borne Diseases on Faba Bean

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This study was undertaken to investigate the seed-borne fungi of faba bean that attack the plants and reduce their yield in Egypt. The results provide a database for further study to control death pathogens. The blotter test methods used surface-sterilized faba bean seeds were tested to detect and isolate death associated seed-borne fungi. The following 8 fungal species belonging to 5 genera were observed and identified as: *Aspergillus niger* (Van Tieghem), *Alternaria alternata* (Fr.) Keissler, *Fusariurn oxysporum* (Schlechlendahl), *F. semitectum* (Berkeley and Ravenel), *F. solani* (Mart.) Sacc., *F. moniliforme (verticillioides)* (Sheld), *Rhizoctonia solani (*Kuhn.).

The pathogenicity test revealed that the most commonly isolated fungi from pre- and post-emergence damping-off and stunted seedlings were *R. solani, F. moniliforme, F. oxysporum F. solani.* These fungi significantly reduced the photosynthetic pigments in faba bean leaves. *R. solani,* followed by *F. moniliforme, F. oxysporum* caused the greatest reduction in chlorophyll content and significantly reduced total phenols content when compared with the other tested fungi.

*In vitro, a*ll tested fungicides indicated that increasing concentrations of the tested fungicides have gradually decreased the fungal linear growth of the tested pathogenic isolates. All pathogenic fungi were sensitive to Premis, followed by Topsin-M, Maxim, Rizolex-T, Vitavax 70 while, *F. semitectum* was sensitive to Vitavax 70, while, *F. moniliforme* was sensitive to Rizolex-T. On the other hand, using antagonistic bioagents significantly reduced the *in vitro* linear growth of all examined fungi, where *B. subtilis* and Plant Guard (4 m/L) were the most effective bioagents *B. subtilis* followed by Plant Guard.

Scanning infestation of faba bean seeds with tested root-rot pathogens decreased the percentages of protein and carbohydrates content into three tested faba bean seeds of cvs. Giza-843, Misr-1 and Sakha-1 comparing with uninfested seeds (control) at all incubation days which ranged between 10-30 days.

Keywords: *Alternaria alternata*, *Aspergillus niger*, bioagent, damping-off, faba bean, fungicides, *Fusariurn* spp., *Rhizoctonia solani* and root-rot.

Faba bean (*Vicia faba* L.) is an important legume crop in Egypt, and many parts of the world. Seeds are excellent source of protein which accounts for (20-25%), calcium (0.15%), phosphorus (0.50%), lysine (1.5%) and mediionine-cystine (0.5%) of the seed dry weight. The seeds are also an excellent source of complex carbohydrates, dietary fibber, choline, lecithin, minerals and secondary metabolites (phenolics and levo-dlihydroxy-phenylalainne (L-DOPA), which is precursor of neurotransmitter dopamine and is naturally found in seedlings, green pods and beans) (Rabey *et al.*, 1992). It is a popular breakfast food and also used as vegetable green or fresh canned. Broad bean is important crop for soil improvement and is used as break crop in cereal rotation to keep the soil fertile and productive through nitrogen fixation. In much of the world, the name broad bean is used for the large-seeded cultivars grown for human food. Horse bean and field bean refer to cultivars with smaller, harder seeds (more like the wild species) used for animal feed, although their stronger flavour is preferred in some human food recipes, such as falafel. In Egypt, faba bean (*V. faba* L) is considered as the most common fast food item in the Egyptian diet, eaten by rich and poor alike (El-Sayed *et al*., 1982).

Faba bean seeds and plants are infected with many fungal pathogens which caused considerable yield losses (Mahmoud, 1996 and Neergard, 1997). In this respect, root-rot and damping-off are the most important fungal diseases affecting. In this respect (Abdel-Hafez, 1988, Sepulveds, 1991, El-Morsy *et al*., 1997, Akem and Bellar, 1999, Rauf, 2000, Ahmed, 2005 and Ahmed, 2013) isolated *Fusarium oxysporum and F. solani* f.sp. *fabae*, *Rhizoctonia solani*, and *Macrophomina phaseolina* from wilted and rotten roots in different parts of the world and are considered the most important widespread diseases. All stages of faba bean growth are subjected to numerous injuries and stresses that interfere with their growth and development. There are many seed-borne fungi, while a number of fungi are serious pathogens on flowers and maturing seeds. These pathogens reduce the yield of seed qualitatively and quantitatively. Other fungi, including saprophytes and very weak parasites, may lower the quality of seeds. The most common seed-borne fungi listed on faba bean are *Ascochyta fabae*, which causes leaf and pod spot, *Botrytis cinerea*, the cause of grey mould: *Botrytis fabae*, the cause of chocolate spot *Fusarium* sp., the cause of foot rot and wilts and *Rhizoctonia solani*, the cause of damping-off of seedlings. Seed abortion, shrunken seed discoloration, reduction in germination capacity and physiological alterations in seed are the symptoms caused by these pathogens (Neergard, 1997). As for pathogenicity of root-rot and damping-off pathogens of faba bean many researchers (Omar, 1986, Wang and Chai, 2000 and Kurmut *et al*., 2002) confirmed the abilities of *R solani*, *F. oxysporum*, *F. moniliforme* and *F. solani* and *Verticillium dahliae* as pathogen in infecting faba bean causing root-rot and diseases.

Regarding the effect of bioagents against root-rot pathogens, (Mathew and Gupta, 1998) emphasized the abilities of *Trichoderma viride, Trichoderma koningii, T. harzianum, T. virens* and *Bacillus subtilis* in inhibiting the linear growth of root-rot fungi like *R. solani* and *F. solani* on faba bean and other legume crop. Also, El-Gindy (2003) mentioned that *T. harzianum, T. lignourm* and *Bacillus subtilis* affected significantly the average diameter of *B. fabae* colonies than the control.

On the other hand, Ibrahim (2005) found that *T. hamatum* and *T. harzianum* inhibited in vitro the growth of two isolates of *F. oxysporum* f.sp. *fabae*. Abd-El-Khair *et al.* (2011) mentioned that the antagonistic effect of four *Trichoderma* species, *i.e. T. album, T. hamatum, T. harzianum* and *T. viride*, were tested against *F. solani* and *R. solani in vitro*, in greenhouse and in field. *In vitro* tests, all *Trichoderma* spp. significantly reduced the mycelial growth of two pathogenic fungi. In greenhouse experiment, *T. album, T. hamatum, T. harzianum* and *T. viride*, as soil treatments, significantly reduced the pre- and post-emergence damping off disease incidence under artificial infection with *F. solani* and *R. solani*. The aim of this work is to evaluate the efficacy of antagonists, fungicides in controlling root-rot pathogens *in vitro.*

Materials and Methods

Seed samples:

Seed of three cultivars *i.e.*, Giza-843, Misr-1 and Sakha-1 were obtained from Legume Research Department, Field Crops Research Institute, Agricultural Research Centre.

Isolation, purification and identification of the fungal pathogens:

Hundred seeds were used for isolation from three cultivars; seeds were disinfected by soaking in 1% sodium hypochlorite solution for 2 minutes, then washed several times with sterilized water and dried between two sterilized filter papers. Ten seeds representing were plated onto glass Petri dish (7 cm) over potato dextrose agar containing 40% ppm streptomycin sulphate to avoid any bacterial growth (Christensen, 1957). All dishes were incubated at 25±2ºC with a daylight regime of alternating cycles of near ultraviolet (UV) light for 12 hrs and 12 hrs darkness for 8 days. Seeds were examined every day. Any fungal growth was transferred and purified using hyphal tip and/or single spore techniques. Developing fungi were transferred to PDA slant (Dhingra and Sinclari, 1995). Fungal cultures (15 days old) were identified according to their morphological and microscopical characters as described by (Gilman, 1957; Ram, 1970; Barnett and Hunter, 1972 and Sneh *et al*., 1991). Also, identification was confirmed at the Dept. of Fungal Taxonomy, Plant Pathol. Res. Inst., ARC. Giza, Egypt.

Pathogenicity test:

Pathogenicity tests were carried out under greenhouse condition at **(**Agricultural Research Centre. Giza, Egypt. On 2012 growing season). All fungal isolates tested for their pathogenic potentialities on faba bean cvs. under greenhouse conditions to select the highly pathogenic isolates. Pots (25cm) were sterilized by dipping in 5% formalin for 5 min and then left in open air till dryness. Soil (clay loam soil) sterilization was accomplished with 5 % formalin, mixed thoroughly, covered with plastic sheet for one week and then the plastic sheet was removed in order to complete formalin evaporation (Whitehead, 1957). Soil infestation with each individual fungus was carried out at the rate of 3 % of soil weight (El-Sayed, 1999). Inocula were prepared by growing fungi on sand barley (SB) medium (25g clean sand, (75g) barley and enough water to cover the mixture**).** Flasks contained

sterilized medium were inoculated with each particular fungus and incubated at 25°for two weeks. Potted soil was watered daily for a week to enhance fungal growth**.** Soil of control pots was mixed with the same amount using sterilized sand-barley (SB) medium. Ten seeds were surface sterilized using sodium hypochlorite 5% for 2 min, washed several times with sterilized water, before sowing. Three replicates with a total 30 seeds were used for each treatment, Three faba bean cultivars.

Disease assessment:

Percentages of pre- and post-emergence damping-off as well as healthy survived plants were determined 15 and 30 days after sowing, respectively, using the formula as described by El-Helaly *et al.* (1970).

$$\text{Pre-emergence damping–off (\%)} = \frac{\text{No. of un–germinated seeds}}{\text{No. of planted seeds}} \times 100$$

$$\text{Post-emergence damping–off (\%)} = \frac{\text{No. of dead seedlings}}{\text{No. of planted seeds}} \times 100$$

Laboratory studies:

In vitro evaluation of some fungicides on the growth of the pathogenic fungi:

This experiment was conducted to study the effect of some fungicides, *i.e.* Maxim, Premis, Topsin–M70, Rizolex–T and Vitavax–T70, at different concentrations on the linear growth of pathogenic fungi which were selected according to their pathogenic capabilities after the pathogenicity tests. The fungicides were tested at different concentrations, *i.e*. 0, 1, 5, 10, 25, 100, 200 and 400 ppm based on their active ingredient. In this respect, sterilized PDA medium was mixed with any of the tested concentration immediately before solidification and poured into plates (7 cm). Plates with poisoned or un–poisoned PDA medium were inoculated, at the centre with equal discs (4 mm) taken from the margin of 7 days–old cultures of the above mentioned pathogenic fungi and incubated at 28ºC. Four plates were used for each treatment as replicates. Linear growth of each tested fungus was measured daily until the mycelial growth completely covered the surface of the medium in the control treatment (un–poisoned medium) and the averages of the two perpendicular diameters of the fungal growth in mm were calculated as described by (Mahrous, 1974).

Evaluation of some commercial bioagents on growth of the tested pathogenic fungi:

In this experiment, the commercial bioagents, *i.e*. Plant Guard (each ml contains about $30x10^6$ spore of *Trichoderma harzianum*) and Rizo-N (each gram contains about $30x10^6$ cfu of *Bacillus subtilis*) were used to test their effect on the linear growth of the tested pathogenic studied fungi. Two equal amounts, *i.e.* 100 µl Plant Guard or 100 µg of Rizo-N were spotted (for the first) or streaked (for the second) at two opposite sides on the surface of (PDA) medium and apart equal distances from the peripheral side of the plates. Treated as well as un–treated plates (9 cm Φ) were inoculated simultaneously at their centre (between the two spots or streaks of the tested bioagent) each with a disc of any other tested. The inhibition percent was calculated using the formula of (Abd El-Moity, 1985) as follows:

$$\text{Reduction in linear growth (\%)} = \frac{R1-R2}{R1} \times 100$$

Whereas:

R1 = the radius of control growth

R2 = the radius of inhibited growth of pathogenic fungus.

Four plates were used as replicates for each treatment and all plates were incubated at 25º C for about 5–7 days until plates of control treatment (without bioagent) were covered by the mycelial growth of any of the tested fungi. Then, the average linear growth of tested fungi was recorded in mm.

Greenhouse experiments:

Effect of some fungicides and commercial bioagents on disease incidence of faba bean under greenhouse conditions:

The effect of some fungicides and commercial bioagents on root-rot incidence expressed as faba bean dead plants under greenhouse conditions was investigated. In this study, loamy soil was infested with 3% inoculum level of any of the tested fungal inoculum and distributed in sterilized pots (25 cm) as previously mentioned.

a. Effect of different fungicides on the incidence of root-rot:

Five different fungicides were used as seed treatments. These fungicides were Premis (3ml/kg seeds), Maxim (5ml/kg seeds), Topsin–M70, Rizolex–T and Vitavax–T70 (3 g/kg seeds). Seed dressing was carried out by mixing the fungicides with seeds plus glue suspension as sticker the treated seeds were sown in pots (25 cm) at the rate of 10 seeds/pot., faba bean seeds, free of tested fungicides, sown in potted soil infested with any of the tested pathogenic fungi, were kept as check (control).

b. Effect of Plant Guard and Rizo-N bioagents on root-rot incidence:

Two commercial bioagents Plant Guard (*T. harzianum*) and Rizo-N (*Bacillus subtilis)* were tested. In this respect, seeds of faba bean were mixed with the bioagents at the rate of 3g and 3 ml/kg seeds, respectively, and sown at the rate of 10 seeds/pot. Three replicates for each treatment were used. The incidence of root-rot disease (dead plants) of faba bean caused by the tested pathogens was determined 30 days after sowing for all the mentioned greenhouse studies.

c. Determination of photosynthetic pigments

Chlorophyll (A), chlorophyll (B) and carotenoids were determined according to the method suggested by Wettestien (1957).

Determination of protein (%) and carbohydrates (%) content:

Artificial infection of faba bean:

Fifteen gram of healthy seeds from each cvs. Giza-843, Misr-1, and Saka-1of faba bean were surface disinfested with 2% aqueous solution of sodium hypochlorite as described by (Seenappa *et al.,* 1981). The disinfested seed were then transferred to 250 ml sterile Erlenmeyer flasks and moistened to reach a moisture content of 25% by adding sterile distilled water according to (Christensen, 1967). Each flask was inoculated with 2 ml of concentrated spore suspension from 10 days old PDA culture of the fungi *F. moniliforme, F. semitectum, F. solani, F. oxysporum* and *R. solani*. After inoculation, the flasks were gently shaken to distribute the inoculum

evenly. The inoculated and uninoculated treatments were stored at 85% RH (26±2°C) for 30 days in a humidity chamber as described by (Seenappa *et. al.*, 1981). At the end of the incubation period Protein (%) and Carbohydrates (%) were determined at 0 times, 10, 20 and 30 days after inoculation. Seed samples were ground to a fine powder using a laboratory mill with 0.5 mm and using the flour in determined to Protein (%) (N X 6.25) according to (A.O.A.C., 1984), and Carbohydrates % were determined according to the method described by (Dubois *et al.*, 1956).

Determination of phenolic compounds:

Phenolic compounds were determined using the colorimetric method of analysis described by (Bary and Thorpe, 1954).

Statistical analysis:

All the aforementioned experiments which carried out under Lab., greenhouse conditions were performed in a complete randomized block design. All data were analyzed according to (Snedecor and Cochran, 1989).

Results

Isolation, purification, identification and frequency of fungi associated with faba bean seeds:

Isolation trials from faba bean seeds resulted in several fungi belonging to 5 genera and 8 species. The isolated fungi were purified and identified as *(Alternaria alternata* (Fr.) Keissler, *Aspergillus niger* van Tieghem, *Fusarium moniliforme* J. Sheld, *F. semitectum* Berk & Rav, *F. solani* (Mart.) Sacc. emend. Snyder & Hansen, *F. oxysporum* (shlechlendahl), and *R. solani* Kuehn) in addition to some unidentified fungi. The obtained data Table (1) reveal that the mean of isolates obtained from faba bean seeds of cvs. Giza-843, Misr-1 and Sakha-1 were 70, 60 and 60 isolates, respectively. Out of them, *R. solani* produced the highest number of colonies (42, 33 and 29 isolates) with the highest frequency being 60.0, 55.0 and 48.2% it was followed by *F. moniliforme* and *F. oxysporum* from seeds of cvs. Giza-843, Misr-1 and Sakha-1, respectively. Meanwhile, *F. semitectum*, and *F. solani* were more frequent from seeds of cvs. Giza-843, Misr-1 and Sakha-1, respectively.

Table 1. Number and frequency of the isolated fungus from faba bean seeds

Isolated fungus	Giza-843*		Misr-1		Sakha-1		Mean
	No.	Fre.	No.	Fre.	No.	Fre.	
A. alternata	2	2.9	1	1.7	2	5.0	3.2
A. niger	1	1.4	3	5.0	2	5.0	3.8
F. moniliforme	8	11.4	7	11.7	9	15.0	12.7
F. semitectum	6	8.6	5	8.3	5	8.3	84.0
F. solani	4	5.7	4	6.7	5	8.3	6.9
F. oxysporum	7	10.0	7	11.7	8	13.2	11.6
R.. solani	42	60	33	55.0	29	48.2	54.4
Total	70	100	60	100	60.	100	100

* No. = Number of isolates. Fre.= Frequency (%) of the isolated fungi.

Generally, all isolated fungi from cv. Sakha-1 except, *R. solani* were less in their frequency than on both cvs. Giza-843 and Misr-1 when compared with cvs. Giza-843 and Misr-1. Also, *A. niger* and *A. alternata* recorded less frequency on cvs. Giza-843, Misr-1 and Sakha-1 (being 1.4, 5 and 5% in case of *A. niger* and 2.9, 1.7 and 5% in case of *A. alternata*), respectively.

Pathogenicity tests:

Data in Table (2) indicate that *R. solani* was the highest pathogenic fungus among all of the tested fungi where it caused the highest infection of pre-emergence damping-off on both faba bean cvs. Giza-843, Misr-1 and Sakha-1. In this respect, *R. solani* caused the highest pre-emergence damping–off percentage followed by *F. moniliforme, F. semitectum, F oxysporum* and *F. solani.*

Table 2. Pathogenicity test of the isolated fungi from faba bean seeds under greenhouse condition

Tested fungus	Giza-843			Misr-1			Sakha-1		
	Pre (%)	Post (%)	Mean	Pre (%)	Post. (%)	Mean	Pre (%)	Post. (%)	Mean
F. moniliforme	13.3	20.0	33.3	10.0	23.3	32.2	13.3	20.0	33.3
F. semitectum	13.3	13.3	33.3	10.0	20.0	33.3	10.0	23.3	33.3
F. solani	10.0	16.6	33.3	10.0	20.0	33.3	10.0	20.0	33.3
F. oxysporum	10.0	16.6	33.3	10.0	23.3	33.2	10.0	23.3	33.3
R. solani	13.3	26.6	33.3	20.0	33.3	32.2	16.6	26.6	35.5
Control	00.0	00.0	33.3	00.0	00.00	33.3	00.0	00.0	33.3
Mean	9.98	15.5	33.3	10.0	19.98	32.92	9.98	18.9	33.6
L.S.D. at 5%	Pre			Post					
cv. Giza-843	7.31			6.99					
cv. Misr-1	NS.			8.02					
cv. Sakha-1	5.66			7.77					

Regarding, the post emergence damping-off, it is clear that the post infection ranged from 26.6 to 20.0% in case of faba bean cv. Giza-843 and 33.3 to 23.3% in case of cv. Maser-1and as well as 26.6 to 20.0% in case of cv. Sakha-1. *Rhizoctonia solani* and *F. moniliforme* were the most virulent pathogens at this disease stage meanwhile; *F. solani* was the least virulent one.

As for the plant survival, the results indicate that the highest survival (%) in case of cvs. Giza-843, Misr-1 and Sakha-1 infection with *F. solani.* Also, it is clear from the results that the means survived faba bean plants indicate that *R. solani* followed by *F. moniliforme* were the highly pathogenic fungi at most tested pathogens whereas *F. solani* was the least one in this respect on the tested faba bean cultivars.

Greenhouse studies:

Effect of treating faba bean seeds with some fungicides on the percentage of dead plants, 30 days after sowing:

Data in Table (3) show that treating faba bean seeds of cvs. Giza-843, Misr-1 and Sakha-1 with some commercial fungicides (Maxim, Premis, Topsin-M,

Table 3. Effect of treating faba bean seeds with some fungicides on the percentage of dead plants, 30 days post sowing in the greenhouse

Tested fungus at 3% inoculum	Dead seedlings (%) of three faba bean cultivars						
	Maxim	Premis	Topsin-M	Rizolex-T50	Vitavax-70	Control-1*	Mean
Giza-843							
F. moniliforme	10.0	13.3	10.0	6.6	3.3	20.0	10.51
F. semitectum	6.6	13.3	10.0	10.0	6.6	16.6	10.53
F. solani	6.6	10.0	6.6	3.3	3.3	16.6	7.73
F. oxysporum	6.6	16.6	10.0	6.6	6.6	20.0	11.10
R. solani	13.3	16.6	13.3	10.0	6.6	23.3	13.85
Control-2	00.0	00.0	00.0	00.0	0.0	00.0	00.00
Mean	7.18	11.63	8.31	6.08	4.4	16.08	8.93
Misr-1							
F. moniliforme	10.0	13.3	10.0	10.0	3.3	20.0	11.10
F. semitectum	10.0	13.3	13.3	13.3	6.6	20.0	12.75
F. solani	10.0	13.3	13.3	3.3	6.6	20.0	11.08
F. oxysporum	6.6	13.3	10.0	6.6	6.6	23.3	11.06
R. solani	13.3	13.3	10.0	10.0	6.6	23.3	12.75
Control-2	00.0	00.0	00.0	00.0	0.0	00.0	00.00
Mean	8.31	11.10	9.43	7.20	4.95	17.76	9.79
Sakha-1							
F. moniliforme	6.6	13.3	10.0	10.0	3.3	20.0	10.53
F. semitectum	10.0	13.3	13.3	13.3	6.6	23.3	13.20
F. solani	6.6	13.3	10.0	10.0	3.3	20.0	10.53
F. oxysporum	10.0	13.3	13.3	6.6	3.3	23.3	11.63
R. solani	13.3	13.3	13.3	10.0	6.6	23.3	13.30
Control-2**	00.0	00.0	00.0	00.0	0.0	00.0	00.00
Mean	7.75	11.1	9.98	8.32	3.85	18.32	9.87
L.S.D. at 5%	Fungi (F)=1.8 Treatments (T)= 4.41 Cultivar (C)= Ns. F x T= 3.12 F x C= 3.12 T x C= 7.63						

* Control-1= Infested soil with fungi without seed dressing.
** Control-2= Seeds treated with fungicides only without infestation.

Rizolex-T50 and Vitavax-70) before sowing in infested soil with the pathogenic fungi decreased significantly the dead plants comparing with the highest percentages of dead plants in the soil infested only with the pathogenic fungi (control-1).

As for Giza-843, the dead faba bean seedlings percentages were high in the infested soil with the pathogenic fungi reached from 16.6 to 23.3 %. In this respect, the increased percentage of dead plants was due to infestation the soil with *R. solani* followed *F. oxysporum*, *F. moniliforme*, *F. semitectum* and *F solani* caused a percentage of dead plants reaching (23.3, 20, 20, 16.6 and 16.6). On the other hand, treating cotton seeds cv. Giza-843 with Maxim, Premis, Topsin-M, Rizolex-T and Vitavax-70 reduced the infection of faba bean seedlings 3.3-6.6% in the case of Vitavax70 while it was 3.3-10.0% with Rizolex-T treatment. Also, it is clear from

the obtained results that treating faba bean seeds with Vitavax70 was the better fungicides than Maxim, Premis, Topsin-M, Rizolex-T in reducing infection of faba bean seedlings where the average of dead plants were 4.4, 7.18, 11.63, 8.31 and 6.08%, respectively.

Regarding Misr-1, the same trend was true where the infections of faba bean seedlings were high in soil infested with any of the pathogenic fungi, being 20.0-23.3%. Additionally, treating faba bean seeds cv. Misr-1 with Maxim, Premis, Topsin-M, Rizolex-T and Vitavax-70 gave similar results to those of cv. Giza-843. In this respect, treating faba bean seeds cv. Misr-1 with Vitavax-70 was better than Maxim, Premis, Topsin-M and Rizolex-T treatment in reducing the percentages of dead plants, being 4.95, 8.31, 11.10, 9.43 and 7.20%, respectively. While, dead seedlings due to pathogens inoculation and fungicidal treatments recorded decreases in variable rates according to the pathogen and fungicides.

Regarding Sakha-1, the same trend was true where the infection of faba bean seedlings were high in soil infested with any of the pathogenic fungi, being 20.0-23.3%. As well as, treating faba bean seeds cv. Sakha-1 with Maxim, Premis, Topsin-M, Rizolex-T and Vitavax-70 gave similar results to those of Giza-843 and Misr-1. In this respect, treating faba bean seeds cv. Sakha-1 with Vitavax-70 was better than Maxim, Premis, Topsin-M and Rizolex-T in reducing the percentages of dead plants, being 3.85, 7.75, 11.10, 9.98 and 8.32%, respectively. Meanwhile, using fungicides reduced these percentages to 7.18, 11.63, 8.31, 6.08 and 4.4% for Giza-843 and 8.31, 11.10, 9.43, 7.20 and 4.95% for Misr-1and as well as 7.75, 11.10, 9.98, 8.32, and 3.85 for Sakha-1 in case of using Maxim, Premis, Topsin-M, Rizolex-T and Vitavax-70, respectively.

Effect of Rizo-N and Plant Guard on the dead seedlings, 30 days after sowing under greenhouse conditions:

Data in Table (4) show that treating faba bean seeds cvs. Giza-843, Misr-1 and Sakha-1 with commercial antagonists (Rizo-N or Plant Guard) before sowing in infested soil with the pathogenic fungi decreased significantly the dead plants comparing with the highest percentages of dead plants in the soil infested only with the pathogenic fungi (control-1).

As for Giza-843, the dead faba bean seedlings percentages were high in the infested soil with the pathogenic fungi where they ranged from 16.6 to 23.3%. In this respect, the highest percentage of dead plants due to infestation of the soil with *R. solani* recorded 23.3% followed by 20.0% for *F. oxysporum* and 16% for *F. moniliforme*, *F. semitectum* and *F. solani*. On the other hand, treating faba bean seeds cv. Giza-843 with Rizo-N or Plant Guard reduced the infection of faba bean seedlings by 10.0-16.6% in the case of Rizo-N, while it was 6.6-10.0% when treated with Plant Guard. Also, it is clear from the obtained results that treating faba bean seeds with Plant Guard was better than Rizo-N in reducing infection of faba bean seedlings where the average of dead plants recorded 7.62 and 9.98%, respectively.

Regarding Misr-1, the same trend was true where the infections of faba bean seedlings were high in soil infested with any of the pathogenic fungi being 20.0-23.3%. As well as, treating the seeds of faba bean cv. Misr-1 with Rizo-N or

Table 4. Effect of bioagents Rizo-N and Plant Guard on the dead seedlings, 30 days after sowing in greenhouse

Tested fungus	Dead seedlings (%) of three faba bean cultivars			
	Rizo-N	Plant Guard	Control-1*	Mean
Giza-843				
F. moniliforme	13.3	10.0	16.6	13.3
F. semitectum	10.0	10.0	16.6	12.2
F. solani	10.0	10.0	16.6	12.2
F. oxysporum	10.0	6.6	20.0	12.2
R. solani	16.6	10.0	23.3	16.63
Control-2	00.0	00.0	00.0	00.0
Mean	9.98	7.76	15.52	11.08
Misr-1				
F. moniliforme	13.3	10.0	20.0	14.43
F. semitectum	13.3	6.6	20.0	12.2
F. solani	13.3	10.0	20.0	11.1
F. oxysporum	10.0	6.6	23.3	13.3
R. solani	13.3	10.0	23.3	15.53
Control-2	00.0	00.0	00.0	00.0
Mean	10.53	7.2	17.76	11.83
Sakha-1				
F. moniliforme	13.3	10.0	20.0	14.43
F. semitectum	13.3	10.0	23.3	15.53
F. solani	10.0	6.6	20.0	12.2
F. oxysporum	10.0	10.0	23.3	14.43
R. solani	10.0	6.6	23.3	13.3
Control-2**	00.0	00.0	00.0	00.0
Mean	9.43	7.2	18.31	11.64
L.S.D. at 5%	Bioagents (B)= 1.52	Fungi (F)= 2.15	B x F= 3.72	

* and ** As described in footnote of Table (3).

Plant Guard antagonists gave similar results to those of Giza-843. In this respect, treating faba bean seeds cv. Misr-1 with Plant Guard was better than Rizo-N treatment in reducing the percentages of dead plants, being 7.2 and 10.53, respectively. As for Sakha-1 the same trend was true where the infections of faba bean seedlings were high in soil infested with any of the pathogenic fungi being 20.0-23.3%. As well as, treating the seeds of faba bean cv. Sakha-1 with Rizo-N or Plant Guard gave similar results to those of Giza-843. In this respect, treating faba bean seeds cv. Sakha-1 with Plant Guard was better than Rizo-N treatment in reducing the percentages of dead plants, being 7.2 and 10.53, respectively. Meanwhile, using antagonists reduced these percentages to 7.76 and 9.98% for Giza-843; 7.2 and 10.53% for Misr-1and as well as 7.2 and 9.43% for Sakh-1, in case of using Plant Guard and Rizo-N, respectively.

Chemical changes in some plant components of faba bean detached leaves due to infection with root-rot pathogens:

Changes of chlorophyll (A), (B) and carotenoids:

Regarding phenols, it is clear from data in Table (5) that infestation the soil with root-rot pathogens affected positively the content of total, free and conjugated phenols in leaves of faba bean plants cvs. Giza-843. Maser-1 and Sakha-1. In this respect, the lowest amount of phenols as mg/g fresh weight (total, free and conjugated phenols) was recorded in case of *F. solani*. Meanwhile, the highest increase in both cvs. (Giza-843, Maser-1 and Sakha-1) was recorded in the case of infestation the soil with *R. solani* followed by *F. moniliforme* and *F. semitectum*, respectively. It is clear also that all the determined phenols (total, free and conjugated phenols) were higher in cv. Sakha-1 than those determined in cv. Giza-843 and Misr-1.

Table 5. Effect of the pathogens on the phenols in faba bean detached leaves, 30 days post sowing in soil

Tested fungus	Phenols (mg/g fresh weight)								
	Free			Conjugated			Total		
	Giza-843	Misr-1	Sakha-1	Giza-843	Misr-1	Sakha-1	Giza-843	Misr-1	Sakha-1
F. moniliforme	3.54	3.67	3.38	1.74	1.34	1.63	5.28	5.01	5.01
F. semitectum	4.13	3.38	3.38	2.04	1.78	1.78	6.17	5.16	5.16
F. solani	3.24	2.94	3.10	1.60	1.18	1.35	4.84	4.12	4.45
F. oxysporum	3.54	2.94	2.23	1.74	1.18	1.63	5.28	4.12	4.86
R. solani	5.03	4.12	4.85	2.62	2.21	2.25	7.65	6.33	7.10
Control	2.36	2.64	2.94	1.32	1.19	1.48	3.68	3.83	4.42

Data in Table (6) reveal that infestation of the soil with the tested root-rot pathogens affected negatively the content of chlorophyll when determined in leaves of faba bean seedlings as mg/g fresh weight of both tested cultivars after 30 days of sowing. In this respect, all the tested root-rot pathogens decreased the content of chlorophyll A and B and the total chlorophyll in faba bean leaves comparing with uninfested soil (control) of the three cvs. Giza-843, Maser-1 and Sakha-1. The highest decrease in chlorophyll A and B as well as total chlorophyll was recorded in case of infestation of the soil with *Fusarium solani, F. moniliforme, F. semitectum, F. oxysporum,* and *R. Solani,* respectively, comparing with uninfested soil (control) of the three cultivars. The results also indicate that the carotenoids content did not change markedly in the leaves of grown faba bean plants comparing with control where all the determined carotenoids ranged from 0.96 to 1.02 mg/g fresh weight.

Effect on protein and carbohydrates content of infested faba bean seeds:

Data in Table (7) reveal that infestation of faba bean seeds with any of the tested root-rot pathogens, *i.e. F. moniliforme, F. semitectum, F. solani, F. oxysporum* and *R. solani,* affected negatively protein and carbohydrates contents of the seeds. In this respect, all the tested root-rot pathogens decreased the percentages of protein and carbohydrates content into the tested faba bean seeds of cvs. Giza-843, Maser-1 and Sakha-1 comparing with the uninfested seeds (control) at any incubation period, *i.e.* 10, 20 and 30 days. It is clear also that increasing incubation period from

Table 6. Effect on chlorophyll and carotenoids content in the leaves of faba bean, 30 days post sowing in infested soil with root-rot pathogens

Treatment (mg/g fresh weight)		Pathogenic fungi					
		F. moniliforme	*F. semitectum*	*F. solani*	*F. oxysporum*	*R. solani*	Control
Chlorophyll A	Giza-843	2.21	2.43	2.19	2.61	2.82	4.62
	Misr-1	2.14	2.52	2.13	2.53	2.74	4.48
	Sakha-1	2.15	2.49	2.14	2.54	2.76	4.51
Chlorophyll B	Giza-843	1.30	1.43	1.28	1.53	1.66	2.72
	Misr-1	1.21	1.32	1.19	1.42	1.53	2.52
	Sakha-1	1.24	1.36	1.22	1.46	1.57	2.59
Total	Giza-843	3.51	3.86	3.47	4.14	4.48	7.34
	Misr-1	3.35	3.84	3.32	3.95	4.27	7.00
	Sakha-1	3.39	3.85	3.36	4.00	4.33	7.10
Carotenoids	Giza-843	0.98	0.98	0.98	0.98	1.00	1.02
	Misr-1	0.96	0.96	0.96	0.96	0.96	0.98
	Sakha-1	0.98	0.98	0.98	0.98	0.97	1.00

Table 7. Effect of treating faba bean seeds with the root-rot pathogens on protein and Carbohydrates content on incubation at 25°C

Tested fungus	Day after inculcation					
	Protein (%)			Carbohydrates (%)		
	Giza-843	Misr-1	Sakha-1	Giza-843	Misr-1	Sakha-1
10 days after inculcation						
F. moniliforme	33.7	29.6	30.7	41.2	42.3	39.6
F. semitectum	34.2	30.2	31.1	41.8	42.3	39.7
F. solani	34.8	30.6	31.4	41.8	42.9	39.8
F. oxysporum	34.2	30.4	31.2	41.8	42.6	39.8
R. solani	33.4	29.2	30.3	40.9	41.9	39.4
Control	35.6	31.9	32.6	43.6	44.7	42.4
20 days after inculcation						
F. moniliforme	32.2	28.4	29.3	40.10	38.6	31.8
F. semitectum	33.1	29.0	30.0	40.2	40.6	38.3
F. solani	33.6	29.3	30.2	40.4	41.2	38.4
F. oxysporum	33.0	29.3	30.1	41.4	41.6	38.7
R. solani	32.1	28.3	29.2	39.6	38.2	31.1
Control	35.6	31.9	32.6	43.5	44.7	42.4
30 days after inculcation						
F. moniliforme	31.3	27.8	27.8	39.0	37.2	30.6
F. semitectum	32.1	17.9	28.7	39.1	39.2	37.1
F. solani	32.4	28.2	28.8	39.8	39.8	37.2
F. oxysporum	32.2	28.3	29.0	40.1	40.1	37.2
R. solani	30.8	27.1	27.1	38.3	37.1	29.8
Control	35.6	31.9	32.6	43.5	44.7	42.4

10 to 30 days decreased gradually the of protein and carbohydrates contents for all treatments compared with the un-infested seeds (control). The highest decrease was recorded in the case of seed infestation with *R. solani* and *F. moniliforme* with all periods.

Laboratory studies:

A- Effect of some fungicides on the growth of the tested fungi:

This experiment was conducted to study the effect of different concentrations of some fungicides on the linear growth of *R. solani, F. moniliforme, F. semitectum, F. oxysporum* and *F. solani.*

Data in Table (8a) indicate that all the tested fungicides affected the linear growth of *R. solani* to values ranged from 4 to 68 mm comparing with the un-treated (control). In this respect, Premis, Maxim and Topsin-M were the best effective fungicides on growth of *R. solani*. It is clear also, that Topsin-M was the highest effective one at the concentration of 5-400ppm where the resulted linear growth was only 9 mm followed by Premis at concentration of 5-400ppm (13 to 9 mm) and Maxim at 10-400 ppm (10 mm).

Table 8a. Effect of some fungicides on the linear growth (mm) of *R. solani*, 5 days post inoculation at 28ºC

Fungicide	Linear growth of *R. solani* (mm) at different concentrations (ppm)							Mean
	Control	10	25	50	100	200	400	
Maxim	70	10	10	10	10	10	10	17.0
Premis	70	9	9	9	9	9	9	16.8
TopsinM70	70	9	9	9	9	9	9	20.8
RizolexT50	70	44	42	36	25	19	16	41.1
VitavaxT70	70	68	64	61	42	41	9	54.5
Mean	70.0	28.0	28.8	25.0	19.0	17.6	10.6	30.1
L.S.D. at 5% for: Concentration (C)= 0.11 Fungicides (F)= 0.09 C x F= 0.27								

On the other hand, Maxim and Premis were more effective than Topsin-M at concentration of 1 ppm. Moreover, the least effective fungicide on the linear growth of *R. solani* was Vitavax-T70 especially at the tested concentrations ranged from 1 to 200 ppm where the average of the linear growth was 54.5 mm. It is clear that increasing the concentration from 1 to 400 ppm increased gradually the effect of the tested fungicides in reducing the growth of *R. solani.*

Data in Table (8b) indicate that Maxim and Premis were the best effective fungicides on linear growth of *F. moniliforme* where the resulted linear growth was only 9 mm at all the tested concentrations which ranged between 1-400 ppm. Meanwhile, Topsin-M was also effective at the tested concentrations ranged from 5-400 ppm where its linear growth was only 9mm. On the other hand, Rizolex-T, Vitavax-T70 were less effective than the first three fungicides especially at the tested concentrations ranged from 1-50 ppm. It is clear also that increasing the concentrations of the tested fungicides from 1 to 400ppm increased gradually the

Table 8b. Effect of some fungicides on the linear growth (mm) of *Fusarium moniliforme*, 5 days post inoculation at 28ºC

Fungicide	Linear growth of *R. solani* (mm) at different concentrations (ppm)							Mean
	Control	10	25	50	100	200	400	
Maxim	70	9	9	9	9	9	9	15.8
Premis	70	9	9	9	9	9	9	15.8
TopsinM70	70	9	9	9	9	9	9	18.2
RizolexT50	70	44	18	9	9	9	9	28.4
VitavaxT70	70	26	25	9	9	9	9	27.0
Mean	70	19.4	14.0	9.0	9.0	9.0	9.0	21.1
L.S.D. at 5 % for	Concentration (C)= 0.13 Fungicides (F)= 1.1 C x F= 0.32							

effect on the average of resulted linear growth. Additionally, Rizolex-T and Vitavax-T70 were the least effective fungicides on linear growth of tested *F. moniliforme*, respectively.

Results in Table (8c) indicate that Premis followed by Topsin-M were the highest effective fungicides on linear growth of *F. solani* where they gave the least average of linear growth (15.8 and 18.6 mm), respectively. Premis fungicide gave the least average of the linear growth (9 mm) at all the tested concentrations, *i.e.* 1 to 400 ppm. Meanwhile, Topsin-M gave the same average of linear growth (9 mm) at the tested concentrations ranged from 5-400ppm. On the other hand, Vitavax-T70 followed by Rizolex-T were the least effective fungicides on the linear growth of *F. solani*. Also, increasing the concentrations from 1-400 increased gradually the effect of tested fungicides.

Table 8c. Effect of some fungicides on the linear growth (mm) of *Fusarium solani* 5 days post inoculation at 28ºC

Fungicide	Linear growth of *R. solani* (mm) at different concentrations (ppm)							Mean
	Control	10	25	50	100	200	400	
Maxim	70	47	42	40	35	31	9	42.1
Premis	70	9	9	9	9	9	9	15.8
TopsinM70	70	9	9	9	9	9	9	18.6
RizolexT50	70	52	49	47	39	28	24	46.6
VitavaxT70	70	62	53	52	32	18	16	47.8
Mean	70	35.8	32.4	31.4	24.8	19.0	13.4	34.2
L.S.D. at 5 % for	Concentration (C)= 0.06 Fungicides (F)= 0.05 C x F= 0.15							

Results in Table (8d) reveal clearly that Premis followed by Topsin-M were the best effective fungicides on growth of *F. semitectum in vitro* where they gave the least average of growth (20.7 and 22.0 mm) respectively. Meanwhile, Rizolex-T was the least effective one on the growth of *F. semitectum*. Also, increasing the concentration from 1 to 400 ppm gradually increased the effect of tested fungicides.

Table 8d. Effect of some fungicides on the linear growth (mm) of *Fusarium semitectum* 5 days post inoculation at 28ºC

Fungicide	Linear growth of *R. solani* (mm) at different concentrations (ppm)							Mean
	Control	10	25	50	100	200	400	
Maxim	70	29	29	28	28	25	9	30.6
Premis	70	22	10	9	9	9	9	20.7
TopsinM70	70	9	9	9	9	9	9	22.0
RizolexT50	70	48	42	38	37	24	15	42.6
VitavaxT70	70	46	43	33	9	9	9	34.4
Mean	70	30.8	26.6	23.4	18.4	15.2	10.2	30.1
L.S.D. at 5 % for	Concentration (C)= 0.1 Fungicides (F)= 0.08 C x F= 0.24							

Results in Table (8e) indicate that Premis followed by Topsin-M were the highest effective fungicides on linear growth of *F. oxysporum*, where they gave the least average of linear growth (15.8 and 18.6 mm) respectively. Premis fungicide gave the least average of the linear growth (9 mm) at all the tested concentrations, *i.e.*, 1 to 400 ppm. Meanwhile, Topsin-M gave the same average of linear growth (9 mm) at the tested concentrations ranged from 5-400ppm. On the other hand, Vitavax-T70 followed by Rizolex-T were the least effective fungicides on the linear growth of *F. oxysporum*. Also, increasing the concentrations from 1-400 increased gradually the effect of tested fungicides.

Table 8e. Effect of some fungicides on the linear growth (mm) of *Fusarium oxysporum*, 5 days post inoculation at 28ºC

Fungicide	Linear growth of *R. solani* (mm) at different concentrations (ppm)							Mean
	Control	10	25	50	100	200	400	
Maxim	70	47	42	40	35	31	9	42.1
Premis	70	9	9	9	9	9	9	15.8
TopsinM70	70	9	9	9	9	9	9	18.6
RizolexT50	70	52	49	47	39	28	24	46.6
VitavaxT70	70	62	53	52	32	18	16	47.8
Mean	70	35.8	32.4	31.4	24.8	19.0	13.4	34.2
L.S.D. at 5 % for	Concentration (C)= 0.06 Fungicides (F)= 0.1 C x F= 0.15							

B- Effect of some antagonists on the growth (mm) of the tested fungi:

Data in Table (9) show the effect of the tested bioagents in this study on the linear growth of all the tested root-rot pathogens. In this respect, Rizo-N was better than Plant Guard in its effect on growth of *R. solani*, *F. moniliforme* and *F. semitectum* as well as comparing to control treatment Meanwhile, Plant Guard was better only on its effect on growth of *F. solani* and *F. oxysporum.*

Table 9. **Effect of two antagonists on the linear growth (mm) of the tested root-rot fungi, 5 days after incubation at 28ºC**

Tested fungus	Bioagent			
	Control	Rizo-N	Plant Guard	Mean
R. solani	70	43	50	54.3
F. moniliforme	70	45	53	56.0
F. semitectum	70	47	52	56.3
F. solani	70	39	34	47.6
F. oxysporum	70	36	34	46.7
R. solani	70	42	44.6	52.2

D i s c u s s i o n

Faba bean *(Vicia faba* L.) is one of the most important legume crops in Egypt. It is susceptible to a number of fungal diseases which decrease production and quality of seeds. Due to the lack of information regarding seed-borne diseases of the crop in Egypt, researchers studied the seed-borne fungi of faba bean stated that, the fungal pathogens causing damping-off root-rot, wilt and chocolate spot diseases are the most important fungal diseases affecting faba bean production in Egypt (Mahmoud, 1996). Sum of 190 isolate of different fungi were isolated from seeds of faba bean plants showed root-rot and wilt symptoms of three cvs. Giza-843, Misr-1 and Sakha-1 cultivated in the Egyptian Governorates. These isolates were identified as *Rhizoctonia solani, Fusarium oxysporum, F. solani, F. semitectum* and *F. moniliforme. Rhizoctonia solani* followed by *F. moniliforme* were the most frequent fungi in the three cultivars. The highest number of isolated fungi was recorded in cv. Giza-843 followed by cvs. Misr-1 and Sakha-1, respectively. These results are similar to those obtained by many researchers (Sepulveda, 1991; E1-Morsy *et al.*, 1997; Akem & Bellar, 1999; Ahmed, 2005 and Ahmed 2013) who isolated *F. oxysporum, F. solani* f.sp. *fabae, R. solani* and *Macrophomina phaseolina* from wilted and rotten roots of faba bean grown in different parts of the world. Also, Mohamed *et al.* (1986) indicated that the highest frequency of ***B. fabae*** was recorded in Menoufya and Kafr-El-Seikh Governorates during season 1998/1999. Isolation trials revealed *Aspergillus flavus, A. niger, A. ochraceus, Penicillium digitatum, P. italicum, Alternaria alternata, Botrytis fabae, Cephalosporium* sp., *Cladosporium cladosporioides, Epicoccum nigrum, F. oxysporum, F. semitectum, F. solani, F. verticillioides (moniliforme), R. solani, Rhizopus stolonjfer, Stemphylium globuljferum, Trichothecium roseum* and *Verticillium dahliae* (Abdel-Hafez, 1988; Dubey and Patel, 2000 and Rauf, 2000).

Pathogenicity of 15 fungal isolates, representing 2 genus and 5 species, *i.e. R. solani. F. moniliforme. F. oxysporum* followed by *F. semitectum* and *F. solani*, on three faba bean cultivars, *i.e.* Giza-843, Misr-1 and Sakha-1, revealed that all tested isolates could infect the seed of tested faba bean cultivar causing pre- and post-emergence damping-off and reduced survived plant percentages. These findings are in agreement with those obtained by (Omar, 1986 and Simay, 1992).

As for *in vitro* antagonistic studies, obtained results indicated that applying the bioagents significantly reduced the linear growth of all examined fungi where they varied the positive role of *Trichoderma* spp. and *Bacillus* spp. *In vitro* and *in vivo* controlling of root-rot and chocolate spot pathogens as well as their effects on plant growth characters was extensively studied by many researchers (Mahmoud *et al.*, 2004; El-Gammal, 2005 and Ibrahim, 2005) who concluded that the success of *T. harzianum, T. hamatum* and *B. subtilis,* in controlling *Botrytis fabae,* spore germination and chocolate spot development as well as *F. oxysporum* f.sp. *fabae* was varied. On the other hand, the antagonistic forms which appeared in the interaction between *T. harzianum* with *R. solani, F. oxysporum.*

As for using fungicides *in vitro* results indicated that increasing concentrations of the tested fungicides decreased the fungal linear growth of the tested pathogenic isolates. Vitavax-T70 was the most effective fungicide in reducing fungal linear growth compared with the other fungicides at any corresponding concentration. All pathogenic fungi *R. solani, F. moniliforme, F. semitectum, F. oxysporum* and *F. solani* were sensitive to Vitavax, Premis, Maxim, Topsin-M and Rizolex T50 respectively. These results are in harmony with those of (El-Fiki, 1994) who recorded that treating seeds of *Vicia faba* with Vitavax-T, Quinolate V4X or Rizolex decreased significantly pre- and post-emergence damping-off while, spraying the faba bean plants with Benlate + chlorothalonil mixture was the best for controlling chocolate spot disease *(Botrytis fabae).* Therefore, E1-Gindy (2003) confirmed the efficacy of many different fungicides in controlling root-rot and chocolate spot pathogens of faba bean in vitro and in vivo. Also, the results of (El-Gammal, 2005) are in harmony with the obtained findings where he found that Dithane M-45 and Tridex Polyram-DF were more effective than Kocide-l0l when used for controlling *B. fabae* on faba bean plants (cv. Giza-40).

Pathogenicity tests showed that *R. solani F. moniliforme, F. oxysporum, F. semitectum* and *F. solani* were most isolated fungi from faba bean seeds which showed decay, pre- and post-emergence damping-off. Concentrations of chlorophyll A, chlorophyll B and carotenoid in infected plants with above-mentioned pathogens showed significant reductions, while total phenols in diseased plants increased. Obtained results indicated that there is a correlation between disease incidences and concentration of total phenols as well as chlorophyll A, chlorophyll B and carotenoid, in plant tissue. The use of pathogen-free seed is a must to insure high protection for faba bean seeds.

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تأثير بعض المبيدات الفطرية والمركبات الحيوية فى مقاومة الأمراض المحمولة على بذور الفول البلدي

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عزلت ١٩٠ عزلة (تمثل مختلف الفطريات المحمولة على البذرة) من بذور الفول البلدي أصناف جيزة-٨٤٣ ، مصر-١ وسخا-١. هذه العزلات تسبب الذبول و أعفان الجذور و قد عرفت تلك العزلات على أنها رايزوكتوبيا سولانى ، فيوزاريوم مونيليفورم ، فيوزاريوم سيميتيكتام ، فيوزاريوم أوكسيسبورم و فيوزاريوم سولانى على التوالى. و كان فطر رايزوكتوبيا سولانى أكثرا لفطريات المعزولة تكرارا تلاه فى ذلك فطر، فيوزاريوم مونيليفورم وفطر، فيوزاريوم سيميتيكتام فى الثلاث أصناف.وقد سجل أكبر عدد تلك الفطريات من صنف الفول البلدي جيزة ٨٤٣ وكان أقلها صنف الفول البلدي مصر-١ على التوالى. أظهر اختبار العدوى المرضية لخمس العزلات من الفطريات المسببة للذبول وعفن الجذور والتى تتبع جنسين مختلفين تم عزلها من الثلاث أصناف أن جميع العرلات يمكنها أن تصيب جذور نبتات الفول البلدي أصناف جيزة - ٨٤٣ ، مصر-١ وسخا-١ مسببة موت البادرات سواء قبل أو بعد الظهور فوق سطح التربة مما أثر على انخفاض نسبة النباتات المتبقية.

أظهرت عزلات الفطريات رايزوكتوبيا سولانى ، فيوزاريوم مونيليفورم وفيوزاريوم سيميتيكتام ضراوة مرضية حيث تسببت فى حدوث إصابة كلية (موت للبادرات قبل وبعد الظهور فوق سطح التربة) كما قللت بشدة نسبة النباتات الحية المتبقية.

المبيدات الفطرية المستخدمة يصحبها تناقص تدريجي فى النمو الخطى للفطريات الممرضة تحت الدراسة فى حين قد تفوق مبيد بريمس على باقي المبيدات المستخدمة.

المركبات الحيوية التجارية المختبرة قللت نمو مسببات أعفان الجذور المختبرة ومن أفضلها ريزو-ن الذى كان أكثر فعالية من البلانت جارد فى تخفيض النمو الخطى لجميع الفطريات المختبرة.

عدوى بذور أصناف الفول البلدي (جيزة-٨٤٣، مصر-١و سخا-١) بمسببات أعفان الجذور وهى رايزوكتوبيا سولانى ، فيوزاريوم مونيليفورم ، فيوزاريوم سيميتيكتام ، فيوزاريوم أوكسيسبورم و فيوزاريوم سولانى قللت من النسبة المئوية لمحتوى البذور من البروتين الكربوهيدرات مقارنة (الكنترول) فى كل فترات التحضين التى تراوحت بين ١٠و ٣٠ يوماً. و قد لوحظ أن محتوى ا لبذور من البروتين الكربوهيدرات يتناقص تدريجياً بزيادة فترة التحضين من ١٠ إلى ٣٠ يوماً نتيجة ا لعدوى بكل مسببات المرض السابقة مقارنة بالكنترول. وبلغت أعلى نسبة إ نخفاض لمحتوى البروتين الكربوهيدرات فى البذور المعداة بفطر الرايزوكتوبيا سولانى ، فيوزاريوم مونيليفورم عند كل فترات تحضين البذور المعداة لكل الأصناف(جيزة- ٨٤٣، مصر-١ وسخا-١).

زراعة نباتات الفول البلدي-٨٤٣ ، مصر-١ وسخا-١ فى تربة معداه بمسببات أعفان الجذور وتقدير محتوى الأوراق من الفينولات(الكلية ,الحرة والمرتبطة) قد سجلت أعلى نسبة فى كمية الفينولات السبقة (ملليجم /جم) من الوزن الغض لأوراق الأصناف المستخدمة عندما زرعت لبذور فى تربة معداه بريزوكتونيا سولانى ، ، فيوزاريوم سيميتيكتام ، فيوزاريوم مونيليفورم , على التوالى . بينما سجلت أقل نسبة الفينولات فى تربة معداه فيوزاريوم سولانى .لوحظ أن محتوى الفينولات المقدرة أعلى دائما فى أوراق الصف سخا-١ يليه صنف جيزة-٨٤٣ وأقلها صنف مصر-١ .