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EFFICACY OF ANTAGONISTS, NATURAL PLANT EXTRACTS AND FUNGICIDES IN CONTROLLING WILT, ROOT ROT AND CHOCOLATE SPOT PATHOGENS OF FABA BEAN IN VITRO

BY

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ABSTRACT

Seventy one isolate of different soil-borne fungi as well as, eight *Botrytis* isolates were isolated from wilted, rotten roots and spotted leaves of faba bean plants cv. Giza-716 cultivated in the three Egyptian governorates of north Delta, i.e. Minufiya, Gharbia and Kafr El-Sheikh. These isolates were identified as *Rhizoctonia solani, Verticillium albo-atrum, Fusarium oxysporum, F. solani, F. semitectum* and *F. moniliforme*. *R. solani* followed by *V. albo-atrum* were the most frequent fungi in the three governorates. The highest number of isolated fungi was recorded in Menoufya governorate followed by Kafr El-Sheikh and Gharbia governorates, respectively. Also, *Botrytis* isolates were identified as *Botrytis fabae* Sard and scored its highest number in Menoufya governorate followed by Gharbia and Kafr El-Sheikh governorates, respectively. Pathogenicity test of 18 isolates of root isolates, (represent 6 different fungi of the three governorates) revealed that all tested isolates could infect the roots of faba bean (Giza-716 cv) causing pre and post emergence damping-off and reduced the survived plants. The isolates of *F. oxysporum* (Isolate-2) followed by *R. solani* (Isolate-3), *F. solani* (Isolate-2), *F. moniliforme* (Isolate-3) and *V. albo-atrum* (Isolate-1) were the most virulent ones. Also, the eight tested isolates of *Botrytis fabae* could infect faba bean plants (cv. Giza-716) with significant differences among them. *B. fabae* (Gharbia isolate) was the most aggressive isolate causing chocolate spot severity.

All tested plant extracts i.e., garlic, onion and caraway have significantly reduced the linear growth of all the tested fungi with variations among the tested plant extracts. Garlic extract was the most effective plant extract followed by onion and caraway. On the other hand, using antagonistic bio-agents *in vitro* reduced significantly the linear growth of all examined fungi, where *T. koningii* and *T. harzianum* were the most effective bio-agents followed by *B. subtilis*. Scanning electron microscope (SEM) of the interaction sites between *Trichoderma harzianum* and the pathogenic fungi revealed different types of parasitism in the form of coiled hyphae, pincer shaped hyphae, hook and pincer shaped hyphal branches, hooked parallel hyphae looking for penetration, ring hyphae and adhesive shaped hyphae as well as appressorium-like bodies as cleared in cases of *R. solani, F. oxysporum* and *B. fabae*. All tested fungicides, *in vitro* 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 Benlate, while, *V. albo-atrum* was sensitive to Vitavax 200, while, *F. moniliforme* was sensitive to Rizolex-T and Monceren. Meanwhile, all the tested fungicides at all the tested concentrations affected significantly the growth of *B. fabae*, where Dithane-M45 was the most effective fungicide followed by Benlate and Copper Acrobat respectively.

Key words: faba bean, soil-borne fungi, chocolate spot, plant extracts, bio-agents, bio-agents and fungicides

INTRODUCTION

Faba bean (*Vicia faba* L.) is a legume crop with high nutritional value. It contains about 18.5 and 37.8% protein (El-Sayed *et al.*, 1982). Faba bean plants infected with many fungal pathogens which caused a considerable yield losses (Mahmoud, 1996). In this respect, root-rot, damping-off and chocolate spot diseases are the most important fungal diseases affecting faba bean production in Egypt. In this respect, Sepúlveda (1991), El-Morsy *et al.* (1997), Akem and Bellar (1999) and Hugar (2004) isolated *Fusarium oxysporum* and *F. solani* f.sp. *fabae*, *Rhizoctonia solani*, *F. oxysporum* f.sp. *fabae*, *Fusarium oxysporum* and *Macrophomina phaseolina* from wilted and rotten roots of faba bean in different parts of the world as well as considered them the most important and widespread fungal diseases observed at all locations. Meanwhile, all of Morsy (2000), Daboor (2001), and Abo-Baker (2002) isolated *Ascochyta fabae*, *Botrytis fabae*, *B. cinerea*, *Uromyces fabae* [*U. viciae-fabae*], *Alternaria* spp, *Cercospora* and *Stemphylium* spp from spots of the faba bean plants in different parts of the world as well as most of them considered *B. fabae* and *B. cinerea* as the most important and widespread fungi causing chocolate spot disease on faba bean. El-Gammal (2005) surveyed the distribution of causal organisms of chocolate spot disease in 126 faba bean fields at 8 Governorates during two successive seasons. He found that Botrytis isolates were the most frequent in their number. *B. fabae* isolates were 141 isolates with frequency % 55.5 whereas *B. cinerea* isolates were 113 isolates only with frequency % 44.5 when identified 254 Botrytis isolate. The highest isolation number of *B. fabae* was recorded at Kafr-El Sheikh and Menuofia being 13 isolates for each Governorate during season 1998/1999, in the same season, the fungus was not detected in Gharbia, Dakahlia, Sharkia, Qualubia and Beni-Swief Governorates. Meanwhile, the highest isolation number of *B. cinerea* was recorded in Sharkia Governorate.

As for pathogenicity of root rot and chocolate spot pathogens of faba bean, 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* in infecting faba bean plants causing root rot and wilt diseases. They verified the ability of *Botrytis fabae* and *B. cinerea* for infecting faba bean plants causing chocolate spot disease. On the other hand, Metwaly (2004) revealed that all isolates of *B. fabae* and *B. cinerea* were capable for infecting faba bean plants causing chocolate spot but they varied in their virulence. *B. fabae* isolates were more severe than that of *B. cinerea*. *B. fabae* isolates of Beheira Governorate were the most virulent isolates, followed by isolates of Minufyia, Domiat

Governorates, respectively. On the other hand, pathogenicity test of *Rhizoctonia solani* isolates exhibited that *R. solani* isolated from Kafer El-Sheikh governorate was the most virulent isolate from Sharkia governorate.

As for the effect of natural plant extracts in controlling root rot and chocolate spot pathogens, Michoil and El-Khateeb (1985) and Gaafar *et al.* (1989) found that garlic extract gave the best results in controlling both damping-off and root rot diseases with superiority of garlic extract effect over onion extract. Also, Heweidy and Mohamed, Fatma (1997) found that eight crude extracts of garlic cloves (*Allium sativum* L.) and henna leaves (*Lawsonia inermis* L.) have *in vitro* positive effect on reducing mycellial growth and spore germination of *B. fabae* the causal pathogen of chocolate spot disease of faba bean (*Vicia faba* L.). El-Gindy (2003) found that different concentrations of aniseed and caraway juices significantly inhibited the growth of *Botrytis fabae* isolate. Coriander and fenugreek had the best effect on the fungal rate.

Regarding the effect of bio-agents against root rot and chocolate spot pathogens, all of, Mathew and Gupta (1998) Hazarika and Das (1999) 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, F. solani* on faba bean and other legume crops. Also, El-Gindy (2003) mentioned that *T. harzianum, T. lignorum* 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 *Fusarium oxysporum* f.sp. *fabae* isolate.

Concerning the effect of fungicides, El-Fiki (1994) recorded that treating seeds of *Vicia faba* with Vitavax-T, Quinolate V_4X or Rizolex decreased significantly pre- and post-emergence damping-off while, spraying the faba plants with Benlate + chlorothalonil mixture was the best for controlling chocolate spot disease (*Botrytis fabae*). Also, all findings of Vadhera *et al.* (1997) and El-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. Moreover, El-Gammal (2005) found that Dithane M-45 and Tridex Polyram-DF were more effective than Kocide-101 in controlling *B. fabae* on faba bean as well as the best concentration was 200 ppm of all tested fungicides.

This work aimed to clear the efficacy of antagonists, natural plant extracts and fungicides in controlling wilt, root rot and chocolate spot pathogens in vitro.

MATERIALS AND METHODS

1- Isolation and identification of the causal organisms:

a. Sampling and isolation from infected seedlings and roots:

Faba bean roots of rotten roots and whole plants were collected from different localities that cultivated with faba bean in Egypt, *i.e.* Gharbia (Tanta), Kafr El-Sheikh (Kafr El-Sheikh) and Minufiya (Sers El-Layian), Governorates were used as samples of isolation.

Infected parts were cut into small pieces, washed thoroughly with running water to remove any adhering soil particles. These pieces were surface sterilized by immersing in 5% sodium hypochlorite solution for 2 min, followed by 70% ethanol for 2 min, then washed several times in sterilized water then dried within sterilized filter papers. Four surface sterilized pieces were aseptically transferred onto potato dextrose agar medium (PDA) containing 40 ppm streptomycin sulphate to avoid any bacterial contamination. Plates were incubated at 25°C for 3 -7 days and observations were recorded (Christensen, 1957). Hyphal-tips of grown fungi were transferred individually to new PDA plates (Riker and Riker, 1936) and then identified according to their morphological and microscopical characters as described by Gilman, (1957) and Jens *et al.* (1991). Identification was confirmed by the Department of Mycology, Plant Pathology Institute, Agricultural Research Center, Giza, Egypt.

b. Sampling and isolation from infected faba leaves:

Samples of naturally infected faba bean leaves (*Vicia faba* L.) collected from the aforementioned localities in Egypt were used for isolation of chocolate spot pathogens. Infected leaflets having symptoms of chocolate spot disease were cut into small pieces, each with single lesion of the concerned disease. Infected tissues were surface sterilized by immersing them in 5% sodium hypochlorite solution for 2 min and dried between double layers of sterile filter paper. The samples were plated on ready PDA plates. Four pieces were put in each plate then the plates were incubated at 20°C for one week. Pure isolates were obtained using single spore or hyphal tip techniques. The causal organisms were identified by the aid of Department of Mycology, Plant Pathology Institute, Agricultural Research Center, Giza, Egypt.

2- Pathogenicity tests of:

a- Wilt and root rot pathogens:

Pathogenicity tests were carried out under greenhouse conditions at (Sers El-Layian Agricultural Research Station at 1999 growing season).

All fungal isolates isolated from rotten roots of faba bean were tested for their pathogenic potentialities on susceptible faba bean cv Giza-716 under greenhouse conditions in order to select the highly pathogenic isolates.

Pots (25 cm Φ) 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 3.5% of soil weight (El-Sayed, 1999). Inocula were prepared by growing fungi on sand-barley (SB) medium (25 g clean sand, 75 g barley and enough water to cover the mixture). Flasks contained sterilized medium were inoculated with each particular fungus and incubated at 25°C 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 of sterilized sand-barley (SB) medium. Ten faba bean seeds were surface sterilized using sodium hypochlorite 5% for 2 min, washed several times with sterilized water, before sowing. Three replicates with a total of 30 seeds were used for each particular treatment.

Disease assessment:

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

b- Chocolate spot pathogen:

At the first, all isolates of *Botrytis fabae* from spotted leaves of faba bean were tested for their pathogenic potentialities on susceptible faba bean cv Giza-716 under greenhouse conditions in order to select the highly pathogenic isolate.

Seeds of Giza-716 were sown in pots (25 cm Φ), filled with clay loam soil (10 kg soil/pot), at the rate of ten seeds/pot. Spores of *Botrytis fabae* were obtained from 12 day-old culture of each isolate, which grown on FBLA medium (50 g faba bean leaves +30 g sucrose +20 g sodium chloride and 20 g agar in one liter of distilled water) as described by Leach and Moore, (1966)] at 20°C. Spores were separated using a camel brush, counted using a haemocytometer slide, then adjusted to $5x10^5$ spores/ml, and used immediately. Spore suspension was sprayed on 35 day-old healthy faba bean plants with an atomizer to obtain fine mist on the inoculated plants. The check plants were sprayed with sterilized water only to serve as control. Plants were left under polyethylene bags for 24 hours then were removed and plants were kept under greenhouse conditions. Chocolate spot symptoms appeared 7 days after inoculation were scored as infection type and disease severity was assessed according to the 1 - 9 scale of Bernier *et al.* (1984).

$$\text{Disease severity \%} = \frac{(n \times v)}{9\,N} \times 100$$

Where:

(n)= Number of plants in each category.
(v)= Numerical values of symptoms category.
(N)= Total number of plants.
(9)= Maximum numerical value of symptom category.

Table (1): The infection type scale of faba bean chocolate spot disease (Bernier *et al.* 1984).

Disease rating	Host status	Description
1	Highly resistant	No disease symptoms, or very small, specks.
3	Resistant	Few small discrete lesions.
5	Moderately resistant	Some coalesced lesions with some defoliation.
7	Susceptible	Large coalesced sporulating lesions, 50% defoliation, some dead plants.
9	Highly susceptible	Extensive lesions on leaves, stems, and pods, sporulation, stem girdling, blackening and death of more than 80% of plants.

3. Factors affecting faba bean chocolate spot, wilt and root-rot pathogens in vitro:

a. Effect of natural plant extracts:

250 g of each of onion bulbs, garlic cloves and caraway seeds were mixed with 50 ml of sterilized water (El-Shami *et al.*, 1985). The mixture of each plant extract was blended and filtered using Seitz apparatus. The obtained stock solution of each plant extract was considered as 100% concentration. Dilutions of the plant extracts, *i.e.* 0, 25, 50, 75 and 100% were prepared. Three ml of any concentration of the tested plant extracts was poured in each sterilized Petri plate (90 mm Φ) then followed by PDA medium, plant extracts and PDA medium were gently mixed. The plates were inoculated with an equal disc (5 mm Φ) of each of the tested fungi then incubated at 28°C for *R. solani, F. oxysporum, F. moniliforme, F. solani, F. semitectum* and *V. albo-atrum* and at 20°C for *B. fabae*. Control plates were supplied with 5 ml of sterilized water only. Three replicates were used for each treatment. Linear growth of each one of the tested fungi was measured when the mycelial growth of the control treatment completely covered the surface of the medium.

b. Effect of antagonists on fungal linear growth of tested pathogens:

The antagonistic microorganisms such as *Trichoderma harzianum, T. koningii* and *Bacillus subtilis* which isolated previously from the phylloplane of faba bean leaves (El-Sayed, 2006) were tested for their antagonistic abilities. In this respect, Petri plates containing PDA medium were inoculated with a disc (5 mm Φ) taken from 7 day-old cultures of the pathogenic fungi taken from 10 day-old culture of *B. fabae*. The pathogenic fungi were inoculated at one side, whereas the opposite side was inoculated with either disc of each antagonistic fungus *T. harzianum* and *T. koningii* or with streaking for antagonistic bacterium *B. subtilis*. Plates only inoculated with pathogenic fungi at one side, 5 mm from the plate edge were kept as control. Three replicates were used for each treatment. Plates were incubated at 28°C *R. solani, F. oxysporum, F. moniliforme, F. solani, F. semitectum* and *Verticillium albo-atrum* and at 20°C for *B. fabae*. Linear growth of the tested fungi was measured when pathogenic fungi have completely covered the surface of the medium in the control treatment. The inhibition percent was calculated using the formula of Abd El-Moity, (1985) as follows:

$$\text{Reduction in linear growth (\%)} = \frac{R_1 - R_2}{R_1} \times 100$$

Where:

R_1 = the radius of control growth

R_2 = the radius of inhibited growth

c. Scanning electron microscope (SEM) of the interaction between *Trichoderma harzianum* and tested pathogens.

From the interaction sites between *T. harzianum* and *F. solani, F. oxysporum* and *Botrytis fabae* isolates, disc of 8 mm in diameter covered with *Trichoderma* hyphae and the pathogens were prepared for SEM examination. The samples were immersed in 5% glutaraldehyde in 0.1 M phosphate buffer pH 7.2, washed in the same phosphate buffer, dehydrated by their passage through graded

aqueous ethyl alcohol series (10, 30, 50, 75 and 95%) then placed in 100% ethanol at room temperature for few minutes. They were then dried with a critical point dryer unit mounted on aluminum stubs with silver glue and coated with gold-palladium using anion sputtering unit. The samples were then examined under scanning electron microscope, SEM unit at Fac. Agric., Ain-Shams University Manzali, *et al.* (1993).

d. Effect of some fungicides on wilt, root rot and chocolate spot pathogens:

Five fungicides namely; Benlate (Benomyl-50), Rizolex-T (Tolclofos methyl+thiram), Monceren (Pencycuron-25), Vitavax-T (carboxin -75 Thiram) and Primes (Triticonazole) were tested against root rot pathogens meanwhile some other fungicides, *i.e.* Benlate, Dithane-M45, Copper oxychloride-50 and Copper Acrobat, were tested against *Botrytis fabae* as chocolate spot pathogen. Different concentrations were made from each fungicide, *i.e.* 0, 5, 10, 25, 50, 100, 200 and 400 ppm. Five ml of each concentration from each fungicide was poured to Petri plates (90 mm Φ), then followed by PDA medium then Petri plates were gently mixed. Plates were then inoculated with tested pathogens and then incubated at 20°C in case of *Botrytis fabae* and at 28°C for the tested root rot pathogens. Three replicates were used for each concentration. Linear growth of tested fungi was measured when the pathogenic fungi completely covered control treatment by taking the average of two perpendicular diameters (in mm).

RESULTS

1- Isolation and identification of the causal organisms:

a. From infected roots:

Data in Table (2) indicate that seventy one isolate of different soil-borne fungi were isolated from wilted and rotten roots of faba bean plants (Giza-716 cv), showed root-rot and wilt symptoms, cultivated in three Egyptian governorates of north Delta, *i.e.* Menoufya, Gharbia and Kafr El-Sheikh. These isolates were identified as *Rhizoctonia solani* (18 isolate), *Verticillium albo-atrum* (15 isolates), *Fusarium oxysporum* (9 isolates), *Fusarium solani* (7 isolates), *Fusarium semitectum* (6 isolates) and *Fusarium moniliforme* (4 isolates) respectively. Meanwhile, 12 isolates were not identified from the three governorates. As for the frequency % of the isolated fungi in the three governorates, *R. solani* followed by *V. albo-atrum* were the most frequent fungi in the three governorates. Also, the highest number of isolated fungi were recorded in Menoufya followed by Kafr El-Sheikh and Gharbia, respectively.

b. From infected leaves:

Eight Botrytis isolates were isolated from faba bean leaves (Giza-716 cv), showed chocolate spot disease symptoms, collected from three different governorates in north Delta (as mentioned before). These isolates were identified as *Botrytis fabae* Sard. according to their morphological features.

Data in Table (2) indicate that *Botrytis fabae* was recorded in all tested governorates. The highest number of isolates was scored in Menoufya governorate (4 isolates) followed by Gharbia and Kafr El-Sheikh (2 isolates for each).

Table (2): Isolated fungi from rotten and wilted roots as well as from spotted leaves of faba bean and their frequency (%) in three different locations.

Fungal isolates	Gharbia		Kafr El-Sheikh		Menoufya		Total
Root-rot Fungi	*F	**F%	F	F%	F	F%	
Rhizoctonia solani	4	21.05	6	26.08	8	27.59	18
V. albo-atrum	4	21.05	5	21.74	6	20.69	15
F. oxysporum	3	15.79	2	8.70	4	13.79	9
F. solani	2	10.53	2	8.70	3	10.34	7
F. semitectum	1	5.26	2	8.70	3	10.34	6
F. moniliforme	1	5.26	1	4.30	2	6.89	4
Unknown fungi	4	21.05	5	21.74	3	10.34	12
Total	19		23		29		71
Chocolate spot							
B. fabae	2		2		4		8

*F= frequency number of isolated fungi **F% = frequency% of isolated fungi

2- Pathogenicity tests:

a- Wilt and root rot pathogens:

In this experiment, 18 isolates only of root isolates, which represent 6 different fungi were tested for their pathogenic potentialities (three isolates of the same fungus representing three different governorates).

Results presented in Table (3) reveal that all tested isolates could infect the roots of faba bean (Giza-716 cv) causing pre and post emergence damping off and thus reduced the survived plants. The highest pre- emergence damping-off infection % (20.0%) was recorded by *R. solani* (Isolate-3) isolated from Menoufya followed by *F. oxysporum* (Iso-2) and *F. solani* (Isolate-2) isolated from Kafr-El Sheikh then *F. moniliforme* (Isolate-3) and *V. albo-atrum* (Isolate-1) respectively. On the other hand, the isolates *F. moniliforme* (Isolates-1&2), *F. semitectum* isolates (Isolates-1&3) and *V. albo-atrum* isolate (Isolates-2) were not able to cause any pre- emergence damping-off infection %. As for post-infection%, *F. oxysporum* (Isolates2) followed by *R. solani* (Isolate-3), *F. solani* (Isolate-2), *F. moniliforme* (Isolate-3) and *V. albo-atrum* (Isolate-1) were the highest in this regard respectively. Also, all tested isolates without exception caused post-emergence damping-off ranging between 3.3 - 23.3%.

It is concluded from the results that the isolates *i.e., F. oxysporum* (Iso-2) followed by *R. solani* (Isolate-3), *F. solani* (Isolate-2), *F. moniliforme* (Isolate-3) and *V. albo-atrum* (Isolate-1) were the more virulent ones.

b- Chocolate spot pathogen:

Data in Table (3b) indicate that the eight tested isolates of *Botrytis fabae* were able to infect faba bean (cv. Giza-716) causing different levels of disease severity with significant differences among the eight tested isolates. In this respect, *B. fabae* isolated from Gharbia was the highest aggressive isolate compared with the other tested isolates, which gave 52.52% chocolate spot severity. Meanwhile, *B. fabae* isolated from Menoufya gave the lowest disease severity.

Table (3a): Pathogenicity test of 18 root rot and wilt fungi of isolates isolated from faba bean roots (cv. Giza-716) collected from three Governorates.

Isolated fungi	Isolation localities	*Pre-%	**Post%	***Sur-%
F. moniliforme (1)	Gharbia	0.0	3.3	96.7
F. moniliforme (2)	Kafr-El Sheikh	0.0	6.7	93.3
F. moniliforme (3)	Menoufya	10.0	13.3	76.7
F. semitectum (1)	Gharbia	0.0	3.3	96.7
F. semitectum (2)	Kafr-El Sheikh	10.0	6.7	83.3
F. semitectum (3)	Menoufya	0.0	3.3	96.7
F. solani (1)	Gharbia	3.3	6.7	90.0
F. solani (2)	Kafr-El Sheikh	13.3	16.7	70.0
F. solani (3)	Menoufya	6.7	10.0	83.3
F. oxysporum (1)	Gharbia	6.7	10.0	83.3
F. oxysporum (2)	Kafr-El Sheikh	16.7	23.3	60.0
F. oxysporum (3)	Menoufya	3.3	6.7	90.0
R. solani (1)	Gharbia	3.3	6.7	90.0
R. solani (2)	Kafr-El Sheikh	6.7	6.7	86.6
R. solani (3)	Menoufya	20.0	16.7	63.3
V. albo-atrum (1)	Gharbia	10.0	13.3	76.7
V. albo-atrum (2)	Kafr-El Sheikh	0.0	3.3	96.7
V. albo-atrum (3)	Menoufya	3.3	10.0	86.7
Control		0.0	0.0	100.0
L.S.D. 0.05		17.8	10.7	14.4

*Pre-%= Pre emergence damping off infection

**Post-% = Post emergence damping off infection

***Sur-% = Survived plants

Table (3b): Pathogenicity test of 8 *Botrytis fabae* isolates on faba bean (cv. Giza-716) under greenhouse conditions.

Botrytis isolates	Isolation localities	*D. S (%)
Botryits fabae (1)	Gharbia	52.52
Botryits fabae (2)	Gharbia	14.07
Botryits fabae (1)	Kafr El-Sheikh	5.18
Botryits fabae (2)	Kafr El-Sheikh	15.55
Botryits fabae (1)	Menoufya	2.22
Botryits fabae (2)	Menoufya	15.18
Botryits fabae (3)	Menoufya	21.74
Botryits fabae (4)	Menoufya	17.77
Control		3.37

*D.S (%) = Disease severity % according to **Bernier *et al.* (1993).**

3. Factors affecting faba bean chocolate spot and root-rot pathogens in vitro:

As mentioned before in the pathogenicity test experiments, six isolates from faba bean roots *i.e. Fusarium solani* (2), *Fusarium moniliforme* (3), *Rhizoctonia solani* (3), *Verticillium albo-atrum* (1), *F. semitectum* (2), *F. oxysporum* (2) as well as one isolate of *Botrytis fabae* of Gharbia Governorate were chosen to complete the further studies based on their pathogenic abilities.

a. Effect of natural plant extracts:

Data in Table (4) indicate that garlic extract inhibited linear growth of *F. solani*, *F. moniliforme*, *R. solani*, *F. semitectum*, *F. oxysporum* and *B. fabae* at all tested concentrations with the exception of the concentration 25% while, *V. albo-atrum* was the lowest affected one. On the other hand, onion extract inhibited the linear growth of *F. solani*, *V. albo-atrum*, *F. oxysporum* and *F. semitectum* at concentration rates 75, 75, 100 and 100%, respectively, while it gradually reduced linear growth of remained fungi. Caraway extract inhibited the linear growth of *V. albo-atrum*, *F. oxysporum* and *Botrytis fabae* at concentration rate 100% for the three fungi. Generally, all plant extracts used were significantly reduced linear growth of all tested fungi. However, variations among the tested plant extracts against the examined fungi were recorded. Garlic extract was the most effective plant extract followed by onion and caraway. The most sensitive fungi to garlic was *F. semitectum* and *F. oxysporum*, but the most sensitive fungi with onion extract were *F. solani*, *F. oxysporum* and *V. albo-atrum*, while the most sensitive fungus to caraway extract was *B. fabae*.

b. Effect of antagonists on fungal linear growth of tested pathogens:

Data in Table (5) reveal that the tested antagonists have significantly reduced linear growth of all tested fungi compared to control. In general, *T. koningii* and *T. harzianum* were the most effective bio-agents followed by *B. subtilis* where they recorded the highest percentages of reduction. Also, all tested pathogenic fungi varied clearly in their reaction to *T. harzianum*, where the highest reduction% was recorded with *F. oxysporum*, *V. albo-atrum* and *F. solani*, respectively. Meanwhile, the lowest reduction% was recorded with *F. semitectum*. On the other hand, *T. koningii* was different in its reaction with the tested pathogenic fungi, where the highest reduction% was recorded with *F. semitectum*, *R. solani*, *F. moniliforme*, *F. oxysporum* and *V. albo-atrum*, respectively. Also, using *B. subtilis* as a natural antagonist was not highly effective in reducing the growth of tested pathogenic fungi comparing with *T. harzianum* and *T. koningii* where it reduced the growth to levels below 50% except with *V. albo-atrum* (52.6%). As for, *B. fabae*, all tested antagonists reduced the growth of the fungus. In this respect, *T. harzianum* and *T. koningii* were the best while *B. subtilis* was the least effective one.

c. Scanning electron microscope (SEM) of the interaction between *Trichoderma harzianum* and tested pathogens.

The illustrated results in Fig. (1 a,b & c) show that scanning electron microscope (SEM) is a very useful research tool for examining the interaction sites between *Trichoderma harzianum* and the pathogenic fungi *i.e. F. oxysporum*, *F. solani* and *Botrytis fabae* which, infecting faba bean plants. In Fig.

(1a) *Trichoderma* hyphae coiled around the host hyphae of *F. oxysporum* consisting different types of parasitism in form of coiled hyphae (shape 1), pincer hyphae (shape 2) and ring hyphae (shape 3). Meanwhile, Fig. (1b) illustrate the parasitism of *T. harzianum* on *F. solani* where it coiled around the host hyphae consisting of coiled hyphae (shape 1), hook and pincer shaped hyphal branches (shape 2) and hooked parallel hyphae looking for penetration or coiling sites (shape 3).On the other hand, Fig. (1c) illustrate the parasitism of *T. harzianum* on *Botrytis fabae* in form of adhesive hyphae which penetrate and degrade the host hyphae as well as appresorium-like bodies (shape 1), coiled shaped hyphae around the hyphal host (shape 2) and parallel hyphae which penetrate the mycelium and spores of *Botrytis* (shape 3).

Table (4): Effect of natural plant extracts at different concentrations on linear growth (mm) then tested root and chocolate spot pathogens.

Tested isolates	Average linear growth (mm) on media contained different concentrations (%) of prepared extracts				
	Garlic				
	0	25	50	75	100
R. solani	90.0	26.6	00.0	00.0	00.0
F. solani	90.0	37.3	00.0	00.0	00.0
V. albo-atrum	90.0	45.0	30.7	16.7	00.0
F. oxysporum	90.0	00.0	00.0	00.0	00.0
F.moniliforme	90.0	18.3	00.0	00.0	00.0
F. semitectum	90.0	00.0	00.0	00.0	00.0
Botrytis fabae	90.0	46.7	24.7	00.0	00.0
L.S.D. at 1%	3.53				
	Onion				
R. solani	90.0	60.7	53.3	30.0	8.30
F. solani	90.0	41.7	31.7	00.0	00.0
V. albo-atrum	90.0	41.7	33.3	18.3	00.0
F. oxysporum	90.0	36.7	30.0	00.0	00.0
F.moniliforme	90.0	51.7	40.0	30.0	16.6
F. semitectum	90.0	62.3	47.7	37.3	00.0
Botrytis fabae	90.0	75.0	54.7	30.7	3.3
	5.49				
	Caraway				
R. solani	90.0	65.0	43.3	35.0	18.3
F. solani	90.0	60.0	48.3	41.7	23.3
V. albo-atrum	90.0	63.3	51.7	40.0	00.0
F. oxysporum	90.0	90.0	47.3	38.3	00.0
F.moniliforme	90.0	71.7	61.7	45.0	33.3
F. semitectum	90.0	61.7	47.7	40.7	21.7
Botrytis fabae	90.0	90.0	38.3	00.0	00.0
L.S.D. at 1%	6.30				

Table (5): Effect of the tested antagonists on linear growth of faba bean root and chocolate spot pathogens.

Tested pathogens	% Reduction in linear growth of root pathogens		
	T. harzianum	*T. koningii*	*B. subtilis*
R. solani	70.0	81.1	43.3
V. albo-atrum	78.1	75.9	52.6
F. oxysporum	79.6	76.6	34.1
F. solani	76.3	73.0	36.3
F. semitectum	64.1	86.3	30.7
F. moniliforme	74.8	77.2	47.8
B. fabae	76.7	64.8	33.7

LSD at 1% for Interaction FxA = 8.74

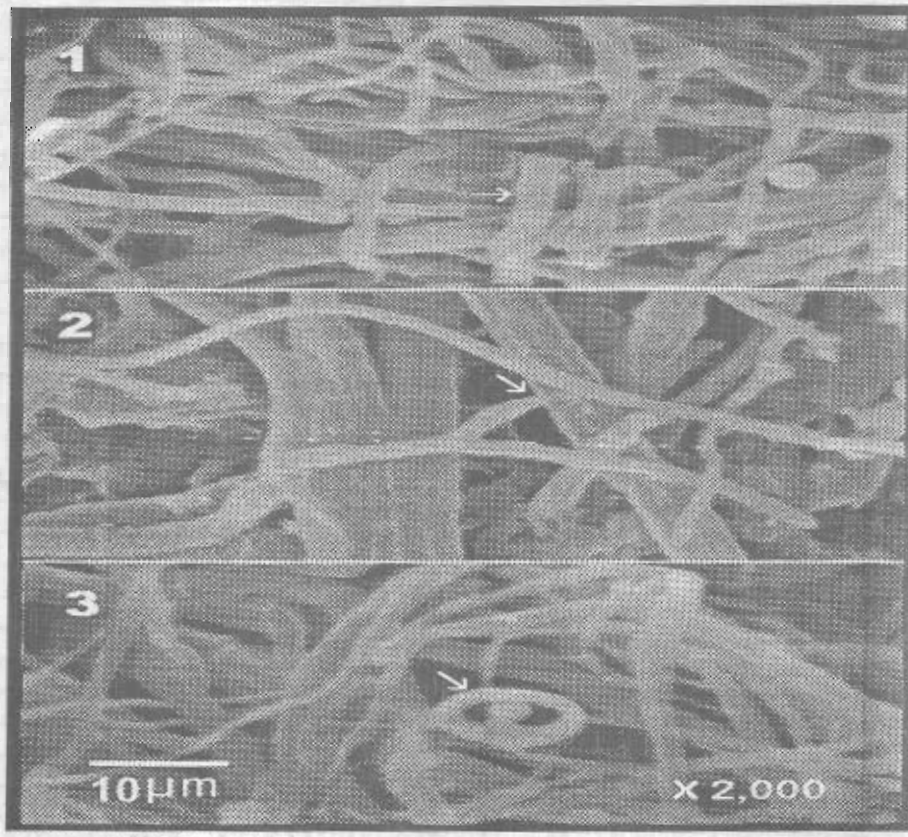

Fig. (1a): Scanning electron micrograph showing different types of *Trichoderma* parasitism on *F. oxysporym* hyphae (2000X).
1-*Trichoderma* hyphae coiled around the host hyphae of *F. oxysporum*.
2- Pincer shape hyphae of *Trichoderma* around the host hyphae.
3- Ring shape hyphae of *Trichoderma*.

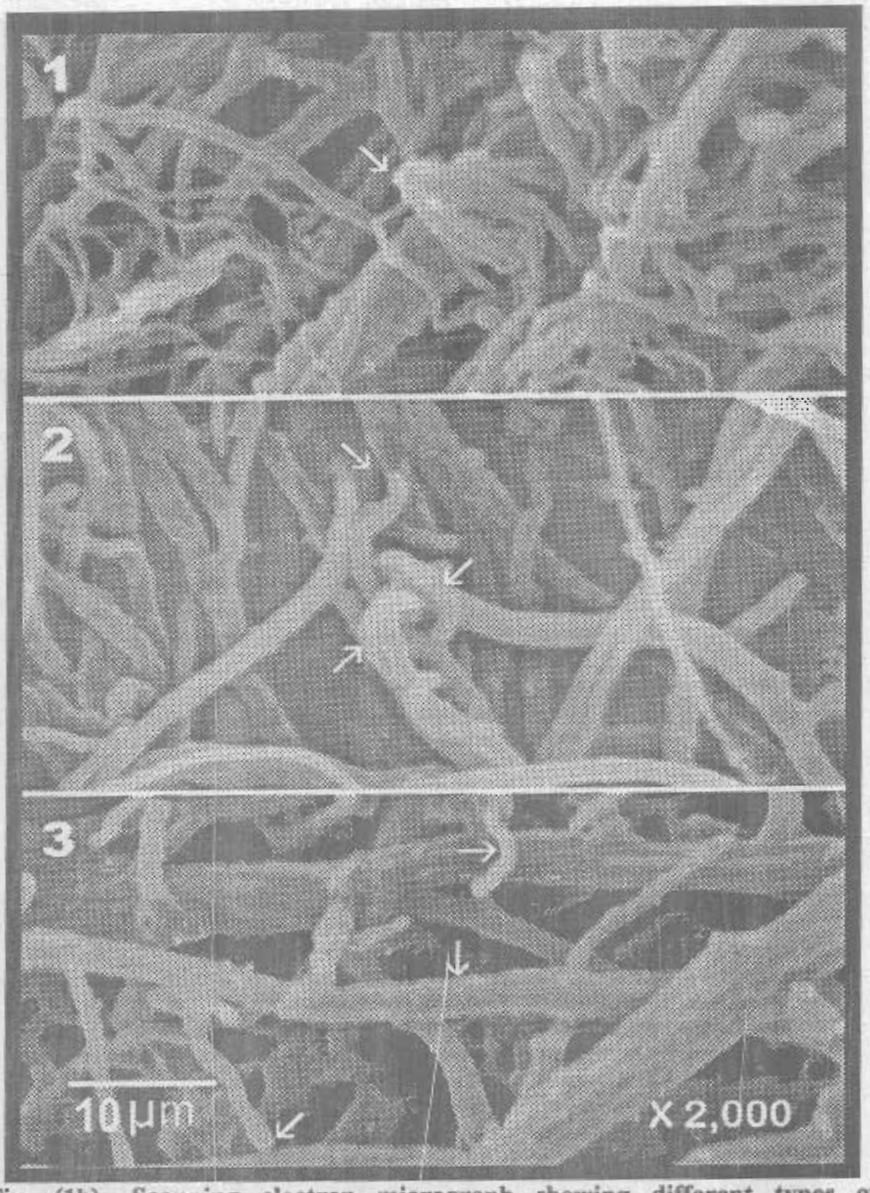

Fig. (1b): Scanning electron micrograph showing different types of *Trichoderma* parasitism on *F. solani* hyphae (2000X).

1- *Trichoderma* hyphae coiled around the host hyphae of *F. solani*.

2- Formation of hook and pincer shape from hyphal branches of *Trichoderma* around the host hyphae.

3- Hooked parallel hyphae of *Trichoderma* which looking for penetration or coiling sites.

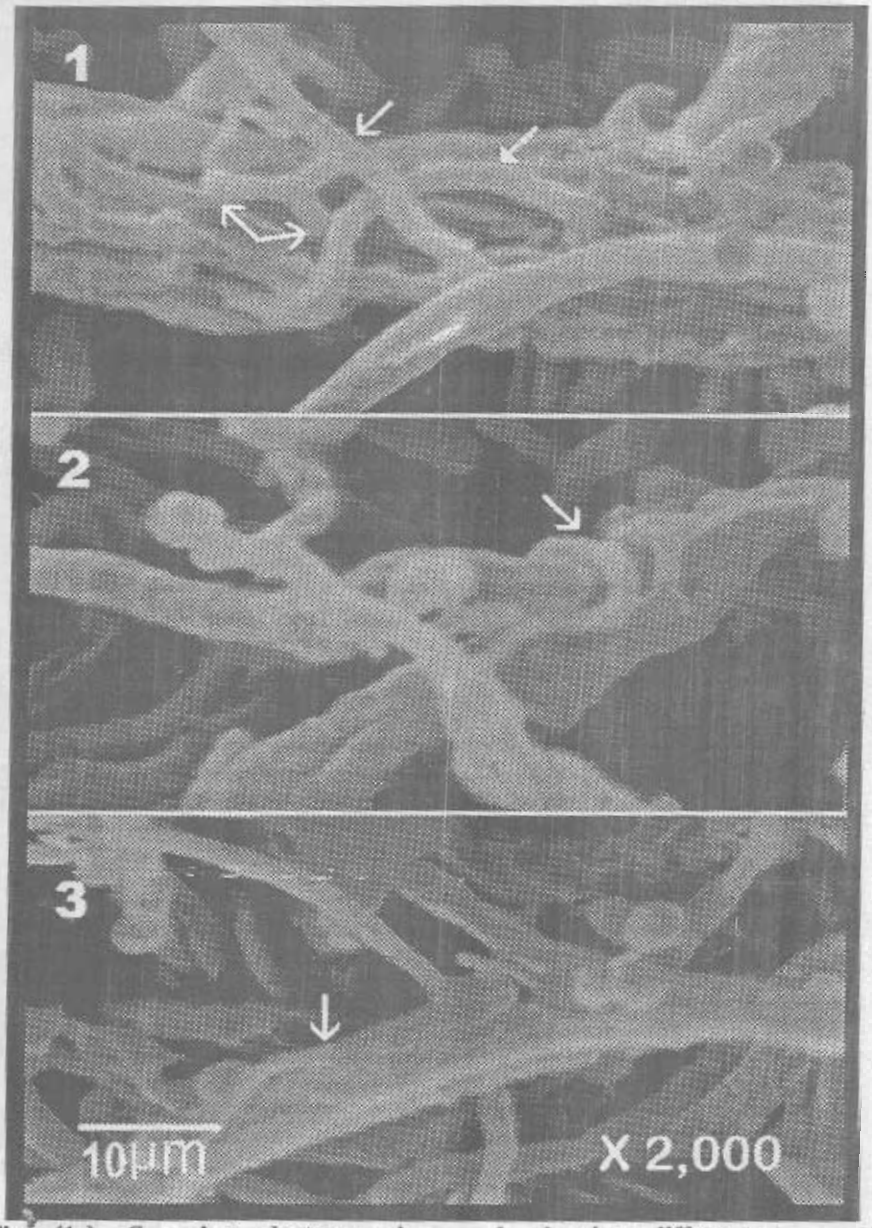

Fig. (1c): Scanning electron micrograph showing different types of *Trichoderma* parasitism on *B. fabae* hyphae (2000X).

1- Adhesive hyphae of *Trichoderma* as well as its appresorium-like bodies

2- *Trichoderma* hyphae coiled around the host hyphae of *B. fabae*.

3- Parallel hyphae which penetrate the mycelium and spores of *Botrytis*.

d. Effect of some fungicides on root rot and chocolate spot pathogens:

Data in Table (6a, b,c,d,e & f) revealed a significant reduction of fungal linear growth as a result of using the examined fungicides. Moreover, increasing concentrations of tested fungicides decreased gradually the fungal linear growth of the tested pathogenic isolates to reach its maximum reduction with the higher concentration used. In this respect, at 400 ppm concentration for any fungicide, no growth was recorded for all tested fungi. It is pronounced also that there were variations among the fungicides in their efficacy on the tested pathogenic fungi where Benlate was the most effective fungicide in reducing fungal linear growth compared with any other fungicides tested at any corresponding concentration. All pathogenic fungi were sensitive to Benlate, while, *V. albo-atrum* was sensitive to Vitavax-200, meanwhile, *F. moniliforme* was sensitive to Rizolex-T and Monceren.

Table (6a): Effect of different fungicides on growth of *R. solani.*

Fungicides	Linear growth (mm) at different concentrations (ppm)							
	0	10	25	50	100	200	400	Mean
Vitavax-200	90	90	90	77	13	0	0	52
Monceren	90	90	90	77	38	0	0	55
Rizolex-T	90	90	79	53	50	26	0	56
Primes	90	90	83	67	61	52	0	63
Benlate	90	90	75	43	30	18	0	49
Mean	90	90	83.4	63.4	33.6	24	0	

LSD at 1% for	Fungicides (A)	Concentration (B)	Interaction (AB)
	1.46	2.16	4.82

Table (6b): Effect of different fungicides on growth of *F. solani.*

Fungicides	Linear growth (mm) at different concentrations (ppm)							
	0	10	25	50	100	200	400	Mean
Vitavax-200	90	90	84	71	32	22	0	56
Monceren	90	90	81	72	53	32	0	60
Rizolex-T	90	90	83	57	32	21	0	53
Primes	90	88	79	68	51	38	0	59
Benlate	90	72	49	28	18	10	0	38
Mean	90	86	75	59	37	25	0	

LSD at 1% for	Fungicides (A)	Concentration (B)	Interaction (AB)
	1.28	1.80	4.06

Table (6c): Effect of different fungicides on growth of *F. moniliforme*.

Fungicides	Linear growth (mm) at different concentrations (ppm)							
	0	10	25	50	100	200	400	Mean
Vitavax-200	90	90	78	67	42	16	0	55
Monceren	90	87	59	24	20	12	0	42
Rizolex-T	90	85	65	27	21	0	0	41
Primes	90	88	82	62	51	38	0	59
Benlate	90	52	32	0	0	0	0	25
Mean	90	81	63	36	26	13	0	

LSD at 1% for	Fungicides (A)	Concentration (B)	Interaction (AB)
	2.80	1.99	4.44

Table (6d): Effect of different fungicides on growth of *F. oxysporum*.

Fungicides	Linear growth (mm) at different concentrations (ppm)							
	0	10	25	50	100	200	400	Mean
Vitavax-200	90	84	80	45	0	0	0	43
Monceren	90	90	80	70	49	0	0	54
Rizolex-T	90	90	82	67	53	17	0	57
Primes	90	86	71	63	51	42	0	58
Benlate	90	78	52	0	0	0	0	31
Mean	90	86	73	49	27	12	0	

LSD at 1% for	Fungicides (A)	Concentration (B)	Interaction (AB)
	1.59	1.76	3.93

Table (6e): Effect of different fungicides on growth of *F. semitectum*.

Fungicides	Linear growth (mm) at different concentrations (ppm)							
	0	10	25	50	100	200	400	Mean
Vitavax-200	90	90	87	60	43	21	0	56
Monceren	90	90	90	58	73	28	0	61
Rizolex-T	90	90	81	28	42	22	0	51
Primes	90	88	81	72	62	46	0	63
Benlate	90	86	81	66	51	36	0	59
Mean	90	88	84	57	54	31	0	

LSD at 1% for	Fungicides (A)	Concentration (B)	Interaction (AB)
	2.05	2.24	5.01

Table (6f): Effect of different fungicides on growth of *V. albo-atrum*.

Fungicides	Linear growth (mm) at different concentrations (ppm)							
	0	10	25	50	100	200	400	Mean
Vitavax-200	90	86	69	51	31	17	0	49
Monceren	90	90	82	57	37	21	0	54
Rizolex-T	90	90	81	28	42	22	0	50
Primes	90	88	81	72	62	46	0	58
Benlate	90	60	42	32	18	10	0	36
Mean	90	83	71	48	38	23	0	

LSD at 1% for	Fungicides (A)	Concentration (B)	Interaction (AB)
	2.44	2.60	5.70

As for the effect of four different fungicides against *Botrytis fabae* growth, the results in Table (6g) reveal that all tested fungicides at all concentrations affect significantly the growth of *B. fabae* comparing to check treatment. Moreover, increasing the fungicide concentration levels from 0 to 400ppm increased gradually the reduction of linear growth. On the other side, Dithane-M45 was the best effective fungicide followed by Benlate and Copper Acrobat respectively in reducing fungal growth *B. fabae* while, Copper Oxychloride was the lowest one in this respect.

Table (6g): Effect of different fungicides on growth of *B. fabae*.

Fungicides	Linear growth (mm) at different concentrations (ppm)							
	0	10	25	50	100	200	400	Mean
Benlate	90	33	7	0	0	0	0	18.6
Copper oxychloride	90	83	58	42	12	0	0	40.7
Dithane-M45	90	26	0	0	0	0	0	16.6
Copper Acrobat	90	53	36	3	0	0	0	25.6
Mean	90	48.8	25.3	11.3	3.0	0	0	

LSD at 1% for	Fungicides (A)	Concentration (B)	Interaction (AB)
	3.1	2.7	5.4

DISCUSSION

Faba bean (*Vicia faba* L.) is one of the most important legume crops. It is infected with many fungal pathogens causing considerable yield losses where 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 71 isolate of different soil-borne fungi were isolated from roots of faba bean plants showed root-rot and wilt symptoms of cv. Giza-716 cultivated in the three Egyptian governorates of north Delta, i.e. Minufiya, Gharbia and Kafr

El-Sheikh. These isolates were identified as *Rhizoctonia solani, Verticillium albo-atrum, Fusarium oxysporum, F. solani, F. semitectum* and *F. moniliforme*. *R. solani* followed by *V. albo-atrum* were the most frequent fungi in the three governorates. The highest number of isolated fungi was recorded in Menoufya governorate followed by Kafr El-Sheikh and Gharbia governorates, respectively. These results are similar to those obtained by Sepúlveda (1991), El-Morsy *et al.* (1997), Akem and Bellar (1999) and Hugar (2004) isolated *Fusarium oxysporum* and *F. solani* f.sp. *fabae*, *Rhizoctonia solani*, *F. oxysporum* f.sp. *fabae*, *Fusarium oxysporum* and *Macrophomina phaseolina* from wilted and rotten roots of faba bean in different parts of the world as well as considered them the most important and widespread fungal diseases observed at all locations. On the other hand, eight *Botrytis* isolates were isolated from faba bean leaves (Giza-716 cv) showing chocolate spot symptoms in the some North Delta governorates. These isolates were identified as *Botrytis fabae* Sard. *B. fabae* was recorded in all inspected governorates and scored the highest number in Menoufya governorate followed by Gharbia and Kafr El-Sheikh governorates, respectively. These results are in harmony with the findings of Mohamed *et al.* (1986) and Morsy (2000) who isolated 4 isolates of *B. cinerea* and of 3 *B. fabae* from faba bean fields of Beheira, Kafr El-Sheikh and Sharkia governorates. He added that *B. fabae* isolated from Behera governorate was the most virulent. Moreover, Daboor (2001) isolated 7 isolates of *Botrytis* spp. from different growing areas of faba bean in Egypt, of which 4 isolates were of *B. cinerea* and 3 of *B. fabae*. Furthermore, Abo-Bakr (2002) isolated *B. fabae* and *B. cinerea* from different faba bean growing areas in Egypt and found that *B. fabae* isolates were the more aggressive than *B. cinerea*. Also, Bretag and Raynes (2004) reported that chocolate spot, caused by *B. fabae* and *B. cinerea* is the most important disease of faba beans in Victoria and South Australia. In addition, El-Gammal, (2005) indicated that the highest frequency of *B. fabae* was scored in Menoufya and Kafr- El Seikh governorates during season 1998/1999.

Pathogenicity test of 18 isolates of root isolates, (represent 6 different fungi of the three governorates) revealed that all tested isolates could infect the roots of faba bean (Giza-716 cv) causing pre and post emergence damping-off and reduced the survived plants. The isolates of *F. oxysporum* (Isolate-2) followed by *R. solani* (Isolate -3), *F. solani* (Isolate-2), *F. moniliforme* (Isolate-3) and *V. albo-atrum* (Isolate-1) were the most virulent ones. These findings are in agreement with those obtained by Omar (1986) and Simay (1992). In this respect also, Metwaly (2004) found that *R. solani* isolated from Kafer El-Sheikh governorate was the most virulent isolate than that of Sharkia governorate. Also, the results indicated that the eight tested isolates of *Botrytis fabae* were able to infect faba bean plants (cv. Giza-716) causing different levels of disease severity with significant differences among the eight tested isolates. *B. fabae* (Isolate-1) that isolated from Gharbia governorate was the highest pathogenic. In this respect, Wang and Chai (2000), Abo-Baker (2002) confirmed the virulent of *B. fabae* more than *B. cinerea* and its role in restricting development of faba bean in China and Egypt. Moreover, Metwaly (2004) found that *B. fabae* isolates of Beheira governorate were the most aggressive isolates, followed by isolates of Menoufya and Domiat governorates, respectively.

As for the factors affecting faba bean damping-off and chocolate spot diseases, *in vitro* studies, the results indicated that all the tested plant extracts have significantly reduced the linear growth of all the tested fungi. However, variations among the tested plant extracts against the examined fungi were recorded. Garlic extract was the most effective plant extract followed by onion and caraway. Meanwhile, the most sensitive fungus to garlic was *Fusarium solani* and *F. moniliforme*, but the most sensitive fungus with onion extract were *F. solani*, and *V. albo-atrum*, while the most sensitive fungus to caraway extract was *B. fabae*. These findings could be interpret in light of the results of Michoil and El-Khateeb (1985) and Gaafar *et al.* (1989) who found that garlic extract gave the best results in controlling both damping-off and root rot diseases with superiority of garlic extract effect over onion extract. This mentioned power of garlic extract in inhibition the growth development of the tested pathogenic fungi or decreasing the mortality of diseases of faba bean plants might be attributed to volatile organic compounds consisting of linear chain aldehydes, allyl sulfides and disulfides as mentioned by Binchi *et al.* (1997). Also, Heweidy and Mohamed, (1997) found that crude extracts of garlic cloves (*Allium sativum* L.) and henna leaves (*Lawsonia inermis* L.) *in vitro* reduced mycelial growth and spore germination of *B. fabae* Sard,. While, El Gindy (2003) verified that aniseed and caraway juices at different concentrations have significantly inhibited the growth of *Botrytis fabae* but coriander and fenugreek had the best effect in this respect.

On the other hand, using antagonistic bio-agents *in vitro* reduced significantly the linear growth of all examined fungi, where *T. koningii* and *T. harzianum* were the most effective bio-agents followed by *B. subtilis* Also, there were variations among the tested fungi in relation to their reaction to *T. harzianum*, where the highest reduction% was recorded with *F. oxysporum* followed by *V. albo-atrum* and *F. solani* respectively. As for, *B. fabae*, all the tested antagonists reduced the growth of the fungus. In this respect, *T. harzianum* and *T. koningii* were the best in this regard, while *B. subtilis* was the least effective one. On the other hand, scanning electron microscope (SEM) of the interaction sites between *Trichoderma harzianum* and the pathogenic fungi illustrated the antagonistic behavior, where *T. harzianum* coiled around the host hyphae of the tested pathogenic fungi consisting different types of parasitism in form of coiled hyphae, pincer shaped hyphae, hook and pincer shaped hyphal branches, hooked parallel hyphae looking for penetration, ring hyphae and adhesive shaped hyphae as well as appressorium-like bodies as cleared in cases of *R. solani*, *F. oxysporum* and *B. fabae*. These results are in harmony with the findings of Mathew and Gupta (1998) and El Gindy (2003), where they verified the positive role of *Trichoderma* spp and *Bacillus* spp. in controlling wilt, root rot and chocolate spot infection and their pathogens *in vitro* and *in vivo* as well as their positive effect on plant growth characters. In this respect, of Mahmoud *et al.* (2004), El-Gammal (2005) and Ibrahim, (2005) verified 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* isolate. On the other hand, the antagonistic forms which appeared in the interaction between *T. harzianum* with *R. solani*, *F. oxysporum* or *B. fabae* in this

study are in similar in most cases to those obtained by El-Habbaa (1997) who studied the parasitism of *Trichoderma* on *B. cinerea.*

Concerning the effect of fungicides, *in vitro* results indicated that increasing concentrations of the tested fungicides decreased gradually the fungal linear growth of the tested pathogenic isolates. Benlate was the most effective fungicide in reducing fungal linear growth compared with the other fungicides at any corresponding concentration. All pathogenic fungi were sensitive to Benlate, while, *V. albo-atrum* was sensitive to Vitavax 200, while, *F. moniliforme* was sensitive to Rizolex-T and Monceren. On the other hand, all the tested fungicides at all the tested concentrations affected significantly the growth of *B. fabae,* where Dithane-M45 was the most effective fungicide followed by Benlate and Copper Acrobat respectively. These results are in harmony with those of El-Fiki (1994) who recorded that treating seeds of *Vicia faba* with Vitavax-T, Quinolate V_4X or Rizolex decreased significantly pre- and post-emergence damping-off while, spraying the faba plants with Benlate + chlorothalonil mixture was the best for controlling chocolate spot disease (*Botrytis fabae*). Therefore, El-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-101 when used for controlling *B. fabae* on faba bean plants (Giza-40).

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كفاءة إستخدام عوامل التضاد الحيوى والمستخلصات النباتية الطبيعية والمبيدات الفطرية فى مقاومة المسببات المرضية للذبول وأعفان الجذور والتبقع الشيكولاتى على الفول البلدى تحت ظروف المعمل

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عزلت ٧١ عزلة (تمثل مختلف الفطريات القاطنة للتربة) من نباتات الفول البلدي صنف جيزة-٧١٦ مصابة بالذبول وأعفان الجذور من ثلاث محافظات في شمال ووسط الدلتا هي المنوفية والغربية وكفر الشيخ. وقد عرفت تلك العزلات على أنها رايزوكتونيا سولاني ، فيرتيسليوم البواترام ، فيوزاريوم أوكسيسبورام ، فيوزاريوم سولاني ، فيوزاريوم سيميتيكتام و فيوزاريوم مونيليفورم على التوالي. وكان فطر رايزوكتونيا سولاني أكثر الفطريات المعزولة تكرارا تلاه في ذلك فطر فيرتيسليوم البواترام في الثلاث محافظات. وقد سجل أكبر عدد من تلك الفطريات في محافظة المنوفية ثم محافظة كفر الشيخ و كان أقلها في محافظة الغربية على التوالي. عزل ايضا ثماني عزلات من فطر بوترايتس من أوراق الفول البلدي (صنف جيزة ٧١٦) تبدي الأعراض المميزة لمرض التبقع الشيكولاتي من ثلاث محافظات بشمال ووسط الدلتا عرفت على أنها لفطر بوترايتس فابى وكانت أكثر العزلات عدداً من محافظة المنوفية يليها الغربية ثم كفر الشيخ على التوالي. أظهر إختبار القدرة الإمراضية لـ ١٨ عزلة من الفطريات المسببة للذبول وعفن الجذور والتي تتبع ٦ أجناس فطرية مختلفة تم عزلها من الثلاث محافظات أن جميع العزلات يمكنها أن تصيب جذور نبات

الفول البلدي صنف جيزة ٧١٦ مسببة موت للبادرات سواء قبل أو بعد الظهور فوق سطح التربة مما أثر بالانخفاض على نسبة النباتات المتبقية. وقد أظهرت عزلات الفطريات فيوزاريوم أوكسيسبورام (٢) يليها رايزوكتونيا سولاني (٣) و فيوزاريوم سولاني (٢) و فيوزاريوم مونيليفورم (٣) ثم فيرتيسليوم ألبوأترم (١) ضراوة عن بقية العزلات الأخرى حيث تسببت في حدوث إصابة كلية (موت للبادرات قبل وبعد الظهور فوق سطح التربة) كما قللت بشدة نسبة النباتات الحية المتبقية. كما أشارت النتائج أن كل الثماني عزلات لفطر بوترايتس فابى كانت قادرة على إصابة الفول البلدي صنف جيزة ٧١٦ مسببة إصابة بدرجات مختلفة من الشدة بفروق معنوية بين الثمانية عزلات المختبرة. وكانت العزلة رقم واحد لفطر بوترايتس فابى والمعزولة من محافظة الغربية من أشد العزلات إحداثاً لمرض التبقع البني (الشيكولاتي) في الفول.

وأشارت النتائج إلى أن مستخلصات جميع النباتات المختبرة (ثوم – بصل – كراوية) قد قللت وبدرجة ملحوظة من نمو جميع الفطريات موضع الدراسة. وكان مستخلص الثوم من أكثر المستخلصات المختبرة فعالية يليه مستخلص البصل ثم الكراوية. كما أدى استخدام الفطريات المضادة إلى تقليل نمو الفطريات الممرضة وكان الفطران ترايكودرما كونينجاي وترايكودرما هارزيانم أكثر فعالية يليهما بكتريا باسيلس سبتلس. كما أوضحت الصور الملتقطة بالميكروسكوب الإلكتروني الماسح وجود تداخل بين فطر ترايكودرما هارزيانم والفطريات الممرضة فيوزاريوم سولاني وفيوزاريوم أوكسيسبورام وبوترايتس فابى حيث قام الفطر المضاد ترايكودرما هارزيانم بتكوين حلقات خيطية تلتف حول خيوط الفطريات الممرضة بأشكال مختلفة منها ما يشبه الخطاف أو الكماشة إلى جانب الهيفا الملتصقة والممصات التي أرسلتها داخل خيوط الفطريات الممرضة فيوزاريوم سولاني وفيوزاريوم أوكسيسبورام وبوترايتس فابى. وقد لوحظ أن التزايد التدريجي في تركيزات المبيدات الفطرية المستخدمة يصحبه تناقص تدريجي في النمو الخطي للفطريات الممرضة موضع الدراسة. وفي حين أظهرت جميع الفطريات الممرضة حساسية للمبيد بنليت أظهر فطر فرتسليوم ألبو أترام حساسية للمبيد فيتافاكس ثيرام ، أما فطر فيوزاريوم مونيليفورم فقد كان حساسا لمبيدات رايزوليكس-ت ومونسيرين. ومن ناحية أخرى كانت جميع المبيدات الفطرية المستخدمة فعالة ضد فطر بوترايتس فابى إلا أن مبيد الدايثان-م٤٥ تفوق على غيره في التأثير على نمو الفطر يليه في ذلك البنليت وأكروبات النحاس.