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EFFICACY OF ANTAGONISTIC FUNGAL AND BACTERIAL BIOAGENTS AGAINST FABA BEAN DAMPING-OFF DISEASE

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ABSTRACT: Faba bean damping-off caused by *Rhizoctonia solani* Kühn and *Fusarium solani* Mart are considered the most destructive diseases. All tested pathogenic fungal isolates of *F. solani* and *R. solani* that have been collected from various locations at nine governorates were clearly varied in their virulence onto faba bean plants (cv. Giza-429) under greenhouse conditions. *F. solani* El-Nubaria (Fs_4) and *R. solani* El-Menia (R_{20}) were the most virulent isolates. To evaluate the genetic diversity of the most aggressive isolates of *R. solani* and *F. solani* (9 isolates for each one), were investigated using random amplified polymorphic DNA (RAPD) technique. The most effective antagonistic bacterial and fungal isolates were identified by testing the ability of these isolates to utilize different carbon sources and amino acids using Biolog-System Technique. All tested bioagents (*Trichodema hamatum*$_{-2}$, *T. harzianum*, *Bacillus subtilis*-$_1$ and *B. mylotquefaciens*$_{-1}$), culture filtrates, biocides (Bio-Arc and Bio-Zeid) and chemical fungicides (Rizolex-T, Vitavax-200 and Moncut) significantly reduced the percentages of damping-off and increased the percentage of survived plant compared with control. The most effective treatments for reducing these diseases were obtained by applying fungicides followed by both of active bioagents and biocides. Meanwhile, culture filtrates of bioagents were the least effective treatments. Rizolex-T and Moncut were the most effective fungicides against *F. solani* and *R. solani*.

Key words: Faba bean, damping-off, biolog system, biological control, biocides.

INTRODUCTION

Faba bean (*Vicia faba* L.) is considered as one of the most important legume crops in Egypt. The economic importance of faba bean cultivation is due to its richness in protein (28%), carbohydrates (56%), vitamins (B and C), minerals (iron, zinc, and calcium) and some other components (**Abdel-Monaim, 2013**). Thus, it is a valuable source of food for human and feed for animal. In addition, faba bean helps in improving soil fertility through nitrogen fixation **(Elgilany *et al*., 2007).** Therefore, improving the productivity of this crop is one of the priorities in agricultural policy in many countries. Faba bean is subjected to attack by several foliar and soil borne diseases **(Eisa *et al*. 2006; Abd-El-Kader *et al*., 2015)**, during its various growth stages from seedling to maturity.

Soil borne fungal pathogens, *i.e*. *Fusarium solani*. and *Rhizoctonia solani* are considered the most important pathogens, involved in damping-off and root-rot of faba bean which reduced yield qualitatively and quantitatively **(Al-Abdalall, 2010 ; Habtegebriel and Boydom, 2016)**.

Molecular characterization by Random Amplified Polymorphic DNA (RAPD) technique have been used as a tool in genetic mapping, molecular taxonomy, genetic diversity and diagnosis of several pathogenic fungi such as *Fusarium* spp. and *R. solani* (**El-Fadly *et al*., 2008; Indira *et al*., 2011; Mohamed, 2015**). RAPD, analysis may be useful in studying

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forma specials, races of *F. oxysporum* and quantification of genomic DNA of *R. solani* in soil and plant (A**bd-El-Salam *et al.*, 2003 and 2007**).

The bacterial and fungal isolates were characterized according to their ability to utilize different carbon sources and amino acids (**Bochner, 2003; Seidl *et al.,* 2006**). Different control methods have been used for controlling soil borne diseases. Fungicides are widely used as seed or soil treatment to depress various root-rot and wilt diseases (**Bahaa El-Din, 2005; Emhemed, 2015).** Rhizolex-T, Vitvax-200, Benlate and Topsin-m70 were the most effective treatments for controlling *R. solani, F.oxysporum, F. solani, Sclerotium rolfsii* and *F. moniliforme* **(Eisa *et al*., 2006)**.

Biological control is one of the most promising and safe measure in field of plant protection **(Abou-Zeid *et al*., 2002; Mokhtar *et al*., 2011)**. Antagonistic microorganisms as an alternative approach to fungicides and as biocontrol agents, which produce a wide variety of antifungal compounds play a major role in controlling many soil-borne fungal diseases **(Abd-El-Kader *et al*., 2015).** *Trichoderma* spp. and *Bacillus* spp. are recognized primarily as an antagonist against pathogenic fungi, it also has been documented as effective biological control agents of root-rot and wilt diseases of crop plants caused by *R. solani*, *Fusarium* spp. and *Sclerotium rolfsii* **(Mclean *et al*., 2004 ; Dubey *et al*., 2007).**

Plants treated with *Trichoderma* spp. and *Bacillus* spp. can produce extracellular antibiotics metabolites or enzymes (proteases, chitinases and glucanases), phytotoxic substances, deposition of callose-enriched wall appositions and pathogenesis-related proteins on the inner surface of cell walls **(Harman, 2006 ; Fernando *et al.*, 2007)**. Moreover, some strains might enhance plant growth and development (**Howell *et al*., 2000**), induce systemic and localized resistance in plants, and suppression several plant pathogens (**El-Hassan *et al.,* 2004** and **Abd-El-Khair *et al.,* 2011**). The pathogenic fungi varied in their reactions against enzymes and toxic substances that produced by different antagonists **(Rajeev and Mukhopadhyay, 2001)**.

The present work was carried out for identification the isolated pathogens involved in damping off and root-rot of faba bean and differentiation between them based on pathogenicity test and molecular characterization techniques. Evaluation of the most effective bioagents, culture filtrates, biocides, and chemical fungicides in controlling damping off disease under greenhouse condition. The most effective antagonistic isolates of fungi and bacteria were identified using biolog system technique.

MATERIALS AND METHODS

Isolation and Identification the Causal Pathogens

Samples of infected faba bean plants showing damping-off and root-rot symptoms were collected from various locations that represented different governorates of Egypt for isolation of the associated fungi. Diseased faba bean in each sample was cut into small pieces, washed carefully with running tap water and sterilized by immersing in 2% sodium hypochlorite for 2 minutes, then subsequently rinsed with sterilized water and dried with sterilized filter paper. Sterilized diseased samples were cut into small pieces and placed on potato dextrose agar (PDA) medium in Petri dishes and incubated at 26±2°C, for five days. The isolated fungi were purified according to **Abdel-Monaim (2013)** and identified according to their morphological features using the descriptions of **Gilman (1957), Booth (1971)** and **Singh (1982)** at the Unit of Identification of Microorganisms, Plant Pathology Research Institute (PPRI), Agricultural Research Center (ARC), Giza, Egypt.

Pathogenicity Test of the Isolated Fungi Under Greenhouse Conditions

The pathogenicity test of 40 isolates of *Rhizoctonia solani* and *F. solani* were carried out in pot experiment under greenhouse conditions on faba bean susceptible cultivar (Giza-429 cv.), which obtained from Legume Crops Dept, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. Inocula of the pathogenic fungi were grown on sorghum grain sand medium (25% clean sand and 75%

sorghum grains and sufficient amount of distilled water to cover the mixture and autoclaved at 121°C for 30 min) and then incubated for 15 days at 26±2°C. Sterilized pots (20 cm diameter) were filled with sterilized clay soil and infested individually with *R. solani* and *F. solani* isolates, at the rate of 3 and 5% of soil weight (*W/W*) per pot, respectively (**Abou-Zeid *et al*., 2002**). Fungal inocula of the tested isolates were mixed with the soil, then irrigated and left 7 days to enhance fungal growth. The pots were arranged in a complete randomized block design. The control was treated with the same amount of fungal free autoclaved sorghum sand medium without fungal inocula **(Whitehead, 1957)**. Faba bean seeds (Giza-429 cv.) were sown at the rate of five seeds/pot. Three replicates were used for each treatment.

Disease incidence was recorded as the percentage of pre- and post- emergence damping-off as well as survived plants 15, 30 and 60 days after planting, according to **El-Helaly *et al*. (1970) and Ahmed (2005),** using these formula:

Pre-emergence (%) =

$$\frac{\text{No. planted seeds - No. emerged seedlings}}{\text{Total No. planted seeds}} \times 100$$

Post-emergence (%) =

$$\frac{\text{No. dead seedlings after emergence}}{\text{Total No. planted seeds}} \times 100$$

Plant survival (%) =

$$\frac{\text{No. planted seeds - No. (pre + post)}}{\text{Total No. planted seeds}} \times 100$$

Molecular Characterization of Pathogenic Fungi using Random Amplified Polymorphic DNA (RAPD) Analysis

RAPD technique was used to evaluate the genetic diversity of the most aggressive pathogenic isolates of *R. solani* and F. *solani* isolates (9 isolates of each one), which obtained from nine governorates using three primers (MWG, Germany; Table 1). DNA isolation and extraction procedure of **Zimand *et al*.** (**1994**) was followed. Quantification and gel documentation (Gel Documentation and Analysis Systems, Uvitec, Cambridge, UK) were carried out in laboratory of Biotechnology according to **Abd-El-Salam *et al.* (2007)**.

Isolation and Identification of the Antagonistic Microorganisms

Different antagonistic microorganisms were isolated from rhizosphere of healthy faba bean plants, collected from naturally heavily infested field with the pathogens **(Abou-Zeid *et al*., 2003b)**. The most effective bacterial and fungal isolates were identified according to the ability of these isolates to utilize selected panel of different carbon and amino acids sources using Biolog-Micro Plats™ **(Anonymous, 1993 and 2010)**, belonging to the Identification of Microorganisms Unit, Plant Pathology Research Institute, A.R.C., Giza, Egypt.

Effect of Faba Bean Seed Treatments with Fungicides, Biocides, Bioagents, and Their Culture Filtrates on Damping-Off Disease Under Greenhouse Conditions

The efficiency of three fungicides (Rizolex-T, Vitavax-200 and Moncut 25%), four antagonist's microorganisms (*T. harzianum*-$_1$, *T. hamatum*$_{-2}$, *Bacillus subtilis*-$_2$ and *B. amylotquefaciens*-$_1$) and their culture filtrates compared with two biocides (Bio-Arc and Bio-Zeid) were tested for controlling *F. solani* El-Menia-FS_4 and *R. solani* El-Nubaria-R_{20}, under greenhouse conditions.

Inocula of the pathogenic (Fs_4 and R_{20}) fungi were grown on sorghum grain sand medium for 15 days at 25±2°C. The antagonistic fungi (10 days old) and bacteria (3days old) were grown on PDA broth medium and Luria-Bertani (LB) broth medium, respectively. Culture filtrates of the antagonistic fungi and bacteria were prepared as described by **Mukherjee *et al.* (1995) and Ahmed (2005)**.

This experiment was carried out using sterilized pots (20 cm diameter) containing sterilized clay soil under greenhouse conditions. Soil infested with individual *R. solani* and *F. solani* isolates, at the rate of 3 and 5% (*W/W*), respectively (**Abou-Zeid *et al*., 2002**), then irrigated and left 7 days to enhance fungal growth. Tested fungicides and biocides were applied at the rate of 2.5 and 3 g/kg, respectively as seed dressing. The antagonistic spore suspension

Table 1. RAPD polymorphic decamer primers

Primer	5'-Sequence-3'	G+C (%)
1	GGTCCCTGAC	70
2	TGCCGAGCTG	70
3	GGGTAACGCC	70

(6×10^6 spore/ml) for *Trichoderma* spp. or bacterial cell suspension (10^8 cfu cell/ml) as mentioned by **Abou-Zeid *et al.* (2003a).** Antagonistic culture by filtrates were used as seed socking **(Ahamed, 2005)**. Five faba bean seeds (Giza-429 cv.) were sown per pot, and three replicates were used for each treatment. Untreated seeds were sown in infested soil as control treatment. Disease incidence was recorded as mentioned before.

Statistical Analysis

Statistical analysis was done using complete randomize block design for analyzing the obtained data according to Computer Statistical Package (CO-STATE) originated by **Anonymous (1989)**.

RESULTS AND DISCUSSION

Isolation and Identification of the Causal Pathogens

The isolated fungi, which were found to be associated with damping-off symptoms of faba bean plants collected from various locations at different governorates, were purified and identified as *R. solani* Kühn and *F. solani* (Mart), Sacc.

Pathogenicity Test of the Isolated Fungi Under Greenhouse Conditions

Pathogenicity tests showed that, all tested pathogenic fungal isolates of *F. solani* and *R. solani* were pathogenic and clearly varied in their virulence on faba bean plants (cv. Giza-429) under greenhouse conditions.

In this respect, results in Table 2 reveal that *F. solani*, El-Nubaria-Fs_4 was the most significant virulent isolate, which recorded 73.3, 20.0 and 6.7% for pre-, post-emergency damping-off and survived plants, respectively. On the other hand, Kafr-Shokr-Fs_{10} was the least virulent isolate as it recorded 20, 13.33 and 66.67% for pre-, post-emergency damping-off and survived plants, respectively.

Results in Table 3 indicat that *R. solani*, El-Menia-R_{20} and Sidy-Salem-R_3 isolates showed the highest significant percentage of pre- and post-emergence damping-off and the lowest surviving plants, respectively, which recorded 53.3, 40.0 and 6.67% and 73.3, 13.3 and 13.33%, respectively. Kafr-Shokr-R_{10} was the least virulent isolate showing 20.0, 6.67 and 73.3% for pre- and post-emergence damping-off and survival plants, respectively.

These results are in accordance with those reported by **Aly *et al.* (2010) and Al-Abdalall (2010)** who found that *R. solani*, *F. solani* and *Sclerotium rolfsii* found to be the most important destructive soil-borne pathogenic fungi to roots of many plants such effect might be due to the synergistic action between polygalactuonase and oxalic acid produced by these pathogenic fungi **(Bateman and Beer, 1964).** Similar results were obtained by **Mahmoud *et al.* (2007)** and **Ahmed *et al.* (2009).** They reported that their pathogenic isolates varied in their virulence. Whereas **Ahmed (2005)** and **Iraildes *et al.* (2011**) reported that *R. solani* isolates were found to be the most severe and destructive pathogen for faba bean plants, which survive between crops as sclerotia or as fungal mycelia in the soil.

Molecular Characterization of Pathogenic Fungi Using RAPD Technique

Molecular techniques using random amplified polymorphic DNA (RAPD) technique have been used to evaluate characterization and variation of the genetic diversity of 18 pathogenic fungal isolates of *R. solani* and *F. solani* (9 isolates of each one) which obtained from different governorates using three primers. Results shown in Figs. 1 and 2, exhibited that the dendrogram

Table 2. Pathogenicity of twenty *Fusarium solani* isolates on faba bean plants (Giza-429 cv.) under greenhouse conditions

Fusarium solani					
Isolate code No.	**Governorate**	**Location**	**Damping-off (%)**		**Surviving Plants (%)**
			Pre*	**Post***	
Fs_1	**Kafr-El Sheikh**	Kleen	46.67	13.3	40.0
Fs_2		Sakha	53.3	33.3	13.3
Fs_3		Sidy-Salem	46.67	20.0	33.3
Fs_4	**Beheira**	Nubaria	73.3	20.0	6.67
Fs_5		Etay-El Baroud	46.67	20.0	33.3
Fs_6		Kafr-El Dowwar	46.67	13.3	40.0
Fs_7	**Gharbia**	Gemmeiza	46.67	26.67	26.67
Fs_8		Kafr-El Zayat	40.0	0.0	60.0
Fs_9	**Qalubia**	Toukh	33.3	20.0	46.67
Fs_{10}		Kafr-Shokr	20.0	13.3	66.67
Fs_{11}	**Sharkia**	Faquos	46.67	0.0	53.3
Fs_{12}		Hehia	40.0	26.67	33.3
Fs_{13}	**Dakahlia**	Meet-Ghamr	46.67	13.3	40.0
Fs_{14}		Aga	46.67	6.67	46.67
Fs_{15}	**Menuofia**	El-Bagour	66.67	13.3	20.0
Fs_{16}		Serce-Alian	33.3	20.0	46.67
Fs_{17}	**Menia**	Menia	66.67	6.67	26.67
Fs_{18}		Sides	60.0	6.67	33.3
Fs_{19}	**Beni-Suef**	Beni-Suef	66.67	20.0	13.3
Fs_{20}		Biba	53.3	6.67	40.0
Control			0	0	100
LSD $_{0.05}$		-	16.87	14.67	18.41

Pre*: Pre-emergency damping-off Post*: Post-emergency damping-off

Table 3. Pathogenicity of twenty *Rhizoctonia solani* isolates on faba bean plants (Giza-429 cv.) under greenhouse conditions

Rhizoctonia solani					
Isolate code No.	**Governorate**	**Location**	**Damping-off (%)**		**Surviving Plants (%)**
			Pre	**Post**	
R_1		Kleen	40.0	26.67	33.33
R_2	**Kafr-El Sheikh**	Sakha	46.67	13.3	40.0
R_3		Sidy-Salem	73.3	13.3	13.33
R_4		Nubaria	40.0	40.0	20.0
R_5	**Beheira**	Etay-El Baroud	40.0	13.33	46.67
R_6		Kafr-El Dowwar	53.33	13.3	33.33
R_7	**Gharbia**	Gemmeiza	40.0	20.0	40.0
R_8		Kafr-El Zayat	46.67	0.0	53.3
R_9	**Qalubia**	Toukh	40.0	0.0	60.0
R_{10}		Kafr-Shokr	20.0	6.67	73.3
R_{11}	**Sharkia**	Faquos	26.67	20.0	53.3
R_{12}		Hehia	20.0	13.3	66.67
R_{13}	**Dakahlia**	Meet-Ghamr	53.3	20.0	26.7
R_{14}		Aga	46.67	13.33	40.0
R_{15}	**Menuofia**	El-Bagour	46.67	6.67	46.67
R_{16}		Serce-Alian	33.3	13.3	53.3
R_{17}	**Beni-Suef**	Sides	60.0	6.67	33.33
R_{18}		Beni-Suef	53.3	6.67	40.0
R_{19}		Biba	40.0	6.67	53.3
R_{20}	**Menia**	Menia	53.3	40.0	6.67
Control			0	0	100
LSD $_{0.05}$		**-**	13.54	15.16	19.36

Pre*: Pre-emergency damping-off Post*: Post-emergency damping-off

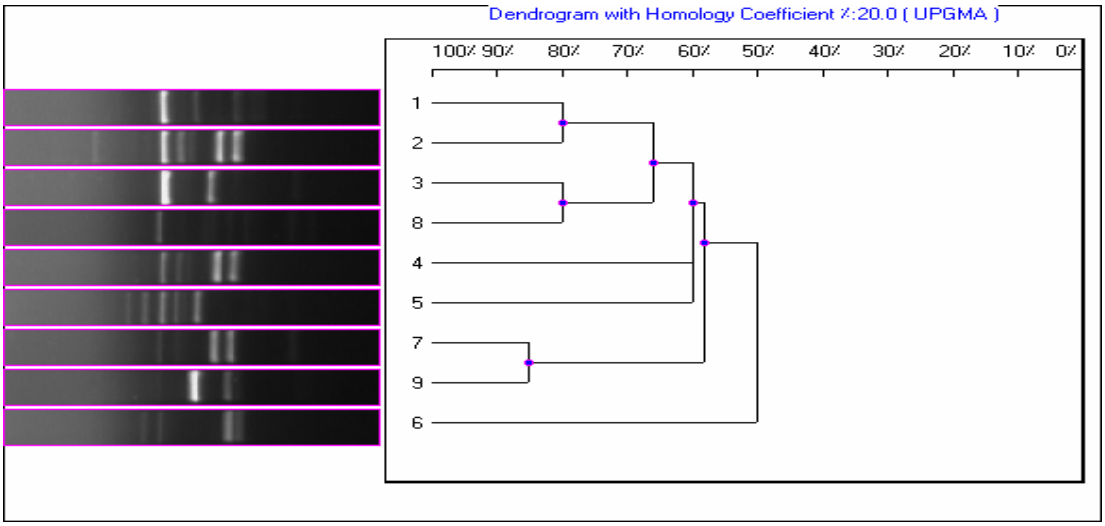

Fig. 1. RAPD-PCR and dendrogram cluster analysis of nine *Fusarium solani* isolates using the random primer-$_3$ (GGGTAACGCC) similarity values are indicated, and final linkages for the sub-clusters are marked

1- Sakha isolate 2-El-Nubaria isolate 3-Gemmeiza isolate

4-Toukh isolate 5-Hehia isolate 6-Meet-Ghamr isolate

7-El-Bagour isolate 8-El-Menia isolate 9-Beni-Suef isolate

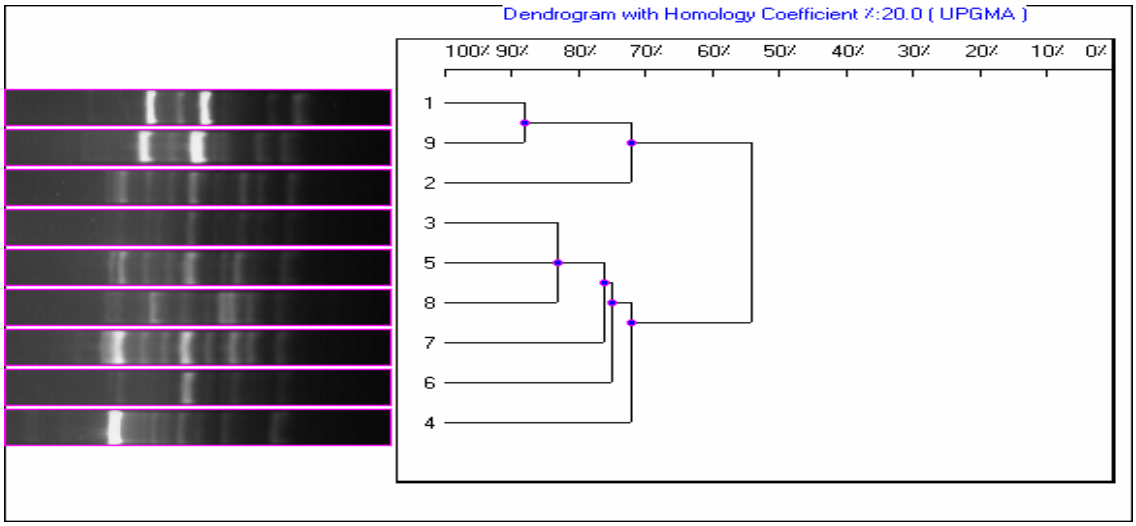

Fig. 2. RAPD-PCR and dendrogram cluster analysis of nine *Rhizoctonia solani* isolates using the random primer-$_2$ (TGCCGAGCTG) similarity values are indicated, and final linkages for the sub-clusters are marked

1-Sidy-Salem isolate 2-El-Nubaria isolate 3-Gemmeiza isolate

4-Toukh isolate 5-Faquos isolate 6-Meet-Ghamr isolate

7-El-Bagour isolate 8-Sides isolate 9-El-Menia isolate

generated by UPGMA analysis divided the isolates into main and sub-main clusters which different in between. These differences corresponded to their pathogenic characteristics.

Results in Fig. 1 show that from the three used primers only primer-$_3$ (GGGTAACGCC), had success in differentiation and amplification among *F. solani* isolates which divided into two main clusters with similarity 50.0% in between. The major cluster was divided into clusters (similarity level = 59.0%). The first one was divided in two sub clusters (similarity level = 67.7), the similarity between isolates No.1 and 2 was 80.0% while, it was 79.0% between isolate No. 3 and 8. The second cluster was for isolate No. 4, while the third cluster was for isolate No. 5. It is clear also that similarity among the isolates of first, second and third clusters were being 60.0%. Meanwhile in the fourth cluster, the similarity between isolates No. 7 and 9 was 86.0%, which revealed similarity 59.0% between them and other isolates in the major main cluster. On the other hand, isolate No.6 was distinguished as single isolate in the minor cluster.

Results in Fig. 2 reveal that, primer-$_2$ (TGCCGAGCTG), was successful in amplification among *R. solani* isolates which generated by UPGMA analysis for two main clusters with similarity 54.0% in between. The major cluster was divided into four sub clusters. In the first cluster, the similarity between isolates No.3, 5 and 8 was 83.0% while, it being 76.5% between them and isolate No. 7 in the second cluster. The third cluster was for isolate No.6, which being 75.0% similarity between them and the isolates of the second, and the third clusters. The fourth cluster was isolate No.4, which revealed similarity 71.0% between them and the other isolates of the major cluster. On the other hand, the minor cluster was divided into two sub clusters with similarity valued 72.0%. In the first cluster, the similarity between isolates No. 1 and 9 was 88.0%, which revealed similarity amounted 72.0% between them and isolate No.2 in the second cluster.

These results are in line with those reported by **Kini *et al.* (2002)** and **Indira *et al.* (2011)**. They reported that molecular techniques, RAPD have been used as a tool in genetic mapping, molecular taxonomy, evolutionary studies, diagnosis of several fungal species characterization and variation of pathogenic fungi isolates *R. solani*, which obtained from different governorates. In this respect characterize strains of many *Fusarium* spp. carried out by **El-Fadly *et al.* (2008)** and **Arif *et al.* (2012)**, explained the genetic diversity of fusarium wilt and characterizing of pathogenic and non-pathogenic isolates of *F. solani* and *F. oxysporum.*

Isolation and Identification of the Antagonistic Microorganisms

In our previous work, twenty-five bacterial and thirty fungal antagonistic isolates were obtained from faba bean rhizosphere and assayed for their antagonistic activity against two virulent pathogenic fungi (*F. solani* El-Nubaria-Fs$_4$ and *R. solani* El-Menia-R$_{20}$) *in vitro* and *in vivo* under greenhouse conditions **(Khalifa, 2016).**

The most effective antagonistic bacterial and fungal isolates were identified by test the ability of these isolates to utilize different carbon sources and amino acids using Biolog-System Technique. The obtained bacterial isolates were belonged to five species, which identified as *B. subtilis, B. licheniformis, B. leavolactieus, B. amylotquefaciens* and *B. cereus*. Meanwhile, the tested antagonistic fungi were identified as *T. harzianum*, *T. hamatum, T. viride*, *T. atrreoviride, T. virens* and *T. aureoviride*.

Utilization of carbon sources and amino acid by various bacterial isolates

All 7 bacteria isolates utilized only 6 carbon and amino acid sources (D-Fructose, α-D-Glucose, Maltose, D- Mannose, Sucrose and D-Trehalose) and unutilized twelve carbon sources (Inulin, D-Arabitol, L-Fucose, M- Inositol, Lactulose, D-Melezitose, α-Methyl-D Galactoside, α-Methyl-D Mannoside, L-Rhamnose, Sedoheptulosan, Putrescine and α-D-Glucose-1-Phosphate), while other sources gave varying degrees of utilization by individual isolate.

Utilization of carbon sources and amino acid by various fungal isolates

The fungal isolates were differ in utilized of carbon sources and amino acid (C and A).

Twenty-six carbon and amino acid sources (Amygdalin, D-Arabitol, Arbutin, D-Galactose, D-Galacturonic acid, D-Glucosamine, α-D-Glucose, Lactulose, Maltose, D-Mannitol, D-Melezitose, D-Melibiose, α-Methyl-DGlucoside, D-Raffinose, Salicin, D-Sorbitol, Stachyose, Sucrose, D-Trehalose, L-Alanine, L-Alanyl-Glycine, L-Asparagine, L-Glutamic acid, L-Ornithine, L-Pyroglutamic acid and L-Serine) were utilized by all Trichoderma isolates. While, all isolates unutilized only one (C and A) was (α- Cyclodextrin). On the other hand, other carbon sources gave varying degrees of growth with individual isolates.

The results of **Leroux (2003) and Druzhinina** and **Kubicek, (2005)** emphasized these obtained results. Also, the bacterial and fungal isolates were subjected to their characterization according to their physiological characteristics due to the ability of these isolates to utilize different carbon sources and amino acids, described by **Bochner (1989 and 2003), Druzhinina and Kubicek (2005), Druzhinina *et al.* (2006)** and **Seidl *et al.* (2006)**.

Effect of Faba Bean Seed Treatments with Bioagents, Culture Filtrates, Biocides and Fungicides on Control of Damping-off Disease *In vivo*

Results in Table 4 indicate that, all treatments of the most active bioagents (*Trichodema hamatum*$_{-2}$, *T. harzianum, Bacillus subtilis*-$_1$ and *B. mylotquefaciens*$_{-1}$), their culture filtrates, biocides (Bio-Arc and Bio-Zeid) and fungicides (Vitavax-200, Rizolex-T and Moncut), were effective in controlling *F. solani* El-Menia-FS$_4$ and *R. solani* El-Nubaria-R$_{20}$, under greenhouse conditions. The most effective treatments for reducing these diseases were obtained by applying fungicides followed by both of active bioagents and biocides. Meanwhile, culture filtrates of bioagents were the least effective treatments. Rizolex-T and Monocut were the most effective fungicides in reducing disease incidence and gave the highest percentage of surviving plants by 93.33 and 86.67% ; 86.67; 86.67%, for *F. solani* and *R. solani*, respectively compared with control, which recorded 13.3 and 6.67% survived plants of the two pathogens, respectively.

These results are in harmony with those obtained by **Eisa *et al.* (2006), Mokhtar *et al.* (2011), Abd-El-Kader *et al.* (2015) and Emhemed (2015)**. They reported that application of fungicides Rhizolex-T, Vitvax-200, Benlate, Topsin-m70 and Monceren were the most effective treatments for controlling wilt and root-rot diseases caused by *R. solani, F. solani, F. oxysporum, Sclerotium rolfsii* and *F. moniliforme* under greenhouse conditions.

Bio-Arc and Bio-Zeid were the most effective in decreasing percentage of wilt, damping-off and root-rot followed by *T. hamatum, T. harzianum, T. viride* and *B. subtilis* (**Bahaa El-Din, 2005; Salama, 2006; Abdel-Monaim, 2013**). Also, the different antagonists (*T. harzianum*, *T. viride*, *B. subtilis* and *Rhizobium* spp.) and their cultural filtrates were the most effective against *R. solani*, *F. oxysporum* and *F. solani* (**El-Hassan *et al.*, 2004; Mazen, 2004; Mazen *et al*., 2008**). The pathogenic fungi varied in their reaction against different antagonists due to their owns defense mechanism against enzymes and toxic substances that produced by different antagonists. The biocontrol agents antagonism pathogen through antibiosis, competition, mycoparasitism or other form of direct exploitation (**Rajeev and Mukhopadhyay, 2001**). Some products of bioagents (*T. harzianum* and *B. subtilis*) stimulated plant growth and reduced population density of plant pathogens (**Abou-Zeid *et al*., 2003b),** produce mycolytic enzymes, siderephores (pyoverdin) and several secondary metabolites with antimicrobial activity, such as diacetylphloroglucinol, oomycin A, phenazine-1-carboxylic acid, pyoluteorin, pyrrolnitrin and hydrogen (**Fernando *et al.*, 2007**).

In conclusion the usage DNA (RAPD) analysis has been used to evaluate characterization and variation of the genetic diversity of *R. solani* and *F. solani*. Differentiation among antagonistic isolate according to utilize carbon and amino sources acids was clarified using Biology system. Controlling using the effective bioagents and their culture filtrates, chemical fungicides and biocides as seed socking were significantly reduced faba bean damping- off under greenhouse conditions.

Table 4. Effect of different treatments (bioagents, biocides and fungicides) on faba bean damping-off disease caused by *R. solani* and *F. solani* under greenhouse conditions

Treatment	*F. solani* (Fs_4)			*R. solani* (R_{20})		
	Pre (%)	Post (%)	Surviving plants (%)	Pre (%)	Post (%)	Surviving plants (%)
***Bacillus subtilis*-1**	20.00	6.67	73.30	20.00	13.33	66.67
***B. amylotquefaciens*-1**	33.33	0.00	66.67	33.33	6.67	60.00
Trichodema harzianum	20.00	13.33	66.67	26.67	20.00	53.33
***T. hamatum*-2**	20.00	6.67	73.33	20.00	6.67	73.30
***Bacillus subtilis*-1 (C.F.*)**	40.00	20.00	40.00	53.33	13.33	33.33
B. amylotquefaciens*-1 C.F.	40.00	26.67	33.33	40.00	20.00	40.00
***T. harzianum* (C.F.*)**	33.33	40.00	26.67	33.33	40.00	26.67
***T. hamatum*-2 (C.F.*)**	40.00	13.33	46.67	60.00	0.00	40.00
Bio-Arc	20.00	13.33	66.67	20.00	13.33	66.67
Bio-Zeid	26.67	13.33	60.00	26.67	13.33	60.00
Vitavax-200	13.33	0.00	86.67	13.33	6.67	80.00
Rizolex-T	6.67	0.00	93.33	0.00	13.33	86.67
Moncut	13.33	0.00	86.67	13.33	0.00	86.67
Control	46.67	40.00	13.30	60.00	33.30	6.67
LSD $_{0.05}$	13.66	13.65	17.87	14.59	15.48	14.60

Pre*: Pre-emergency damping-off Post*: Post-emergency damping-off C.F.*: Culture filtrates

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فاعلية عــوامل المكافحة الحيوية الفطرية والبكتيرية ضد مرض موت البادرات فى الفول البلدى

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تعتبر أمراض موت البادرات المتسببة عن فطرى رايزوكتونيا سولانى وفيوزاريوم سولانى من أخطر وأهم الأمراض التى تصيب الفول البلدى، وقد أتضح أن العزلات التي تم جمعها من تسع محافظات مختلفة قد اختلفت فيما بينها فى قدرتها المرضية عند إختبارها على صنف الفول البلدى الحساس جيزة-٤٢٩ تحت ظروف الصوبة، كانت عزلتى النوبارية والمنيا للفطرين فيوزاريوم سولانى (Fs_4) و رايزوكتونيا سولانى (R_{20}) هما الأكثر ضراوة، تم تقييم الاختلاف في التنوع الجيني عن طريق التقنيات الجزيئية باستخدام تكنيك RAPD -DNA بين أكثر العزلات الممرضة ضراوة بواقع ٩عزلات لكل من فطرى رايزوكتونيا سولانى وفيوزاريوم سولانى، تم تعريف أهم عوامل المكافحة الحيوية الفطرية والبكتيرية بإستخدام تقنية البيولوج بإستخدام مصادر الكربون والنتروجين المختلفة، وكانت أكثر عوامل المكافحة الحيوية فاعلية هى الفطر (ترايكودرما هاماتم-٢، والفطر ترايكودرما هارزيانم ، والبكتريا باسيلس ستلس-١ والبكتريا باسيلس أميلوليكيوفاشنس-١)، ثم تلاها راشح المزرعة، ثم المبيدات الحيوية (بيوأرك و بيوزيد) كما أظهرت المبيدات الفطرية المختبرة (ريزولكس - فيتا فاكس - مونكت) فعالية في تقليل الاصابة بموت البادرات على الفول، وكانت المبيدات الفطرية أفضل المعاملات للحد من هذه الأمراض تلاها المعاملة بكل من عوامل المقاومة الحيوية والمبيدات الحيوية بينما كانت رواشح عوامل المقاومة الحيوية هى الأقل فاعلية، وقد كان مبيدى الريزولكس والمونكت الأكثر تأثيراً ضد فطرى الرايزوكتونيا سولانى وفيوزاريوم سولانى.

الكلمات الإسترشادية: الفول البلدى، موت البادرات، نظام البيولوج، المكافحة الحيوية، المبيدات الحيوية.

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