J. Plant Prot. and Path., Mansoura Univ., Vol. 1 (12): 937 - 948 , 2010

APPLICATION OF CERTAIN RESISTANCE INDUCERS FOR CONTROLLING FABA BEAN WILT DISEASE UNDER GREENHOUSE AND FIELD CONDITIONS

Asran, M. R. [*] and Nashwa M. A. Sallam [**]

[*] **Dept. of Plant Pathology, Faculty of Agriculture, Sohag Univ., Sohag, Egypt**

[**] **Dept. of Plant Pathology, Faculty of Agriculture, Assiut Univ., 71526, Assiut, Egypt**

ABSTRACT

Fusarium oxysporum f.sp. *fabae* (Schlecht) Snyder &.Hansen (FOF) is a serious pathogen on faba bean causing wilt disease. In greenhouse experiments, the three tested isolates of the pathogen were differed in their virulence on faba bean cultivars (Giza 29, Giza 4 and Misr 1). Isolate (FF1) was the most virulent one. Faba bean cultivars reacted differently to the infection by FOF isolates. While cv. Misr 1 was the most susceptible one, cvs Giza 29 and Giza 4 showed moderate resistance. *In vitro* testes, the resistance inducers ascorbic acid (AS) and acetic acid (AA) reduced dry weight of the pathogen followed by salicylic acid (SA) and oxalic acid (OA). Effectiveness of the tested resistance inducers in controlling the disease was tested under greenhouse and field conditions. All applied treatments protected faba bean seedlings against FOF in greenhouse. The most effective treatments were AS and AA treatments, however in field experiments, seed treatments by OA, AA, AS and SA reduced the disease incidence. Almost tested treatments significantly increased cellulose content in treated plants except OA treatment compared with untreated plants. The highest reduction in disease incidence was obtained with AS, AA and SA treatments. Seed treatment with AA and AS significantly increased pectin content in treated plants compared with untreated ones. Faba bean seed treatment with 1 % (AS) exhibited the highest content of lignin, while, seed treatment with 0.25 % (AA) exhibited the lowest lignin levels.

Keywords: Faba bean wilt, resistance inducers (RIs), *Fusarium oxysporum* f.sp. *fabae* lignin, cellulose

INTRODUCTION

Fusarium wilt disease of faba bean (*Vicia faba* Linn.) incited by *Fusarium oxysporum* f.sp. *fabae* is an economically important disease in Egypt as well as in many other countries around the world (Khalifa, 1997 and Nehal, El-Mougy, 2001). It is responsible for great reduction in faba bean production and severs economic losses (Abdel-Kader *et al*., 2002).

Certain chemicals, such as salicylic acid, oxalic acid, acetic acid and ascorbic acid were reported to induce systemic acquired resistance (SAR) in plants against certain plant pathogens (Abo-Elyouser *et al*., 2009 and Oostendorp *et al*., 2001). Salicylic acid (SA) played an important role in protection of some crops against wilt disease (Zhang and Reddy 2001). These synthetic chemical compounds can trigger plant responses against pathogens. Salicylic acid inhibited, *in vitro* growth of *F. oxysporum*, the causal agent of onion rot (El-Ganaieny *et al*., 2002). Also, different concentrations of SA or OA were significantly inhibited the linear growth of *F. oxysporum* f.sp.

lycopersici, the causal agent of tomato wilt disease (Eraky, Amal, *et al*., 2007).. Hammerschmidt and Smith-Becker (1999) reported that SA affects a variety of biochemical and molecular events in plants associated with induction of disease resistance.

Certain biochemical changes occurring after application of the inducing agents can act as markers for induced systemic resistance (Schönberck *et al*., 1980). These include accumulation of phytoalexins and reinforcement of cell wall polymers with deposition of lignin (Thangavelu *et al*., 2003).

The aim of the present study was measure the pathogenicity of different *F. oxysporum* f. sp. *fabae* isolates on various faba bean cultivars and to evaluate some resistance inducers against the mycelial growth of wilt pathogen *in vitro*. Furthermore, the application of certain resistance inducers for controlling faba bean wilt under greenhouse and field conditions were also evaluated. The contents of lignin, cellulose and pectin in treated plant tissues were also determined.

MATERIALS AND METHODS

Isolation and identification of the causal pathogen

Naturally wilted faba bean plants were collected from different localities of Assiut Governorate in 2007 growing season. The plant roots were cut in small pieces, thoroughly washed with tap water, surface sterilized for two minutes with 2% sodium hypocholorite solution, then rinsed several times in sterilized distilled water and dried between folds of sterilized filter papers. The surface sterilized root samples were plated onto Potato Dextrose Agar (PDA) medium and incubated at 27°C. After 4-5 days of incubation period, the developed fungal colonies were picked up and purified by hyphal tip and single spore technique. Identification of the fungal isolates was carried out according to the morphological characteristics of mycelia and spores as described by Nelson *et al*., 1983.

Preparation of inoculum

The fungal isolates were cultured on potato dextrose agar (PDA) medium and incubated under a 12 h light/dark cycle at 25±1°C. Inoculum of *Fusarium oxysporum* f.sp. *fabae* isolates was prepared on autoclaved barley medium (75g washed dried barley grains, 100 washed dried coarse sand and 75 ml tap water) in 500 ml glass bottles. Each bottle was inoculated with five discs (0.7 cm in diameter) of 14-day-old cultures of the pathogen. Bottles were incubated at 25±1°C for 15 days. For each isolate, the contents of 20 bottles were thoroughly mixed in a plastic container and used as a source of inoculum. Inocula of each isolate were added to sterile soil in pots (30 cm diameter) at the rate of 3 % w/w (~10^6 cfu g^{-1}) two weeks before planting. Pots containing non infested soil were used as control. The most virulent fungal isolate and most susceptible cultivar were chosen for further studies under greenhouse and field conditions.

Reaction of faba bean cultivars to FOF infection under greenhouse conditions

Isolates were examined for their pathogenic potentiality to induce wilt of the faba bean cultivars under greenhouse conditions in the growing

season 2008. Cultivars (Giza 29, Giza 4 and Misr 1) were evaluated for their resistance to wilt disease. Seeds of each cultivar were surface sterilized in 2 % sodium hypochlorite solution for 3 min., rinsed in sterile distilled water and then air-dried. Seeds were planted in plastic pots 30 cm diameter (2.4 kg soil), filled with a pasteurized mixture of soil and sand (4:1 W/W). Six seeds were sown in pot and these pots were irrigated each 3days. Experimental design was split plot with five replicates of each treatment, where, cultivars were in the main plots and the fungal isolates were in the subplots. Severity of wilt was determined after 60 days according to Abdou *et al*. (2001) using a rating scale of 0 to 5 as following: 0 = neither root discoloration nor leaf yellowing, 1 = 1-25 % root discoloration or one leaf yellowed, 2 = 26-50 % root discoloration or more than one leaf yellowed, 3 = 51 – 75 % root discoloration plus one leaf wilted, 4 = up to 76 % root discoloration or more than leaf wilted, and 5 = completely dead plants. For each replicate a disease index (DI) similar to that described by Liu *et al*., (1995) was calculated as follows: DI% = $\sum$(1A +2B + 3C + 4D + 5E)/ 5T X 100 where, A, B, C and D are the number of plants corresponding to the numerical grade, 1, 2,3,4 and 5 respectively and 5T is the total number of plants (T) multiplied by the maximum grade 4, where T= A+B+C+D+E.

In vitro, screening for inhibitory effect of resistance inducers on growth of the pathogen.

Resistance inducers (RIs) salicylic acid (SA), oxalic acid (OA), acetic acid (AA) and ascorbic acid (AS) were obtained from El-Naser Company, Egypt. The effect of SA, OA, AA and AS on the dry weight of *Fusarium oxysporum* f.sp. *fabae* isolates was tested *in vitro*. They were dissolved in ethanol and added singly to Potato Dextrose liquid medium at concentrations of 1% and 0.25%. The flasks were inoculated, individually by fungal isolates using 0.7 mm fungal growth discs taken from 14 days old cultures and incubated at 25 ºC for 7 days on an orbital shaker at 200 rpm. Flasks without acids were used as control (Galal and Abdou,1996). The growth of each fungal isolates was determined as mycelial dry weight of biomass (g). Five replicates were used for each treatment and the experiment was conducted twice.

Greenhouse Experiments

Trials were carried out in the greenhouse of Plant Pathology Dept. Faculty of Agriculture, Assiut Univ. Pots were sterilized by immersing in 5% formalin solution for 15 min., then air drayed. Meanwhile, soil was wetted by the same solution, then covered with a polyethylene sheet for 7 days to retain the gas and left to dry for 2 weeks until all traces of formaldehyde disappeared. Pathogen inocula were added separately to the potted soil at a rate of 3% (w/w) and mixed thoroughly with the soil 2 weeks before planting. Each pot was seeded with six seeds of faba bean cv Misr 1 previously impregnated in each of tested inducers for 30 min. at two concentrations (1 and 0.25 %). Five replicates were used for each particular treatment. Pots containing only infested soil were used as control.

Field Experiments.

Field trials were conducted at the Experimental Farm of Faculty of Agriculture, Assiut University, Assiut, Egypt in 2009 and 2010 growing winter

seasons. Field plots (2 x 2.5 m) comprised into three rows and 15 holes per row arranged in a completely randomized block design. Three plots were used as replicates for each treatment as well as for the untreated control treatment. Each row was infested with 150 g of the *Fusarium oxysporum* f. sp. *fabae* isolate (FF1) two weeks before planting (10 g per hole). Faba bean seeds cv. Misr 1, were sown (3 seeds per hole). Application of resistance inducers was carried out as in greenhouse experiments. Disease incidence (Liu *et al*., 1995) was evaluated after two months from planting. From each row, 10 plants were used (30 plant replicate^{-1}) for evaluating DI%. All plots were arranged in a completely randomized block design.

Determination of pectin, cellulose and lignin.

Ten plants from each replicate were selected randomly and the main fractions of the cell wall including, pectin, cellulose and lignin were determined following the methods described by Galbriath and Shields (1981) and Selvendran and O'Neill (1987).

Cell wall fractionation.

This procedure was conducted essentially according Dever *et al*. (1968), Galbriath and Shields (1981) and Selvendran and O'Neill (1987). Pectin and cellulose fractions were estimated by anthorne-sulphuric acid method.

Pectin fraction.

Wall preparations were first extracted twice with 0.5% ammonium oxalate-oxalic acid (pH 4) at 90 ºC for 24 hours. Ammonium oxalate solution precipitates calcium ions, which connect the glycosidic bounds in pectin molecules, and thus it becomes water-soluble. Extractions were combined and designated as "pectin fraction".

Cellulose fraction.

The residues from the above alkaline extractions were dried and suspended in 72% H_2SO_4 and kept at 0-4^{o}C for 48 hours with occasional stirring, then suspension diluted with distilled water to final concentration of 1% H_2SO_4 and autoclaved for 2 hours. The supernatant contained the acid hydrolyzed cellulose (Selvendran and O'Neill, 1987).

Lignin fraction.

Lignin was determined by the method described by Liyama and Wallis (1988), as follows: 10-15 mg of samples were weighed into 4 ml brown vials and 20 ml of acetyl bromide in glacial acetic acid (1:3 v/v) containing perchloric acid (70%, 0.08 ml) was added. After digestion, the samples were transferred, with the aid of acetic acid, to 50 ml volumetric flasks containing 2 M sodium hydroxide (5ml) and acetic acid (12 ml). The flasks were carried out in triplicate on duplicate sample of root material.

The lignin contents were calculated from the equations:

Absorbance value = ODs- ODb/concentration of sample (gl^{-1})

Lignin content= 33.6 absobance-11.1 g kg^{-1} dry matter

Where ODs is optical density of sample and ODb optical density of blank.

tatistical analysis.

All experiments were repeated twice. Analysis of variance (ANOVA) was carried out using MSTAT-C program. The Least Significant Difference

(LSD) at P≤0.05 was applied to detect differences among treatments. (Gomez and Gomez, 1984).

RESULTS

Isolation and identification of the pathogen

Three isolates of FOF (FF1, B6 and FR1) were isolated from naturally diseased faba bean plants showing wilt symptoms grown in the fields of Assiut Governorate, Egypt. The isolates were identified according to their morphological traits (eg., colony and conidia) and all were grouped to the species of *F. oxysporum* f.sp. *fabae* (Nelson *et al*., 1983).

Reaction of certain bean cultivars to pathogens

Data in Fig.1 show that all isolates of FOF proved to be pathogenic to faba bean plants and showing wilt symptoms on all tested cultivars. However, they differed in their virulence to cause wilt. Isolate FF1 caused the highest disease severity in all tested cultivars followed by isolates B6 and FR3. Data also show that faba bean cultivars differed in their resistance to wilt disease caused by the three *Fusarium oxysporum* f.sp. *fabae* isolates. The mean disease index of wilt in the different cultivars decreased in the following order: Misr1 (67.62%), Giza 4 (57.25%) and Giza 29 (53.44%). On bases of this result, isolate FF1 and cv. Misr 1 were used in the following experiments.

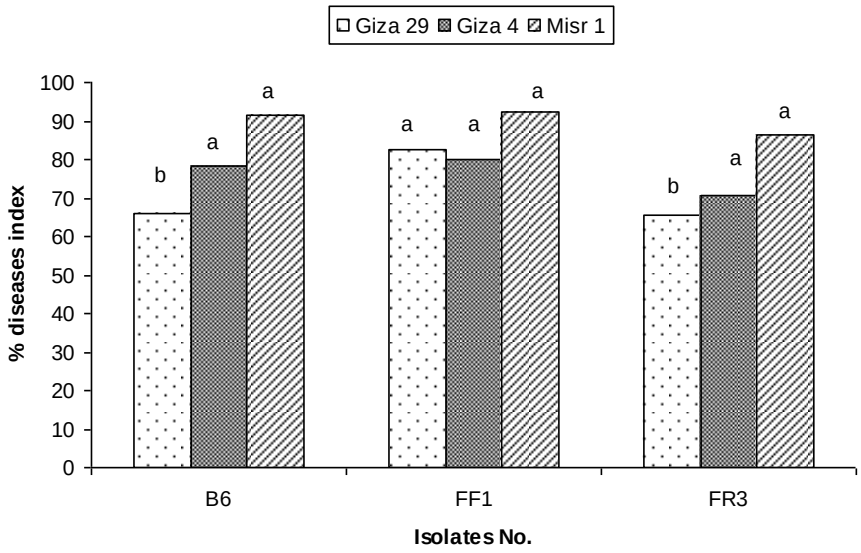

Fig.1: Reaction of faba bean cultivars to infection by *F. oxysporium* f. sp. *fabae* isolates under greenhouse conditions. Different letters indicate significant differences among treatments within the same color column according to least significant difference test (P = 0.05).

Effect of resistance inducers (RIs) on Fusarium mycelial growth

Data presented in table 1 show that all tested treatments reduced dry weight of *F. oxysporium* f.sp. *fabae* isolates, except treatment with AS at 0.25% concentration. In general, the highest concentration of tested chemicals (1%) was superior in their effect on reduction of mycelial growth. The highest inhibition in the mycelial dry weight was observed with FOF isolates (FF1) and (FR1) treated by AA and OX at concentration of 1%. In case of isolate (B6), the highest inhibition in the mycelial dry weight was observed by isolate (B6) treated by OX, AA and SA at concentration 1%. There is no significant different between FOF isolates in tested acids.

Table 1: Effect of resistance inducers (RIs) on mycelial dry weight (gm/ml culture medium) of *F. oxysporium* f.sp. *fabae* isolates

Treatments	Fungal isolates			Mean
	FF1	FR1	B6	
Control	0.19	0.17	0.10	0.15
OX (1%)	0.04	0.04	0.04	0.04
OX (0.25%)	0.05	0.06	0.05	0.05
SA (1 %)	0.09	0.07	0.03	0.06
SA (0.25%)	0.13	0.11	0.05	0.10
AA (1%)	0.02	0.03	0.06	0.04
AA (0.25%)	0.05	0.15	0.06	0.09
AS (1%)	0.10	0.06	0.07	0.07
AS (0.25%)	0.12	0.14	0.09	0.12
Mean	0.09	0.09	0.06	

L.S.D. 0.05 % Isolates 0.04 Treatments 0.06 Interaction 0.10

Whereas (SA) salicylic acid, (OA)oxalic acid, (AA) acetic acid and (AS) ascorbic acid

Effect of RIs on bean wilt disease.

Greenhouse experiments

Data in Fig.2 indicate that seed treatments with different resistance inducers significantly decreased disease index in comparison with the check treatment. The applied inducers were varied in their effect on percentage of disease index. Seed treatments with AS showed the highest reduction in disease index followed by SA and OX treatments, while, AA showed the lowest effective one.

Filed experiments

Data in Fig. 3 indicate that seed treatments by oxalic acid, acetic acid, ascorbic acid and salicylic acid reduced faba bean wilt. The reduction was from 61.9 in untreated plants to 13.8- 21.8 % in treated ones in 2009 growing season and from 66.9% in untreated plants to 16.9 – 23.8% in 2010 growing season. In both tested seasons, the most effective treatments were AA followed by OX and AS treatments, while, the lowest effective treatments were SA treatments. Data indicate also that the high concentration caused the highest decrease in disease incidence for all chemical inducers.

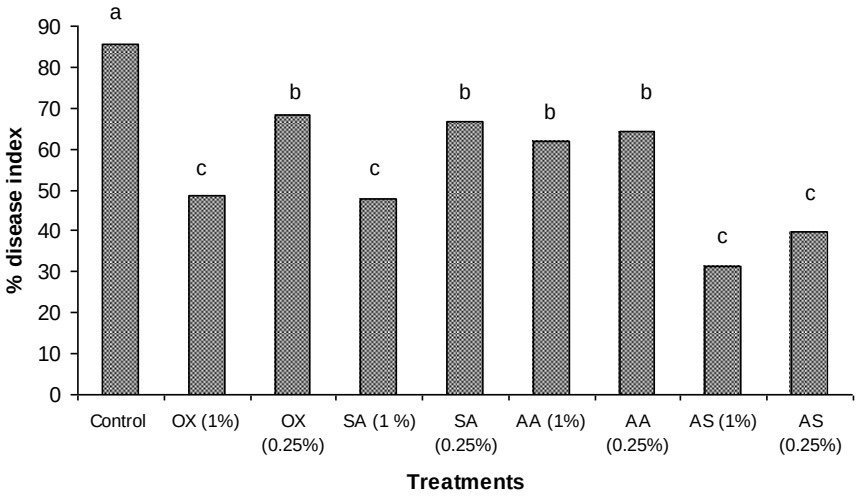

Fig. 2: Effect of resistance inducers (RIs) on faba bean wilt disease under greenhouse conditions during 2008 growing season. Whereas (SA) salicylic acid, (OA)oxalic acid, (AA) acetic acid and (AS) ascorbic acid. Different letters indicate significant differences among treatments according to least significant difference test (P = 0.05).

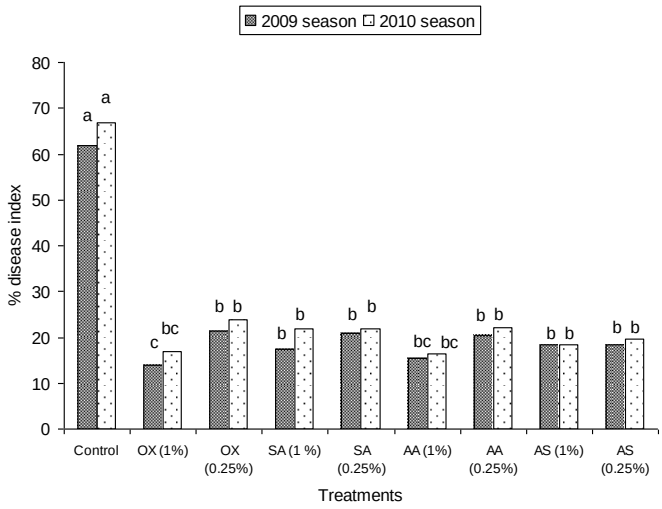

Fig 3: Effect of resistance inducers (RIs) on faba bean wilt under field conditions during 2009 and 2010 growing seasons. Whereas (SA) salicylic acid, (OA)oxalic acid, (AA) acetic acid and (AS) ascorbic acid. Different letters indicate significant differences

among treatments within the same color column according to least significant difference test (P = 0.05).

Contents of cellulose, pectin and lignin in treated faba bean plants

Effect of the tested resistance inducers on cellulose, pectin and lignin contents of faba been roots was estimated. Results in Table 2 indicate that, almost all treatments significantly increased cellulose content in treated plants compared with untreated plants except in case of oxalic acid treatments. In general the highest increase was obtained with ascorbic acid followed by acetic acid and then salicylic acid treatments. In case of pectin content, results indicated that, only, seed treatment with 1% acetic acid or 1% ascorbic acid significantly increased pectin content in treated plants compared with untreated ones. In plants treated with oxalic acid, salicylic acid, ascorbic acid and acetic acid, the lignin contents ranged from 33.13 to 47.83. The highest content of lignin (47.83%) was found in plants treated with ascorbic acid 1%. The lowest levels of lignin (33.13) were found in host tissue treated by acetic acid 0.25%.

Table 2: Cellulose, pectin and lignin contents in bean roots of cv. E1 treated by chemical resistant inducers to faba bean wilt disease

	Cell wall contents (% of dry weight of root)		
Treatments	Cellulose	Pectin	Lignin
Control (untreated plants)	30.10*	2.67	30.50
OX (1%)	33.13	3.20	41.00
OX (0.25%)	32.10	3.00	40.00
SA (1 %)	40.13	3.30	44.13
SA (0.25%)	36.20	3.00	39.13
AA (1%)	44.00	4.06	41.17
AA (0.25%)	40.13	3.30	33.13
AS (1%)	44.17	4.20	47.83
AS (0.25%)	41.87	3.30	41.17
L. S. D. 0.05	4.59	1.04	2.60

*** % of dry weight of root**

Whereas (SA) salicylic acid, (OA)oxalic acid, (AA) acetic acid and (AS) ascorbic acid

Discussion

Three isolates of *F. oxysporium* f.sp. *fabae*, (FOF), B6, FF1 and FR3 were isolated from naturally wilted faba bean plants. They caused wilt disease to the plants of the three faba bean cultivars tested (Giza 29, Giza 4 and Misr 1). Isolates were differed in their virulence to cause wilt. The isolate FF1 caused the highest wilt incidence for the tested cultivars. Faba bean cultivars differed in their resistance to wilt disease caused by the three FOF isolates. Isolate FF1 was the most virulent isolate and faba bean cv. Misr 1 was the most susceptible one. These results are in harmony with those reported by Khalifa (1997) and Abdel-Kader *et al*., (2002).

Using chemicals to induce resistance against plant pathogens have been reported previously (Abo-Elyousr *et al*., 2005; Asran 2005; Eraky, Amal *et al*., 2007;Yalpani *et al*., 1991). Results reported herein indicated that SA,

OA, AA and AS significantly inhibited dry weight of *F. oxysporium* f.sp. *fabae*. Also, the high conc. (1 %) had higher inhibitory effect on fungal dry weight than the lower one (0.25 %). These results are in agreement with Mahmoud (2005) who found that application of SA and AS *in vitro* significantly inhibited the mycelial growth of *F. oxysporum.*

The resistance inducers, SA, OA, AA and AS were applied individually to test their efficacy in controlling faba bean wilt disease caused by FOF isolate FF1 under greenhouse and field conditions. In greenhouse experiments as well as in the field, all applied treatments protected faba bean seedlings against FOF and the most effective treatments were AS treatments. Many researchers suggested that SA and OA could induce systemic resistance in plants, inhibit catalase enzyme, enhance the PR gene expression of chitinases which could hydrolyze the wall of many fungi (Davis *et al*., 2002; Narusaka *et al*., 1999). The role of SA, AA, OA and AS in induction of SAR or ISR against certain plant pathogens was widely accepted by Spletzer and Enyedi (1999) against *Alternaria solani* in tomato ; Carl *et al*., (2005) against CMV on cucumber, pepper and tobacco ; Mahmoud (2005) against *F. oxysporum* on sugar beet and Eraky, Amal *et al*., (2007) against *F. oxysporum* f.sp*. lycopersici* on tomato.

In the present work, the tested treatments of OX, SA, AA, and AS resistance inducers increased accumulation of cellulose, pectin and lignin interacted faba bean plants compared with diseased untreated ones. These results are in agreement with Abo-Elyousr et. al.(2009). This may be due to accumulate of polysaccharides that are the principal components of cell walls which represent a physical barriers (Carpita and McCann, 2000) or reservoirs of antimicrobial proteins and secondary metabolites that inhibit the growth and invasion of many pathogens (Darvill and Albershein, 1984; De Lorenzo and Ferrari, 2002; Thomma et. al., 2002).

REFERENCES

Abdel-Kader, M.M., Nehal, S. El-Mougy and Ashour, A.M.A. 2002. Suppression of root rot incidence in faba bean fields by using certain isolates of *Trichoderma.* Egypt. J. Phytopathol*.* 30,12-25

Abdou, S., Abd-Alla, H.M. and Galal, A.A. 2001. Survey of sesame root rot/wilt disease in Minia and their possible control by ascorbic and salicylic acids. Assuit J. Agric. Sci. 32,135-152.

Abo-Elyouser, K.A.M., Sallam, N.A. and Asran, M. 2005. Accumulation of defence-related enzymes and phenols in bean plants in relation to induction of systemic resistance against common blight caused by *Xanthomonas campestris* pv. *phaseoli*. Assuit J. Agric*.* Sci. 36,107-119.

Abo-Elyouser, K.A.M.., Hashem, M. and Ali, E.H. 2009. Integrated control of cotton root rot disease by mixing fungal biocontrol agents and resistance inducers. Crop Protect*.* 28, 295-301.

Asran, M. R. 2005. Antifungal activity of chitosan against *Fusarium graminearum* root rot and seedling blight diseases of maize (*Zea mize* L.). Assuit J. Agric. Sci. 36,85-97.

Carl, N.M., Lee, K. Catherine, A., Wong, S. and Caor, J.P. 2005. Salicylic acid-induced resistance to cucumber mosaic virus in squash and *Arabidopsis thaliana*: Contrasting mechanisms of induction and antiviral action. Molecular Plant-Microbe Interact. 18,428-434.

Carpita, N.C. and McCann, M. 2000. The cell wall. In: Buchanan, B. (Ed.), Biochemistry and Molecular Biology of Plants. American Soci. Plant Physiologists. 52-108.

Darvill, A. and Albersheim, P. 1984. Phytoalexins and their elicitours- a defence against microbial infection in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 35,243-275.

Davis, J. M., Wu, H., Cooke, J. E. K., Reed, J. M., Luce, K. S., and C. H., Michler. 2002. Pathogen challenge, salicylic acid and jasmonic acid regulate expression of chitinases gene homologs in pine, Molecular Plant-Microbe Interact.15, 380-387.

De Lorenzo, G. and Ferrari, S. 2002. Polygalacturonase-inhibiting proteins in defence against phytopathogenic fungi. Curr. Opin. Plant Biol. 5:295-299.

Dever, J.E., Bandurski, R.S. and Kivilaan, A. 1968. Partial chemical characterization of corn root cell wall. Plant physiol. 43,50-56.

El-Ganaieny, R.M.A., El Sayed, A.M. and Gebraiel, M.Y. 2002. Induction resistance to Fusarial diseases in onion plants by treatment with antioxidants. Assuit J. Agric. Sci. 33,133-147.

Eraky, Amal, M.I, Abd El Hak, O. and Fahmy, F.G. 2007. Efficiency of salicylic acid and oxalic acid for controlling Fusarium wilt disease of tomato. Assuit J. Agric. Sci. 38,97-110.

Galal, A.A .and Abdou. El. S., 1996. Antioxidants for the control of fusarial diseases in cowpea. Egypt. J. Phytopathol. 24, 1-12.

Galbriath D.W. and Shields, B.A., 1981. Analysis of the initial stages of plant protoplast development using 33258 Hoechst: reactivation of the cell cycle. Physiol. Plant 51,380-386.

Gomez, K. A. and Gomez, A. A.1984. Statistical Procedures for Agriculture Research, 2nd ed. John Willey. New York, 680 p.

Hammerschmidt, R. and Smith-Becker, J.A. 1999. The role of salicylic acid in disease resistance. In: Agrawal, A.A. Tuzun, S. Bent, E. (Eds). Induced Plant Defenses Against Pathogens and Herbivores APS Press, St. Paul, pp. 37-53.

Khalifa, E.Z. 1997. The efficacy of seed treatment and soil application of *Trichoderma harzianum* on the biological control damping-off and root rot of faba bean caused by *Fusarium solani*. Proc. 8 th Cong. Phytopathol. Cairo, 115-123

Liu, L., Kloepper, J.W., Tuzun, S., 1995. Introduction of systemic resistance in cucumber against *Fusarium* wilt by plant growth-promoting rhizobacteria. Phytopathology, 85, 695-698.

Liyama, K., and Wallis., A.F.A. 1988. An improved acetyl bromide procedure for determining lignin in woods and wood pulps. Wood Sci. Technol. 22,271–280

Mahmoud, A.F.A. 2005. Studies on sugar beat root-rot diseases in Upper Egypt. M.Sc. Thesis, Fac. of Agric., Assiut Univ., Assiut, Egypt.

MSTAT-C, 1991. A Software Program for the Design, Management and Analysis of Agronomic Research Experiments. Michigan State University, p. 400.

Narusaka, Y., Narusaka, N., Horio, T. and Ishii, H. 1999. Comparison of local and systemic induction of acquired resistance in cucumber plants treated with benzothiadiazoles or salicylic acid. Plant cell Physiol., 40,388-395.

Nehal, S. El-Mougy, 2001. Field application of certain biological and chemical approaches on controlling bean wilt disease. Egypt. J. Phytopathol. 29, 69-78.

Nelson, P.E., Toussoun, T. A. and Marasas, W.F.O. 1983 Fusarium Species: An illustrated Manual for identification. The Pennsylvania Stat University Press 193 pp.

Oostendrop, M., Kunz, W., Dietric, B. and Staub, T. 2001. Induced disease in plants by chemicals. Europ. J. Plant Pathol. 107,19-28.

Schönbeck, F., Dehne, H.W. and Beicht, W. 1980. Activation of unspecific resistance mechanisms in plants. J. Plant Dis. Protect. 87:654-666.

Selvendran, R. R. and O'Neill, M.A., 1987. Isolation and analysis of cell wall from plant material. Methods Bioche. Analy. 32,125-153.

Spletzer, M.E. and Enyedi, A.J. 1999. Salicylic acid induced resistance to *Alternaria solani* in hydroponically grown tomato. Phytopathology 89,722-727.

Thangavelu, R., Palanisvami, A., Doraiswamy, S. and Velazhahan, R. 2003. The effect of *Pseudomonas fluorescens* and *Fusarium oxysporium* f.sp. *cubense* on induction of defence enzymes and phenolics in banana. Biol. Plant. 46,107-112.

Thomma, B.P.H.J., Cammue, B.P.A. and Thevissen, K., 2002. Plant defenses. Planta 216,193-202.

Yalpani, N., Silverman, P. Wilson, T.M.A. Kleier, D.A. and Raskin, I. 1991. Salicylic acid is a systemic signal and inducers of pathogenesis-related proteins in virus infected tobacco. Plant cell 3, 809-818.

Zhang, S. and Reddy, M.S. 2001. Lack of induced systemic resistance in peanut to late leaf spot disease by plant growth promoting rhizobacteria and chemical elicitors. Plant Dis. 85, 879-884.

إستخدام بعض المواد المستحثة لمقاومة مرض ذبول الفول البلدى تحت ظروف الصوبة والحقل

محمود رزق الله عسران* و نشوى محمد عاطف سلام**
*** قسم امراض النبات - كلية الزراعة – جامعة سوهاج**
**** قسم امراض النبات - كلية الزراعة – جامعة أسيوط**

يعتبر فطر *Fusarium oxysporum* f.sp. *fabae* من الفطريات الخطيرة التى تصيب نباتات الفول البلدى وتسبب الذبول ، وقد أجرى هذا البحث فى الصوبة والحقل بقسم أمراض النبات – جامعة أسيوط وأمكن التوصل إلى النتائج الآتية:

فى تجارب الصوبة تم استخدام ثلاث عزلات من المسبب المرضى وقد اختلفت هذه العزلات فى قدرتها المرضية على أصناف الفول البلدى المختبرة (جيزة 29 ، جيزة 4 ، مصر 1) فكانت العزلة FF1 أكثر العزلات قدرة مرضية وكذلك اختلفت قابلية أصناف الفول البلدى المختبرة للإصابة حيث كان الصنف مصر 1 أكثر الأصناف قابلية للإصابة بينما أظهر الصنف جيزة 29 والصنف جيزة 4 مقاومة متوسطة.

فى التجارب المعملية انخفض الوزن الجاف للفطر المسبب للمرض عند معاملة البيئة المغذية بحمض الأسكوربك وحمض الخليك يليها المعاملة بحمض السليسليك وحامض الأوكساليك.

تم دراسة تأثير المواد المختبرة على شدة المرض تحت ظروف الصوبة وتحت ظروف الحقل خلال موسمى 2009 ، 2010 حيث أظهرت النتائج أن معاملة بذور الفول بالمواد المختبرة بتركيزات مختلفة أدى إلى انخفاض نسبة الإصابة بدرجة معنوية مقارنة بالبذورغير المعاملة (كنترول).

تم تقدير كمية كلاً من السليلوز واللجنين والبكتين فى جذور النباتات وقد اظهرت النتائج حدوث زيادة معنوية فى محتوى الجذور من السليلوز مع كل المعاملات مقارنة بالنباتات غير المعاملة ماعدا المعاملة بحمض الأوكساليك. كما أدت معاملة البذور بحمض الخليك وحمض الأسكوربك كلاً على حده إلى زيادة معنوية فى محتوى النباتات من اللجنين بالمقارنة بالنباتات غير المعاملة وقد ظهرت أعلى نسبة من اللجنين عند معاملة البذور بحمض الأسكوربك بتركيز 1 % ، بينما كانت أقل نسبة من اللجنين عند معاملة البذور بتركيز 0.25 % من حمض الخليك.

قام بتحكيم البحث

أ.د / ياسر محمد نور الدين شبانة	**كلية الزراعة – جامعة المنصورة**
أ.د /عبد الرازق عبد العليم عبد الرازق	**كلية الزراعة – جامعة أسيوط**