J. Agric. Sci. Mansoura Univ., 33 (1): 231 - 242, 2008

STUDIES ON *Fusarium oxysporum* DISEASES OF SWEET BASIL PLANTS IN ISMAILIA GOVERNORATE

Shaban, W. I. A.
Agric. Botany Dep., Fac. Agric. Suez Canal University

ABSTRACT

Basil plant is one of the most popular medicinally herbs. Vascular wilt disease caused by *Fusarium oxysporum* is considering one of the most important diseases on basil in Egypt. *Fusarium oxysporum* isolates were isolated from all diseased plants showing symptoms of leaf yellowing, stunting, wilted foliage and root rots. All tested isolates of the fungus were found to be pathogenic for basil plants with different degrees. Pathogenicity test with representative isolates indicated that the causal agent is *F. oxysporum* that causes damping off, wilt, crown and root rot on basil seedlings and plants, where, isolate No.(2) showed more virulent. Under greenhouse conditions and artificial infestation, basil cultivars were differed in their susceptibility to infection by the fungus. Double flowers basil cv. showed more resistant for the disease symptoms, followed by Red leaves cv. Two techniques, vegetative or mycelial compatibility grouping and SDS (PAGE) fingerprinting, were showed differences between Fusarium isolates. Bio-Zeid bioproducts was more effective against Fusarium disease incidence. Also, seed dressing by combining of Vitavax-200 and Rizolex-T showed significant effects in controlling the disease incidence and produced the highest value of healthy plants.
Keywords: biological, chemical, control, crown, root, rot, wilt

INTRODUCTION

Sweet basil (*Ocimum basilicum* L., Labiatae) is a cultivated fresh herb grown commercially in the Mediterranean region. It has become popular in the last 10 years and is used, both fresh and dried, as a food spice and in traditional medicine. Fusarium disease caused by *F. oxysporum* f. sp. *basilici*, represents a major problem on this crop in Egypt. Infected basil plants is characterized by one or more of wilt, crown rot and root rot symptoms Hilal *et al*. (1998), with typical including wilting, brown and black discoloration of roots and stems, black lesions on stems and blackening of the vegetative apices Gamliel *et al.* (1996) and Ghebrial (2005). Although the disease has been known since 1956 and occurs in Russia, Italy and France Vergovskii (1956); Grasso (1975) and Mercier and Pionnat (1982) respectively. Seed transmission is believed to be the principal means of dissemination Elmer *et al.* (1994). However, the unavailability of commercially acceptable resistant cultivars dictates the need for identification of resistant germ plasm for breeding purposes. The host range of this disease has been reported to extend to sweet and lemon basil cultivars, whereas six other herbs in the family Lamiaceae were reported to be immune Keinath (1994). However, there is a need to examine a wider selection of cultivars to determine if there are any resistant cultivars in *Ocimum basilicum*. Control of the wilt pathogen is complicated by the limited availability of registered fungicides. The only partially effective compounds are benzimidazoles fungicides, which are seldom applicable even as seed dressings because of frequent residues and inadequate levels of control Garibaldi *et al.* (1997). Low efficacy of chemical

control measures, the limited availability of resistant cultivars Reuveni *et al.* (1997.) and the unsatisfactory level of control sometimes offered by the commercially available formulations of biocontrol agents Keinath (1994) and Minuto *et al.* (1997) boost the urgency for seed and transplant certification procedures on sweet basil. One dimensional polyacrylamide gel electrophoresis of proteins has used extensively for identification and classification at the strain and species level Snider (1973). Root rot and wilt disease of various crops can be controlled by treated with fungicides such as Benlate, Topsin-M 70, Vitavax, Vetavax/Captan, and Vitavax/Thiram Baraka *et al.* (2006). The aims of the present study were to identify the causal agent of basil wilt and examine the genetic activity between the isolates of the pathogen. The sub main objectives of the present investigation were to evaluate several cultivars of basil for their resistance to Fusarium wilt and to evaluate chemical and biological control.

MATERIALS AND METHODS

Isolation and Identification of the causal pathogen:

Diseased basil plants were collected from several basil growing regions in Ismailia Governorate (Sarabium, El-Kassasin, Ismailia and Shark El-Bohirat). The plants showed wilt symptoms were used for isolate potential fungal pathogens. Sections (1cm) from roots, crown and stems of diseased plants were surface sterilized by NaOCl (1%) for 1 min, and rinsed in sterile water. Each segment was then cut into smaller pieces and plated on PDA and incubated at 25 °C for 3-7 days under lights with 12-h photoperiod. Hyphal tip and single spore techniques were prepared for get a pure culture on PDA of fungal isolates. All isolates were identified to species level according to the description of Nelson *et al*., (1983); Moubasher (1993) and Lislie and Summerll (2006) and confirmed in Assiut University Mycological Center (AUMC).

Pathogenicity and disease assessment:

Pathogenicity test was conducted under greenhouse conditions, to confirm that seven isolates of *Fusarium oxysporum* are the causal agent of wilt symptoms on basil plants. Sterilized sorghum medium was used to prepare fungal inoculum. The medium was inoculated with the isolated fungi (*F. oxysporum*, *F. solani* and *Rhizoctonia solani*) and incubated for 15 days at 25°C. Pots (15-cm in diam.) were filled with sand:clay 1:1 (v/v) soil. The potted soil was infested with each fungal isolate at the rate of 1.5% (w/w) from the soil weight. Control pots were inoculated with the equivalent amount of sterile sorghum medium. The infested soil was watered and mixed thoroughly for one week to insure even distribution the inoculum. Ten seeds per pot were sown for each replicate and five replicate were used with each isolate. Another five replicate were used as a control treatment. Pre-, post-emergence damping-off were recorded after 15 and 30 days respectively. Whereas, disease incidence was calculated at 90 days from sowing as infection percentage of wilt and crown rot and disease severity for root rot according to Turner and VanAlfan (1983).

Cultivar reaction to wilt disease:

Reaction of five different basil cultivars to wilt, crown and root rot caused by *F. oxysporum* was determined in pots under greenhouse conditions. Seeds of cultivars were obtained from El-Kassasin Horti. Research Station, Ismailia Governorate. The cultivars tested were: Large and Small leaves, Red leaves, Single and Double red flower. Seeds were germinated in potting mix (peat: vermiculite, 1:1), healthy seedlings (7 to 10 cm tall) were used in this test. A highly virulent isolate of *F. oxysporum* (No.2) was prepared and used in infestation the soil as mentioned before. Five seedlings were transplanted in each pot (20-cm in diam.), three replicates per each cultivar were used. Another set of non infested pots were used as a control. Percentage of infected plants wilt, crown as well as root rot and healthy plants were recorded after 60 days from seedlings sowing as described before.

Mycelial compatibility between *Fusarium* isolates:

Seven isolates of *F. oxysporum* which were previously isolated from diseased basil plants of different locations from Ismailia Governorate were grown in Petri dishes (9cm) containing PDA medium. Isolates were paired in all possible combinations on PDA plates according to Kohn *et al*. (1991). Pairing among isolates was studies to confirm changes and physiological differentiation among isolates. Mycelial reactions were recorded as incompatible when an apparent line of demarcation was observed between the confronting paired isolates. All isolates belonging to the same mycelial compatibility groups were compatible and isolates belonging to different were incompatibility. Each pairing was performed at three times.

Using SDS-PAGE analysis technique:

Isolates of *F. oxysporum* from diverse regions in Ismailia Governorate were used. Mycelium production was carried out by culturing each fungal isolate in 100ml of potato dextrose broth in 500ml Erlenmeyer flasks and incubating at 25°C for ten days. Mycelial mats were filtered under vacuum on a Büchner funnel, rinsed twice with distilled water and blotted dry. Mycelium to be used for protein analyses was freeze-dried and stored at -20°C. Soluble proteins were extracted by grinding 100mg freeze-dried mycelium with pestle and mortar in liquid nitrogen and 5ml buffer solution. The mixture was centrifuged for 20 min. (17000 rpm) and the supernatant collected. The protein content in supernatant was estimated Bradford (1976) with bovine serum albumin as standard protein. Protein content was adjusted to 2 mg/ml per sample. SDS-PAGE was performed by the method described by Laemmli 1970 and modified by Hames 1995, proteins were analyzed on 1.5mm thick and 15cm long gels run in a dual vertical slab unit. From each sample, 25µl of extract was loaded on a polyacrylamide gel. The separation gel (10%) and staking gel (3.5%) were prepared from an acrylamide monomer solution. Electrophoresis was carried out at a constant current of 35mA through the stacking gel, and at 90mA through the separation gel at room temperature. After electrophoresis, the gels were stained by silver nitrate. For cluster analysis of protein banding patterns, the unweighted pair group method using average linkages was used according to methodology of Vauterin and Vauterin (1992).

Disease control:

Chemical and biological control on the most susceptible basil cultivar (Large leave) to wilt disease were determined in this trial. Three bio products namely: Bio-Arc (*Bacillus megaterium*, $30x10^6$ cfu/ml) and Bio-Zeid (*Trichoderma harzianum*, $2.5x10^6$ cfu/ml), that produced by Plant Path. Res. Inst., Agric. Res. Center, Giza, Egypt as well as, Rhizo-N (*Bacilllus subtilis*, $30x10^6$ cfu/ml) produced by El-Nasr Co. Fertilizers (BIO Egypt) were used in this study. Also, two chemical fungicides Rizolex-T and Vitavax-200 at the rate of 3g/L were used individually and together (1.5g/L from each fungicides as combination) as seed dressing before sowing. Seeds were soaked for two hr. in the bioproducts at the rate of 5g/L or in the fungicides as mentioned before. Five pots were infested with the most virulent isolate of *F. oxysporum* (No.2) for using with each treatment. Five pots free from the pathogen were sown by soaked seeds in distilled water to use as control. The pots were sown with seeds at the rate of 10 seeds /pot and irrigated when needed. Percentages of pre-, post-emergence damping-off were recorded after 15 and 30 days respectively. Whereas, percentage of infected plants wilt, crown and root rot as well as healthy plants were recorded after 90 days from sowing as described before.

Statistical analysis:

Obtained data were exposed to the statistical analysis using complete randomized block design described by Gomez and Gomez (1984) and treatment means were compared using L.S.D test at %5.

RESULTS AND DISCUSSION

Isolation from collected wilted basil plant samples yielded the associated of one or more of the following fungi, seven isolates at rate (66.6%) of Fusarium oxysporum, five of them were isolated from internal stems part of wilted plants and two isolates were isolated from roots and crown parts. Colonies of this fungus identified as F. oxysporum based on the presence of growth rate, short monophialides bearing microconidia in false heads, chlamydospores, and characteristically shaped macroconidia according to Nelson *et al.* (1983) and Lislie and Summerll (2006). According to pathogenicity test and symptoms of collected diseased samples (wilt and vascular discoloration) and isolated the pathogen from internal stem parts, the pathogen may be confirm as *F. oxysporum* f sp. *basilici*. This pathogen was previously reported to be the causal agent for basil plants in other countries Gamliel *et al.* (1996) and Chiocchetti *et al.* (1999).Therefore, the pathogen was identified by (Dzidzariya, 1968) as *F. oxysporum* f. sp. *basilicium* and was later confirmed and renamed as *F. oxysporum* f. sp. *basilici* Minuto *et al.* (1994). In addition, other associated fungi were observed with isolation process, two isolates (22.2%) of *F. solani* and one isolate (11.1%) of *Rhizoctonia solani*. Percentage of isolated fungi was presented in (Fig. 1). These pathogens were collected from wilted basil plant confirmed for their pathogenicity.

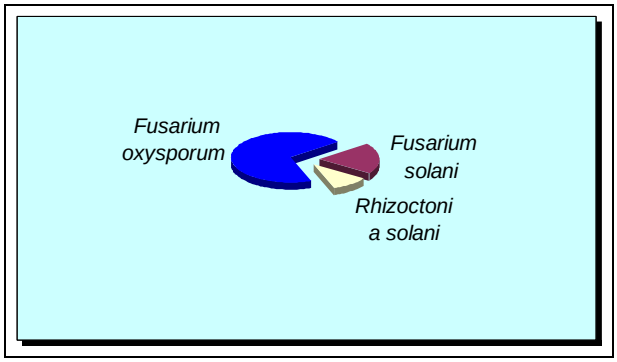
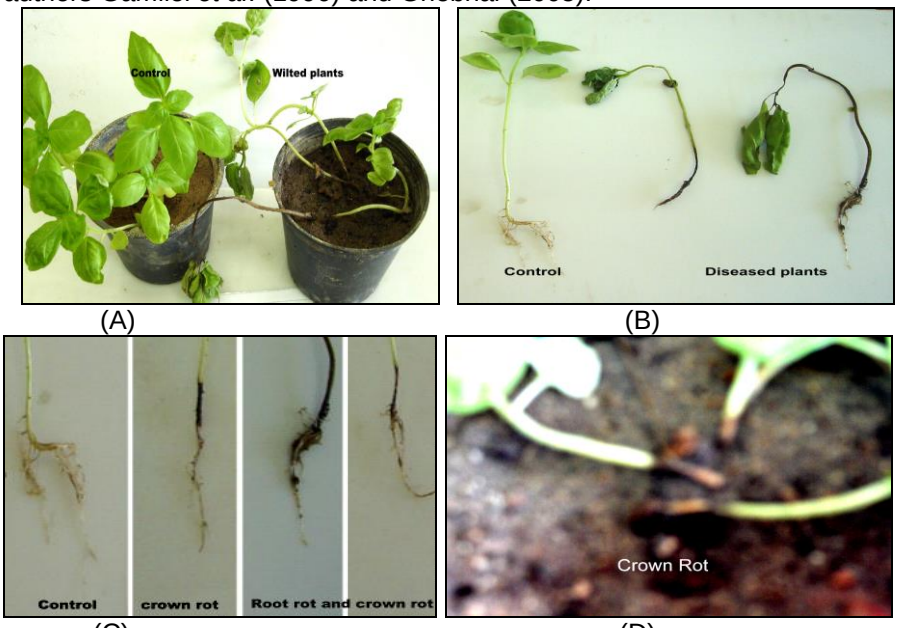


Fig. (1): Percentage of fungi isolated from different parts of diseased basil plant.

Disease symptoms and observations:

Symptoms observed of wilted basil plants were described as follows: Typical symptoms of wilt at all stages of plant growth, including young plants, internal brown or blackening of stems, basal stems (crown) and roots are rotted (Fig. 2: C&D). Leaves showed distortion and often remain attached by the stem (Fig. 2: A&B). In many cases plants eventually died. Appearance and severity of symptoms varied among individual plants and among greenhouses or cultivars. Disease was especially severe in greenhouses with along history of basil cropping. These symptoms were observed and described by many authors Gamliel *et al*. (1996) and Ghebrial (2005).



(A) (B)

(C) (D)

Fig. (2): A,B,C and D Showing Fusarium disease symptoms on basil seedlings.

Pathogenicity test:

All isolates of *F. oxysporum* isolated from wilted basil plants collected from Ismailia Governorate, were found to be the causal agent of Fusarium wilt of basil. Pathogenic isolates originating from various plant parts did not differ in the symptoms they incited on inoculated plants. *F. oxysporum* was re-isolated from artificially infested plants. Isolates of isolated fungi were more or less able to attack basil plants at any stage of growth (Table, 1). Basil plants at all stages of growth were highly vulnerable to attack by isolate No. 2 of *F. oxysporum*, since it gave significantly the low healthy plants percentage (33.3%), and were more *Fusarium* isolates virulent that caused wilt ,crown rot as well as root rot (19.1, 23.8 and 19.1%, respectively), followed by isolate No. 7. Whereas, isolates No. 5 & 6 were less virulent on the basil plants (57.4% & 63.9% of healthy plants, respectively). However, all *F. oxysporum* isolates were able to attack basil plants and caused vascular wilt or crown and root rot at different percentages. Furthermore, as several investigators reported that *F. oxysporum* f. sp. *basilici* (Fob) has long been known to be the main organism causing wilt, crown and root rot of basil. Gamliel *et al.* (1996) and Summerell *et al*., (2006). They indicated that basil wilt caused by *F. oxysporum* f. sp. *basilici* is characterized by both a wilt and rot of the crown as well as roots, with typical including wilting, brown and black discoloration of roots. As for the other pathogens, *F. solani* and *R. solani* were highly virulent to attack basil seedlings during pre- and post-emergence stages in addition root rot stages. However, *R. solani* caused crown rot and root rot (7.3% and 21.7%, respectively). Nevertheless, *F. oxysporum* was not only the most aggressive pathogen, but also the most frequently encountered fungus. For this reason, special attention was paid to this fungus. The pathogenic properties of *F. oxysporum* in this study are in agreement with results obtained by Biris *et al.* (2004); Ghabrial (2005) and Summerell *et al.* (2006).

Table (1): Pathogenicity of isolates representing fungal species associated with basil wilted plants.

Tested isolates	Location	Damping-off %		Diseases symptoms %				Healthy survival %
		Pre-	Post-	Wilt	Crown rot	Root rot	*TIP %	
F.o. 1	**El-Kassasin**	8.0	8.7	17.4	19.6	13.0	36.9	54.3
F.o. 2	**El-Kassasin**	16.0	11.9	19.1	23.8	19.1	54.7	33.3
F.o. 3	**Sarabium**	6.0	8.5	17.0	19.1	14.9	36.2	55.3
F.o. 4	**Ismailia**	8.0	8.7	17.4	19.5	17.7	41.3	50.0
F.o. 5	**Shark el Bohirat**	8.0	6.5	17.4	15.2	8.7	36.1	57.4
F.o. 6	**Ismailia**	14.0	8.3	11.1	16.6	8.3	27.8	63.9
F.o. 7	**El-Kassasin**	10.0	9.3	16.7	17.3	12.5	46.5	44.2
F.s.1	**El-Kassasin**	8.0	14.6	00	00	17.3	17.3	68.1
F.s.2	**El-Kassasin**	12.0	14.6	00	00	15.6	15.6	69.8
R.s.	**El-Kassasin**	18.0	13.3	00	7.3	21.7	29.0	57.7
Control	--	00	0.0	00	00	00	00	100
L.S.D. at 5%	--	1.7	1.4	1.8	2.2	2.1	3.5	5.9

(F.o.) = *Fusarium oxysporum*, (F.s.) = *Fusarium solani*, (R.s.)= *Rhizoctonia solani*
***TIP=Total percent of Infected Plant by wilt, crown rot, root rot or more of one of them**

Data in Table (2) indicate that there is a significant difference in infection susceptibility between the tested basil cultivars. Double flowers basil

cv. showed the lowest percentage of wilt (13.3%), crown rot (13.3%) and root rot (20.3%), in addition, the highest percentage of healthy plants (66.2%) was observed compared with the other cultivars. Whereas, no significant difference between Red leaves and Double flowers basil cvs. on crown and root rot percentages and they showed a moderate resistance for Fusarium disease compared with the other cultivars. On the contrary, Large leaves basil cv. was the more susceptible to wilt (46.6%), crown rot (40.0), root rot (25.6%) and showed the lowest of healthy pants (36.3%) followed by Small leaves basil cv. The variation of response basil cvs. for infected by *F. oxysporum* may be due to mainly factors such as: 1- The virulence of the pathogen that may resisted by a specific gene or polygene. 2- Mechanical or photochemical of host resistance, which also may be due to the cultivars have one or more of control gene. Interaction between these factors may explain the mechanism of host resistance against the pathogen. This is in agreement with the results obtained by Garibaldi *et al*. (1997) who showed that cultivars resistant to Fusarium wilt are being sought by seed companies. The basil cultivars tested by Ghabrial (2005) in Egypt were fond to be varying in their susceptibility to wilt disease caused by *F. oxysporum* f. sp. *basilici*. Also, Reuveni *et al.* (1997) mentioned that there is a limited availability of resistant basil cultivars for controlling Fusarium wilt disease.

Table (2): Varietal reaction of basil plants grown in artificially infested soil against *F. oxysporum* diseases.

Basil cv.	Infection %			Healthy survival %
	Wilt	Crown rot	Root rot	
Large leaves	46.6	40.0	25.6	36.3
Small leaves	33.3	26.6	22.4	38.5
Red leaves	26.6	13.3	21.6	52.2
Single flowers	33.3	33.3	21.5	45.5
Double flowers	13.3	13.3	20.3	66.2
L.S.D. at 5%	4.2	3.5	3.7	4.2

Mycelial compatibility (MC) studies presented in Table (3) show antagonistic and compatibility effect between different *F. oxysporum* isolates. Isolate No. 5 (the less pathogenic isolate) showed antagonistic effect or produce inhibition zone when paired with more of other isolates followed by, isolate No. 4 was produced inhibition zone between four isolates. On the other hand, isolate No. 2 more isolates aggressiveness on basil plants, was the less antagonism when paired with other isolates. Also, self paired between the same isolate not shown interactions. According to the observed results, (MC) may perpetuate discrete genes, on the other hand, aggressiveness level may vary within (MC). The same results are in agreement with Kohn *et al*. (1990) and Elena and Paplomatas (1998). Combinability between isolates or aggressiveness level varied within field populations, geographical origin, taxonomic position of the host from which they were isolated, and they do not appear to be related to pathogenicity for particular host species Chen (1994) and Korolev *et al*. (2000). However, to

draw more definite conclusions, it is necessary to study more isolates from different cultivars and geographic origins. This study demonstrates that different (MC) have distinct pathogenicity and physiology of growth between isolates. Thus, the study can suggest relatedness among certain strains within the pathogen population, shedding light on their evolutionary relationship and often, predicting their pathogenic potential.

Table (3): Mycelial compatibility within *Fusarium oxysporum* isolated from different locations

Isolate No.	1	2	3	4	5	6	7
1	-	-	+	+	+	+	-
2		-	-	-	+	+	-
3			-	+	+	-	+
4				-	+	+	+
5					-	-	+
6						-	+
7							-

(-) No reaction between paired isolates
(+) Strong reaction (inhibition zone) between paired isolates

SDS-PAGE is a rapid method for quantifying comparing and characterizing protein. Cluster analysis of the protein markers data placed the Fusarium isolates into main groups (Fig. 3), according to the previous isolates assignment. Also, data showed differences in the serological relationship among isolates. Within this cluster, there are two main groups at genetic similarity of 75.5% each consisting of several subclusters. The banding patterns of isolate in line 5, which isolates from Shark El- Bohirat, varied greatly from other isolates to justify a separate group (similarity level =75.5%). However, the isolates in line (4&6) have the highest similarity in dissociated protein bands (similarity level =99.8%). At the same time, the isolates in line (1&2) showed the second high similarity according the cluster (similarity level =96%). Clustering analysis indicates that the seven F. oxysporum isolates can be assigned to five protein types. In our study, this technique was used to compare soluble protein dissociated from Fusarium isolates collected from different localities.

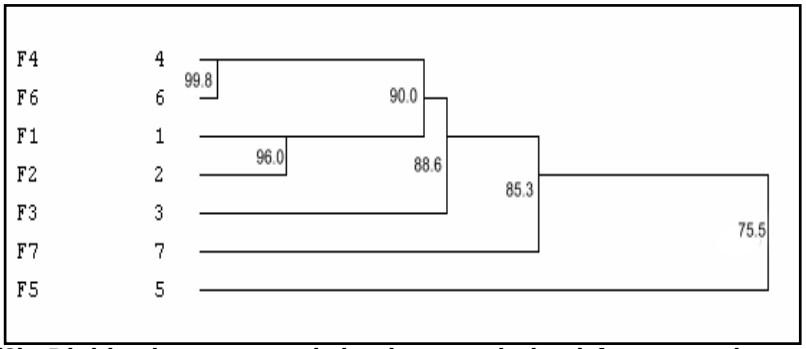


Fig. (3): Digitized pattern and dendogram derived from protein profile analysis of *Fusarium* isolates Using SDS-PAGE analysis.

The genetic similarity between isolates which were incited in the same cluster may due to the isolates growth in closed geographic area and on the same varietal host. These results are agreed with those obtained by Aly *et al.* (2003). The results obtained by SDS-PAGE of whole-cell proteins can discriminate at much the same level as DNA fingerprinting in some cases Priest and Austin (1993).

For different seed dressing treatment were used to control Fusarium wilt disease of basil. Data in Table (4) show a significant reduction in disease incidence as a result of treated seeds by selected fungicides or bioproducts with the control treatment. Adding Bio-Zeid bioproducts as seed dressing greatly reduced wilt, crown and root rot diseases incidence on basil plants and gave the highest percentage (79.6%) of health survival plants. Moreover, seed dressing by combining of Vitavax-200 and Rizolex-T was more effective fungicide treatment in reducing wilt, crown and root rot diseases incidence (10.0, 12.0 and 6.0% respectively), followed by Vitavax-200 (12.2, 10.2 and 8.2% respectively). But no significant differences were observed between the combination and individual effect for Vitavax 200. Also, other bioproducts (Rhizo-N and Bio-Arc) were showed significant effect in controlling wilt, crown and root rot and produced the significant value of healthy plants (68.9 and 68.1% respectively) compared with the control. The findings are consistent with the results of several investigators Hilal and Helmy (1998); Amer and El-Shannawy (2005) and Baraka *et al*. (2006). Other authors have speculated that antagonistic activity of some microorganisms against the plant pathogens may be due to the ability of these agents to grow and sporulate on seed and thereafter to become established in large numbers in the soil Whipps and Lumsden (2001) and McLean *et al.* (2004). Biocontrol of this pathogen may also achieve by any general or specific effect that will temporarily or permanently inactivate the propagules. Antibiosis has been perhaps the most widely recognized mechanism that may bring about inactivation or destruction without germination suppress has been attributed to antagonistic effect of associated microbiota (Wood and Tveit 1955).

Table (4): Efficacy of bioagents and fungicides as seed dressing treatment in controlling Fusarium disease on basil plants.

Seed treatment	Damping-off %		Diseases symptoms %				Healthy survival %
	Pre-	Post-	Wilt	Crown rot	Root rot	***TIP	
Rhizo-N	0.0	4.0	14.6	12.5	8.33	27.1	68.9
Bio-Zeid	0.0	2.0	8.2	10.2	6.1	18.4	79.6
Bio-Arc	0.0	8.0	13.1	15.2	10.9	23.9	68.1
Rizolex-T (I)	0.0	2.0	14.3	14.3	8.2	28.6	69.4
Vitavax 200(II)	0.0	2.0	12.2	10.2	8.2	22.4	75.5
Combined (I+II)	0.0	0.0	10.0	12.0	6.0	22.0	78.0
*Control (I)	18.0	9.7	36.6	24.4	31.7	60.9	29.4
**Control (II)	0.0	0.0	0	0	0	00	100
L.S.D. at 5%	2.8	2.4	2.7	1.2	2.5	3.1	4.6

*** Control (I) = Soil infested with pathogen without fungicides or bioproducts**
**** Control (II) = Non infested potted soil.**
*****TIP=Total Infected Plant by wilt, crown rot, root rot or more one of them**

The biocides effect may be to sensitivity of F. solani and R. solani to an antibiotic complex containing bacilysin and fengymycin produced by B. subtilis (Rhizo-N) Reddy *et al.* (1994). On the other hand, control of this pathogen is complicated by the limited availability of registered fungicides. The only partially effective compounds are benzimidazoles, which are seldom applicable even as seed dressings because of frequent residues and inadequate levels of control Minuto *et al.* (1994) and Garibaldi *et al.* (1997).

REFERENCES

Aly, I.N.; M.A. Abdel-Sattar; K.A. Abd-Elsalam; M.S. Khalil and J.A. Verreet, (2003) Comparison of multi-locus enzyme and protein gel electrophoresis in the discrimination of five *Fusarium* species isolated from Egyptian cottons. African Journal of Biotechnology 2: 206-210.

Amer, G. A. and Z. El-Shennawy, Rania, (2005) Efficiency of formulation biocontrol agents in controlling damping-off and root rot diseases of lupine (*Lupinus termis* L.) under field conditions. Menofyia J. Agric. Res., 30: 853-866.

Baraka, M. A.; S.A. Omar; El-Barougy Ebtehage, and A.H. Zian, (2006) Controlling seedling damping-off, root rot and wilt diseases of lupine (*Lupinus albus* L.). Agric. Res. J. Suez Canal Univ. 6: 57-68.

Biris, D.; D.J Vakalounakis, and E. Klironomou, (2004) Fusarium wilt of basil in Greece: foliar and cultivar evaluation for resistance. Phytoparasitica 32: 160-166.

Bradford, M.M., (1976) A rapid and sensitive for the quantification of microgram quantities of protein utilizing the principle of protein dyebinding. Anal. Biochem. 72: 248-254.

Chen, W. (1994) Vegetative compatibility group of *Verticillium dahlia* from ornamental woody plants. Phytopathology 84, 214-219.

Chiocchetti, A.; S. Ghignone; A. Minuto; M.L. Gullino; A. Garibaldi and Q. Migheli, (1999) Identification of *Fusarium oxysporum* f. sp. *basilici* isolated from soil, basil seed, and plants by RAPD analysis. Plant Dis. 83:576-581.

Dzidzariya, O.M. (1968) Measures for the control of fusariosis of East Indian basil. (In Russian) (Abstr.) Rev. Appl. Mycol. 46:159 (1969).

Elena, K. and E.J. Paplomatas, (1998) Vegetative compatibility groups within *Verticillium dahliae* isolates from different hosts in Greece. Plant Pathol. 47: 635-640.

Elmer, W.H.; R.L. Wick, and P. Haviland, (1994) Vegetative compatibility among isolates of *Fusarium oxysporum* f. sp. *basilici* recovered from basil seeds and infected plants. Plant Dis. 78:789-791.

Gamliel, A.; T. Katan, H. Yunis and J. Katan (1996) Fusarium wilt and crown rot of sweet basil: identification, epidemiology and control. Phytoparasitica 24:2, 1996.

Garibaldi, A.; M.L. Gullino; and G. Minuto (1997) Diseases of basil and their management. Plant Dis. 81:124-132.

Ghebrial, Eman W.R. (2005) Pathological studies and control evaluation of sweet basil disease caused by *Fusarium oxysporum* f. sp. *basilici.* M. Sc. Thesis, Fac. Sci., Cairo Univ., pp. 177.

Gomez, K.A. and A.A. Gomez (1984) Statistical procedures for agricultural research. John Wiley and Sons, Inc. pp. 680.

Grasso, D.S. (1975) Un avvizzimento del basilico de *Fusarium oxysporum*. Inf. Fitopatol. 25:5-7.

Hames, B.D. (1995) One-dimensional polyacrylamide gel electrophoresis. In: gel electrophoresis of protein. A practical approach. Hames, B. D. and Rickwood, D. (eds.) Oxford Univ. Press, New York pp. 1-147.

Hilal, A.A. and A.A. Helmy (1998) Crown and root rot of turfgrasses in Egypt: Identification of the causal pathogen, pathogenicity and biocontrol. Egypt, J. Appl. Sci., 13: 1-18.

Hilal. A.A.; I.M. Harridy; A.M. Abo-Al-Ela; M.A.M. Baioumy and S.A. El Morsy, (1998) Studies on the commonly and newly occurring diseases of seven medicinal plants and yield losses in relation to some agricultural practices in Egypt. Egypt. J. Appl. Sci., 13:41-60.

Keinath, A.P. (1994) Pathogenicity and host range of *Fusarium oxysporum* from sweet basil and evaluation of disease control methods. Plant Dis. 78:1211-1215.

Kohn, L.M.; I. Carbone; and J.B. Anderson (1990) Mycelial interactions in *S. sclerotiorum*. Exp. Mycol. 14:255-267.

Kohn, L.M.; E. Stasovski; I. Carbone; J. Royer and J.B. Anderson, (1991) Mycelial in a compatibility and molecular marked identify genetic variability in field populations of *S. sclerotiorum*. Phytopathology, 81: 480-485.

Korolev, N.; J. Katan, and T. Katan (2000) Vegetative compatibility groups of *Verticillium dahilae* in Israael: their distribution and association with pathogenicity. Phytopathology 90:529-536.

Laemmli, U.K. (1970) Cleaving of the structural protein during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

Lislie J.F. and B.A. Summerll (2006) The Fusarium-Laboratory Manual. Blackwell Publishing pp. 388.

McLean, K.L.; S.L. Dodd; B.E. Sleight; R.A. Hill and A. Stewart (2004) Comparison of the behavior of a transformed hygromycin resistant strain of *Trichoderma atoviride* with the wild-type strain. New Zealand Plant Protection, 57: 72-76.

Mercier, S. and J.C. Pionnat, (1982) Presence en France de la fusariose vasculaire du basilic. C. R. Seances Acad. Agric. Fr. 68:416-419.

Minuto, A.; G. Minuto; Q. Migheli; M. Mocioni and M.L. Gullino (1997) Effect of antagonistic *Fusarium* spp. and of different commercial biofungicide formulations on Fusarium wilt of basil (*Ocimum basilicum* L.). Crop Prot. 16:765-769.

Minuto, G.; A. Garibaldi; and M.L. Gullino (1994) Biological control of Fusarium wilt of basil (*Ocimum basilicum* L.). Brighton Crop Prot. Conf. 2:811-816.

Moubasher A.H. (1993) Soil fungi in Qatar and other Arab countries. The Scientific and Applied Research Center, Univ. of Qatar. 566 pp.

Nelson, P.E.; T.A. Toussoun and W.F.O. Marasas (1983) *Fusarium* species: An illustrated manual for identification. Pennsylvania state Univ. press, Univ. Park, Pennsylvania. USA.

Priest, F.G. and B. Austin, (1993) Modern bacterial taxonomy (2nd ed.) Chapman & Hall, London.

Reuveni, R.; N. Dudai; E. Putievsky; W. H. Elmer; and R. L. Wick, 1997. Evaluation and identification of basil germ plasm for resistance to *Fusarium oxysporum* f. sp. *basilicum*. Plant Dis. 81:1077-1081.

Snider, R.D. (1973) Electrophoresis and taxonomy of phytopathogenic fungi. Bull. Torrey Bot. Club 100: 272-276.

Summerell, B.A.; L.V. Gunn; S. Bullok; L.T. Tesorieo and L.W. Burgess (2006) Vascular wilt of basil in Australia. Australian Plant Pathology. 35: 65-67.

Turner, V. and N.K. VanAlfan (1983). Crown rot of alfalfa in Utah. Phytopathology, 73: 1333-1337.

Vauterin, L. A. and P. Vauterin (1992) Computer-aided objective comparison of electrophoresis patterns for grouping and identification of microorganisms. Eur. Microbiol. 1: 37-41.

Vergovskii, V. I. (1956) Some peculiarities in the presence of fusariosis in basil. Kratk. Otchet. Nauchno Issled. Vseoyus. Inst. Maslichn. Efiromaslichn. Kult. 7:195-197 (c.f. Rev. Appl. Mycol. 38:613, 1959).

Whipps J.M. and R.D. Lumsden (2001) Commercial use of fungi as plant disease biological control agents: status and prospects. CABI Publishing, Wallingford, United kingdom. pp: 9-22.

Wood, R.K.S. and M. Tveit (1955). Control of plant diseases by use of antagonistic organisms. Bot. Rev. 21:441-92.

"دراسات على امراض الفيوزاريوم اكسسبورم علىنباتات الريحان الحلو في محافظة الإسماعيلية"

وليد إبراهيم أمين شعبان

قسم النبات الزراعي - كلية الزراعة- جامعة قناة السويس

نبات الريحان واحد من أكثر الأعشاب الطبية الشعبية. يعتبر مرض الذبول الوعائي المتسبب عن فطر الفيوزاريوم اكسسبورم واحد من أكثر أمراض الريحان في مصر. عزلات فطر الفيوزاريوم اكسسبورمالتى عزلت من جميع النباتات التي أظهرت أعراض الاصفرار و التقزم و ذبول المجموع الخضري و أعفان الجذور. جميع العزلات المختبرة للفطر كانت ممرضة لنباتات الريحان بدرجات متفاوتة. اختبار القدرة المرضية للعزلات المتحصل عليها يشير إلى أن فطر الفيوزاريوم اكسسبورم يسبب موت للبادرات و ذبول وعفن للتاج وعفن للجذور على شتلات ونباتات الريحان وقد وجد ان العزلة رقم(٢) كانت اكثر العزلات فى القدرة المرضية. اختلفت أصناف الريحان في قابليتها للإصابة بالمسبب المرضى تحت ظروف الصوبه والعدوى الصناعية. وجد ان صنف الريحان مزدوج الأزهار أكثر الأصناف مقاومة لكل من الذبول وعفن كل من التاج و الجذور ثم صنف الريحان احمر الأوراق. استخدمت طريقتين (التوافق الميسليومى بين العزلات والفصل الكهربي للبروتين الكلى للفطر) لتوضيح مدى الاختلاف و التشابه بين العزلات. المركب الحيوي بيو-زيد كان أكثر كفاءة في مقاومة حدوث المرض الناتج عن فطر الفيوزاريوم. ووجد أن نقع البذور في خليط مبيدا الفيتافاكس ٢٠٠ و الريزولكس-تى اظهرا تأثير معنوي في مقاومة المرض وإنتاج نسب عالية من لنباتات السليمة