Biological Control of Wilt Disease Caused by *Fusarium oxysporum* in Fennel under Organic Farming System

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ABSTRACT

Alternaria* spp., *Aspergillus flavus*, *A. niger*, *A. terreus*, *Fusarium oxysporum*, *F. solani*, *Penicillium* spp., *Phoma* sp., *Rhizopus nigricans and ***Trichoderma* spp.** were isolated from the rhizosphere and roots and stem bases of fennel plants (***Foeniculum vulgare***), showing wilt symptoms, grown in an organic farm. ***F. oxysporum*** gave the disease symptoms of wilt under greenhouse conditions. Seeds of anise, celery, coriander, fennel and parsley were also susceptible to the crude toxin(s). ***In vitro*** test, ***T. harzianum*** gave the best antagonistic effect against ***F. oxysporum***, followed by ***P. fluorescens*** and ***B. subtilis***. In field experiment, soil treatment revealed that ***T. harzianum*** has decreased the population of the pathogenic fungus, damping off and wilt disease incidence, followed by ***P. fluorescens*** and ***B. subtilis***. The bioagent treatments enhanced some growth parameters, i.e. plant height, number of primary branches, fresh and dry weight of umbels after flowering stage and also the weight of 100 fennel seeds after harvest. This work showed that application of biological control in organic farming was effective against ***F. oxysporum*** causing the wilt disease in fennel.

Key words: Biological control, Fennel, *Fusarium oxysporum*, Medicinal plants, Organic farming.

INTRODUCTION

Fennel (***Foeniculum vulgare***) is one of many medicinal plant species of the family Umbelliferae grown under organic farming system in Egypt. It has a great importance in pharmaceutical industries and food uses. Among the most common pathogenic fungi ***Fusarium*** spp. was oftenly reported on various species of medicinal plants and could be transmitted by seeds **(Machowicz, 2001)**. ***Fusarium oxysporum*** was present in the rhizosphere as well as in the rhizoplane of all non-leguminous plants **(Sharma *et al.*, 2001)**. ***F. oxysporum*, *F. graminearum* (*Gibberella zeae*)** and ***F. moniliforme* (*G. fujikuroi*)** were recorded as pathogens to fennel seeds **(Dal, 1987)**. Fennel seeds are also susceptible to both ***F. oxysporum* f.sp. *cumini*** and its toxins produced on Czapek – Dox medium **(Gour *et al.*, 1988)**. ***Fusarium moniliforme*** was more common than ***F. oxysporum*** in 23 fennel lines. Therefore, seeds were treated before sowing to ensure a good crop **(Randhawa *et al.*, 1995)**. ***Fusarium*** sp. was also isolated from freshly collected fennel seeds and from seeds stored for 4-12 months **(Purohit *et al.*, 1999)**. ***Fusarium oxysporum*, *F. semitectum* (*F. pallidoroseum*)** and ***F. solani*** were the causal agents of wilt, crown rot and root rot disease complex, that kills coriander plants **(Madia *et al.*, 1999)**. ***Fusarium***

avenaceum (G. avenacea), F. culmorum, F. oxysporum and ***F. solani*** were the most common fungi isolated from parsley root cultivars **(Nawrocki *et al.*, 2001).**

Organic agriculture includes broad spectrum agricultural methodologies which are supportive to the environment. Disease and pest control is attained by the encouragement of a balanced / predator relationship, biological and cultural control, and mechanical removal of pests and affected parts. Organic farming gives a prevalent role to prevention rather than to curative interventions **(Torre and Dannarumma, 1999)**. Chemical pest management in herb crops is problematic. Avoiding or minimizing the use of pesticides is especially required with these crops due to the restriction on pesticide residues on the marketable products. Therefore, it is essential to prevent the occurrence of the primary inoculum **(Gullino and Kuijpers, 1994).**

The present study was carried out under organic farming systems to investigate the control of fennel wilt caused by ***F. oxysporum*** using some soil bioagents.

MATERIAL AND METHODS

I. Laboratory tests:

A. Isolation and purification:

Samples of infected fennel plants (showing wilt symptoms) and its rhizosphere soil were collected from the farm cultivated by organic cultivation systems during January of 2002 at El-Hesaniya locality at Sharkiya governorate to isolate the causal organism. Roots and stem bases of infected plants were washed with tap water and cut into small pieces. The plant pieces were sterilized for 2 – 3 minutes in 5% sodium hypochlorite. Then, pieces were rinsed for several times in sterilized water and dried between sterilized filter papers. To isolate the causal organism from rhizosphere soil, the dilution technique was employed. Isolation was carried out on potato dextrose agar (PDA) medium, and the inoculated plates were incubated at 28°C for 5 days. Hyphal tips from the isolated fungi were transferred onto PDA slants for further studies. The isolated fungi were identified based on cultural and morphological characters according to **Gilman (1957), Raper & Fennell (1977). and Nelson *et al.*, (1983)** Frequency occurrence (%) of the isolated fungi in the rhizosphere soil of fennel plant was recorded.

B. Pathogenicity test:

Pathogenicity was tested under greenhouse conditions. Pots (∅ 30 cm.) containing sterilized clay soil were artificially infested with fungal cultures grown on sorghum-sand medium at 28°C for two weeks at the rate of 3% soil weight (w/w). Soil mixed with equal amounts of uninoculated sorghum-sand medium

were used as control. Three replicated pots were used for each treatment. Twenty disinfected fennel seeds were sown in each pot **(Haggag, 1993)**. Disease assessment of pre-emergence, post-emergence and wilt disease on fennel plants were periodically recorded up to 60 day after sowing.

C. Effect of crude toxin(s) on germination of some umbelliferous seeds:

The effect of crude toxin(s) produced by *F. oxysporum* on germination of some umbelliferous seeds was studied *in vitro* test. The fungus was grown on a favourable medium for toxin production consisting of; 2.5g casien; 1.0g ammonium tartarate; 12.0g starch; 12.0g mannitol; 2.0g KH_2PO_4; 1.0 $MgSO_4$ and traces of $FeSO_4$ **(Abd-El-Moity *et al.* 1997).** Flasks containing 25ml of this medium were sterilized, then inoculated with equal disc of fungus. All inoculated flasks were incubated at 28°C. At 5 day intervals, cultures were filtered through filter paper and the filtrate kept in freezer until used. Seed of five plant species namely; anise (***Pimpinella anisum***); celery (***Apium graveolens***), coriander (***Coriandrum sativum***); fennel (***Foeniculum vulgare***) and parsley (***Petroselinum sativum***), obtained from Genetics and Cytology Dept., NRC, were used. Twenty-five seeds of each plant species were treated with crude toxin(s). For control, seeds of each plant species were treated with sterile medium only and sterile water, separately, The treated seeds were incubated for 10 days, then the loss in germination was recorded.

D. Antagonistic effect of bioagents *in vitro* test:

The antagonistic effect of ***Trichoderma harzianum, Bacillus subtilis*** and ***Pseudomonas fluorescens*** (isolated from Egyptian soils and identified by the Plant Pathology Dept., NRC) was tested *in vitro*. The efficacy of bioagents in suppressing the mycelial growth of ***F. oxysporum*** was carried out using the dual culture technique **(Karunanithi and Usman, 1999).** Reduction in the growth of pathogenic fungus due to the antagonistic effect was calculated as a percentage, using the following formula:

$$\text{Growth reduction (\%)} = \frac{\text{Growth in control} - \text{growth in treatment}}{\text{Growth in control}} \times 100$$

II. Field experiment:

The efficacy of ***T. harzianum, B. subtilis*** and ***P. fluorescens*** as a biocide was evaluated as a soil treatment under field conditions, naturally infected with ***F. oxyporum,*** in a farm following the organic cultivation systems of El-Hesaniya locality at Sharkiya governorate. ***T. harzianum*** was grown on potato dextrose broth medium at 28°C for 7 days. Then, the grown cultures were mixed with milled soybean and talc powder mixture (1:1, w:w) to give 3×10^8 propagules/ml **(Abd-El-Moity *et al.*, 1997 and Abd-El-Khair & El-Mougy, 2003).** Bacterial bioagents were grown on nutrient glucose (1%) broth medium at 28°C for 72h

(Schaad, 1980). Then the bacterial suspension was adjusted to be about 3 x 10^8 cfu/ml **(Ziedan, 1998 and Abd-El-Khair & El-Mougy, 2003).**

The field experiment, consisted of plots 8 m^2 in area (4x2m) with three replicates (plots) for each treatment as well as control, was designed in a complete randomized block. Each plot comprised of 5 lines with 50 cm between hills. Inoculum (100 g biocide-mixture/1 m^2) of each bioagent was mixed with the top 20cm of the soil surface at planting sites **(Abd-El-Kader, 1997).** Seeds were sown at the beginning of November 2002. Fennel plants were fertilized according to organic systems using compost. Furrow irrigation was followed and plants were thinned before stalk differentiation to two plants/hill. Disease assessment for pre- and post-emergence dumping off and wilt disease were recorded up to 60 days from planting. Frequency occurrence of the different fungi in the rhizosphere of fennel plants grown under bioagent treatments was recorded at flowering stage. Some growth parameters, i.e. plant height, number of primary branches, fresh and dry weights of umbel were also recorded after flowering stage of plant growth. Weight of 100 fennel seeds was recorded after 30 days from harvest.

III. Statistical analysis:

Results obtained were statistically analyzed using normal F-test and the means were compared by L.S.D at 5% level of significant **(Steel and Torrie, 1980).**

RESULTS AND DISCUSSION

1. Laboratory test:

A. Isolation:

Isolation from rhizosphere soil and roots and stem bases of infected fennel plants, collected from organic cultivation farm, revealed the presence of ***Alternaria* spp.**, ***Aspergillus flavus, A. niger, A. terreus, Fusarium oxysporum, F. solani, Penicillium* spp.**, ***Phoma* sp.**, ***Rhizopus nigricans*** and ***Trichoderma* spp.** Data showed the presence of ***F. oxysporum*** and ***F. solani***, which causes the detriment to plant root, were present in the rhizosphere as well as in the roots and stem bases of fennel plant **(Purohit *et al.*, 1999).** The percentage frequency of the root pathogenic fungi and other isolated fungi are shown in Table (1). It is clear that ***F. oxysporum*** was more common than *F. solani* **(Gour *et al.*, 1988).** The isolation results agree with those recorded by **Sharma *et al.* (2001).** They reported that ***A. fumigatus, A. niger, A. ustus, Alternaria tenuis, Curvularia* sp.**, ***Penicillium* spp.**, ***Rhizopus stolonifer, Sordaria* sp.**, ***Aspergillus tamari*** **and** ***Emericola rugulosa*** were isolated from the rnizosphere and rhizoplane of five important spice plants, i.e. coriander, cumin, fennel and pepper. They mentioned that *F. oxysporum* was present in the rhizosphere as well as in the rhizoplane of all non-legumious plants.

B. Pathogenicity:

Pathogenicity test revealed that ***F. oxysporum*** and ***F. solani*** were pathogenic to fennel plants. The percentage of pre-emergence and post-emergence damping off and wilting disease are listed in Table (2). ***F. oxysporum*** was the most virulent in causing pre-emergence damping off and wilting disease than ***F. solani***, while the opposite result was obtained in post-emergence damping off where ***F. solani*** was more aggressive than ***F. oxysporum***. This result agree with that recorded by **Gour *et al.* (1988).** They reported that ***Foeniculum vulgare*** were susceptible to ***F. oxysporum*** **f.sp *cumini.*** Results are confirmed by **Dal (1987),** who reported that the pathogenicity on fennel plant was obtained by inoculation of seeds and roots with ***F. oxysporum***. It was concluded that ***F. oxysporum*** is the main cause of wilt in fennel **(Madia *et al.* 1999).** Other isolated fungi were not pathogenic to fennel plants.

C. Effect of Crude toxin(s) on germination of some umbelliferous seeds:

The produced toxin(s) at different incubation periods also causes loss in germination of the tested umbelliferous seeds ranging from 16 to 88% (Table, 3). It was clear that the seeds of ***Apium graveolens, Coriandrum sativum, Foeniculum vulgure, Pimpinella anisum*** and ***Petroselinum sativum*** were susceptible to toxin(s) produced by ***F. oxysporum*** **(Gour *et al.*, 1988).**

D. Antagonistic effect of bioagents *in vitro* test:

Laboratory test for the antagonistic effect of ***B. subtilis, P. fluorescens*** and ***T. harzianum*** against *F. oxysporum* revealed that the mycelial growth of the tested fungus was decreased being 40.74, 41.30 and 57.71%, comparing with control, respectively (Table 4). No significant differences were recorded between ***B. subtilis*** and ***P. fluorecens*** in their antagonistic effect against the mycelial growth of ***F. oxysporum***. Results showed that ***T. harzianum*** was effective in suppressing the mycelial growth of ***F. oxysporum*** than the bacterial bioagents, this could be due to the fast growth of ***Trichoderma*** when compared with the growth of the pathogenic fungus. ***Trichoderma*** **spp.** also produced non-volatile metabolites with antifungal activity reduced the growth of ***F. oxysporum*** **(Weiming *et al.*, 1999).**

II. Field experiment:

The effectiveness of ***B. subtilis, P. fluorescens*** and ***T. harzianum*** on the pathogenic fungus under field application as a soil treatment was studied as follows:

A. Disease assessment:

Data showed that, all bioagent treatments have significantly reduced the pre-and post-emergence damping off and wilt incidence in comparison to the control (Table, 5). Soil treatment with bioagents showed that ***T. harzianum*** gave

the highest reduction in damping off and wilt incidence followed by ***P. fluorescens*** and ***B. subtilis***, respectively. Therefore, the high suppression of ***T. harzianum*** to the growth of ***F. oxysporum*** may be due to the faster growth of ***Trichoderma* spp.** and production of non-volatile compounds such as ethylene and formic aldehyde as recorded by **Ercole *et al.* (1993)** and **Weiming *et al.* (1999)**. ***T. harzianum*** also produced the chitinolytic enzymes which improved antifungal capacity against ***F. oxysporum*** **(Rey *et al.*, 2000)**. ***B. subtilis*** and ***P. fluorescens*** also play an important role in controlling soil-borne disease by antibiotics and siderophore production **(Roberti and Selmi, 1999)**. It is thus concluded that ***T. harzianum*** can protect the host plant for additional time till reaching harvest due to suppression of disease progress.

B. Frequency occurrence of *F. oxysporum* in the rhizosphere:

Frequency of *F. oxysporum* occurrence was studied comparing with other fungi in the rhizosphere of fennel plants, under organic cultivation systems, at flowering stage in different bioagent treatments are listed in Table (6). This work is aimed to study the effect of bioagents as biocides in decreasing the population of the pathogenic fungus *F. oxysporum* in the rhizosphere. Data show that the frequency occurrence of this fungus was 22.50, 16.48 and 14.56% with ***B. subtilis***, ***P. fluorescens*** and ***T. harzianum*** treatments, respectively, comparing with control being 28.33%. ***T. harzianum*** treatment was decreased the occurrence of *F. oxysporum* followed by ***P. fluorescens*** and ***B. subtilis*** treatments. These results agree with those, which obtained ***in vitro*** test.

C. Effect on some plant parameters:

Plant height, number of primary branches and fresh & dry weight of umbels were decreased which may due to the infection with ***F. oxysporum*** (Table, 7). Application of the bioagents as soil treatment has led to increase growth parameters than the control. Soil treatment resulted that the plant height was 135.0, 117.5 and 112.5 cm in treatments by ***P. fluorescens, B. subtilis*** and ***T. harzianum*** respectively, comparing with 106.5 cm in the control (Table, 7). Data also show that soil treatment with ***T. harzianum*** has produced high fresh and dry weight of umbels, followed by ***B. subtilis*** comparing to ***P. fluorescens*** and control treatments, respectively. The same trend was also obtained in weight of 100 fennel seeds after harvest. These data concluded that the bioagent treatments have increased some plant growth parameters which may due to the production of a growth regulating factor that increase the rate of seed germination and dry weight of shoots and stems of tomato and tobacco plants (**Windham, *et al.*,** 1986).

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Table 1. Percentage occurrence of fungi in infected plant parts and rhizosphere of fennel plants grown under organic cultivation system.

Fungi	Frequency occurrence (%) in	
	Rhizosphere	Infected plants [1]
Alternaria spp.	7.77	12.95
Aspergillus flavus	6.25	12.95
Aspergillus niger	18.21	0.00
Aspergillus terreus	6.29	0.00
Fusarium oxysporum	16.17	28.71
Fusarium solani	10.77	19.13
Penicillium spp.	19.69	14.39
Phoma sp.	3.11	11.87
Rhizopus nigricans	6.74	0.00
Trichoderma spp.	5.00	0.00

(1) Isolation from root and stem bases of wilted plants.

Table 2. Pathogenicity of *Fusarium oxysporum* and *F. solani* on fennel plants under greenhouse conditions.

Fungi	Damping off (%)		Survival (%)	Wilt (%)
	Pre-emergence	Post-emergence		
F. oxysporum	21.67	20.00	58.33	40.00
F. solani	13.33	23.33	63.34	21.15
Control	3.33	0.00	96.67	0.00
L.S.D $_{0.05}$	0.43	0.03	-	0.05

Table 3. Loss in germination of umbelliferous seeds[1] treated with toxin(s) produced by *Fusarium oxysporum* at different incubation periods.

Umbelliferous species	Loss in germination (%)					
	Control		Culture age (days)			
	Water only	Medium only	5	10	15	20
Apium graveolens	12	16	16	28	88	88
Coriandrum sativum	28	28	36	60	84	84
Foeniculum vulgare	28	32	36	40	44	44
Pimpinella anisum	24	28	44	60	76	76
Petroselinum sativum	16	20	28	40	64	80

[1] Twenty five seeds for each species in each treatment.

Table 4. Percentage of reduction in mycelial growth of *Fusarium oxysporum* as affected by bioagents *in vitro* test.

Bioagents / Pathogen	Growth reduction (%)		
	B. subtilis	*P. fluorescens*	*T. harzianum*
F. oxysporum	40.74	41.30	57.71

L.S.D$_{0.05}$ = 5.95

Table 5. Effect of bioagent treatment applied to the soil on disease incidence of fennel plants under field conditions.

Treatments	Damping off (%)		Wilt %
	Pre-emergence	Post-emergence	
B. subtilis	9.63	6.67	12.33
P. fluorescens	8.33	6.67	11.67
T. harzianum	6.67	5.00	8.73
Control	10.83	10.00	18.33
L.S.D $_{0.05}$	0.06	0.17	0.34

Table 6. Percentage occurrence[1] of fungi in rhizosphere of fennel plants under different bioagent treatments.

Fungi	Frequency occurrence (%) of fungi			
	Control	*Bacillus subtilis*	*Pseudomonas fluorescens*	*Trichoderma harzianum*
Alternaria spp.	4.31	4.62	12.29	4.85
A. flavus	7.33	0.00	6.97	2.93
A. niger	9.91	15.29	19.26	14.56
A. terreus	4.31	7.87	6.33	19.42
F. oxysporum	28.33	22.50	16.48	14.56
F. solani	18.88	15.88	10.98	9.71
Penicillium spp.	7.33	21.29	16.39	14.56
Phoma sp.	8.62	2.31	0.00	0.00
R. nigricans	4.31	4.62	5.33	4.85
Trichoderms spp.	6.49	5.62	5.97	14.56

[1] After flowering stage of plant growth

Table 7. Effect of bioagent treatment applied to the soil on some growth parameters of fennel plants under field conditions.

Treatments	Growth parameters				
	After flowering*				
	Plant height (cm.)	No. of primary branches	Weight of umbel (g) Fresh	dry	Weight of 100 seeds (g)
B. subtilis	117.50	5.00	2.26	0.48	0.92
P. fluorescens	135.00	7.00	1.68	0.37	0.86
T. harzianum	112.50	4.00	2.49	0.56	1.08
Control	106.50	3.00	1.56	0.36	0.83
L.S.D $_{0.05}$	1.98	2.10	0.05	0.06	0.01

* Each reading is the average of 10 plants.

الملخص العربى

المقاومة الحيوية لمرض الذبول في الشمر المتسبب عن فطر فيوزاريم أوكسيسبورم في الزراعات العضوية

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[1] قسم أمراض النبات – المركز القومي للبحوث – الدقي – الجيزة – مصر.

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عزلت الفطريات ***Alternaria,* spp** و ***Aspergillus flavus*** و ***A. niger*** و ***A. terreus*** و ***Fusarium oxysporum*** و ***F. solani*** و ***Penicillium* spp.** و ***Phoma* sp.** و ***Rhizopus nigricans*** و ***Trichoderma* spp.** من منطقة الريزوسفير وجذور وقواعد السيقان لنباتات الشمر المصابة بالذبول في الزراعات العضوية . أعطى الفطر ***F. oxysporum*** أعراض مرض الذبول تحت ظروف الصوبة . حيث كانت بذور الينسون والكرفس والكزبرة والشمر والبقدونس أيضاً حساسة للتوكسينات المنتجة في اختبار الأطباق ، أعطى الفطر ***T. harzianum*** أفضل تأثير تضادي ضد الفطر ***F. oxysporum*** تلاه البكتريا ***P. fluorescens*** ثم ***B. subtilis*** في التجربة الحقلية، أوضحت معاملة التربة أن الفطر ***T. harziunum*** أنقص تعداد الفطر المرضي، ومعدل الإصابة بمرض الموت المفاجئ والذبول تلاه البكتريا ***P. fluorescens*** ثم ***B. subtilis*** . حسنت المعاملات بالكائنات الحيوية بعض صفات النمو مثل طول النبات وعدد الأفرع الابتدائية والوزن الغض والجاف للنورات الخيمية عند طور الإزهار وكذلك وزن ١٠٠ بذرة بعد الحصاد. أوضحت نتائج هذه الدراسة أن تطبيق المقاومة الحيوية في الزراعات العضوية ذو فاعلية مناسبة في مكافحة مرض ذبول الشمر الذي يسببه الفطر ***F. oxysporum***