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BIOLOGICAL CONTROL AND DETECTION OF FUSARIUM WILT DISEASE IN "SWEET PEPPER" CAUSED BY *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI* in Egypt

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ABSTRACT: ***This research was conducted at Sakha Agricultural Research Station under greenhouse and field conditions. Fusarium wilt disease of pepper caused by Fusarium oxysporum [FO]. Sixty isolates of [FO] were isolated from different locations in Egypt. The virulence of four isolates of the causal organisms [FO] were examined. The obtained results showed different values of disease severities, however isolate no. (56) which identified as Fusarium oxysporum f. sp. lycopersici [FOL]was the most virulent one showing the highest values of disease severities during growth stages of pepper. The pathogenicity of the four isolates of F. oxysporum f.sp. capsici on California Wonder 300 variety showed that the most degree of wilt diseases incidence at the late period of planting pepper crop. Treated pepper plants by mixture of Trichoderma spp. (T. harzianum, T. viride and T. hamatum), Pseudomonas fluorescens and Bacillus subtilis [commercial bioagent (omega)] compared with fungicide (Rhizolex-T-50%) were highly effective against Fusarium wilt disease, under greenhouse condition. On the other hand, under field condition, the different bioagent showed different degrees of efficacy against [FOL], while it was considered as main causal organisms of wilt on pepper. Antagonistic of microorganisms gave the highest effect to increase the percentage of survival plants and led to reduction in the efficacy of Fusarium wilt incidence during all growth stages of pepper crop. Generally, results showed that using mixture of three Trichoderma isolates gave the highly significantly reduction of disease incidence and increasing in pepper yield. Significant reduction of disease incidence was observed between this treatment either under artificial or natural infested soil compared with other treatments. Data obtained show that positive correlation between adding doses of antagonists and disease incidence reduction than adding one dose of the same antagonists at planting time. Furthermore, adding different fungal antagonistic at any treatment led to significant reduction in wilt incidence and increased pepper yield compared with either bacterial or fungicidal treatment. Protein analysis (extract protein) from pepper plants was used to detect Fusarium oxysporum f.sp. lycopersici [Fol] in Sweet Romy pepper cultivar. Also UPGMA cluster analysis was detected in two clusters, which were indicated that the enhanced resistance of pepper plants to the antifungal effect during growth stages of pepper crop.***

Key Words: ***pepper, biological control, Trichoderma spp, Fusarium oxysporum, detection, UPGMA, chemical control, wilt.***

INTRODUCTION

Pepper (*Capsicum annuum* L.) is one of the most important vegetable crops in many countries. Many soil borne pathogens attack pepper crops. *Fusarium oxysporum* f. sp. *Lycopersici* [FOL] is wide spread and destructive soil born fungi which attack many plant species. They have been reported on pepper everywhere all over the world as well as in Egypt.

***Fusarium oxysporum* is a very common soil borne fungus. The epidemic wilt disease is due to the spread of spores of this pathogen rapidly in long distances by soil transferred irrigation water and contaminated farm equipments. It is also possible that the pathogen spores could spread by wind reported by Nelson *et al.,* 1983. Vascular wilt of pepper caused by *Fusarium oxysporum* f.sp. *lycopersici* is the most dangerous disease in different pepper growing areas (Freire *et al.*, 2000; Apodaca *et al.,* 2004a; Gupta *et al.,* 2005 and Dianez *et al.*, 2007). The typical disease symptoms included yellowing browning, defoliation of the older leaves and wilt of plants. Lower stem discolored and darkened bands appear in infected vascular tissues (Badr *et al.,* 1997; Sameer and Bohra, 2004; Martin *et al.,* 2005 and Goswami *et al*., 2008).**

Control of soil borne and *Fusarium* wilt disease depend on mainly fungicides, such chemicals pollute the environment and causing deleterious effect on humanbeings, animals and all living organisms. Biological control is an alternative method to control soil borne pathogenic fungi (Utkhede and Mathur, 2005 and Jee, 2007).

The biocontrol agents isolates as i.e. *Bacillus subtilis, Pseudomonas fluorescens*, *Trichoderma harzianum*, *T. viridie* and *T. hamatum* were used. These genera and species were reported by many investigators [Liu *et al.,* (2001); Yuan and Zhou (2006); Zaccardelli *et al.* (2006) and Alok *et al*., (2007)]. Many investigators reported that the antagonists (*Bacillus subtilis*, *Pseudomonas fluorescens* and *Trichoderma* spp.) were effective to biocontrol agents against many disease. *Bacillus subtilis* was used by Kumarcsan *et al.,* (2005), Prange and Bishop (2008) and Martinez *et al.,* (2008), they suggested that the antagonistic activity of *B. subtilis* against several pathogenic fungi may be referred to the production of the antibiotics such as surfatin, while Lee *et al.,* (2003); Gullino (2005); Tran (2007) and Pagliaccia *et al.,* (2008) reported that *P. fluorescens* produced (Phenazine-1-caroxilic acid) which is one of the most thoroughly studied biocontrol antibiotic. This product has an activity against most pathogenic fungi (Gullino, 2005). Kay and Stewart (1994) Abo Ellil *et al.,* (1998); Srikant (2006) and Jamiokowska and Wagner (2007) found that *Trichoderma* spp. producing antifungal and antibacterial compound i.e. viridian, antibiotics and dermadin were active against wide range of fungi.

The present study aim to evaluate the antagonistic effect of *Trichoderma* isolates, *Pseudomonas fluorescens* and *Bacillus subtilis* compared with fungicide treatment, application either doses or one dose to reduce wilt

disease incidence caused by [FOL]. As well as, detection of *Fusarium axysporum* f. sp. *lycopersici* of diseased pepper plants by protein analysis technique.

MATERIALAS AND METHODS

Collection of disease samples:

Infected roots, crowns, leaves and stems of pepper plants (sweet and hot pepper) as typical symptoms of *Fusarium* wilt was collected from different pepper crops fields in i.e. Beheira, Sharqiya and Kafr El-Sheikh governorate during the growing season 2005-2006

Greenhouse experiments:

I- Pathogenicity tests:

Four fungal isolates from diseased pepper plants were tested for their pathogenicity using seedlings of pepper cv. California Wonder (300).

Pathogens inocula and soil infestation:

Glass bottles of 500 ml capacity containing 30 g clean sand, 90 g barley and 120 ml water were autoclaved for 30 minutes at 1.5 atm. The bottles inoculated with the tested fungus and incubated at 28^{o}C for 15 days. Pots (25 cm in diameter) were sterilized by dipping in 5% formaline solution for five minutes and left open to dry in air. Pots were filled with sterilized sand loamy soil infested with the fungal inoculum at the rate of 3% soil weight. Infested soil was mixed thoroughly and moistened with water every other day for one week before planting to ensure the distribution and uniformity of the pathogen.

Pepper seedlings surface were sterilized by immersing for one minute in 1/1000 mercuric chloride solution and washed several times with sterilized water. Four surface sterilized seedlings were sown in each pot and irrigated as required. Three replicates, with a total of 12 seedlings were used for each particular treatment. Percentage and severity of wilt disease were estimated after 15, 75, 120 and 150 days.

II. Biological and chemical control:

To find out the most effective methods to control wilt disease incidence. Three different methods were used. These methods were biologically controlled using different antagonists compared with effect of commercial bioagents as non-chemical method and Rhizolex-T as a chemical method under greenhouse with artificial soil infestation conditions of *Fusarium oxysporum* f. sp. *lycopersici.* The most efficient biocontrol agents i.e. [*T. harzianum, T. viride, T. hamatum* and *Pseudomonas fluorescens*], [a commercial bioagent (Omega)] and fungicide (Rhizolex-T) individually or in combination were tested for studying their effect on pepper wilt disease incidence. In the meantime, treatments applied at three times once (at planting date), 15 and 30 days after planting which marked as doses. Pots (25

cm in diameter) and four seedlings per pot were used. Four replicates were accomplished per each treatment. Infested soil with isolate of [FOL] (Check) and non-infested soil (blank) were served as control treatments.

Molecular variability:

The aim of this experiment was to determine the variation of infection on growth stages of pepper plants by using protein analysis technique (SDS-PAGE). This technique gave modern method than other traditional ones for studying a detection of certain stage, whereas *Fusarium oxysporum* f. sp. *lycopersici* gave highly infection in diseased plants compared with healthy plants.

Four samples of different growth stages of healthy and diseased pepper plants which subjected in compatibility test were used. This technique was confirmed at the Department of Biotechnology, Plant Pathology Research Institute, ARC, Giza, Egypt.

Electrophoretic detection of protein by sodium dodecyole sulphate, polyacrylamide gel electrophoresis (SDS-PAGE):

According to Laemmili (1970) method with slight modifications was adopted to use in the present study. The modification, was reduced TEMED from 30 µl to 25 µl and also APS was reduced from 1.5 ml to 1.3 ml.

The protein content in supernatant was estimated according to the method of Bradford (1976) by using bovine serum albumin as a standard protein. Protein content was adjusted to 2 mg/ml per sample.

Field experiment:

This part of study has been carried out to investigate the effect of antagonistic microorganisms of *Fusarium* wilt control of pepper. Seedlings of highly susceptible pepper cv. sweet Romy "Egyptian" were planted on 20th May and the beginning of harvested on the 20th of August, while early yield was considered as the weight of all harvested fruits during the first 30 days of harvesting.

Randomized complete blocks design experiment with three replicates was used in this study. The seedlings were transplanted in the soil of field on one side of the ridge (3.5 meters in the length and 0.75 cm in width). Spacing between plants within the ridge was 50 cm, the plot contained two ridges making an area of 5.25 m^2.

The inoculum amount was 1 L/plot of the antagonistic microorganisms (10^8-10^9 CFU/ml). Different biocontrol agents were applied as doses in three different times i.e. at planting time, 15 and 30 days after sowing.

All the obtained data were statistically analyzed according to the analysis of variance "ANOVA" using the Statistical Analysis System (SAS, 1996).

RESULTS AND DISCUSSION

1. Collection of Fusarium wilt disease samples::

Infected roots and stalks as typical symptoms of wilt, were collected from pepper fields in three Egyptian governorates (Table 1).

Data in Table (1) show that [FO] most frequency in all locations, it reached 100% isolates from two locations i.e. Beheira and Sharqiya while, it reached as 91.66% and 94.44% from samples which collected in Dalangat and Sakha locations, respectively. These results indicate that *F. oxysporum* is the dominant pathogen causing wilt disease as reported by Apodaca *et al.*, 2004 and Jamiokowska, 2008

Table (1): Frequency percentage of *Fusarium oxysporum* isolated from wilted pepper samples collected from different governorates

Governorate	Location	Number of samples	*Fusarium oxysporum* frequency (%)
Beheira	Nubaria	55	100.00
	Dalangat	60	91.66
Sharqiya	Abo-Hamad	70	100.00
Kafr El-Sheikh	Sakha	65	94.44
Mean		62.50	96.52

Greenhouse experiments:

I. Virulence of causal organisms isolates on pepper cv. California Wonder 300 under greenhouse condition:

Four isolates of *Fusarium oxysporum* which caused wilt disease incidence were tested in pot experiment under greenhouse conditions, using pepper cv. California Wonder 300.

Data presented in Table (2) indicated that the least degree of infection to *Fusarium oxysporum* isolates were found at the early period of planting. In general *F. oxysporum* isolate no. (56) from Kafr El-Sheikh caused the highest percentage of infection followed by *F. oxysporum* isolate no. (35), where disease severity recorded 29% and 15% after 75 days of planting, respectively.

Results in Table (2) showed also that all isolates of *Fusarium oxysporum* were pathogenic causing wilt disease to pepper plants. *Fusarium oxysporum* f. sp. *lycopersici* isolate no. (56) also gave the highest infection percent of and wilted plants during all growth stages of pepper plants. The above results clear that the variability of *F. oxysporum* isolates were aggressive and this also was noticed by (Diaz *et al.*, 2005 and Dianez *et al.*, 2007).

Table (2): Virulence of *Fusarium oxysporum* isolates on pepper cv. California Wonder 300 under greenhouse condition (after 15, 75, 120 and 150 days from planting).

Fusarium oxysporum Isolate No.	Disease incidence		Survival plants		Disease severity	Disease index
	No.	%	No.	%		
Seedling stage (15 days)						
9	0.00	0.00	12.00	100.00	0.00	0.00
35	1.00	8.33	11.00	91.67	0.02	2.08
24	0.00	0.00	12.00	100.00	0.00	0.00
56	2.00	16.66	10.00	83.33	0.06	6.25
Control	0.00	0.00	12.00	100.00	0.00	0.00
L..S.D. at 5%	NS		NS			
Flower stage (75 days)						
9	2.00	16.67	10.00	83.33	0.10	10.42
35	4.00	33.30	8.00	66.67	0.25	25.00
24	3.00	25.00	9.00	75.00	0.23	22.92
56	6.00	50.00	6.00	50.00	0.46	45.83
Control	1.00	8.33	11.00	91.67	0.02	2.08
L..S.D. at 5%	1.02		1.02			
Fruit stage (120 days)						
9	2.00	16.67	10.00	83.33	0.14	14.58
35	5.00	41.67	7.00	58.33	0.38	37.50
24	3.00	25.00	9.00	75.00	0.23	22.91
56	7.00	58.33	5.00	41.67	0.58	58.33
Control	1.00	8.33	11.00	91.67	0.04	4.16
L..S.D. at 5%	1.43		1.44			
Fruit stage (150 days)						
9	7.00	58.33	5.00	41.66	0.52	52.08
35	8.00	66.67	4.00	33.33	0.67	66.67
24	7.00	58.33	5.00	41.66	0.54	54.16
56	10.80	83.00	2.00	16.67	0.83	83.30
Control	2.00	16.67	10.00	83.33	0.06	6.25
L..S.D. at 5%	0.97		0.97			

Disease severity = (a x b)/(N x K) (Soleman *et al.,* 1988),
Where: a: Number of infected plants, b: Grade of infection, N: Number of total plants and K: Maximum grade of infection,
Disease index = Disease severity x 100 (Rand and Stevenson, 1999).

II. Biological and chemical control treatments on wilt disease incidence under greenhouse conditions:

The effect of two different control methods i.e. bioagents; and fungicide were used to study their effect on wilt incidence with, using the highly susceptible cv. Sweet Romy under greenhouse conditions.

Data present in Table (3) indicate that the application of biocontrol agents, *Trichoderma spp.*, *Pseudomonas fluorescens* and *Bacillus subtilis* (Commercial omega) significantly decreased with wilted pepper plants. However, using mixture of *T. harzianum, T. hamatum* with *T. viride* led to highly reduction in percentage of infection with wilt disease in comparison with the effect of either *Trichoderma harzianum* or *Pseudomonas fluorescens* alone.

Table (3)

Data in Table (3) also showed that application of bicontrol agents as doses was more effective, where they gave higher numbers and higher percentage of healthy plants than an application by using the dose of the same antagonist.

The fungicide, Rhizolex-T gave significant control of the disease, it increased the percentage of healthy plants and decreased wilted plants after two months of planting (disease severity was 0.0%). However, disease severity as well as disease index was also reduced due to the application of fungicide, Rhizolex with bioagent (omega) compared with either single application of Rhizolex or doses of commercial bioagent (omega) (Table 4). Results also showed that combined treatment significantly reduced wilt disease at early and medium pepper growth stages. These results clear that using integration between the biocontrol agents were as effective as Rhizolex-T in controlling pepper wilt disease. However, Gunasingham and Manjungtha (2008) achieved similar results.

Molecular variability:

The aim of this study was to detect the presence or absence of pathogenic fungus *Fusarium oxypsorum f.sp. lycopersici* in pepper plant Sweet Romy cultivar by using protein marker (225, 150, 100, 75, 50, 35 and 25 kd.) as a standard protein (Fig. 1).

Data presented in Table (5) indicated that the banding patterns of SDS-PAGE protein for soluble protein extracted from pepper plants cv. Sweet Romy, that protein profile of diseased pepper plants Sample (4.) showed high difference in comparison with those of [sample (1.)] (Healthy plants). However, diseased pepper plants samples no. (2.) and (3.) were less difference with healthy plants [sample (1.)].

In addition, results showed different pattern (Presence or absence) in any bands of all tested stages were noticed. Data in Table (5) revealed that the total number of bands detected was (29) with an average of (7.25). The highest number of bands were noticed in sample (1.) [healthy plants (control)] and the sample no. (2.). While the lowest number of bands was in the sample. no. (4.) Fig. (1).

Results in Table (5) also showed that the total number of bands (29 bands) were detected in all tested samples, whereas, the band with (43 kd.) was detected in three samples [no. (1.), no. (2.) and no. (3.)]. While, the four bands with [(70), (58), (24) and (9) kd.] were detected in two samples [no. (1.) and no. (2.)].

UPGMA cluster analysis divided to four stages i.e. [stage (1), stage (2) and stage (3)] of diseased infection plant compared with healthy plants [stage (0)] in two clusters Fig. (2).

The first cluster included the stages i.e. no. (0.), no. (1.) and no. (2.) with similarity (95.27%). On the other hand, the second cluster included the stage no. (3.) with similarity between all tested stages with (90.13%).

Table (4)

In addition, the dendrogram included the two stages no. (1.) and no. (2.) which showed the highest similarity values with (98.36%).

Protein analysis clear the reduction of protein percentage in response to infection with *F. oxysporum f.sp. lycopersici*. It was also noticed that this reduction was increased by time of infection (2-4 months) and this indicate that the infection with wilt pathogen lead to protein degradation. These results were also noticed by Kuku *et al.* (2009).

Field experiment:

Effect of the antagonists on Fusarium wilt disease of pepper under field and naturally soil infestation conditions:

This experiment was designed to evaluate the efficiency of some bicontrol agents as doses in controlling wilt disease incidence of the highly susceptible pepper cv. Sweet Romy "Egyptian" under natural infection at Sakha, Kafr El-Sheikh governorate during 2009 season.

The percentage of survived plants after 120 days of sowing was (93.75%) in *Tricholderma harzianum,* (89.58%) in *Pseudomonas fluorescens* and (100.00%) in the mixture of the three *Trichoderma* isolates Table (6).

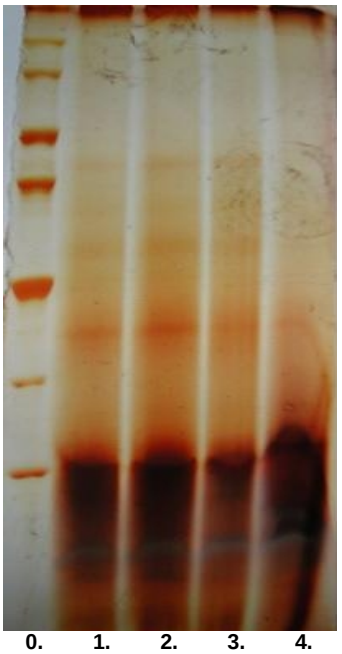
Data presented in Tables (6) indicated that adding different antagonists as three doses, led to significant reduction in disease incidence and also led to increase in early and total yield of pepper plants.

In all cases, total yield as weight of fruit/m^2 was significantly affected by all used antagonists. However mixture of *Trichoderma* isolates treatment produced higher total fruit yield than those single application of *Trihcoderma harzianum* and *Pseudomonas fluorescens* compared with non treated treatments (natural infested soil). The above results showed that controlling wilt disease of pepper using the tested biocontrol agent(s) resulted higher plant survival and significantly higher than the untreated field plots. However, combined application(s) of the biocontrol agents gave much better results. Kim *et al.*, 2008 achieved similar results.

Table (5): Presence (+) versus and absence (-) of SDS-PAGE protein for soluble protein extracted from pepper plants cv. Sweet Romy.

K.Da	Different infection stages and control			
	(1.)	(2.)	(3.)	(4.)
238	-	-	-	+
207	-	-	+	-
163	-	-	-	+
154	-	+	-	-
150	+	-	-	-
106		+	-	-
104	+	-	-	-
86	-	+	-	-
85	-	-	+	-
84	+	-	-	-
70	+	+	-	-
59	-	-	+	-
58	+	+	-	-
49	-	-	+	-
45	-	-	-	+
43	+	+	+	-
28	-	-	-	+
26	-	-	+	-
24	+	+	-	-
20	-	-	-	+
10	-	-	+	-
9	+	+	-	-
7	-	-	-	+
Sum	8	8	7	6

Sample: (1.)*= Healthy plants.
Sample: (2.) = Diseased plants [during (2) month of infection]
Sample: (3.) = Diseased plants [during (3) month of infection]
Sample: (4.) = Diseased plants [during (4) month of infection]



0. 1. 2. 3. 4.

Fig. (1): SDS-PAGE protein for soluble protein extracted from Sweet Romy cultivar.

Sample (0.) = Protein marker
Sample: (1.) = Healthy plants.
Sample: (2.) = Diseased plants [during (2) month of infection]
Sample: (3.) = Diseased plants [during (3) month of infection]
Sample: (4.) = Diseased plants [during (4) month of infection]

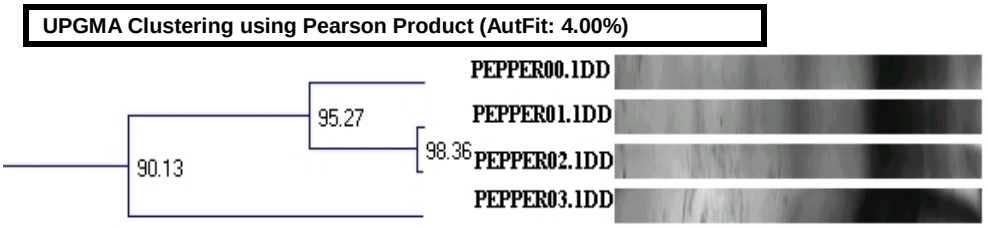


Fig. (2): UPGMA clustering showing a cluster analysis of different infection stages to sweet pepper cultivar.

Table (6): Field application with biocontrol agents as doses for controlling pepper wilt disease (naturally heavily infested soil).

Treatment	Stages	Mean frequency of healthy and infected plants						
		Wilt (infected)						
		Disease incidence		Survival plants		Disease severity	Disease index	Yield/plot (kg/m^2)
		no.	%	no.	%			
Trichoderma harzianum	Seedling stage	0.00	0.00	48.00	100.00	0.00	0.00	-
Trichoderma harzianum + *Trichoderma viride* + *Trichoderma hamatum*	Seedling stage	0.00	0.00	48.00	100.00	0.00	0.00	-
Pseudomonas fluorescens (PF3)	Seedling stage	1.00	2.08	47.00	97.92	0.01	1.56	-
Control (naturally infested soil)		6.00	12.50	42.00	87.50	0.00	9.38	-
Trichoderma harzianum	Flower stage	2.00	4.16	46.00	95.83	0.02	2.56	2.47
Trichoderma harzianum + *Trichoderma viride* + *Trichoderma hamatum*	Flower stage	0.00	0.00	48.00	100.00	0.00	0.00	2.64
Pseudomonas fluorescens (PF3)	Flower stage	3.00	6.25	45.00	93.75	0.03	3.64	2.13
Control (naturally infested soil)		17.00	35.43	31.00	64.58	0.32	32.29	1.76
Trichoderma harzianum	Fruit stage	3.00	6.25	45.00	93.75	0.05	5.20	5.67
Trichoderma harzianum + *Trichoderma viride* + *Trichoderma hamatum*	Fruit stage	0.00	0.00	48.00	100.00	0.00	0.00	6.42
Pseudomonas fluorescens (PF3)	Fruit stage	5.00	10.41	43.00	89.58	0.09	9.37	5.33
Control (naturally infested soil)		32.00	66.67	16.00	33.30	0.62	62.50	3.92
L.S.D. at 5%		1.26		1.26				

*** Doses: The biocontrol agents was applied at 3 times i.e. at planting time, 15 and 30 days after planting.**
Disease severity = a x b/N x K (Soleman *et al.,* 1988),
Where: a: Number of infected plants, b: Grade of infection, N: Number of total plants and K: Maximum grade of infection,
Disease index = Disease severity x 100 (Rand and Stevenson, 1999).
Seedling stage = during two months after sowing, Flowering stage = during three months after sowing, Fruit stage = during four months after sowing

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المكافحة الحيوية وتشخيص مرض الذبول الفيوزاريومى فى الفلفل الحلو المتسبب عن الفطر فيوزاريوم أوكسيسبورم ليكوبرسيسى فى مصر

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الملخص العربي

تم إجراء هذا البحث تحت ظروف الصوبة والحقل بمحطة بحوث سخا ، حيث تم عزل فطر فيوزاريوم أكسيسبورم المسبب لمرض الذبول الفيوزاريومى من نباتات الفلفل المصابة والتى جمعت من أكثر من موقع من بعض محافظات جمهورية مصر العربية وهى البحيرة والشرقية وكفرالشيخ ، ولقد تم الحصول على ما يزيد عن ٦٠ عزلة لفطر *F. oxysporum* والذى ظهر تردده فى جميع مواقع العزل بنسب عالية.

أمكن من خلال اختبارات القدرة المرضية الوصول إلى أشد هذه المسببات الممرضة قدرة على إحداث العدوى وذلك على أربع عزلات منتخبة من الفطر فيوزاريوم أكسيسبورم حيث حققت العزلة رقم (٥٦) شراسة فى إحداث مرض الذبول الفيوزاريومى خلال جميع مراحل نمو الفلفل من طور البادرة وحتى طور النضج الكامل وذلك خلال (أربع أشهر من بداية الزراعة). وتم تعريف هذه العزلة على أنها [Fol] *Fusarium oxysporum f* s.p *lycopersici* وقد أظهرت النتائج تقدم المرض فى المراحل المتأخرة من عمر النبات.

أما فى تجارب الأصص تحت ظروف الصوبة أدى استخدام عزلات كائنات التضاد الحيوى المنتخبة من تجارب التضاد المعملية وهى عبارة عن كائن التضاد الحيوى ترايوكوديرما ، خليط من (تريكوديرما هارزيانم + تريكوديرماهاماتم + تركيوديرما فيردى) ، بكتريا السيدوموناس فلوراسنس مقارنة بالمبيد التجارى الحيوى (أوميجا) والمبيد الفطرى ريزوليكس إلى تحقيق مستويات عالية المعنوية فى مقاومة مرض الذبول الفيوزاريومى وخفض درجات الإصابة به تحت ظروف العدوى الصناعية. أما عن التطبيق تحت ظروف الحقل فقد أدى إضافة كائنات التضاد الحيوى إلى التربه سواء الفطرية أو البكتيرية منها إلى ردود فعل متباينة خلال جميع مراحل نمو

النبات. فقد لوحظ تأثير قوى عند استخدام جميع المعاملات الحيوية المشار إليها فى خفض نسبة الذبول الفيوزاريومى على نباتات الفلفل.

ولقد أثبتت النتائج بشكل عام:

- **أن استخدام خليط من الثلاث أنواع المختلفة لفطر التريكوديرما عند إضافتها فى شكل جرعات فى اعطاء أفضل نتيجة على مستوى جميع المعاملات فى مقاومة الذبول الفيوزاريومى على الفلفل وتحقيق أكبر نسبة من النباتات السليمة والتى بلغت ١٠٠% تحت ظروف الحقل والصوبة..**
- **كذلك سجلت أعلى درجة من المقاومة لمرض الذبول عند إضافة المعاملات المستخدمة فى شكل جرعات متتالية ، واحدة منها مع الزراعة وأثنان بعد الزراعة بفارق خمسة عشر يوما وذلك مقارنة لإضافة المعاملات المستخدمة كجرعة واحدة مع الزراعة.**
- **أظهر استخدام كائنات التضاد الحيوى الفطرية لفطر تريكوديرما سواء عند المعاملة الفردية له أو استخدامه فى شكل خليط إلى إحداث اختزال معنوى فى حدوث المرض خلال جميع مراحل نمو الفلفل علاوة على زيادة ملحوظة فى المحصول بشكل أفضل من استخدام كائنات التضاد الحيوى البكتيرية وذلك تحت ظروف الصوبة والحقل معا.**
- **تم تشخيص وجود المسبب المرضى *Fusarium oxysporum* f.sp. *lycopersici* فى نبات الفلفل الحلو المعداة بالفطر الممرض للوقوف على أهم مراحل تقدم المسبب المرضى داخل النبات وذلك لتحديد الفترات الحرجه فى الاصابة للمحصول ، بما يرتبط بالاشارة الى نوع وكمية ووقت المقاومة.**

Biological control and detection of fusarium wilt ……

M.M. Ammar, Fatma A. Fouad and Enas A. Khalf Allah

Table (3): Effect of the tested biocontrol agents, Rhizolex-T and commercial bioagent (omega) on wilt disease incidence under greenhouse conditions and soil infestation with *F. oxysporum* f.sp. *lycopersici* (1-2) month after planting.

Treatment	Mean frequency of healthy and infected plants											
	Wilt (infected)											
	One month						Two months					
	Disease incidence		Survival plants		Disease severity	Disease index	Disease incidence		Survival plants		Disease severity	Disease index
	No.	%	No.	%			No.	%	No.	%		
F. oxysporum+ *Trichoderma harzianum*	0.00	0.00	16.00	100.00	0.00	0.00	1.00	6.25	15.00	93.75	0.03	3.13
F. oxysporum+ *Trichoderma harzianum* (doses*)	0.00	0.00	16.00	100.00	0.00	0.00	0.00	0.00	16.00	100.00	0.00	0.00
F. oxysporum+ *T. harzianum* + *T. viride* + *T. hamatum*	0.00	0.00	16.00	100.00	0.00	0.00	0.00	0.00	16.00	100.00	0.00	0.00
F. oxysporum+ *T. harzianum* + *T. viride* + *T. hamatum* (doses)	0.00	0.00	16.00	100.00	0.00	0.00	0.00	0.00	16.00	100.00	0.00	0.00
F. oxysporum+ *Pseudomonas fluorescens* (PF3)	0.00	0.00	16.00	100.00	0.00	0.00	1.00	6.25	15.00	93.75	0.06	6.25
F. oxysporum+ *Pseudomonas fluorescens* (PF3) (doses)	0.00	0.00	16.00	100.00	0.00	0.00	1.00	6.25	15.00	93.75	0.05	4.69
F. oxysporum+ Omega (*Bacillus subtilis*)	0.00	0.00	16.00	100.00	0.00	0.00	2.00	12.50	14.00	87.5	0.09	10.93
F. oxysporum+ Omega (*Bacillus subtilis*) (doses)	0.00	0.00	16.00	100.00	0.00	0.00	2.00	12.50	14.00	87.5	0.09	9.37
F. oxysporum+ fungicide (Rhizolex-T 50%)	0.00	0.00	16.00	100.00	0.00	0.00	0.00	0.00	16.00	100.00	0.00	0.00
F. oxysporum+ fungicide (Rhizolex-T 50%) (doses)	0.00	0.00	16.00	100.00	0.00	0.00	0.00	0.00	16.00	100.00	0.00	0.00
F. oxysporum+ *Rhizolex. T* + Omega (*B. subtilis)*	0.00	0.00	16.00	100.00	0.00	0.00	0.00	0.00	16.00	100.00	0.00	0.00
Blank (non infested soil)	1.00	6.25	15.00	93.75	0.03	3.13	4.00	25.00	12.00	75.00	0.18	18.75
Fusarium oxysporum f.sp. *lycopersici* (Check infested soil)	2.00	12.50	14.00	87.50	0.04	4.69	5.00	31.25	11.00	68.75	0.30	29.68
L.S.D. at 5%	0.29		0.29				0.63		0.63			

*** Doses: The biocontrol agents was applied at 3 times i.e. at planting time, 15 and 30 days after planting. Disease severity = a x b/N x K (Soleman *et al.,* 1988),**
Where: a: Number of infected plants, b: Grade of infection, N: Number of total plants and K: Maximum grade of infection,
Disease index = Disease severity x 100 (Rand and Stevenson, 1999).

Table (4): Effect of the tested biocontrol agents, Rhizolex-T and commercial bioagent (omega) on wilt incidence under greenhouse conditions and soil infestation with *Fusarium oxysporum* f.sp. *lycopersici* (3-4) month after planting.

Treatment	Mean frequency of healthy and infected plants											
	Wilt (infected)											
	Three month						Four months					
	Disease incidence		Survival plants		Disease severity	Disease index	Disease incidence		Survival plants		Disease severity	Disease index
	No.	%	No.	%			No.	%	No.	%		
F. oxysporum+ *Trichoderma harzianum*	2.00	12.50	14.00	87.50	0.03	3.13	2.00	12.50	14.00	87.50	0.06	6.25
F. oxysporum+ *Trichoderma harzianum* (doses*)	1.00	6.25	15.00	93.75	0.01	1.56	1.00	6.25	15.00	93.75	0.04	4.69
F. oxysporum+ *T. harzianum* + *T. viride* + *T. hamatum*	1.00	6.25	15.00	93.75	0.01	1.56	1.00	6.25	15.00	93.75	0.03	3.13
F. oxysporum+ *T. harzianum* + *T. viride* + *T. hamatum* (doses)	0.00	0.00	16.00	100.00	0.00	0.00	0.00	0.00	16.00	100.00	0.00	0.00
F. oxysporum+ *Pseudomonas fluorescens* (PF3)	3.00	18.75	13.00	81.25	0.09	9.38	3.00	18.75	13.00	81.75	0.11	10.94
F. oxysporum+ *Pseudomonas fluorescens* (PF3) (doses)	2.00	12.50	14.00	87.50	0.06	6.25	2.00	12.50	14.00	87.50	0.09	9.38
F. oxysporum+ Omega (*Bacillus subtilis*)	3.00	18.75	13.00	81.25	0.14	14.06	4.00	25.00	12.00	75.00	0.16	15.63
F. oxysporum+ Omega (*Bacillus subtilis*) (doses)	2.00	12.50	14.00	87.50	0.09	9.38	3.00	18.75	13.00	81.25	0.14	14.06
F. oxysporum+ fungicide (Rhizolex-T 50%)	1.00	6.25	15.00	93.75	0.03	3.12	2.00	12.50	14.00	87.50	0.11	10.94
F. oxysporum+ fungicide (Rhizolex-T 50%) (doses)	1.00	6.25	15.00	93.75	0.01	1.56	2.00	12.50	14.00	87.50	0.10	10.93
F. oxysporum+ *Rhizolex. T* + Omega (*B. subtilis)*	0.00	0.00	16.00	100.00	0.00	0.00	2.00	12.50	14.00	87.50	0.09	9.38
Blank (non infested soil)	4.00	25.00	12.00	75.00	0.20	20.31	7.00	43.75	9.00	56.25	0.27	39.06
Fusarium oxysporum f.sp. *lycopersici* (Check infested soil)	8.00	50.00	8.00	50.00	0.47	46.88	14.00	87.50	2.00	12.50	0.78	78.13
L.S.D. at 5%	0.76		0.92				0.74		0.75			

*** Doses: The biocontrol agents was applied at 3 times i.e. at planting time, 15 and 30 days after planting.**
Disease severity = a x b/N x K (Soleman *et al.,* 1988),
Where: a: Number of infected plants, b: Grade of infection, N: Number of total plants and K: Maximum grade of infection,
Disease index = Disease severity x 100 (Rand and Stevenson, 1999).