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EFFECT OF ANTAGONISTIC FUNGI ON THE GROWTH AND CONTROL OF *MA CROPHOMINA PHASEOLINA* AND *FUSARIUM OXYSPORUM* THE CAUSAL AGENTS OF CHARCOAL ROT AND WILT OF SESAME

BY

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ABSTRACT

Sesame seedling rot and wilt diseases are considered of the most important diseases that affect yield and seed quality of sesame plants.

Laboratory experiments were carried out to study the effect of five *Trichoderma* spp. isolates on the linear growth (LG) of *M. phaseolina* and *F. oxysporum.* The antagonistic effect of *T. harzianum, T. viride* and *T. hamatum* against the two fungi showed clear suppression in (LG) *by T. harzianum* isolates (Tl, T2, T3 and T4) for the isolates of *hi. phaseolina* (Ml, M5, M8 and M10), respectively. Greenhouse experiments showed that all antagonistic fungi were significantly effective in controlling disease incidence caused by *hi. phaseolina* on survival and healthy plants. The highest percentage of healthy plants was produced by *Trichoderma harzianum*; T2, followed by *T. hamatum* T10. For disease incidence caused by *F. oxysporum,* the highest percentage of healthy plants was produced by *Trichoderma hamatum,* T10 followed by *T. viride* T5 and *T. harzianum,* T3 compared to control treatment.

Key words: Sesame, *Trichoderma* spp., *M. Phaseolina* and *F. oxysporum*

INTRODUCTION

Sesame *(Sesamum indicum* L.) is one of the oldest cultivated plants in the world. Sesame is attacked by some economic diseases *i.e.,* root rot, wilt and damping-off. Also, seed decay is caused by several pathogens among them; *M. phaseolina* and *F. oxysporum* are of great economic importance. These diseases have been increased tremendously during the last few decades (El-Barougy 1990; Gupta & Cheema, 1990; Khalifa, 1997; El-Deeb *et al.,* 1998 and El-Fiki *et al,* 2004). Antagonistic fungi *i.e., Trichoderma harzianum, Trichoderma viride* and *Trichoderma hamatum* were evaluated for their antagonistic effect against fungal growth (Deshmukh and Raut, 1992; Dinakaran and Marimuthu, 1997).

Khalifa (1997) showed that mycelial growth of *F. oxysporum* was inhibited more than *M. phaseolina* by the antagonistic fungi. *Trichoderma* spp. particularly *T. viride* were the most effective in this regard followed by *Gliociadium penicilloides* and *Chaetomium bostrycoides. T. harzianum* followed by *C. bostrycoides* were the best for reducing root-rot and/or wilt disease incidence on sesame and increased percentage of healthy plants compared to other antagonistic fungi.

Ushamalini *et al.* (1997) mentioned that all of *Trichoderma viride, T. harzianum, T. hamatum, T. koningii* and *T. pseudokoningii* inhibited the growth of *M. phaseolina* and *F.oxysporum* using the dual-culture technique but *T. viride* and *T. harzianum* were the most effective.

Siny *et al*. (1981) found that the growth of both *F. oxysporum* and *M. phaseolina,* the causals of wilt and charcoal rot of sesame was inhibited by *Trichoderma* spp. isolated from the rhizosphere region of sesame.

Shin *et al.* (1987) reported that soil treated with *T. viride,* isolated from the rhizosphere soils of sesame plants, reduced damping-off on sesame seedlings. A high density of the antagonist in the soil caused inhibition of sesame seed germination and seedling growth. Different strains of *Trichoderma* spp. were inhibitory agents to varying extents when applied to sesame soil. Normal sesame seedlings of beds treated with the antagonist grew better than those grown in die untreated soil.

Vyas (1994) recorded that application of *T. viride* or *T. harzianum* together with carbendazim reduced dry root-rot disease casued by *M. phaseolina* on soybean plants.

Sankar and Sharma (2001) identified a suitable *T. viride* isolate for controlling root-rot (*M. phaseolina)* in maize. Out of 9 isolates of *T. viride* evaluated in preliminary tests, 2 (MR and 4282) showed superior performance in the laboratory. However, all the nine isolates produced inhibitory volatile substances *in vitro.* In field trials, treating maize seeds with talc-based powder formulations of MR and 4282 at 4,8 and 12g/kg, and carbendazim at 4g/kg, stored for 24 h induced better maize growth and dry matter production over the control. MR isolate 12g/kg produced the maximum shoot length, dry matter and grain weight of 10 cobs, followed by MR at 8g/kg. MR at 12g/kg also produced the maximum 1000-grain weight and yield.

This study was conducted to investigate the efficacy of some bioagents on controlling sesame diseases caused by *M. phaseolina* and *F. oxysporum* under laboratory and greenhouse conditions.

MATERIALS AND METHODS

Laboratory experiments

Effect of some antagonistic fungi on the linear growth of *M. phaseolina* and *F. oxysporum in vitro:*

Investigating the inhibitory effect of some isolates of antagonistic fungi on the linear growth of the four tested isolates of each of *M. phaseolina* and *F. oxysporum* was carried out as reported by Elad *et al.* (1986). In this respect *T. harzianum* (4 isolates), *T. viride* (3 isolates) and *T. hamatum* (3 isolates) were kindly obtained from Integrated Control Research Dept. Agric. Res. Center, Giza, Egypt and were used herein. The antagonistic effects of these fungi were determined by taking two discs (5 mm in diam.) of 7 days-old culture of both antagonistic and the pathogenic fungi (*M. phasseolina* and *F.oxysporum).* Plates were inoculated simultaneously with each opposite the other 1 cm apart from the plate edge in individual plates (9 cm in diam.) contained 20ml PDA medium. In control treatment, the plates were inoculated each with one disc bearing the growth of the same isolates of *M. phaseolina* and *F. oxysporum* in individual treatments. Three plates were used for each particular treatment All plates were incubated at 25C° for 5 days. Percentage of the fungal growth reduction (% GR) was calculated by using the following formula (Papavizas, 1985).

$$\% \text{ GR} = \frac{\text{LR1-LR2 x 100}}{\text{LR1}}$$

Where:
LR1 = linear growth of the pathogen alone.
LR2= linear growth of the pathogen against the antagonistic fungus.

Effect of coating sesame seeds with some antagonistic fungi on seedling-rot and wilt diseases of sesame:

The tested antagonistic fungi *i.e., T. harizanum* (one isolate), *T. hamatum* (one isolate) and *T. viride* (one isolate), each was grown on PDA medium for 10 days at 25°C, then its growth was flooded with sterile-distilled water, scraped with camel brush then filtered through sterilized cheese cloth. The resulted spore suspensions were diluted to contain approx. ($4x10^7$ conidia/ml). A known amount of surface sterilized sesame seeds in plastic bag was thoroughly mixed and shacked slowly for 5 minutes with a mixture consisted of 2 ml spore suspension of the desired antagonistic fungus plus 1 ml of 1% Arabic gum as sticker (Harman *et al.,* 1980). Sesame seeds whether non-treated or treated with antagonistic fungus were sown in potted soils infested by *M. phaseolina* or *F. oxysporum* at the rate of 10 seeds/pot. Three replicates were used for each particular treatment. Percentages of seedling mortality, and plants showing symptoms of charcoal rot or wilt disease were determined as mentioned by Mahdy *et al.* (2007).

Statistical Analysis

The experimental design (s) are completely randomized with three replicates, analysis of variance (ANONA) of the data was performed with MSTAT-C statistical package (A).

Micro computer program was used for the disease management and analysis of the agronomic research experiment. Least significant difference (L.S.D) was used to compare treatment means Gomez and Gomez (1984).

RESULTS

Antagonistic effect of some *Trichoderma* spp. against four isolates *of M. phaseolina:*

The aim of this experiment is to investigate the antagonistic effect of three species of *Trichoderma i.e., T. harzianum, T. viride and T. hamatum* on the linear growth of *M. phaseolina* isolates.

Data presented in Table (1) indicate that the tested antagonistic *Trichoderma* spp. reduced significantly the mycelial growth of the four tested *M. phaseolina* isolates *i.e.,* Ml, M5, M8 and M10. The four isolates of *T. harzianum i.e.,* Tl, T2, T3, and T4 reduced the fungal growth of *M. phaseolina* isolates *i.e.,* Ml, M5, M8 and M10, being 57.9, 59.8, 62.3 and 59.6% on the average, respectively. The highest reduction was recorded in case of *T. harzianum* (T4) against *M. phaseolina* (Ml) followed by (T2) and (Tl), respectively. Data in the same Table also show that *T. viride* isolates *i.e.,* T5, T6 and T7 caused a considerable reduction to the linear growth of the four *M. phaseolina* isolates, being 60.2, 50.6 & 59.4%, respectively. The highest effective isolate was *T. viride* (T7) followed by T5 against *M. phaseolina* (Ml). For *T. hamatum* isolates, T8, T9 and T10 recorded a noticeable reduction in the linear growth, being 61.4, 57.7 and 51.7% on the average, respectively as compared to control.

Table (1): Antagonistic effect of *Trieholderma* spp, on growth reduction

Antagonistic fungi	Isolate/No.	% Reduction in linear growth				Mean, %	Grand mean, %
			M. phaseolina isolates				
		Ml	M5	M8	M10		
T. harzianum	Tl	70.7	44.2	53.3	63.4	57.9	59.9
	T 2	70.8	54.8	52.2	61.3	59.8	
	T3	66.9	61.0	59.2	62.0	62.3	
	T 4	71.5	45.7	59.2	62.0	59.6	
T. viride	T5	70.7	49,4	60.8	59.8	60.20	56.6
	T6	46.8	46.6	48.3	60.6	50.6	
	T7	72.3	44.3	58.3	62.7	59.4	
T. hamatum	T8	75.4	43.4	63.4	63.5	61.4	52.6
	T 9	68.9	46.5	58.3	57.3	57.7	
	T10	74.6	37.8	55.0	39.3	51.7	
Control		**0.0**	**0.0**	0.0	0.0	0.00	0.00
LSD.at 5% for:							
Isolates of the pathogen (Iso) * 1.31				Antagonistic fungi (A) =2.82			
Iso x A = 3.87				Isolates of the Antagonistic fungi (B)=1.90			
Iso x A x B =4.11							

The three antagonistic fungi *i.e., Trichoderma harzianum, T. viride* wed *T. hamatum* showed significant differences in their effect on the growth of *M. phaseolina.* In general, *T. harzianum* caused the highest effect (59.9%), followed by *T. viride* (56.6%) and *T. hamatum* (52.6%) on the average.

As for the ten tested *Trichoderma* isolates *T. harzianum* (T3) followed by *T. hamatum* (T8) and *T. viride* (T5) were the most effective isolates in reducing the growth of *M. phaseolina* isolates.

Antagonistic effect of some *Trichoderma* spp. against four isolates of *F. oxysporum*:

Data shown in Table (2) indicate that all the tested antagonistic *Trichoderma* spp. caused a significant reduction in the fungal linear growth of the four tested isolates of *F. oxysporum i.e.,* F6, F8, F9 and F10. The four isolates of *T. harzianum i.e.,* TI, T2, T3 and T4, supressed the growth of *F. oxysporum* isolates which recorded 63.3, 62.3, 64.5 and 62.7%, on the average, respectively. Whereas, *T. viride* isolates *Le.,* T5, T6 and 17 caused a reduction to the linear growth, being 58.4,60.7 and 57.5 %, respectively on the average, for the tested *F. oxysporum* isolates *i.e.,* F6, F8, F9 and FIO. Regarding to *T. hamatum* isolates, T8, T9 and T10 gave a reduction in the linear growth, being 63.8, 50.9 & 61.6% on the average, respectively with the tested isolates of *F. oxysporum.*

Table (2): Antagonistic effect of *Tricholderma* spp. on growth reduction of 4 *F. oxysporum* isolates *in vitro*.

Antagonistic fungus	Isolate/ No.	%Reduction in the linear growth				Mean	Grand Mean
		F. oxysporum isolates					
		F6	F8	F9	F10		
T. harzianum	Tl	50.0	58.1	73.8	70.8	63.30	63.20
	T 2	51.4	63.2	59.3	75.5	62.30	
	T3	55.7	62.6	70.8	68.9	64.50	
	T 4	53.2	52.6	71.3	73.9	62.70	
T. *viride*	T5	49.9	61.8	50.5	71.3	58.40	58.73
	T6	47.9	59.8	61.0	74.6	60.70	
	T7	52.0	64.1	54.8	59.3	57.50	
T. hamatum	T8	57.6	67.5	63.4	66.6	63.80	58.70
	T9	50.1	49.6	60.0	43.7	50.90	
	T10	62.0	63.4	57.3	63.0	61.60	
Control		0.0	0.0	0.0	0.0	0.00	-

LSD.at 5% for:

Isolates of the pathogen (I so) = 3.12 Antagonistic fungi (A) -3.90

I so x A * 4.58 Isolates of the antagonistic fungi (B)

Isox AxB = 6.11 =2.80

Effect of antagonistic fungi on the incidence of sesame diseases:

In this experiment, 10 species of isolates belonging to three antagonistic *Trichoderma* spp. were used individually in the form of seed coating

to study their effects on the incidence of pre and post-emergence damping-off as well as wilt diseases caused by *M. phaseolina* and *F oxysporum* under greenhouse conditions. The antagonistic fungi were *T. harzianum* (4 isolates), *T. viride* (3 isolates) and *T. hamatum* (3 isolates).

A) Disease incidence caused by *M. phaseolina:*

Data in Table (3) show that all the antagonistic fungi were significantly effective on controlling disease incidence caused by *M. phaseolina* and increasing the survived and the healthy plants.

Table (3): Effect of some antagonistic fungi on the incidence of sesame diseases caused by *M. phaseolina in vivo.*

Antagonistic fungus	Isolate/no.	Disease incidence%				% Efficacy
		Pre emergence	Post-emergence	Mortality*	Total of dead plants	
T. harzianum	Tl	8.7	10.5	9.7	28.9	58.7
	T2	0.0	7.5	4.2	11.7	83.3
	T3	13.3	8.5	8.2	30.0	57.1
	T4	12.5	6.6	11.5	30.6	56.3
T. viride	T5	14.6	8.5	8.5	31.6	54.8
	T6	10.0	12.3	15.0	37.3	46.7
	T?	12.5	13.3	11.2	37.0	47.1
T. hamatum	T8	20.0	4.3	9.5	33.8	51.7
	T9	16.5	19.7	12.3	48.5	30.7
	T10	13.3	0.0	6.7	20.0	71.4
	Control	25.3	30.2	14.5	70.0	
L.SD. at 5%		4.36	3.75	6.19	7.46	

* Plants showing charcoal rot symptoms

Regarding to the percentage of infection due to the tested isolates of *Trichoderma* spp., it's worthy to mention that *7. harzianum* (T2) recorded the least percentage of infection (11.7%). On the other hand, the highest percentage of dead plants (70.0%) was recorded for the untreated control. The highest percentage of efficacy was noticed with *T. harzianum;* (T2), being 83.3%, followed by *T. hamatum* (T10), being 71.4%. Meanwhile, the lowest percentage of efficacy was found with *T. hamatum,* (T9), being 30.7%, compared to control treatment.

B) Disease incidence caused by *F. oxysporum:*

Data in Table (4) indicate that the antagonistic fungi were significantly effective on controlling disease incidence caused by *F. oxysporum* and increasing the survived and healthy plants. The highest percentage of infection was recorded under the effect of *T. viride* (T7), being 46.6%, *7. hamatum* (T9), being 46.1%, and *T. harzianum,* (Tl), being 45.7% respectively without significant differences. The highest percentage of efficacy was obtained by using *7. hamatum* (T10), being 88.1% followed by *T. viride* (T5) and *T. harziamum* (T3), being 73.3 & 64.4%, respectively compared to control treatment.

Table (4): Effect of some antagonistic fungi on the disease incidence of sesame diseases caused by *F. oxysporum in vivo.*

Antagonistic fungus	Isolate/no.	Disease incidence %				%Efficacy*
		Pre-emergence	Port-emergence	Mortality**	Total of dead plants	
T. harzianum	T1	11.6	8.7	25.4	45.7	42.9
	T2	12.5	6.6	13.5	32.6	59.3
	T3	9.0	8.9	10.6	28.5	64.4
	T4	12.8	8.2	19.0	40,0	50.0
T. viride	T5	5.6	3.4	12.5	21.5	73.3
	T6	7.8	10.5	16.4	34.7	56.6
	T7	18.5	19.4	8.7	46.6	41.7
T. hamatum	T8	9.0	12.3	9.3	30.6	61.7
	T9	15.6	20.5	10.0	46.1	42.4
	T10	4.2	0.0	5.3	9.5	88.1
Control		20.5	29.6	29.9	80.0	-
L.SD. at 0.05%		4.63	5.82	7.56	8.93	-

*Efficacy relative to control of total infection.
** Plants showing wilt symptoms.

DISCUSSION

Sesame (*Sesamum indicum* L.) is one of the oldest cultivated plants in the world. Sesame is attacked by serious diseases *i.e.,* root rot, wilt and damping-off. Also, seed decay is caused by several pathogens among them *M. phaseolina* and *F. oxysporum.* Infection with these diseases has been increased tremendously during the last years (EI-Barougy, 1990; Gupta & Cheema, 1990; Khalifa, 1997; El-Deeb *et al.,* 1998 and EI-Fiki *et al.,* 2004). Therefore, the present investigation was conducted to study the biological control methods that can be used to minimize, as possible, yield losses caused by seed and/or soil-borne fungi which attacks sesame plants. Seedling mortality as well as biological control measures were investigated in the present study. The efficacy of the biocontrol agents, *Trichoderma* spp. isolates under lab. conditions showed that the the mode of action by which the antagonistic fungi suppressed the activity of plant pathogens, is that the biocontrol agent contact their host, either by appressorium-like bodies or coiling around host hyphae, then enzymatically digest host cell walls. Antagonistic actions of the tested antagonistic fungi against the different isolates of *M. phaseolina* and *F. oxysporum* are in agreement with those reported by (Dinakaran & Marimuthu, 1997 and Hemeda & Rasmy, 2003).

Studying the efficacy of the biocontrol agents indicated that using *Trichoderma* isolates *i.e., T. hamatum* (T10), *T. viride* (T5) and *T. harzianum* (T3) under greenhouse conditions in soil artificially infested by *F. oxysporum* caused the highest percentage of healthy seedlings. Similar results were recorded against *M. phaseolina* isolates. These results could be interpreted in light of the antagonistic actions of the tested antagonistic fungi against the different isolates of *M. phaseolina* and *F. oxysporum.* These results are in agreement with those reported by (Gaikwad and Kapgate, 1990; El-Khadem *et al.,* 1991; Dinakaran & Marimuthu, 1997; Hemeda and Rasmy, 2003).

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تأثير بعض الفطريات المضادة على تثبيط نمو ومقاومة الفطرين ماكروفومينا فاسيولينا وفيوزاريم أوكسيسبورم المسببة للعفن الفحمى والذبول لنباتات السمسم

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تعتبر الفطريات ماكروفومينا فاسيولينا وفيوزاريم أكسيسبوريم من المسببات الرئيسية لأهم أمراض السمسم وهى العفن الفحمى والذبول على التوالى ولهذا أجريت هذه الدراسة عليها .

تم اختبار التأثير التضادى لأنواع التريكوديرما الثلاثة المختبرة وهى ترايكوديرما هارزيانم ، وترايكوديرما فيردى ، وتريكوديرما هاماتم ضد أربع عزلات من الماكروفومينا فاسيولينا ،وأربع عزلات من الفيوزاريم أكسيسبوريم وظهر أن عزلات T1, T2, T3, T4 تمكنت من تثبيط النمو الطولى لعزلات الماكروفومينا فاسولينا والفيوزاريم أكسيسبوريم .

وعند اجراء تقييم لكفاءة معاملة البذور بالعامل الحيوى تريكودرما بانواعة الثلاث وهى هيرزيانم وفيريدى وهاماتم لمقاومة الفطر ماكروفومينا فاصولينا على نباتات السمسم بالصوبة، كانت أعلى نسبة كفاءة مع عزلات التريكودرما هيرزيانم T2 ثم تريكودرما هاماتم T10. وفيما يختص بمقاومة الأمراض التى يسببها الفطر فيوزاريم اكسيسبوريم كانت أعلى نسبه كفاءة مع استخدام الفطر تريكودرما هاماتم T10 ثم تريكودرما فيريدى T5، على حين تريكودرما هيرزيانم T3 أعطت أعلى نسبه كفاءة بالنسبة لعزلات التريكودرما هيرزيانم بالمقارنه بالكنترول