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Pathogenicity of *Fusarium oxysporum* f.sp. *melonis* to Melon Genotypes (*Cucumis melo* L.) and Its Biocontrol

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Fusarium wilt in melon (*Cucumis melo* L.) is widespread, responsible for serious economic losses in yield of melon in Egypt. Laboratory and greenhouse experiments were performed to evaluate the effects of different biocontrol agents (BCAs) against *Fusarium oxysporum* f.sp. *melonis*. *In vitro*, *Trichoderma viride* and *Gliocladium virens* significantly reduced the mycelia growth of *F. oxysporum* f.sp. *melonis* that produced 86.33 and 86.11% reduction, respectively. Under greenhouse conditions, 1022, caruso, primal and ideal genotype were the best genotypes for tolerant the infestation by *F. oxysporum* f. sp. *melonis* by 60% survival plants when compared with other genotypes. On the other hand, all BCAs tested significantly decreased the disease incidence of *F. oxysporum* f.sp. *melonis*. The treatment with *T. viride* completely suppressed the disease incidence after 15 days from sowing giving 100% survival plants and 96% living plants after 45 days.

Key words: Genotype, Biocontrol agents, Fusarium wilt, Melon.

Melon (*Cucumis melo* L.) is one of the most important fruit crops grown in Egypt. It is highly susceptible to *Fusarium* species when planted in the same field without rotation[1]. Practice intensive agricultural dangerous diseases transmitted through the soil in Egypt, especially *Fusarium* species. Champaco *et al*., [2] confirmed that Fusarium wilt of melon is a destructive vascular disease leading to serious economic losses. *Fusarium* species can occur in soil for several years through chlamydospores formation[3], and is therefore difficult to management. Usually, crop rotation has been established to be an effective strategy to manage various soil borne pathogens, but because *Fusarium oxysporum* f.sp. *melonis* can persist for a long period, the efficacy of crop rotation is restricted as soon as a disease outbreak occurs[4]. Soil solarization is a very effective strategy too but is not easy applicable for intensive vegetable farming systems[5] where time to solarize soil is very limited. Furthermore, this method is often incomplete due to the restrictions the local climate. Also, soil fumigation with chemicals[6,7] is a conventional practice but it must progressively cancel because of environmental concerns and human health[8]. Currently, the use of resistant

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cultivars appears to be the most practical and economically efficient control measure for management of Fusarium wilt of melon and is also a key component in integrated disease management programs[9, 10]. Good progress has been made in the development of high-yielding, with combined complete or partial resistance to Fusarium wilt diseases[10]. However, effectiveness of Fusarium wilt resistance can be curtailed by the occurrence of pathogenic races in *F. oxysporum* f.sp. *melonis*[11,12].

Biological control was an alternative strategy to manage *Fusarium* diseases[13]. Many antagonistic microorganisms have been proved to be active *in vitro* or *in vivo*. *Trichoderma* spp.[14], *Bacillus* species[15], *Aspergillus* species[16] and *Penicillium* spp.[17], are a rare among the extensive lists. The objectives of this work were 1) to evaluate the pathogenicity of *F. oxysporum* f.sp. *melonis* to melon genotypes, 2) to study the efficiency of biocontrol agents against *F. oxysporum* f.sp. *melonis* in melon that has been widespread in Egypt.

MATERIALS AND METHODS

In vitro

Isolation of *Fusarium oxysporum* f.sp. *melonis* antagonistic fungi

Fusarium oxysporum f.sp. *melonis* was isolated from tissue of a diseased cantaloupe plants collected from Daqahliya and Demitta governorate, Egypt. For antagonistic fungi isolation, a rhizosphere soil was sampled from a healthy melon plants, 10-fold series diluted, spread onto the plates containing potato dextrose agar (PDA), incubated at 25±2ºC for 4 days. The isolates that grew rapidly and formed greenish to white concentric circles were transferred to the *Trichoderma*-selective medium rose Bengal agar. The isolates were confirmed as having the same morphotype as on PDA and then stored as purified isolates at 4 ºC in PDA slants. *Trichodermas* sp. were identified by microscopic observations using the identification keys of Overton *et al*.,[18] and Jaklitsch *et al*.,[19]. *Conothyrium minitans* was obtained from Plant Pathology Department, Plant Protection Research, Budapest, Hungary.

Effect of antagonistic fungi on the radial growth of *F. oxysporum* f. sp. *melonis*

The inhibitory effects of *T. viride*, *G. virens* and *C. minitans* on the radial growth of *F. oxysporum* f.sp. *melonis* were studied. All cultures of the antagonistic fungi and the pathogenic fungus were grown on PDA for 7 days. The effect of the antagonistic fungi on the pathogenic fungus was done by using on disc (5 mm.) of the antagonist facing one disc of the pathogen on the PDA surface and relatively closed to the periphery of the Petri plates. All plates were incubated at 25±2ºC for 3 and 10 days. The diameter average of zones of the pathogenic fungus was recorded using the following formula:

$$Inhibition\ \% = \frac{(R - r) * 100}{R}$$

Where, r is the radius of the fungal colony opposite the bacterial colony and, R is the maximum radius of the fungal colony away from the antagonistic fungi colony

In vivo

Pathogenicity test

The pathogenicity of *F. oxysporum* f. sp. *melonis* on 10 genotypes of melon; Regal, Super VIP, C.8, Galia, Mirella, 1022, Caruso, Vicar, Primal and Ideal was studied. Plastic pots (25×30×25 cm.). were filled with autoclaved soil (25% sand+ 50% clay soil +25% peat moss, about 4 kg/ pot) then infested with spore suspension of the pathogenic fungus (25 ml/pot, 1×10^6 spore/ml). The fungus was grown in 250 ml flasks containing 100 ml of potato dextrose broth for two weeks at 25±2ºC. The mycelia in each flask were added to 200 ml of sterilized water in a sterilized blender for 30 seconds. The pots were infested before 8 days from planting, five seeds each pot. Irrigation took place immediately after planting, and repeated every 3 days during the experiment .The planted pots were kept under the plastic house conditions where daily temperature average was 27±2ºC, the experiments contained 5 replicates. Data of disease incidence were recorded after 15 and 45.

Effect of antagonistic fungi on the disease incidence caused by *Fusarium oxysporum* f. sp. *melonis*

The effect of *T. viride*, *G. virens*, and *C. minitans* in suppressing disease incidence caused by *F. oxysporum* f. sp. *melonis* was studied. Plastic pots were filled with autoclaved sandy loam soil as previously mentioned. Sowing was carried out 8 days after inoculation. Five seeds of vicar

genotype were planted (5 replicates). Before planting, seeds were surface sterilized and coated with spores of antagonistic fungi (14 days old). Seeds sterilization were carried out by dipping in 1% sodium hypochlorite for 10 min.; washed with distilled water, then dried under laminar flow. Seeds coated were performed by wetting them with sterile water containing molasses, air dried and then placed on the surface of 14 days-old culture of antagonistic fungi in Petri plates in that conidia were abundant. Control treatment was done by soaking seeds in distilled water, while the chemical treatments were done by Topsin-M 70 2 g/kg and Thiram 3g/kg. Data of the disease incidence was recorded after 15 and 45 days.

RESULTS AND DISCUSSION

In vivo

Effect of antagonistic fungi on the radial growth of *Fusarium oxysporum* f. sp. *melonis*

Data in Table (1) reveal that *T. viride* significantly inhibited the radial growth of *F. oxysporum* f sp. *melonis* which produced 70.39 and 86.33% reduction in the radial growth after 3 and 10 days, respectively when compared to control. This was followed by *G. virens* giving 59.22 and 86.11% suppression in the radial growth of the pathogenic fungus after 3 and 10 days, respectively. Otherwise, *C. minitans* gave the lowest effect in reducing in the radial growth with 50.84% and 54.44 % reduction after 3 and 10 days, respectively. These results agree with some authors who reported that *T. viride* was able to suppress the growth of soil borne pathogenic fungi[21-24]. They found that *T. viride* eliminated *Fusarium* spp. and *F. solani* and. This high antifungal activity of *T. viride* is probably related to secondary metabolites and some enzymes[25] who reported that the activities of Beta-glycosidase and extra cellular chitinase, which are thought to be involved in the mycoparasitic process. Benhamou and Chet,[26] demonstrated that many interactions of *Trichoderma* with fungal pathogens, such as *Trichoderma* had grown parallel to pathogen, grown along the pathogen and grown around the pathogen. After penetration with *Trichoderma* appressorium, normal degradation of plant pathogenic fungi hyphae would take place and they had observed the growth of *Trichoderma* hyphae within the pathogen. On the other hand, Cooney and Lauren,[27] found that the antifungal *Trichoderma* secondary metabolite 6-n-pentyl-2-H-pyran-2-one level significantly increased in the presence of the pathogen.

In the present study, it was noticed that *G. virens* gave moderate to high reduction in the radial growth of *F. oxysporum* f.sp *melonies*. These results agree with Vishwa Dhar *et al*.,[28] and Agarwal *et al*.,[29] they reported that *G. virens* inhibited the mycelial growth of *Fusarium* spp., *Sclerotinia sclerotiotum* and *Rhizoctonia solani*. This antimicrobial of *G. virens* may be attributed to gliotoxin and gliovirin which belong to the epipolythiodioxopiperazine class of peptides[30]. *G. virens* produce copious amounts of gliotoxin within 16 h of growth in liquid culture[31], and the compound can be detected in the rhizosphere[32]. Gliotoxin has received much attention for its role in the biocontrol of soil-borne fungal pathogens[33].

Pathogenicity of *Fusarium oxysporum* f.sp. *melonis* to melon genotypes

After 15 days

Data in Table (2, 3) reveal that there no significant difference in degree of melon genotypes sensitivity to *F. oxysporum* f. sp. *melonis*. Primal genotype was the lowest sensitivity to *F. oxysporum* f. sp. melonis giving 88% survival plants. This was followed by both Caruso and ideal genotype which gave the same result with 84% survival plants. While, the vicar genotype was highly sensitive to infection with the fungus by 72% survival plants.

After 45 day

There were non-significant differences between all melon genotypes for sensitivity *F. oxysporum* f.sp. *melonis*. The 1022, caruso, primal and ideal genotype were the best genotypes for tolerant the infestation by *F. oxysporum* f.sp. *melonis* by 60% survival plants when compared with other genotypes. Whereas, the most genotype sensitivity to *F. oxysporum* f.sp. *melonis* was vicar that produced 48% survival plants (Table 2,3).

In this study, *F. oxysporum* f.sp. *melonis* evaluated was pathogenic to all melon genotypes tested, and have strong pathogenicity. Previous studies on the occurrence and pathogenicity of *Fusarium* spp. were only based on *Fusarium* samples which were isolated from infected plants[34, 35]. However, in this study, *F. oxysporum* f.sp.

melonis obtained from soil was found to be capable of causing disease symptoms in melon plants in Daqahlia and Demitta governorate, North of Egypt.

In Japan, Risser *et al.*,[11] illustrated that *Fusarium oxysporum* f. sp. *melonis* has been divided into four races; 0, 1, 2 and 1, 2 based on pathogenicity to three differential genotype of melon. The resistance genes effective against the respective races have been characterized in many differential possess single dominant resistant genes, *Foml* and *Fom2*, respectively[11]. The race nomenclature corresponds to the resistance genes

Table 1. Effect of antagonistic fungi on the radial growth of *Fusarium oxysporum* f.sp. *melonis*

Antagonistic fungi	After 3 days		After 10 days	
	R.G.	Inh.%	R.G.	Inh.%
Trichoderma viride	26.5[b]	70.39	12.3[c]	86.33
Gliocladium virens	36.5[b]	59.22	12.5[c]	86.11
Coniothyrium minitans	44.0[b]	50.84	41.0[b]	54.44
Control	89.5[a]	0.00	90.0[a]	0.00
LSD	8.78		16.09	

R.G. = Radial growth Inh.% = inhibition%
Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P=0.05).

Table 2. ANOVA of Pathogenicity of *F. oxysporum* f. sp. *melonis* to melon genotypes

		Sum of Squares	df	Mean Square	F	Sig.
Survival plant after 15 days	Between Groups	2.58	9	0.287	0.237	0.987
	Within groups	48.40	40	1.210		
	Total	50.98	49			
Survival plant after 45 days	Between Groups	2.00	9	0.222	0.148	0.998
	Within groups	60.00	40	1.500		
	Total	62.00	49			

Table 3. Pathogenicity of *Fusarium oxysporum* f.sp. *melonis* to melon genotypes

Genotypes	After 15 days			After 45 days		
	No.	Mo.%	Sur.%	No.	Mo.%	Sur.%
Regal	4.0[a]	20.0	80.0	2.8[a]	24.0	56.0
Super VIP	3.8[a]	24.0	76.0	2.8[a]	20.0	56.0
C.8	3.8[a]	24.0	76.0	2.6[a]	24.0	52.0
Galia	4.0[a]	20.0	80.0	2.8[a]	24.0	56.0
Mirella	3.8[a]	24.0	76.0	2.6[a]	20.0	52.0
1022	4.0[a]	20.0	80.0	3.0[a]	20.0	60.0
Caruso,	4.2[a]	16.0	84.0	3.0[a]	24.0	60.0
Vicar	3.6[a]	28.0	72.0	2.4[a]	28.0	48.0
Primal	4.4[a]	12.0	88.0	3.0[a]	28.0	60.0
Ideal	4.2[a]	16.0	84.0	3.0[a]	24.0	60.0
LSD	1.18			1.31		

No. = number of living plants Mo. % = Mortality percentage
Sur. %= Survival Plants
Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P=0.05).

that are overcome. Bouhot,[36] confirmed that race 1, 2 has been sub-divided further into races 1,2w (wilt) and 1,2y (yellows) based on the symptoms that they induces. Lori *et al*.,[37] previously found Japanese cultivars, Amus, Ohi and Ogon 9 that could substitute for the race differential genotypes utilized by Namiki *et al*.,[38,39]. Cultivars Amus, Ohi and Ogon 9 had the same reactions as those of differential cultivars Charantais T, Doublon and CM17187, respectively, to strains of four races[39].

Effect of seeds treatment with antagonistic fungi on the disease incidence

After 15 days, Data in Table (4,5) showed that seed treatment with *T. viride* completely inhibited the melon damping-off caused by *F. oxysporum* f.sp. *melonis* that produced 100% survival plants when compared with non-treated control (40%), while seed treatment with Thiram and Topsin, gave 100% disease control. On the other hand, after 45 days, *T. viride* significantly suppressed the Fusarium wilt caused by the fungus with 92% living plants when compared to controls. Rajappan and Yesuraja,[40] and Dubey,[41] illustrated that *T. viride* gave good result when compared

Table 4. ANOVA of effect of antagonistic fungi on *F. oxysporum* f. sp. *melonis*

		Sum of Squares	df	Mean Square	F	Sig.
After 15 days	Between Groups	36.00	6	6.00	38.182	0.000
	Within groups	4.40	28	0.157		
	Total	40.40	34			
After 45 days	Between Groups	37.143	6	6.190	27.083	0.000
	Within groups	6.400	28	0.229		
	Total	43.543	34			

Table 5. Effect of melon seeds treatment with antagonistic fungi on *Fusarium oxysporum* f.sp. *melonis*

Treatment	15 days			45 days		
	No.	Mo. %	Sur.%	No.	Mo. %	Sur.%
Non-infested	5.0[c]	0	100	5.0[c]	0.0	100.0
Infested	2.0[a]	60	40	1.8[a]	4.0	36.0
Thiram	5.0[c]	0	100	4.6[c]	8.0	92.0
Topsin	5.0[c]	0	100	4.8[c]	4.0	96.0
Trichoderma viride	5.0[c]	0	100	4.6[c]	8.0	92.0
Gliocladium virens	4.4[b]	12	88	4.4[c]	0.0	88.0
Coniothyrium minitans	4.4[b]	12	88	3.6[b]	16.0	72.0
LSD	0.43			0.52		

No. = number of living plants Mo. % = Mortality percentage
Sur. %= Survival Plants
Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P=0.05).

with captan, vitavax and carboxin when the fungus used in integrated control of Fusarium wilt. Suppression in disease incidence and protection of melon seedling against Fusarium wilt was significant with bicontrol agents testing stronger ability in controlling the pathogen compared to control. Results can be understood by synergistic involvement of a several of mechanisms, which might contain stimulation of plant defense system[42]. The synthesis of pathogenesis-linked proteins is one of the most ordinary defense mechanisms triggered in plants following infection with inducing agents[43]. Induced resistance is known as an important mode of *Trichoderma* spp. in plant growth[44]. Salicylic acid produced by *Trichoderma* spp. induced resistance to *B. cinerea* in bean[45]. De Santiago *et al*.,[46] reported that besides, root colonization with *Trichoderma* induced increased peroxidase and chitinase activities in many plants. Furthermore,

Trichoderma spp. secrete volatile secondary metabolites such as ethylene, hydrogen cyanide, aldehyde and ketones which responsible for the suppression of plant pathogens[47,48].

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