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Effect of Some Tunisian *Fusarium solani* f. sp. *cucurbitae* Isolates On Muskmelon Growth and on Crown and Root Rot Severity

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Abstract: In 2005, a crown and root rot, a yellowing and a death of muskmelon plants were observed in some tunisian melon-growing regions. Isolation was made from diseased tissues. Twelve isolates of *Fusarium solani* were identified and used to inoculate melon-seedlings by the root dip method for 1 min. These 12 isolates caused typical symptoms of *Fusarium* crown rot and their pathogenicity indicated that they are identical to *Fusarium solani* f. sp. *cucurbitae*. Disease severity on melon-plants, cv. "Ananas d'Amérique", was estimated by indexes of the leaf damage and crown rot. Analysis of variance revealed significant difference between control and inoculated plants. A significant difference was also observed between some *F. solani* f. sp. *cucurbitae* isolates in causing leaf damage and crown rot and a negative effect was observed mainly on plant fresh weight revealing impact of this pathogen on muskmelon growth.

Key words: *Cucumis melo* L., leaf damage, necrosis index, disease incidence

INTRODUCTION

In Tunisia, 6.150.000 tons of vegetables are produced on 2004. Approximately 7% of this production belongs to Cucurbitaceous species (Anonymous, 2004). Melon (*Cucumis melo* L.) is one of vegetables widely cultivated of this group with approximately 115.857 tons of production (Jebari *et al*., 2004). However, important economic losses were noted on this crop due to the development of a severe root rot and vine decline disease during the last decades. These problems were caused by some soilborne fungi, in particular *Fusarium* and *Verticillium* species (Martyn, 1983; El Mahjoub and Ben Khedher, 1987; Jabnoun-Khiareddine *et al*., 2006). Symptoms, including leaf yellowing and die back of the crown leaves, cortical rot of the taproot and lateral roots and discolouration of the vascular system in the root and crown, were similar to *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *melonis* and *Fusarium* crown and root rot caused by *Fusarium solani* f. sp. *cucurbitae* (Champaco *et al*., 1993).

During 2005 and 2006 growing seasons, melon plants, showing symptoms of root rot, were frequently observed. Isolation from diseased fragments showed presence of *F. solani*. Thus, the objectives of this research were to identify the *forma specialis* and to study the incidence of *Fusarium solani* f. sp. *cucurbitae* on muskmelon plant growth.

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MATERIALS AND METHODS

Fungal Isolates

Twelve isolates of *F. solani* were obtained during 2005 and 2006, from tunisian melon-growing areas, by isolation from melon plants showing typical symptoms of crown and root rot. Surface disinfected diseased fragments were placed on Potato Dextrose Agar (PDA) containing 300 mg L^{-1} of streptomycin sulfate and incubated at 25°C for 4-5 days in the dark (Table 1). Single-spore cultures of all isolates were stored at -20°C.

For plant inoculation, mycelium was taken from the edge colony of each isolate and placed in Erlenmeyer flasks containing 150 mL of Potato Dextrose Broth (PDB) and incubated at 25°C for 5 days in a rotary incubator (120 rpm). The liquid culture was filtered and the conidial suspension was adjusted to 10^7 conidia mL^{-1} by a Malassez cystometer.

Plant Material

Seeds of the melon cultivar "Ananas d'Amérique", the most cultivated cultivar in Tunisia, were disinfected by immersion for 2 min in fresh 10% Ethanol. They were then rinsed in sterile water and placed on moistened cotton wool in Petri dishes in the dark at 27±2°C. The germinated seeds were planted in an autoclaved peat and incubated in a growth chamber at 26±1°C by day and 20±1°C by night with 14 h photoperiod.

Pathogenicity Tests

Pathogenicity of the isolates was established by dipping seedlings, at the expansion of the first-true leaf, for 1 min in the inoculum suspension adjusted at 10^7 conidia mL^{-1} (Jacobson and Gordon, 1988). Five seedlings were inoculated with every isolate and five seedlings dipped in sterile distilled water served as controls. The whole were transplanted to plastic pots (0.7l) filled with an autoclaved mixture of peat and perlite (3:1) and placed in a growth chamber at 26±1°C by day and 20±1°C by night with 14 h photoperiod. They were irrigated regularly and fertilized with the nutrient solution of El Mahjoub (1985) (per liter): 0.225 g of KNO_3, 0.3 g of KH_2PO_4, 0.225 g of NH_4Cl, 0.45 g of $MgSO_4,7H_2O$, 0.3 g of $(NH_4)_2SO_4$, 1.2 g of $Ca(NO_3)_2$, 0.045 g of EDTA Na, Fe and other oligo-elements.

Symptoms, as described by Champaco *et al*. (1993), traduced by leaf yellowing, crown or cortical rot were first observed 20 days after inoculation. Fungal isolates implicated in typical symptom development, determined via re-isolations made from rotted roots and crown of inoculated melon seedlings, were considered pathogen and then attributed to the forma specialis cucurbitae.

Table 1: Isolates of *Fusarium solani* from some melon cultivars and their geographic origin

Isolates	Origin	Cultivars
I.1.05	Bizerte	Ananasd'Amérique
I.3.05	Bizerte	Blanco
I.6.05	Bizerte	Gold Mayen
I.8.05	Monastir	Fakous
I.9.05	Monastir	Pancha
I.13.05	Bizerte	Jaune Canari
I.19.05	Monastir	Fakous
I.22.05	Bèjà	Maazoul
I.24.05	Monastir	Asli
I.25.05	Monastir	Galaawi
I.26.05	Monastir	Fakous
I.27.05	Monastir	Fakous

Disease Incidence on the Melon Growth

Disease severity was assessed via an index of leaf damage (ILD) where a scale of 0-4 was used every 3-4 days: 0 = asymptomatic leaf, 1 = leaf wilted, 2 = leaf with yellowing, 3 = leaf with necrosis, 4 = dead leaf. Isolate's virulence were expressed as an index of leaf damage (ILD) which is calculated per muskmelon plant (Béye and Lafay, 1985):

$$ILD = \Sigma \text{ notes/max}$$

ILD : Index of leaf damage.
Σ notes : Total notes.
Max : 4 times of the developed-leaves number.

Another scale of 0-3 was used, 48 days after inoculation, to assess disease severity on crown necrosis or rots: 0 = asymptomatic crown, 0.5 = some necrotic traces, 1 = necrosis of 1/3 of the crown, 2 = necrosis of 2/3 of the crown, 3 = necrosis of more than 2/3 of the crown.

Plant height and fresh weight was also recorded 48 days after inoculation. Pathogen isolations were done at the end of the bioassay.

Statistical Analysis

Five plants were used for each elementary treatment (isolates and control). Analysis of variance were conducted for each dependant variable to determine the disease severity and the effect of isolates on plants development. Data are arranged by completely randomized design where treatments (seedlings inoculated by each of 12 *Fusarium* isolates and control seedlings) are the only fixed factor. They were analyzed using SPSS and subjected to analysis of variance and Fisher's least significant difference test LSD (at $p \leq 0.05$).

RESULTS

Pathogenicity Tests of *F. solani*

Several fungal species were isolated from rotted root and crown melon samples on PDA, but *Fusarium* species, especially *F. solani*, were predominant. In pathogenicity tests, *F. solani* isolates used were pathogenic by inducing yellowing, 20 days after inoculation and death of inoculated seedlings. Similar symptoms developed in naturally diseased plants. This result showed that it is the forma specialis *cucurbitae* race 1. The fungus was re-isolated from rotted roots and crown of all inoculated seedlings.

Incidence of *F. solani* f. sp. *cucurbitae* on the Development of Muskmelon Plants
Effect of *F. solani* f. sp. *cucurbitae* Inoculation on the Leaf Damage Index

The incidence of 12 *F. solani* f. sp. *cucurbitae* isolates from muskmelon was studied by the root dip method inoculation of seedlings. On the basis of analysis of variance of disease severity from seedlings inoculated with some isolates, statistically significant differences were found in disease severity among some isolates and between healthy and some diseased plants (Table 2). Twenty to twenty four days after inoculation, I.24.05, I.26.05 and I.19.05 were found to be the most virulent isolates. Their leaf damage index reached 0.2627, 0.2166 and 0.2168, respectively. However, I.6.05, I.8.05 and I.22.05 had the least ILD. with 0.025, 0.0332 and 0.0166, respectively. No significant difference between indexes of inoculated plants was observed from 28 days after inoculation (Table 2). At the end of the bioassay, I.6.05 appeared the most pathogenic with an ILD of about 3.5; but I.22.05 was the least with 0.3642.

Table 2: Evolution of the Index of Leaf Damage (ILD) for muskmelon plants, cv. "Ananas d'Amérique", inoculated by *F. solani* f. sp. *cucurbitae* isolates observed 20 days after inoculation

Days after inoculation	Index of Leaf Damage (ILD)						
	Control	I.1.05	I.3.05	I.6.05	I.8.05	I.9.05	I.13.05
20	0[c]	0.0332[bc]	0.0791[bc]	0.025[bc]	0.0332[bc]	0.0625[bc]	0.1748[abc]
24	0[c]	0.1332[bc]	0.125[bc]	0.0582[c]	0.05[c]	0.185[bc]	0.2373[bc]
28	0.0334[b]	0.0875[ab]	0.1309[ab]	0.0957[ab]	0.09[ab]	0.2458[ab]	0.22[ab]
32	0.0668[b]	0.1559[ab]	0.1976[ab]	0.13[ab]	0.09[ab]	0.4481[a]	0.3332[ab]
36	0.12[b]	0.185[ab]	0.1916[ab]	0.1366[b]	0.255[ab]	0.4572[a]	0.3334[ab]
40	0.119[ab]	0.053[b]	0.1632[ab]	0.1366[ab]	0.2826[ab]	0.3886[a]	0.4543[a]
44	0.12[b]	0.20[b]	0.3187[ab]	0.4706[ab]	0.1856[b]	0.375[ab]	0.673[a]
48	0.18[b]	0.7332[ab]	0.645[ab]	3.5[a]	0.3152[b]	0.554[ab]	0.755[ab]

Table 2: Continued

Days after inoculation	Index of Leaf Damage (ILD)						
	Control	I.19.05	I.22.05	I.24.05	I.25.05	I.26.05	I.27.05
20	0[c]	0.2168[ab]	0.0166[c]	0.2627[a]	0.0332[bc]	0.2166[ab]	0.1082[abc]
24	0[c]	0.3124[ab]	0.0416[c]	0.475[a]	0.1957[bc]	0.3209[ab]	0.217[bc]
28	0.0334[b]	0.3041[ab]	0.0891[ab]	0.3568[ab]	0.1791[a]	0.21[ab]	0.288[ab]
32	0.0668[b]	0.2792[ab]	0.1925[ab]	0.3875[ab]	0.2082[ab]	0.4125[ab]	0.308[ab]
36	0.12[b]	0.4057[ab]	0.2145[ab]	0.4032[ab]	0.1834[ab]	0.464[a]	0.217[ab]
40	0.119[ab]	0.459[a]	0.44[a]	0.3736[ab]	0.1954[ab]	0.3316[ab]	0.2641[ab]
44	0.12[b]	0.496[ab]	0.399[ab]	0.519[ab]	0.5136[ab]	0.549[ab]	0.3642[ab]
48	0.18[b]	1.3956[ab]	0.3642[b]	0.6428[ab]	0.814[ab]	1.231[ab]	1.6718[ab]

In.05: *F. solani* f. sp. *cucurbitae* isolates.[z]Within lines, means followed by the same letter(s) are not significantly different ($p \leq 0.05$) according to SNK test

Table 3: Effect of inoculation by different *F. solani* f. sp. *cucurbitae* isolates on muskmelon plant growth (cv. "Ananas d'Amérique"), observed 48 days after inoculation

Treatments	Parameters		
	Crown rot index	Plant height (mm)	Fresh weight (g)
Control	0.00[c]	53.5[a]	7.40[a]
I.1.05	1.00[abc]	32.0[ab]	1.538[c]
I.3.05	0.50[bc]	36.8[ab]	2.56[bc]
I.6.05	0.833[abc]	31.6[ab]	0.936[c]
I.8.05	0.666[bc]	46.8[ab]	5.40[ab]
I.9.05	0.50[bc]	36.6[ab]	5.40[ab]
I.13.05	2.00[a]	31.6[ab]	1.934[c]
I.19.05	0.1667[bc]	36.2[ab]	4.134[bc]
I.22.05	0.333[bc]	25.4[b]	3.20[bc]
I.24.05	1.50[abc]	32.8[ab]	1.96[c]
I.25.05	0.333[bc]	32.8[ab]	3.00[bc]
I.26.05	1.667[ab]	34.4[ab]	1.48[c]
I.27.05	0.1667[bc]	36.8[ab]	2.38[bc]

[z]Within column, means followed by the same letter(s) are not significantly different ($p = 0.05$) according to SNK-test

Effect of *F. solani* f. sp. *cucurbitae* Inoculation on the Crown Rot Index

Analysis of variance revealed significant difference between control and inoculated plants. A significant difference was observed between some isolates in causing crown rot (Table 3). The most important crown rot index was caused by the isolate I.13.05. A significant necrosis was observed on crown of plants inoculated with isolates I.24.05, I.26.05, I.1.05 and I.6.05. However, these symptoms were not present on non-inoculated plants (Table 3).

Effect of *Fusarium* Crown and Root Rot on Melon Plant Height

Significant difference was observed between noninoculated and inoculated plant heights. Moreover, variance analysis showed a significant difference between *F. solani* f. sp. *cucurbitae* isolates. In fact, I.22.05 had the most important height reduction with 53% comparatively to control plants. Furthermore, isolates I.1.05, I.6.05 and I.13.05 reduced plant height by 40 to 52% (Table 3).

Effect of *Fusarium* Crown and Root Rot on Plant Fresh Weight

Significant decreases in the fresh weight of muskmelon plants inoculated with *F. solani* f. sp. *cucurbitae* were noted compared to control plants (Table 3). In fact, all inoculated melon plants showed varying degree of fresh weight reduction. Isolate I.6.05, I.1.05 and I.26.05 seemed to have the most important effect in reducing weight by 87.35, 80 and 79.22%, respectively when compared to the noninoculated control. I.13.05 and I.24.05 also revealed very aggressive. However, I.8.05 and I.9.05 had the least effect on muskmelon plant fresh weight (Table 3).

DISCUSSION

Twelve *F. solani* isolates were obtained from crown and root rot affecting melon plants in Tunisia. They are responsible of a cortical rot at the stem base and the upper portion of the taproot causing yellowing and wilting of leaves. These symptoms were identical that those described by Martyn (1996) and Armengol *et al.* (2000) caused by *F. solani* f. sp. *cucurbitae* race 1 (Tousson and Snyder, 1961; Messiaen *et al.*, 1991). This pathogen caused important damage to muskmelon traduced by leaf alteration, crown and root rot development and plant fresh weight losses. In the same way, the negative effect was observed by Boughalleb *et al.* (2005) on watermelon in Tunisia.

This pathogenic effect of *F. solani* is due to the secretion of a broad range of hydrolytic enzymes involved in penetration and colonization of host plant tissues during infection (Walton, 1994). Thus, isolates of *F. solani* f. sp. *cucurbitae* produce similar lesions on all plant parts once the cuticule and epidermis have been broken (Hawthorne *et al.*, 1994). Annis and Goodwin (1997) reported that the importance of cell wall degrading enzymes to pathogenicity might be different in each plant-microbe interaction. It has been also proposed that cell wall depolymerases can function as signals that trigger various plant physiological processes such as the induction of plant defence responses (Walton, 1994).

In this study, the incidence of different *F. solani* f. sp. *cucurbitae* isolates, obtained from diverse tunisian regions, was showed on susceptible muskmelon seedlings. A difference in their pathogenicity was observed. This result confirmed that obtained by Nagao *et al.* (1994). Although the fungus causing *Fusarium* crown and root rot of muskmelon has been taxonomically classified as *Fusarium solani* f. sp. *cucurbitae*, many researchers was indicated that this fungus has a broader Cucurbitaceae host range than only muskmelon (Blancard *et al.*, 1991; Jebari *et al.*, 2004). For this reason and as *F. solani* is a soilborne pathogen surviving in the soil and in some vegetable debris (Messiaen *et al.*, 1991), its control revealed very necessary as it affected plant weight, growth and consequently production of an important culture in Tunisia. Thus, the research of some chemical and biological control measures were necessary.

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