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Screening and pathogenicity of soil-borne fungal communities in relationship with organically amended soils cultivated by watermelon in Tunisia

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Abstract

Soil mycoflora analysis from cucurbits showed remarkable diversity A total of 180 soil samples were collected from experimental field treated with four doses of organic amendments (0, 20, 40, 60 tons per hectare) and cultivated by five cucurbits (watermelon, grafted watermelon, muskmelon, grafted muskmelon and squash) to determining number of soil-borne fungi. This study aimed to isolate and identify the mesophilic and thermophilic fungi from soil and to evaluate their pathogenicity. The highest number of CFUs was recorded in the fertilized soil samples with 60 tons per hectare, compared with control and amended soil with 20 tons for mesophilic (30°C) or thermophilic (45°C) fungi. The CFUs values of soil samples fertilized with 60 tons/ ha of organic manure varied from 23.554 x 10^5 per gram of soil (melon seedlings) to 18.598 x 10^5 (grafted watermelon seedlings), for the total number of mesophilic fungi, and from 23.697 x 10^3 (melon seedlings) to 9.993 x 10^3 (grafted watermelon seedlings) for all the identified thermophilic fungi. The use of 60 tons per hectare of organic amendments was the most suppressive dose, with 0.34% of the total number of mesophilic pathogenic fungi.. Thirty-six species belonging to 17 genera were identified for the two fungi types. The genera with the highest number of species were *Aspergillus* sp. (9) and *Penicillium* sp. (4). According to pathogenicity test, *Fusarium solani*, *F. oxysporum*, *Macrophomina phaseolina* and *Rhizoctonia solani* are the most virulent on watermelon plants.

Key words: Soil mycoflora, CFUs, mesophilic and thermophilic fungi, pathogenicity.

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Introduction

The soil represents the main reservoir of microorganisms. It is inhabited by a wide range of microorganisms including bacteria, fungi, algae, viruses and protozoans. There are 1-10 million microorganisms per gram of soil, in which, fungi and bacteria are the most prevalent microbes (Kumar et al., 2013). Furthermore, Hawksworth et al. (1995) reported that fungi are an important component of the soil microbiota and constitute the most important soil biomass than bacteria depending on soil depth and nutrient conditions. Fungi are among the most diverse group of organisms on earth (Hammond, 1995). Fungi are present in the soil as mycelium, conidia, ascospores, chlamydospores or sclerotial bodies (Luangsa et al., 2004). Temperature is one of environmental factors that play a decisive role in the distribution, diversity, survival, microbial growth in soils (Paul & Clark, 1996). Fungi are divided according to their tolerance to temperature in mesophilic and thermophilic fungi (Kumar et al., 2013). During the last four decades many species of thermophilic fungi sporulating at 45°C have been reported. Therefore, thermophilic fungi are those that have a maximum temperature for growth at or above 50°C and a minimum temperature for growth at or above 20°C (Salar & Aneja, 2007). Defined as having better growth at 45°C than at 25°C, thermophilic fungi have been isolated from a wide variety of artificial and natural substrates including compost, soil, herbivore dung, bird and alligator nests and living animals (Tansey 1971, 1973, 1975, 1977; Fergus & Sinden, 1969; Cooney & Emerson, 1964). Usually mostly of the mesophilic fungi grow at temperature ranging from 20°C to 37°C (Maheshwari, 2011). The role of the diverse soil organisms could be beneficial or not beneficial and soil fungi fulfill both roles and are ubiquitous. Some soil fungi are potential pathogen to both plants and other organisms. These pathogens are responsible of reduced crop production, disease or death of plants (Kumar et al., 2013). Cucurbit plants are affected by a number of plant pathogens including fungi which cause root and crown rot. These pathogenic fungi are recorded in almost all *Cucurbitaceae* growing areas of the world. In Tunisia, Boughalleb and El Mahjoub (2006), showed the presence of a fungal complex including *Fusarium solani*, *F. oxysporum*, *Macrophomina phaseolina*, *Rhizoctonia solani* and *Monosporascus cannonballus*. These pathogens were isolated from infected plants of watermelons. Diseased plants were characterized by yellowing of the leaves, stem necrotic lesions, phloem discolorations, and collapse. There are many pathogens capable of producing these vine decline symptoms in *cucurbits* (Boughalleb et al., 2005, Mahmoud, 2016). The *Rhizoctonia* canker caused by *R. solani* Kühn can damage different parts of plant (Kumar et al., 2013). *R. solani* causes seed decay, pre-emergence and post emergence damping-off of seedlings, hypocotyl and root necrosis, stem rot, foliar blight, root and fruit rots, crown rot, and stem canker (Aiello et al., 2012). This disease leads either to premature plant death and/or decreased yield (Garciá-Jiménez et al., 1999; Bruton, 1998; Tu et al., 1996). *M. phaseolina* (Tassi) Goid. is a soilborne fungus causing the charcoal rot disease

on more than 500 plant species from more than 100 families (Mahmoud & Budak 2011; Srivastava et al., 2001; Mihail, 1992; Wyllie, 1988), including economically important hosts such as *cucurbits* (Safarnezhad, 2004). Despite its wide host range, only one species has been identified in the genus *Macrophomina* (Wyllie, 1988). *M. phaseolina* induces diseases on a range of crops, ranging from seedling blight, root and stem rot, wilt, and pre- to post-emergent damping off, which result in decreased stem height, girth, root and head weight, or death, of affected plants (Raut, 1983). The objectives of this investigation were to (*i*) isolate and identify the fungi community from soil samples cultivated by 5 cucurbits and treated with 4 doses of organic amendments; (*ii*) determine the frequency of the fungi (thermophilic and mesophilic) at different temperature ranges (30°C and 45°C), and (*iii*) study the pathogenicity of *F. oxysporum*, *F. solani*, *M. phaseolina* and *R. solani* isolated from soil samples.

Materials and methods

Soil sampling: Soil samples were collected 90 days after planting from the experimental field amended with 4 doses of organic manure (0, 20, 40, 60 tons per hectare) and cultivated by five cucurbits (watermelon, grafted watermelon, muskmelon, grafted muskmelon and squash). Samples were taken with a soil probe at 10-20 cm depth. A total of 180 soil samples were collected for the present research aimed to identify different fungi with their prevalence ratio in different plots.

Microbiological analysis: The number of soil-borne fungi was determined by the dilution-plate method according to Bagley and Seidler (1978). 10 g of soil was diluted in 90 ml sterile distilled water. Serial of dilutions were made from 10^{-1} to 10^{-7} (Rapilly, 1968). A volume of 0.1 ml of each dilution was transferred aseptically into Petri dishes containing PDA. The plates were incubated in the dark at 30°C and 45°C. The count of fungal population is determined by counting the colonies: nly Petri dishes containing between 30 and 300 colonies at two successive dilutions are retained. The number of colony forming units (CFU) was calculated as followed: CFU / g of soil = ((Total No. of colonies)/(0.1 x (Number of Petri dishes considered for the first dilution retained + 0.1 x Number of Petri dishes considered for the second dilution retained) x dilution factor) (Mouria et al., 2012). The species identification is carried by observing the macroscopic (growth, color, aspect of the colony) and microscopic characterization (mycelium, conidiophore, conidia, resistance structures, sexual form), after a series of subculturing until purification of the fungus using the blue cotton as a mounting liquid and with reference to different identification keys. The relative frequency (Rf) of the fungal specie was evaluated using the formula: Rf = (n/N) × 100, which n = the number of colonies presented by each species; N = the total number of colonies of all species (Mouria et al., 2012).

Greenhouse experiment: Thirteen isolates collected from soil were used in this experiment are *F. oxysporum* (Fo1, Fo2, Fo3 and Fo4), *F. solani* (Fs1, Fs2 and Fs3), *M. phaseolina* (Mp1, Mp2 and

Mp3) and *R. solani* (Rs1, Rs2 and Rs3). Pathogenicity of strains was evaluated by artificial inoculation on watermelon (cv. *Crimson sweet*). *F. oxysporum* and *F. solani* were grown in PDA for 4 days. The conidia densities were calculated with hemocytometer and adjusted to 10^6 conidia ml^{-1}. Each plantlet of Crimson sweet was inoculated with 10 ml of each spores suspension for each *F. oxysporum*, *F. solani R. solani* and *M. phaseolina* strains. Watermelon seedlings were inoculated at the cotyledon and four-true-leaves stage. Control plants were treated with 10 mL of sterile distilled water. The inoculated plants in parallel with controls were kept in greenhouse, with a temperature ranging from 25 to 31°C. For each fungal species one cucurbit plant randomly distributed with 5 plants per replicate (3 replicates) in each treatment. Parameters measurements were determined after 30 days of inoculation (Baixia et al., 2014; Ferniah et al., 2014). The re-isolation was conducted from the infected plants to confirm the Koch's postulates.

Evaluation parameters: Three parameters were used for disease assessment. The first one is disease severity index (DSI) used for evaluate the disease rating scale as presented in Table 1 for each pathogen. Isolates causing no symptoms (0-0.3 DSI) were considered avirulent; isolates causing mild symptoms (0.4-1.9 DSI) were considered less virulent; isolates causing moderate symptoms (2-2.9 DSI) were classified as moderately virulent; isolates with severe symptoms (3-3.9 DSI) were considered virulent and isolates responsible of decline (4-5 DSI) were highly virulent (Sneh et al., 2004). The second is the percent of mortality (%) recorded only for *Fusarium* spp. by using the following formula: Mortality (%) = (Total No. of dead plants / Total No. of plants) x 100 (Khanzada et al., 2012). The third evaluation parameter is the wilting percent (%) was considered also only for *Fusarium* spp by using the following formula: Wilting (%) = (Total No. of wiltered plants / Total No. of plants) x 100 (Ferniah et al., 2014). Others Horticultural measurements noted on plants were recorded for above-ground (stem + leaf) and below ground (root) fresh and dry weights. The weights were determined for nine representative plants for each isolate. After determining the fresh weight of above- and below-ground portions, the plants samples were placed in an oven at 60°C for 48 hrs to determine the dry weight (Heitholt, 1989). The length of the plant and the main roots was measured (cm) using a flat rule. Root volume (cm^3) was determined by the immersion method as described by Musick et al., (1965) by comparing levels of the water before and after immersing the whole root in a known volume of water.

Data analysis: The disease severity index, percentage of mortality (%) and percent of wilting (%) were analyzed with the GENMOD procedure using the multinomial distribution and the cumulative logit as link function, and means of the values were separated by χ^2 test at $P < 0.05$ using SAS program (SAS Institute, Cary, NG). The other variables were compared by analysis of variance (ANOVA) and means of the values were separated with Duncan test at $P < 0.05$ using SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA).

Table 1: Disease severity of watermelon plants inoculated with *F. oxysporum*, *F. solani*, *M. phaseolina* and *R. solani*.

Fungi	Rating scale	Reference
F. oxysporum	0=healthy plant; 1=plant with brown vessels in the first internode region, without other visible symptoms; 2=plant with brown vessels up to the height of the first leaf, with yellowing of at least one leaflet; 3=plant showing vessel browning up to half of the stem length, with yellowing of two or more leaves; 4=plant showing vessel browning nearly to the leader shoot, with most leaves wilted, except the leader shoot; 5=dead plant or plant showing vessel browning and wilted leaves up to the leader shoot.	Tokeshi and Galli (1966), modified by Silva & Bettiol (2005).
F. solani	0=no infection; 1=slight infection; 2=moderate infection; 3=slightly severe infection; 4=severe infection; 5=dead leaves / shisham plants.	Rajput et al., (2008).
M. phaseolina	0=symptomless; 1=1 to 3% of shoot tissues infected; 2=10% of shoot tissues infected; 3=25% of shoot tissues infected; 4=50% of shoot tissues infected; 5=more than 75% of shoot tissues infected.	Ravf & Ahmad, (1998).
R. solani	0=healthy roots; 1=secondary roots are rotten; 2=secondary root and part of taproot is rotten; 3=taproot is rotten; 4=taproot and crown are rotten; 5=death of plant.	Ahed et al., (2013).

Results

Effect of crop and organic amendment on community of mesophilic and thermophilic soilborne fungi: Soil-borne fungi were recovered from all experimental fields.. Statistical analysis indicated that the interaction between doses of organic amendments and crops was significant for thermophilic fungi (P<0.05) and not for mesophilic fungi (P= 0.761). In the case of mesophilic fungi a significant effects (P= 0.018) of crops and organic amendments doses were detected (P<0.05). The data of colony-forming units (CFU) per gram of soil for thermophilic fungi showed that crops amended with different organic doses differed significantly (P<0.05) (Table 2). The highest number of CFUs was recorded in the soil samples amended with 60 tons per hectare of organic manure. The CFUs values varied from 23.554 $x10^5$ (melon seedlings) to 18.598x 10^5 (grafted watermelon seedlings) colony-forming units (CFUs) per gram of soil for mesophilic fungi, and from 23.697 x 10^3 (melon seedlings) to 9.993 $x10^3$ (grafted watermelon seedlings) CFU per gram of soil for thermophilic fungi (Table 2). For example for squash seedlings, the total population fungal recovered from soils ranged from 5.834x 10^5 to 21.061 x 10^5 CFU per gram of soil for mesophilic fungi, and from 5.044 x 10^3 to 14.665 x 10^3 for thermophilic fungi (Table 2).

Mycological analysis: A total of 223.68 $x10^5$ CFU/g fungal communities of soil representing 17 genera and 36 species were identified at the two temperatures (30 and 45°C). Total number of fungi was ranged from 34.55 x 10^5 CFU/g soil (*Aspergillus fumigatus*) to 0.0009x 10^5 CFU/g of soil (*Humicola lanuginosa*). Different thermophilic and mesophilic

fungi at the different experimental fields are presented in Table 3. The mesophilic fungi were more frequent (221.96 x10^5 CFU/g of soil) compared to thermophilic fungi (172.67x 10^3 CFU/g of soil). *Aspergillus glaucus*, *H. lanuginosa*, *Gliocladium virens*, *G. virides*, *G. catenulatum*, *Paecilomyces victoriae*, *Scytalidium thermophilum*, *Arthrinium Kunze* and *Endosporestible* spp. were obtained only at 45°C, but *A. fumigatus*, *A. nidulans*, *A. flavus* and *A. brevipes* was recovered for the two incubation temperatures (30 and 45°C). The genera with the highest species number were *Aspergillus* (54.8%) and *Penicillium* (17.24%). The total population number of *Aspergillus* spp. was 121.566x 10^5 CFU/g soil, followed by *Penicillium* spp. (38.58x 10^5 CFU/g of soil). While, the genus with the lowest number of species were *Paecilomyces* spp. with 0.03x 10^5 CFU / g of soil (Table 3). The percentage of fungal isolated from the different experimental fields indicate that *A. fumigatus* (15.45%) and *A. flavus* (11.47%) presented the highest percentage, followed by *A. terreus* (9.73%), *Chaetomium globosum* (8%) (Table 3).

Table 2: Concentration of fungal community in response to crops and organic amendments.

Crops	Treatment (tons per hectare)	Fungal concentration[a]	
		Mesophilic fungi (10^5 CFU / g soil)	Thermophilic fungi (10^3 CFU / g soil)
Watermelon	0	5.072 *b*[b]	4.354 *d*
	20	6.039 *b*	5.606 *c*
	40	7.128 *b*	7.143 *b*
	60	19.434 *a*	10.564 *a*
P-value[c]	-	0.001	0.000
Muskmelon	0	5.718 *c*	5.452 *b*
	20	7.748 *c*	5.923 *b*
	40	11.746 *b*	7.393 *b*
	60	23.554 *a*	23.694 *a*
P-value	-	0.000	0.000
Grafted watermelon	0	5.196 *c*	6.061 *c*
	20	6.394 *c*	6.364 *c*
	40	10.372 *b*	7.2844 *b*
	60	18.598 *a*	9.9934 *a*
P-value	-	0.000	0.000
Grafted muskmelon	0	4.827 *c*	5.295 *c*
	20	7.788 *c*	6.566 *c*
	40	12.508 *b*	10.246 *b*
	60	22.184 *a*	18.584 *a*
P-value	-	0.000	0.000
Squash	0	5.834 *d*	5.044 *c*
	20	9.028 *c*	6.366 *c*
	40	13.274 *b*	9.34 *b*
	60	21.061 *a*	14.665 *a*
P-value	-	0.000	0.000

[a] The number of colony forming units (CFU) was calculated as followed: CFU / g of soil = ((Total No. of colonies)/(0.1 x (Number of Petri dishes considered for the first dilution retained + 0.1 x Number of Petri dishes considered for the second dilution retained) x dilution factor) (Mouria et al., 2012) *(means of nine values per fungal species).* [b]Duncan's Multiple Range Test, values followed by different superscripts are significantly different at $p \leq 0.05$. [c] Probabilities associated with individual F tests.

Table 3: Mean numbers of mesophilic (35°C), thermotolerant and thermophilic (45°C) fungus.

Fungus	Frequency of fungi and incubation temperature					
	30°C		45°C		Total number[a]	
	10^5 CFU / g soil	%*	10^3 CFU / g soil	%	10^5 CFU / g soil	%
Aspergillus terreus	21.76	9.8	-	-	21.76	9.73
A. fumigatus	34.39	15.49	16.15	9.35	34.55	15.45
A. nidulans	7.014	3.16	5.22	3.02	7.07	3.16
A. pseudoelegans	16.46	7.42	-	-	16.46	7.36
A. flavus	25.15	11.33	51.36	29.75	25.66	11.47
A. niger	15.98	7.2	-	-	15.98	7.14
A. brevipes	0.65	0.29	0.345	0.2	0.65	0.29
A. glaucus	-	-	3.4	1.97	0.034	0.02
A. parasiticus	0.327	0.15	7.5	4.34	0.402	0.18
Penicillium italicum	17.33	7.81	-	-	17.33	7.75
P. digitatum	12.6	5.68	-	-	12.6	5.63
P. janthinellum	4.64	2.09	-	-	4.64	2.07
P. purpurascens	4.01	1.81	-	-	4.01	1.79
Chaetomium globosum	17.89	8.06	-	-	17.89	8
Trichoderma harzianum	4.23	1.91	-	-	4.23	1.89
T. viride	1.28	0.58	-	-	1.28	0.57
Humicola grisea	5.62	2.53	-	-	5.62	2.51
H. insolens	0.17	0.08	-	-	0.17	0.08
H. lanuginosa	-	-	0.09	0.05	0.0009	0.0004
Gliocladium penicillioides	2.41	1.09	-	-	2.41	1.08
G. virens	-	-	2.6	1.51	0.026	0.01
G. virides	-	-	12.6	7.3	0.126	0.06
G. catenulatum	-	-	7.9	4.58	0.079	0.04
Paecilomyces victoriae	-	-	3	1.74	0.03	0.01
Colletotrichum gleosporioides	0.75	0.34	-	-	0.75	0.34
Cladosporium cladosporioides	16.67	7.51	-	-	16.67	7.45
C. herbarum	0.86	0.39	-	-	0.86	0.38
Scytalidium thermophilum	-	-	25.2	14.59	0.252	0.11
Arthrinium Kunze	-	-	30.3	17.55	0.303	0.14
Endosporestible spp.	-	-	7	4.05	0.07	0.03
Fusarium oxysporum	3.69	1.66	-	-	3.69	1.65
F. solani	0.95	0.43	-	-	0.95	0.43
Rhizoctonia solani	1.5	0.68	-	-	1.5	0.67
Macrophomina phaseolina	1.953	0.88	-	-	1.953	0.87
Alternaria alternata	3.47	1.56	-	-	3.47	1.55
Sclerotinia sclerotiorum	0.206	0.09	-	-	0.206	0.09
Total	221.96	100	172.67	100	223.68	100

[a] Total number =30 and 45°C, *The relative frequency (Rf) of the fungal specie was evaluated using the formula: Rf = (n/N) × 100, which n = the number of colonies presented by each species; N = the total number of colonies of all species. *(Means of nine values per fungal species).*

Population diversity of mesophilic fungi in the soil: The total of mesophilic fungi recovered from the 180 soil samples at 30ºC on PDA ranged from 0.17 x10^5 (for *H. insolens*) to 34.39 x10^5 CFU per gram of soil (for *A. fumigatus*). The percentage of mesophilic fungi were 0.08% and 15.49%, respectively and 27 fungal species belonging to 13 genera were recovered. From these fungi, *Aspergillus* with 8 species was the most frequent especially *A. fumigatus* (34.39

x10^5 CFU/g soil) and *A. flavus* (25.15 x10^5 CFU/g of soil); *F. oxysporum* and *A. alternata* had the highest values with 1.66% and 1.55%, respectively. Moreover, *P. italicum* and *P. digitatum* were found more frequent with values of 17.33 10^5 and 12.6 10^5 CFU/g of soil, respectively. The percentage of pathogenic fungi isolated from the soil samples exhibited that *M. phaseolina* and *S. sclerotiorum* revealed to be moderately virulent (0.88 and 0.09%, respectively) (Table 3). The pathogenic fungi were *Rhizoctonia solani*, *Macrophomina phaseolina*, *Fusarium oxysporum* and *F. solani*, which are the most recurrent in the untreated soils (78.2%) and those amended by 20 tons of organic amendments per hectare (19.3%) (Table 4).

Table 4: Population diversity of thermophilic and thermotolerant fungi in the soil.

Fungus	Treatment (tons per hectare)[a]				*P-value* [c]
	0	20	40	60	
F. oxysporum	2.47 a[b]	0.95 b	0.20 c	0.04 c	0.000
F. solani	0.72 a	0.22 b	0 c	0 c	0.000
R. solani	0.95 a	0.48 b	0 c	0 c	0.000
M. phaseolina	1.37 a	0.5 b	0.05 c	0 c	0.000

[a] Soil samples were collected 90 days after planting from the experimental field amended with 4 doses of organic manure (0, 20, 40, 60 tons per hectare) and cultivated by 5 cucurbits (watermelon, grafted watermelon, muskmelon, grafted muskmelon and squash) (Means of three soil samples per treatments). [b]Duncan's Multiple Range Test, values followed by different superscripts are significantly different at $p \leq 0.05$. [c]Probabilities associated with individual F tests.

Population diversity of thermophilic and thermotolerant fungi in the soil: Thermophilic and thermotolerant fungi recovered from the soil samples and incubated on PDA at 45ºC ranged from 0.09 x10^3 (*H. lanuginosa*) to 51.36 x10^3 CFUs per gram of soil (*A. flavus*). The percentages were comprised from 0.05 to 29.75%, respectively. The genera with the highest value and number of species were *Aspergillus* (6 species), with *A. flavus* (51.36 x10^3 CFU/g of soil (29.75%)) and *A. fumigatus* (16.15x 10^3 CFU/g of soil (16.15%)) which are the most frequent. *A. Kunze* and *S. thermophilum* emerged with high percentage of 17.55 (30.3 x10^3 CFU/g of soil) and 14.59% (25.2 x10^3 CFU/g of soil), respectively (Table 3).

Pathogenicity test of *Fusarium solani*, *F. oxysporum*, *Macrophomina phaseolina* and *Rhizoctonia solani* and their response to plant growth: The results of pathogenicity test *Fusarium* isolates exhibited high degree of pathogenicity according to the measured disease severity parameter. *F. oxysporum* isolates were virulent with DSI values varying from 3.78 and 4.22 (control=0). Watermelon seedlings inoculated with *Fusarium solani*, presented DSI values ranged from 3.89 and 4.22 (control=0). Thirty days after inoculation, necrotic lesions appeared on leaves and roots. Roots became semitransparent, shrunken,

water-soaked, and easily disintegrated. All isolates of *Fusarium* spp. caused disease symptoms typical of *Fusarium* wilt and collar rot on cucurbit. The same plant mortality and plant wilting percentage were detected in plants inoculated with Fo4 (*F. oxysporum*) and Fs3 (*F. solani*) isolates (100%) (Table 5).

Table 5: Pathogenicity and plant growth on watermelon of various isolates of *F. oxysporum*, *F. solani*, *R. solani* and *M. phaseolina* isolated from soil.

Fungi	Treatments	Disease assessment and plant parameters[a]									
		DSI	PW (%)	PM (%)	FWA (g)	DWA (g)	FWB (g)	DWB (g)	RV (cm^3)	PL (cm)	RL (cm)
R. Solani	Control	0 c[b]	-	-	14.35 a	2.1 a	1.06 a	0.12 a	6.94 a	36.83 a	3.18 a
	Rs1	3.22 b	-	-	11.45 b	0. 85 b	0.41 b	0.06 b	3.97 b	18.44 b	1.74 b
	Rs2	3.44 b	-	-	9.44 c	0.47 c	0.35 b	0.03 c	3.67 b	19.39 b	1.57 b
	Rs3	4.33 a	-	-	8.57 c	0.40 c	0.47 b	0.06 b	4.41 b	15.22 b	1.73 b
P. value[c]		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F. oxysporum	Control	0 b	0 b	0 b	15.27 a	1.83 a	1.23 a	0.17 a	7.19 a	33.17 a	4.4 a
	Fo1	4.22 a	88.89 a	77.78 a	11.45 b	1.15 b	0.54 b	0.105 a	4.48 b	20 b	2.16 b
	Fo2	3.78 a	100 a	66.67 a	10.22 c	0.99 b	0.52 b	0.07 a	4.06 bc	21.33 b	2.02 b
	Fo3	4.00 a	88.89 a	66.67 a	9.10 d	1.05 b	0.49 b	0.22 a	4.05 bc	18.83 bc	2.1 b
	Fo4	4.22 a	100 a	100 a	8.59 d	1.01 b	0.48 b	0.14 a	3.56 b	15.78 c	2.26 b
P. value		0.000	0.000	0.000	0.000	0.000	0.000	0.242	0.000	0.000	0.000
F. solani	Control	0 b	0 b	0 b	14.22 a	2.32 a	1.6 a	0.15 a	6.78 a	34.18 a	4.47 a
	Fs1	4.00 a	88.89 a	77.78 a	12.7 b	1.99 ab	0.85 b	0.11 b	4.38 b	24.24 b	2.42 b
	Fs2	3.89 a	100 a	88.89 a	13.22 ab	1.87 ab	0.58 b	0.11 b	3.25 c	18.64 c	2.64 b
	Fs3	4.22 a	100 a	100 a	12.29 b	1.65 b	0.81 b	0.11 b	4.47 b	18.31 c	2.68 b
P. value		0.000	0.000	0.000	0.022	0.28	0.000	0.004	0.000	0.000	0.000
M. phaseolina	Control	0 b	-	-	15.01 a	2.28 a	0.90 a	0.13 a	6.75 a	38.56 a	4.06 a
	Mp1	4.00 a	-	-	12.19 b	1.48 b	0.65 ab	0.08 b	5.01 b	19.98 b	1.86 b
	Mp2	3.78 a	-	-	12.55 b	1.4 b	0.34 b	0.07 b	4.43 b	21.33 b	1.76 b
	Mp3	4.11 a	-	-	10.65 c	1.49 b	0.75 b	0.05 b	4.28 b	19.27 b	2.21 b
P. value		0.000	0.000	0.002	0.000	0.000	0.035	0.016	0.000	0.000	0.000

[a] Disease severity index (DSI) was evaluated using the disease rating scale: isolates causing no symptoms (0-0.3 DSI) were considered avirulent; isolates causing mild symptoms (0.4-1.9 DSI) were considered less virulent; isolates causing moderate symptoms (2-2.9 DSI) were classified as moderately virulent; isolates with severe symptoms (3-3.9 DSI) were considered virulent and isolates responsible of decline (4-5 DSI) were highly virulent (Sneh et al., 2004). (*Means of five plants per replicate (three replicates)*). [b]Duncan's Multiple Range Test, values followed by different superscripts are significantly different at $p \leq 0.05$. [c]Probabilities associated with individual F tests.

The results revealed a different level of aggressively between pathogens; values for all horticultural parameters (fresh weight of above-ground (stem + leaf), dry weight of above-ground (stem + leaf), fresh weight of below ground (root), dry weight of below ground (root), root volume, plant length and main roots length) were recorded for the control were important compared to the plants inoculated. Significant differences were noted for fresh and dry weight of above-ground (FWA, DWA), fresh weight of below ground (FWB), main roots length (RL), plant length (PL) and root volume (RV) in the case of *F. oxysporum* isolates ($P<0.05$), FO4 was the virulent isolate. Similarly, for *F. solani* isolates, and FS3 was aggressive. *R. solani* exhibited different level of pathogenicity. The highest disease severity index (4.33) was induced by Rs3. The isolates of *R. solani* were able to cause characteristic symptoms of *Rhizoctonia* root rot on watermelon plants. One month after inoculation of *R. solani* isolates, fresh

and dry weight of above-ground (stem + leaf), fresh and dry weight of below ground (root), root volume, plant length and main roots length were highly significantly reduced (P<0.05) (Table 5). The pathogenicity test demonstrated that *M. phaseolina* revealed being pathogenic on watermelon plants. Variations in pathogenicity level were indicated among the three isolates. Mp1 (4) and Mp3 (4.11) were highly virulent according to thedisease severity index, while, Mp2 was tolerant (3.78). These isolates (Mp1, Mp2 and Mp3) were responsible of characteristic charcoal rot symptoms on plants. Premature yellowing of the top leaves followed by premature leaf drop occurred. Numerous small, black sclerotia were easily visible on the roots were observed. Data of plant growth response to *M. phaseolina* indicated that all plant growth indicators differed significantly (P<0.05) on plants development (Table 5). After symptom development, the inoculated pathogens were re-isolated from randomly selected plants. The fragment infection tissue was incubated in Petri dishes containing PDA. Further Koch's postulates are confirmed for *F. solani*, *F. oxysporum*, *M. phaseolina* and *R. solani* isolates.

Discussion

Isolation of mesophilic and thermophilic fungi from different experimental field treated with 4 organic amendments doses and cultivated by 5 cucurbits was investigated in the present paper. The dilution plate technique was found to be suitable in this study. The main finding is organic amendment proved to be an important factor affecting the mycofloral population (60 tons per hectare of organic amendments). Cwalina-Ambroziak and Wierzbowska (2011) reported that the fertilizers could affect the populations of soil fungi. The highest fungal community was obtained from the soil amended with dried and pelleted sewage sludge or municipal green waste compost. The pathogen-antagonistic fungi were more often isolated from the soil fertilized with farm yard manure or organic fertilizers than the unfertilized or NPK-nourished soil. Other researchers reported that the fungi population was influenced by the compound of the organic amendment (Luna Ramos et al., 2015; Swer et al., 2011; Bonanomi et al., 2007). Furthermore, Pandey et al., (2006) revealed that for the phytosanitary safety of crops, it is necessary to maintain dynamic changes of fungal populations in the soil. Organic fertilizers added to soil induce favorable conditions for the development of antagonistic fungi. The use of organic amendments such as animal manure has been proposed, both for conventional and biological systems of agriculture, to improve soil structure and fertility (Lonacrir et al., 2008; Cavigelli & Thien, 2003; Conklin et al., 2002; Magid et al., 2001) and decrease the incidence of disease caused by soil borne pathogens (Noble & Coventry, 2005; Litterick et al., 2004). Different complementary mechanisms have been proposed to explain the suppressive capacity of organic amendments and enhance activities of antagonistic microbes (Hoitink & Boehm, 1999). The data of colony-forming units (CFU) per g soil showed a significant thermophilic (P<0.05) and mesophilic fungi (P= 0.018). Hackl et al., (2000) indicated that the plant species could have an effect on

the population and species composition of the soilborne fungi. In relation to genus detected, the prevalence of *Aspergillus* and *Penicillium* in the soil samples is consistent with the reports of Ghatora et al., (2006), Martin et al., (2004), Gaddeyya et al., (2012) and Onyimba et al., (2014). Based on their experiments, Wittling et al. (1996) reported that natural fertilizers and composts inhibited the development of *Fusarium* spp., *Pythium* and *Phytophthora*. Tsror Lakhim et al., (2001) and Lazarovitz et al., (2007) demonstrated that the infection of potato by *R. solani* and *Streptomyces scabies* is less severe in soil fertilized with cattle manure. Mills et al., (2002) mentioned that less severe infection of *solanaceous* plants by *P. capsici*, *Alternaria solani* and *Septoria lycopersici* were obtained in soil amended with composted plant waste. Soil-borne plant pathogens such as *Fusarium* spp., *R. solani* and *M. phaseolina* could be responsible of yield and quality reduction in cucurbit crops. Most of the pathogens identified in this study have the ability to grow on a wide range of substrates and have efficient mechanisms for dispersal. The result of pathogenicity test demonstrated that all of the selected isolates were pathogenic on watermelon plants. The isolates exhibited different degrees of pathogenic levels. Typical symptoms of each pathogen to those produced in natural field conditions were observed on inoculated watermelon plants. Femandez et al., (2006) reported that great variability in pathogenicity was recognized among isolates from different host species. Su et al., (2001) found high pathogenicity levels variation of *M. phaseolina*. Manici et al., (1995) demonstrated the pathogenicity of *M. phaseolina* on eight plant species including melon. Meinhardt et al., (2001) reported that pathogenicity studies clearly showed that *R. solani* isolates bean could cause disease in a number of different plants and that the isolates virulence varied depending on the host. In the same sense Bolkan and Ribeiro (1985), mentioned that *R. solani* isolates collected from different hosts from various regions of Brazil varied in their virulence on six different indicator plants. The pathogenicity of *Rhizoctonia* was tested in growth chamber on watermelon (cv. Crimson Sweet). All isolates were pathogenic and showed significant differences on the disease incidence and severity (Aiello et al., 2012). For *Fusarium* species the present study revealed their pathogenicity on watermelon plant. These results are in agreement with previous investigation (Zhao et al., 2014; Chehri et al., 2011; Mehl & Epstein, 2007; Boughalleb et al., 2005). Tunisia being agricultural state, proper steps must be taken to avoid diseases and damage to the crop due to fungal mycoflora, for saving economy of the country.

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