Isolation of Pathogenic and Non-Pathogenic Fungi from Cucurbits Roots Grown in Soil Amended with Organic Manure in Tunisia

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Abstract

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This investigation examined the effect of organic manure application (20, 40 and 60 tons/ha) on fungal communities in the rhizosphere of cucurbit plants (watermelon, grafted watermelon, melon, grafted melon, and squash) grown under field conditions. Prevalent fungal species were identified as *Cladosporium cladosporioides* (0.83×10^5 CFU/g soil) and *Alternaria alternata* (0.22×10^5 CFU/g soil). *Sclerotinia sclerotiorum*, *Cladosporium herbarum*, *Scytalidium thermophilum*, and *Arthrinium* sp. were also consistently detected. Pathogenicity test via artificial inoculation on watermelon leaves in greenhouse revealed that the most virulent pathogens were *S. sclerotiorum* (SC2) with level of lesion severity reached 80.72%, and *A. alternata* (Aa2) 73.76%. To assess potential biocontrol agents, 24 soilborne-fungal antagonists were evaluated for their inhibitory activity against six pathogens using direct contact method. *Trichoderma harzianum* displayed the strongest mycelial growth inhibition rate (48.99-85.42% against various pathogens), followed by *Humicola grisea* (32.21-80.68%) and *Gliocladium catenulatum* (26.64-83.66%). Finally, the *in vivo* efficacy of four fungal antagonists against six pathogens was tested in a randomized complete block design following preventive and curative treatments on watermelon leaves. Both *T. harzianum* and *T. viride* significantly reduced disease incidence caused by the six phytopathogens.

Keywords: Antagonistic fungi, Cucurbitaceae, fungal communities, pathogenic fungi.

Introduction

Plant-microbe-soil interactions play a vital role in maintaining plant health and productivity of field and horticultural crops (Boughalleb-M'Hamdi *et al.,* 2018). Due to the limitation in the effectiveness of fungicides and lack of successful plant-based resistance, enhancement of soil-based natural disease suppression could be an effective option to control diseases, especially when achieved in the field through crop and/or soil management practices (Rhouma *et al.,* 2016; 2019).

Both beneficial and pathogenic fungi interact with plants during all phases of their growth. Plants actively interact with fungi (Sarkar *et al.,* 2009) and reported to have developed sophisticated tactics to control the composition and behavior of their fungal environment (Berg & Smalla, 2009). The influence of fungi on plants can be affected by soil characteristics, and management techniques that change soil characteristics may be utilized to maximize the advantage of adding inoculants. The management of soil organic matter (SOM) appears to be essential for managing soil microorganisms, since SOM regulates a variety of soil parameters (Manlay *et al.,* 2007). The methods that most significantly affect SOM include conservation tillage practices and adding organic materials to the soil (Ellouze *et al.,* 2014).

Organic amendments, such as animal manures, were commonly used in the past for agricultural production due to their value as fertilizers and their ability to improve plant health (Boughalleb-M'hamdi *et al.,* 2017; De Corato, 2020; Okon *et al.,* 2022). Organic matter has a longer-lasting effect, especially if large volumes are used, although they have a higher impact on microbially mediated soil structuring (Bamdad *et al.,* 2021; Ellouze *et al.,* 2014; Hoffland *et al.,* 2020; Urra *et al.,* 2019).The rhizosphere is home to the soil fungi that have the biggest impact on plants, and it appears that plants can control these fungi to enhance soil health and the effectiveness of annual cropping systems. In this situation, crop rotation can be employed as a fundamental tactic to broaden the rhizosphere's diversity and limit the encroachment of diseases (Ellouz *et al.,* 2014).

Organic soil amendments serve a dual purpose in promoting soil health. Firstly, they directly increase soil organic carbon (SOC) content. Secondly, they can exert both direct and indirect effects on soilborne pathogens (Bamdad *et al.,* 2021). The increased SOC content fosters beneficial soil biota, while potentially suppressing pathogens through competition for resources or production of inhibitory compounds. This combined approach makes organic amendments a valuable tool for sustainable agricultural practices (Jayaraman *et al.,* 2021; Larkin, 2015; Vida *et al.,* 2020; Walters & Bingham, 2007).

By encouraging antagonistic native microorganisms against soil-borne pathogens through direct antagonism, antibiosis, parasitism, or competition for resource acquisition, these suppressive soils function similarly to biocontrol agents. These traits, however, cannot be transferred from one soil to another (Bonanomi *et al.,* 2018; Schlatter *et al.,* 2017; Panth *et al.,* 2020).

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Biological control of soil-borne pathogens has received increasing attention as a promising supplement or alternative to chemical control, using potential microorganisms, which is also ecology-conscious and environment-friendly (Kumar *et al.,* 2015; Richard *et al.,* 2022). An interaction that leads to bio-control includes antibiosis, competition, induction of host resistance, production of growth-stimulating factors, and predation (Bhatt *et al.,* 2015).

Among all the microbial biocontrol agents (BCA), *Trichoderma* spp. are ones of the most commonly used worldwide as a safe BCA. *Trichoderma harzianum*, *T. virens* and *T. viride* are the most common (Boughalleb-M'Hamdi *et al.,* 2018; Rhouma *et al.,* 2018). However, the main biocontrol mechanisms exhibited by *Trichoderma* in confrontation with fungal pathogens are mycoparasitism and antibiosis (Boughalleb-M'Hamdi *et al.,* 2018; Dana *et al.,* 2001; Rhouma *et al.,* 2018).

Alternaria species have a wide host range causing leaf spots and blights on many plant parts (Khan *et al.,* 2014). *Sclerotinia sclerotiorum* (Lib.) De Bary is a necrotrophic fungal pathogen with broad ecological distribution, with long-time survival of the sclerotia in the soil (Bhuiyan *et al.,* 2012). Fungi of the genus *Cladosporium* are common in many areas of the world, and their spores can be found in the air, soil, and water. However, some genera are pathogens of various plants and people, particularly *C. cladosporioides* and *C. herbarum* which are the most common aeroallergens next to *Alternaria* spp. (Khan *et al.,* 2014). The genus *Arthrinium* Kunzehas been most extensively treated by Ellis (1971) but additional species or varieties have been described by many researchers (Larrondo & Calvo, 1990). *Scytalidium thermophilum* is an important thermophilic fungus in the production of mushroom compost. They are believed to contribute significantly to the quality of the compost (Vos *et al.,* 2017).

This investigation focused on the influence of varying organic manure application rates (20, 40, and 60 t/ha) on fungal community composition within the rhizosphere of watermelon (grafted and non-grafted), melon (grafted and non-grafted), and squash plants cultivated under field conditions. The population densities were quantified using the serial dilution plate technique. Thus, this analysis aimed to determine how different doses of organic amendments affect the abundance of some phytopathogens. Moreover, the antifungal activity of antagonist's fungal species was evaluated against *Sclerotinia sclerotiorum*, *Alternaria alternata*, *Cladosporium herbarum*, *C. cladosporioides*, *Scytalidium thermophilum*, and *Arthrinium* spp. under both controlled laboratory conditions (*in vitro*) and within a plant-based system (*in vivo*).

Materials and Methods

Effect of soil manure on fungal community in the cucurbit plants rhizosphere

A field experiment investigating the impact of organic manure application on microbial communities was conducted. The experimental design was a completely randomized design with four organic manure application rates (0, 20, 40, and 60 t/ha) and five cucurbit crops (watermelon, grafted watermelon, melon, grafted melon, and squash). Soil samples (500 g) were collected 90 days after planting, using a steel soil auger at a depth of 10-20 cm. To ensure representativeness, a total of 180 samples were collected with a randomized sampling approach. Each sample consisted of three subsamples pooled from a single replicate, with three replicates per treatment. Sterile polyethylene bags were used to collect and transport the soil samples to the laboratory for further analysis.

Microflora analysis

Soil-dilution plate method was used (Johnson & Curl, 1972). One gram of soil sample was suspended in 10 mL of sterilized-distilled water. Before the soil settles, one mL of the suspension with a sterile pipette was transferred to 9 mL of distilled water. Then, this dilution step continued until the 10^{-7} dilution (Mouria *et al.,* 2012). One mL of each dilution was spread over the surface of a PDA agar plateand incubated at 28°C in the dark for 4-7 days (Boughalleb-M'hamdi *et al.,* 2017). After incubation, all of the plates were carefully examined, and differences in colony size and shape were noted. The number of fungal colonies was counted and recorded. Only plates with 30-300 colonies per plate were counted and retained. Population densities were expressed in terms of Colony Forming Unit (CFU) per gram of soil. Three replicates (three Petri dishes/replicate) were undertaken for each treatment and the experiments were repeated twice.

The total number of CFU was calculated by using the following formula (Boughalleb-M'hamdi *et al.,* 2017; Mouria *et al.,* 2012):

$$\text{CFU/g of soil} = \frac{\text{Total No. of colonies}}{\begin{array}{c}0.1 \times (\text{No. of petri dishes considered for the first retaining dilution}) + 0.1 \times (\text{No. of petri dishes considered for the second retaining dilution})\end{array}} \times \text{dilution factor}$$

The contribution rate of different fungal species were calculated by using the following formula (Boughalleb-M'hamdi *et al.,* 2017; Mouria *et al.,* 2012):

$$\text{Frequency of fungi (\%)} = \frac{\begin{array}{c}\text{Total No. of CFU of an individual species}\end{array}}{\begin{array}{c}\text{Total No. of CFU of all species}\end{array}} \times 100$$

Fungal species were identified based on phenotypic characteristics including colony morphology (growth, color, appearance), microscopic morphology (mycelium, conidiophore, conidia, resistance structures, sexual structures), and comparative analysis with identification keys. Repeated subculturing and using cotton blue as a mounting medium facilitated fungal purification before identification (Nagamani *et al.,* 2006).

Isolated fungi were identified down to the species level using a phenotypic approach on standard media. Identification keys were employed as follows: Wanger *et al.* (2017) for *Penicillium* spp.; Domsch *et al.* (1980); Agarwal & Hasija (1986) and Samson *et al.* (2004) for *Aspergillus* spp.; Krebs (1972) and Dugan (2006) for dematiaceous hyphomycetes; Pikovskaya (1948) for *Fusarium* spp.,

Oladapo *et al.* (2020) for miscellaneous fungi; and Anwar *et al.* (2022) for Ascomycetes. Author abbreviations follow Milagres *et al.* (1999). The classification system used is the latest system appearing in the 10th edition of Ainsworth and Bisby's Dictionary of the Fungi (Abd Alhakim *et al.,* 2022). Fungal names were verified using the Index Fungorum database.

Pathogenicity test on watermelon plants

Eighteen fungal isolates obtained from the soil samples were used for pathogenicity testing: *Sclerotinia sclerotiorum*, *Alternaria alternata*, *Cladosporium herbarum*, *C. cladosporioides*, *Scytalidium thermophilum*, and *Arthrinium* sp. Each fungal species was represented by three isolates (Aa1, Aa2, and Aa3 for *A. alternata*, Ch1, Ch2, and Ch3 for *C. herbarum*, Cc1, Cc2, and Cc3 for *C. cladosporioides*, St1, St2, and St3 for *S. thermophilum*, Ak1, Ak2, and Ak3 for *Arthrinium* sp.and Ss1, Ss2, and Ss3 for *S. sclerotiorum*). Pathogenicity was assessed through artificial inoculation on watermelon (cv. Crimson sweet) seedlings grown in pots containing a pasteurized soil-peat mix (1:2). At 30 days old plants, healthy leaves were washed with sterilized water and inoculated with a 40 µl droplet of spore suspension (10^5 conidiam/L) on the upper surface. A complete randomized design with three replicates (five leaves per replicate) per treatment was performed. Negative controls received only sterilized water. Following inoculation, plants were maintained in a controlled greenhouse environment (28±2°C). Disease severity (lesion size) was visually assessed ten days post-inoculation and calculated as a percentage using the formula:

$$N = (T/C) \times 100$$

where N represents lesion severity, T is the infected leaf area, and C is the total leaf area (Boughalleb-M'hamdi *et al.,* 2017, 2018; Rhouma *et al.,* 2018).

***In vitro* interactions of phytopathogens with antagonistic fungi**

Antifungal activity of twenty-four antagonist species isolated from soil (Table 1) on radial mycelia growth of six fungal pathogens was determined by confrontation technique by direct contact on culture medium. This method consisted of placing in the same Petri dish containing PDA, five agar pellets (6 mm in diameter), four of them with antagonist plugs equidistants (3 cm) and one with the pathogen (in the middle of the Petri dish). Plates were incubated at 25±2°C for six days (Benhamou & Chet, 1996). The evaluation of the mycelial growth inhibition (%) was determined following the formula:

$$I\ (\%) = (1- C_n/C_o) \times 100$$

where C_n is the mean diameter values of the pathogen colonies in the presence of the antagonist and C_o the mean diameter values of the control (Benhamou & Chet, 1996).

Table 1. Collection and origin of antagonist's fungal species used in direct contact method.

Isolate code	Antagonistic fungi*	Mesophilic/ thermophilic fungi	Temperature (°C)	Host rhizosphere soil
AT1	*Trichoderma viride*	Mesophilic	30	Watermelon
AT2	*Humicolagrisea*	Mesophilic	30	Grafted watermelon
AT3	*Trichoderma harzianum*	Mesophilic	30	Watermelon
AT4	*Humicolainsolans*	Mesophilic	30	Melon
AT5	*Penicillium purpurascens*	Mesophilic	30	Melon
AT6	*Penicillium italicum*	Mesophilic	30	Grafted melon
AT7	*Penicillium janthinnelum*	Mesophilic	30	Grafted watermelon
AT8	*Penicillium digitatum*	Mesophilic	30	Grafted watermelon
AT9	*Aspergillus terreus*	Mesophilic	30	Watermelon
AT10	*Aspergillus fumigatus*	Mesophilic	30	Watermelon
AT11	*Aspergillus nidulans*	Mesophilic	30	Watermelon
AT12	*Chaetomiumglobosum*	Mesophilic	30	Watermelon
AT13	*Aspergillus pseudoelegans*	Mesophilic	30	Squash
AT14	*Aspergillus flavus*	Mesophilic	30	Grafted melon
AT15	*Aspergillus niger*	Mesophilic	30	Grafted melon
AT16	*Aspergillus brevipes*	Mesophilic	30	Squash
AT17	*Aspergillus glaucus*	Thermophilic	45	Watermelon
AT18	*Gliocladiumvirides*	Thermophilic	45	Squash
AT19	*Gliocladiumcatenulatum*	Thermophilic	45	Watermelon
AT20	*Aspergillus parasiticus*	Mesophilic	30	Grafted watermelon
AT21	*Gliocladiumpenicillioides*	Mesophilic	30	Watermelon
AT22	*Paecilomycesvictoriae*	Thermophilic	45	Watermelon
AT23	*Humicolalanuginosa*	Thermophilic	45	Melon
AT24	*Gliocladium virens*	Thermophilic	45	Melon

*These antagonists were collected and identified previously (Boughalleb-M'hamdi *et al.,* 2017).

***In vivo* efficacy of four antagonists against six pathogens**

A randomized complete block design with three replicates (three plants per replicate) was used to evaluate the *in vivo* efficacy of four antagonist isolates (*Trichoderma viride*, *T. harzianum*, *Penicillium italicum* and *Aspergillus niger*) against six fungal pathogens (*S. sclerotiorum*, *A. alternata*, *C. herbarum*, *C. cladosporioides*, *S. thermophilum*, and *Arthrinium* sp.) on watermelon (cv. Crimson sweet) leaves. The experiment consisted of preventive and curative treatments. In the preventive treatment, antagonist conidial suspensions were applied as droplets onto leaves (six leaves per plant) before pathogen inoculation. For the curative treatment, antagonist application occurred 24 hours after pathogen inoculation (Matrood *et al.,* 2022). Spore suspensions of both pathogens and antagonists were adjusted to a concentration of 10^6 spores/mL using a hemocytometer and applied as 40 µl droplets to the leaves. Controls were performed by inoculating the plant with the pathogen only in case of positive control and with distilled water only as negative control. Treated plants were maintained in a greenhouse for 30 days. Disease assessment relied solely on disease incidence (DI), a visually determined percentage calculated using the formula (Ahmed, 2017):

$$DI\ (\%) = \frac{\text{Total No. of symptomatic leaves}}{\text{Total No. of leaves}} \times 100$$

Statistical analysis

The population assessment of phytopathogenic fungi (*S. sclerotonium*, *A. alternata*, *C. herbarum*, *C. cladosporioides*, *S. thermophilum* and *Arthrinium* sp.), the level of lesion severity, the disease incidence and the mycelial growth inhibition were analyzed using the multinomial distribution and the cumulative logit as link function. These variables were compared by analysis of variance (ANOVA) and means of the values were separated with Duncan multiple range test at $P < 0.05$. Statistical analyses were conducted using SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Effect of soil manure on fungal community in the cucurbit plants rhizosphere

Mycoflora population data revealed significant diversity between crops, fungal population and the interaction between them ($P<0.01$). Fungal community distribution in analyzed soils determined the prevalence of the two mesophilic fungal species *Cladosporium cladosporioides* (0.83×10^5 CFU/g soil) and *Alternaria alternata* (0.22×10^5 CFU/g soil) showing highest frequency rate of 70.34 and 18.64%, respectively (Tables 2 and 3).

Six different fungal species were isolated from all soil samples and the fungal concentration differed significantly ($P<0.05$). The highest isolation rate was recorded for *C. cladosporioides* with values of 6.28 and 1.47% in watermelon rhizosphere untreated and amended with 40t/ha, respectively. In melon rhizosphere with no organic manure, the value was 3.69%. *Alternaria alternata* isolation rate was 1.35% detected in watermelon without organic manure and 0.52% when soil was amended with 20t/ha, and 0.35% when soil was amended with 40 t/ha. *Scytalidium sclerotiorum* was isolated only from watermelon with soil amended with 20t/ha (0.21%). Both *C. cladosporioides* and *S. thermophilum* were obtained from grafted watermelon and grafted melon rhizosphere with values of 1.21 and 0.1%, respectively (Tables 2 and 3).

Arthrinium sp. was mostly detected from squash rhizosphere with isolation rate of 0.09% (60 t/ha) and 0.3% (20 and 40 t/ha) in grafted watermelon rhizosphere. However, results revealed that both *Cladosporium herbarum* and *S. sclerotiorum* werenot detected in melon, grafted melon and grafted watermelon rhizospheres. The data of colony-forming units (CFU) per g of soil showed that the lowest population of pathogenic fungi was recorded in experimental units which were treated with organic manure at doses of 40 and 60t/ ha (Tables 2 and 3). However, mycoflora population was most prevalent in untreated and amended soils with the lowest dose of organic manure (Tables 2 and 3).

Table 2. Comparison of the frequency of different mesophilic and thermophilic fungi.

	Fungi occurrence	
Fungi	**Per 10^5 CFU/ g soil**	**Frequency %**
A. alternata (mesophilic fungus)	0.22 b	18.64 b
C. cladosporioides (mesophilic fungus)	0.83 a	70.34 a
C. herbarum (mesophilic fungi)	0.05 c	4.24 c
S. sclerotiorum (mesophilic fungi)	0.01 c	0.85 c
Arthrinium sp. (thermophilic fungi)	0.06 c	5.08 c
S. thermophilum (thermophilic fungi)	0.01 c	0.85 c
Total	1.18	100

Values followed by the same letters in the same column are not significantly different based on Duncan's multiple range test at P=0.05.

Pathogenicity test on watermelon plants

There was a significant difference between the pathogenicity of the six pathogens tested in comparison with the control ($P<0.05$) (Table 4). The high level of lesion severity (%) was recorded by *S. sclerotiorum* isolate (SC2) with 80.72%, *A. alternata* Aa2 (73.76%), *C. cladosporioides* Ccl1 (68.16%) and *C. herbarum* isolates (Ch1) with the value of 67.3%. The lowest level of leaf infection was generated by the two *S. thermophilum* isolates (Sth3 and Sth2) with values of 3.97 and 5.23%, respectively (Table 4).

Table 3. Concentration of pathogenic fungi *A. alternata*, *Arthrinium* sp., *C. cladosporioides*, *C. herbarum*, *S. sclerotiorum* and *S. thermophilum* in relation to crops infected and doses of organic amendments added to the soil.

		Organic manure doses (tons per hectare)			
Fungus	**Crops**	**0**	**20**	**40**	**60**
C. cladosporioides	Watermelon	6.28±0.97 aaAb	1.17±0.37 bAB	1.47±0.40 bA	0.00±0.00 c
	Grafted watermelon	0.00±0.00 bD	1.21±0.20 aA	0.00±0.00 bB	0.00±0.00 b
	Muskmelon	3.69±0.35 aB	0.94±0.33 bBC	0.00±0.00 cB	0.00±0.00 c
	Grafted muskmelon	0.00±0.00 bD	0.82±0.25 aC	0.00±0.00 bB	0.00±0.00 b
	Squash	1.09±0.38 aC	0.00±0.00 bD	0.00±0.00 bB	0.00±0.00 b
C. herbarum	Watermelon	0.31±0.34 aA	0.10±0.22 bAB	0.04±0.02bA	0.06±0.03 bA
	Grafted watermelon	0.00±0.00 B	0.00±0.00 B	0.00±0.00 B	0.00±0.00 B
	Muskmelon	0.00±0.00 bB	0.16±0.21 aAB	0.00±0.00 bB	0.00±0.00 bB
	Grafted muskmelon	0.00±0.00 B	0.00±0.00 B	0.00±0.00 B	0.00±0.00 B
	Squash	0.09±0.10 abB	0.20±0.25 aA	0.00±0.00 bB	0.01±0.02 bB
A. alternata	Watermelon	1.35±0.19 aA	0.52±0.36 bA	0.14±0.11 cBC	0.17±0.08 cA
	Grafted watermelon	0.00±0.00 bC	0.15±0.13 aBC	0.05±0.05 bC	0.00±0.00 bC
	Muskmelon	0.41±0.37 aB	0.00±0.00 bC	0.35±0.30 aA	0.05±0.02 bB
	Grafted muskmelon	0.30±0.35 aB	0.00±0.00 bC	0.00±0.00 bC	0.00±0.00 bC
	Squash	0.35±0.27 aB	0.30±0.11 aB	0.28±0.22 aAB	0.09±0.04 bB
S. sclerotiorum	Watermelon	0.00±0.00 b	0.21±0.13 aA	0.00±0.00 b	0.00±0.00 b
	Grafted watermelon	-	-	-	-
	Muskmelon	-	-	-	-
	Grafted muskmelon	-	-	-	-
	Squash	-	-	-	-
S. thermophilum	Watermelon	0.03±0.02 abB	0.02±0.02 bcB	0.05±0.03 aA	0.00±0.00 c
	Grafted watermelon	-	-	-	-
	Muskmelon	0.00±0.00 bB	0.05±0.01 aA	0.00±0.00 bB	0.00±0.00 b
	Grafted muskmelon	0.10±0.08 aA	0.00±0.00 bC	0.00±0.00 bB	0.00±0.00 b
	Squash	-	-	-	-
Arthrinium sp.	Watermelon	0.00±0.00 bB	0.05±0.02 aB	0.00±0.00 bB	0.00±0.00 bB
	Grafted watermelon	0.03±0.01 bB	0.06±0.02 aB	0.00±0.00 cB	0.01±0.00 8cB
	Muskmelon	-	-	-	-
	Grafted muskmelon	0.01±0.02 bB	0.00±0.00 bB	0.03±0.01 aB	0.00±0.00 bB
	Squash	0.29±0.25 aA	0.30±0.21 aA	0.30±0.15 aA	0.09±0.04 bA

Mean values of fungal concentrations followed by the same small letters in the same column (same manure dose and different crops) are not significantly different according to Duncan's multiple range test at P=0.01.
Mean values of fungal concentrations followed by the same capital letters in the same row (same crop with different manure doses) are not significantly different according to Duncan's multiple range test at P=0.01. - = Not tested.

In vitro interaction of phytopathogens with antagonistic fungi

Results obtained showed that all biological fungi possess antifungal activity against tested pathogenic fungi. The highest antifungal activity was obtained in *Trichoderma harzianum* treatment as the inhibition rate for the pathogenic fungi ranged between48.99% (*Cladosporium herbarum*) to 85.42% (*S. sclerotiorum*), and for *Humicola grisea* (A2); values recorded between 32.29% (*C. herbarum*) and 80.68 % (*Arthrinium* sp.). For *Gliocladium catenulatum* (A19), results showed also a mycelial growth reduction between 26.64% (*Sclerotinia sclerotiorum*) and 83.66% (*Arthrinium* sp.). Antifungal activity for *Aspergillus glaucus* (A17) ranged from 25.16% (*C. herbarum*) to 82.2% (*Arthrinium* sp.), and for *Aspergillus parasiticus* (A20) values ranged from 27.81% (*C. herbarum*) to 79.58% (*Arthrinium* sp.);for *Penicillium italicum* (A6) from 50.03% (*A. alternata*) to 78.25% (*Arthrinium* sp.).The lowest biocontrol activity was observed after a confrontation with *Aspergillus fumigatus* and *Chaetomium globosum*, and the mycelial growth inhibition rate ranged from 36.21% (*C. herbarum*) to 65.03% (*S. thermophilum*), and from 41.03% (*C. herbarum*) to 69.7% (*Arthrinium* sp.). In this study, the tested pathogens were affected by the antagonists and revealed that the maximum mycelial growth inhibition was recorded by *Arthrinium* sp. with the highest values between 80.68 and 83.66%, followed by *S. sclerotiorum* (83.55-85.42%), and *S. thermophilum* (69.6-71.48%). Whereas, *C. herbarium* was the least mycelial growth inhibition 0f 18.17% (*Gliocladium viride*) and 60.15% (*Aspergillus brevipes*) (Table 5).

In vivo efficacy of four antagonists against six pathogens

The preventive and curative application of antagonist showed same results when disease incidence reduction was assessed (Table 6). *T. harzianum* and *T. viride* significantly decreased the DI of watermelon plants inoculated with *Arthrinium* sp. (75-66% reduction) and *S. thermophilum* (70-64% reduction). *T. harzianum* produced the best results, with reduction in disease incidence between 73-69% (*S. sclerotiorum*) and 80-76% (*C. cladosporioides*) (Table 6).

Table 4. Pathogenicity test on watermelon plants.

Fungi	Treatments	Lesion severity (%)
Sclerotinia sclerotiorum	Control	0.00±0.00 ba
	Sc1	5.48±0.77 b
	Sc2	80.72±1.38 a
	Sc3	8.91±0.61 b
Alternaria alternata	Control	0.00±0.00 c
	Aa1	34.13±0.96 b
	Aa2	73.76±1.04 a
	Aa3	22.94±0.70 b
Cladosporium herbarum	Control	0.00±0.00 b
	Ch1	67.30±1.28 a
	Ch2	22.03±0.95 b
	Ch3	25.99±1.63 b
Cladosporium cladosporioides	Control	0.00±0.00 c
	Ccl1	68.16±1.13 a
	Ccl2	11.21±0.81 c
	Ccl3	25.16±0.52 b
Scytalidium thermophilum	Control	0.00±0.00 b
	Sth1	22.26±0.92 a
	Sth2	5.23±0.62 b
	Sth3	3.97±0.29 b
Arthrinium sp.	Control	0.00±0.00 c
	Ak1	48.82±0.32 a
	Ak2	29.09±1.21 b
	Ak3	8.62±0.43 c

Values followed by the same letter for isolates of the same pathogen are not significantly different according to Duncan's multiple range test at P=0.01.

Discussion

In order to show how soil amendments can either increase or decrease fungal diversification, the current study focused on the fungi that were found in various cultivated experimental plots with watermelon, grafted watermelon, melon, grafted melon, and squash. Results obtained showed that *C. cladosporioides* and *A. alternata* predominated regardless of the organic manure amounts applied and the presence of cucurbit crops. These soil samples were used to isolate fungal species using the serial dilution method, which showed the maximum number of species belonging to distinct fungal genera. Similar results were documented earlier by Kumar *et al.* (2015).

Organic manure is crucial for improving the soil's structure and water-holding ability. Additionally, it improves nutrient-depleted soil's natural ability to control pathogens that are transmitted via the soil and helps restore soil structure, both of which boost the biological fertility of the soil (Akanmu *et al.*, 2021; Bonanomi *et al.*, 2007; Chukwuka *et al.,* 2020).

Several isolated species were involved in strong fungal associations and have dominant adaptive features as primary colonizers probably due to their capacity for the rapid invasion of the available substrate (Bhatt *et al.,* 2015). The distribution of fungi has been reduced with the augmentation of the amendment. When the soil was amended with 40 and 60t/ha, the fungi concentration was comprised between 0 and 1.47% and from 0 to 0.17%, respectively. Boughalleb-M'Hamdi *et al.* (2017) noted that *Aspergillus* (9 species) was the most prominent among samples collected from an experimental field in Chott Meriem (Sousse, Tunisia).

Table 5. Antagonists effect on mycelial growth inhibition rate of six different pathogens under *in vitro* conditions.

Isolate code	*S. sclerotiorum*	*A. alternata*	*C. herbarum*	*C. cladosporioides*	*S. thermophilum*	*Arthrinium* sp.
AT1	83.55±0.94 ab	60.30±1.78 a	53.01±1.79 ab	60.81±1.16 ab	71.14±0.53 a	76.78±0.62 ab
AT2	63.39±1.02 abc	60.32±1.44 a	32.21±1.49 ab	49.92±0.52 abcde	65.13±0.97 abc	80.68±0.61 a
AT3	85.42±0.88 a	51.32±1.25 abc	48.99±0.99 ab	67.83±0.66 a	62.81±1.28 abc	78.20±0.77 a
AT4	60.19±1.18 abc	42.30±1.48 abcd	57.87±1.19 a	51.29±1.10 abcde	59.02±1.24 abc	77.91±1.06 a
AT5	49.08±1.53 abc	41.03±1.15 abcd	43.42±1.84 ab	43.05±2.03 abcde	55.98±1.32 abc	78.07±0.43 a
AT6	69.15±1.11 ab	50.03±1.35 abcd	54.46±1.51 ab	61. 62±0.87 ab	69.46±0.91 ab	78.25±0.58 a
AT7	47.58±1.45 abc	33.34±0.72 bcd	37.63±1.69 ab	54.46±0.96 abcd	69.36±0.48 ab	80.91±0.63 a
AT8	41.29±1.57 bc	38.47±0.84 abcd	44.83±1.47 ab	57.97±0.79 abc	61.03±1.15 abc	76.27±0.95 ab
AT9	41.80±1.56 bc	43.63±0.52 abcd	46.72±1.41 ab	49.99±0.83 abcde	67.48±0.35 ab	70.96±0.96 ab
AT10	46.25±1.47 abc	42.34±0.16 abcd	36.21±1.38 ab	56.12±0.92 abcd	65.03±0.99 abc	63.56±1.33 b
AT11	45.57±1.55 abc	33.32±0.77 bcd	33.29±1.15 ab	65.60±1.11 ab	71.48±0.60 a	69.67±0.99 ab
AT12	46.15±1.62 abc	41.03±1.15 abcd	30.58±1.17 ab	54.13±1.29 abcde	67.58±0.71 ab	69.70±0.85 ab
AT13	48.94±1.11 abc	43.63±0.52 abcd	50.91±0.91 ab	54.94±0.55 abcd	61.61±0.85 abc	72.14±0.97 ab
AT14	55.82±1.31 abc	57.73±1.60 ab	48.64±1.10 ab	46.48±1.06 abcde	45.97±1.30 bc	73.05±1.34 ab
AT15	62.06±1.23 abc	43.61±0.91 abcd	60.09±1.31 a	63.07±0.79 ab	61.47±0.77 abc	77.88±0.79 a
AT16	39.66±1.70 bc	50.07±0.63 abcd	60.15±1.14 a	51.03±1.17 abcde	63.69±0.90 abc	76.55±0.63 ab
AT17	46.12±1.71 abc	51.34±0.68 abc	25.16±1.39 ab	45.83±1.55 abcde	53.52±1.41 abc	82.20±0.42a
AT18	37.31±1.74 bc	44.95±1.28 abcd	18.17±1.23 b	31.21±1.15 de	57.62±1.02 abc	73.99±0.78 ab
AT19	26.64±1.78 c	47.49±0.30 abcd	29.65±1.37 ab	32.67±1.36 cde	42.02±1.87 c	83.66±0.60 a
AT20	48.77±1.51 abc	51.99±0.58 abc	27.81±1.04 ab	45.37±0.95 abcde	56.65±1.42 abc	79.58±0.52 a
AT21	57.54±1.62 abc	32.07±1.17 cd	54.18±1.70 ab	39.82±1.19 bcde	67.58±0.71 ab	78.01±0.52 a
AT22	51.86±1.50 abc	29.51±1.41 cd	44.17±1.44 ab	28.50±1.11 e	70.66±1.24 a	71.22±0.85a b
AT23	47.88±1.87 abc	26.91±0.62 cd	43.90±1.31 ab	45.08±1.09 abcde	63.11±1.25 abc	76.63±0.96 ab
AT24	65.16±1.51 abc	25.64±1.12 d	42.17±1.56 ab	51.29±1.10 abcde	69.60±0.70 ab	73.93±0.44 ab
P-value	≥0.05	≥0.05	≥0.05	<0.05	≥0.05	≥0.05

Mean values followed by the same small letters in the same column are not significantly different according to Duncan's multiple range test at P=0.05.

Table 6. Disease incidence recorded by watermelon seedlings inoculated with *A. alternata*, *C. cladosporioides*, *C. herbarum*, *S. sclerotiorum*, *Arthrinium* sp. and *S. thermophilum* and treated preventively and curatively with four antagonist *in vivo* assays. Positive and negative controls were performed by inoculating plants with only the pathogen or only distilled water, respectively.

Treatment	*A. alternata*	*C. cladosporioides*	*C. herbarum*	*S. sclerotiorum*	*Arthrinium* sp.	*S. thermophilum*
Preventive treatment						
Positive control	96.30±0.20 aAa	90.74±0.32 aA	90.74±0.20 aA	92.59±0.20 aA	64.81±0.32 aB	44.44±0.26 aC
Negative control	3.70±0.20 e	7.41±0.20 e	1.85±0.20 e	5.56±0.03 d	7.41±0.19 d	7.41±0.30 b
Trichoderma viride	40.74±0.20 cAB	42.59±0.19 cAB	44.44±0.34 cA	33.33±0.26 cB	22.22±0.26 cC	14.81±0.19 bC
T. harzianum	24.07±0.19 dA	18.52±0.19 dAB	20.37±0.19 dAB	25.93±0.32 cA	18.52±0.20 cdAB	11.11±0.26 bB
Penicillium italicum	72.22±0.37 bAB	62.96±0.32 bBC	68.52±0.20 bAB	81.48±0.28 bA	50.00±0.26 bC	53.70±0.28 aC
Aspergillus niger	75.93±0.19 bA	72.22±0.26 bAB	87.04±0.32 aA	75.93±0.33 bA	51.85±0.42 abC	53.70±0.46 aBC
Mean	52.16	49.07	52.16	52.47	35.8	30.86
Curative treatment						
Positive control	98.15±0.19 aA	94.44±0.26 aA	96.30±0.19 aA	96.30±0.20aA	72.22±0.34 aB	50.00±0.34 aC
Negative control	5.56±0.26 e	9.26±0.32 f	3.70±0.19 e	7.41±0.20 e	9.26±0.20 d	9.26±0.28 b
Trichoderma viride	44.44±0.26 c AB	46.30±0.20 dA	48.15±0.20 cA	37.04±0.19 cB	25.93±0.19 cD	18.52±0.28 bD
T. harzianum	27.78±0.26 dA	22.22±0.26e AB	24.07±0.19 dA	29.63±0.20 dA	22.22±0.26 cAB	14.81±0.20 bB
Penicillium italicum	77.78±0.26 bAB	64.81±0.28 cC	74.07±0.19 bB	85.19±0.19 bA	55.56±0.26bD	57.41±0.19 aCD
Aspergillus niger	79.63±0.28 bA	81.48±0.28 bA	92.59±0.40 aA	81.48±0.28 bA	59.26±0.28 bB	61.11±0.34 aB
Mean	55.56	53.09	56.481	56.17	40.74	35.19

Mean values of disease incidence followed by the same small letters in the same column, or same capital letters in the same row, are not significantly different according to Duncan's multiple range test at P=0.01.

Among *Aspergillus* genera, only *A. fumigatus* (34.65 × 10^5 CFU/g soil) and *A. flavus* (26.28 × 10^5 CFU/g soil) were frequently isolated. Moreover, *P. italicum* and *P. digitatum* were found more frequent with values of 17.33 × 10^5 and 12.6×10^5 CFU/g soil, respectively. Some studies demonstrated that changes in soil microbial communities across space are often strongly correlated with differences in soil chemistry (Jenkins *et al.,* 2009). In particular, it has been shown that the composition, and in some cases diversity, of soil communities is often strongly correlated with soil pH level. Environmental factors such as the soil pH, moisture, temperature, organic carbon, and nitrogen play an important role in the mycoflora distribution (Kumar *et al.,* 2015).

Suppressive soils may be effective in reducing disease due to either high total microbial activity or the presence of particular antagonistic species (Bhatt *et al.,* 2015). Boughalleb-M'Hamdi *et al.* (2017, 2018) showed that *C. gloeosporioides* was the most recurrent species in the untreated soil with a percentage varying from 55.56 to 100% and soil amended by 20 tons of organic amendments per hectare (from 0 to 44.44%). The percentage of pathogenic fungal isolated from soil indicates that watermelon and grafted watermelon had the highest value of 0.29 and 0.18 10^5 CFU/soil, respectively, followed by muskmelon and squash with the lowest value (0.08 and 0.09 10^5 CFU/soil, respectively). These differences could be related, to the species and density, shrubs and overlaying vegetation, to the pedologic characteristics of the forest, and the fungi interactions. Many studies highlight clear correspondence between microfungal community composition and vegetation types (Ramos *et al.,* 2015).

Reeleder (2003) identified biological parameters as being the best predictors of the capacity of soils for disease suppression, including antagonist fungi, particularly *Trichoderma* spp. Wittling *et al.* (1996) reported that natural fertilizers and composts inhibited the development of *Fusarium* spp., *Pythium* spp., and *Phytophthora* spp. Lazarovitz *et al.* (2007) demonstrated that the infection of potatoes 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. Other researchers noted that the fungi population was influenced by the compound of the organic amendment (Ramos *et al.,* 2015). These are the main factors affecting the fungal population that was very high in the soil samples analyzed. The mycofloral analysis was in agreement with other studies such as Bhatt *et al.* (2015). This finding indicated that a reservoir of thermophilic and thermotolerant fungi always coexists (Kumar *et al.,* 2015).

For pathogenicity data, similar results were reported by many researchers (Bhuiyan *et al.,* 2012; Khan *et al.,* 2014; Larrondo & Calvo, 1990; Vos *et al.,* 2017) who studied the pathogenicity of *Sclerotinia sclerotonium*, *Alternaria alternata*, *Cladosporium herbarum*, *Cladosporium cladosporioides*, *Scytalidium thermophilum* and *Arthrinium* sp. in the field and *in vitro* screening. They reported that lesion severity was highly correlated with the disease symptoms (Bhuiyan *et al.,* 2012; Khan *et al.,* 2014; Larrondo & Calvo, 1990; Vos *et al.,* 2017).

In the study of *in vitro* interactions of pathogens and antagonists, twenty-four antagonists were tested *in vitro* for preliminary screening to look for the potential of biological control agents against *S. sclerotiorum*, *A. alternata*, *C. herbarum*, *C. cladosporioides* and *S. thermophilum* and *Arthrinium* sp. A considerable variation was observed between, as well as within the fungal and antagonists with regard to the hyphal interaction and subsequent events to the inhibition of pathogen growth. The results of dual culture indicated that the higher antifungal effect on the growth of the pathogens was obtained with *T. harzianum* and *G. catenulatum*.

T. harzianum has a high inhibitory effect against the different isolated fungi, with several biological modes like mycoparasitism and antibiosis. *Trichoderma* spp. have been

developed into several commercial biological control products used in field crop and greenhouse systems and are known to control numerous soil-borne fungi, such as *Sclerotinia sclerotiorum* (Lib.) de Bary. *Sclerotium rolfsii* showed the highest tolerance to *T. harzianum* and *T. virens* (Bhuiyan *et al.,* 2012). Bosah *et al.* (2010) reported that *A. niger* reduced the *S. rolfsii* mycelial growth but with *Penicillium* it was ineffective.

Filamentous fungi, *Trichoderma* and *Gliocladium* genera, are well studied and are efficient in the biocontrol of different phytopathogens such as *Alternaria, Botrytis*, *Colletotrichum, Fusarium, Phytophthora, Pythium, Rhizoctonia, Sclerotinia,* and V*erticillium* (Anith *et al.,* 2002; Boughalleb *et al.,* 2005). Many *Trichoderma* species are strong opportunistic invaders, fast-growing, prolific producers of spores, and powerful antibiotic producers (Bhatt *et al.,* 2015). Because there is some degree of host-specificity in biocontrol agents even at the sub-species level, this may partially account for the reported inconsistent performance of biocontrol agent preparations. A single biocontrol agent is not likely to be active in all soil environments or against all pathogens that attack the host plant (Ramos *et al.,* 2015). Selection of biocontrol agents as well as understanding the mechanisms involved in the antagonistic effect on plant pathogens are important in designing effective and safe biocontrol strategies.

It can be concluded from this study that organic amendments may influence plant disease development through a multi-faceted approach. By improving plant nutrient availability and enhancing plant biological and physicochemical features, organic amendments may indirectly reduce crop disease susceptibility. Additionally, organic amendments can directly impact the soil microbial community, potentially promoting the activity and colonization of beneficial microorganisms around plant roots. These beneficial microbes can act as antagonists towards plant pathogens, further suppressing disease incidence and severity. Ultimately, a healthy and balanced soil microbiome fostered by organic amendments can increase crop yields through enhanced disease resistance. This research highlighted the ecological importance of soil health in managing plant diseases through promoting a diverse and active soil microbial community by adding organic amendments.

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الملخص

رحومة، عبد الحق، ابتسام بن سالم، محمود محمدي ونعيمة بوغالب محمدي. 2025. عزل الفطور الممرضة وغير الممرضة من جذور القرعيات المزروعة في تربة مصحّحة بأسمدة عضوية في تونس. مجلة وقاية النبات العربية، 43(4):453−463.

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بحثت هذه الدراسة في تأثير تطبيق السماد العضوي (بمعدل 20، 40 و 60 طن/هكتار) على مجتمعات الفطور في محيط جذور نباتات القرعيات (البطيخ الأحمر، البطيخ الأحمر المُطعّم، البطيخ، البطيخ المُطعّم والكوسا) المزروعة في الحقل. تمّ تشخيص الأنواع الفطرية السائدة، وهي: *Cladosporium cladosporioides* (0.83×10^5 وحدة مكونة للمستعمرة/غ تربة) و *Alternaria alternata* (0.22×10^5 وحدة مكونة للمستعمرة/غ تربة). كما تمّ الكشف عن وجود الفطور *Sclerotinia sclerotiorum* و *Cladosporium herbarum* و *Scytalidium thermophilum* و *Arthrinium* sp. بشكل دائم. كشف اختبار القدرة الإمراضية عن طريق التلقيح الاصطناعي على أوراق البطيخ تحت ظروف صوبة زراعية أن العزلتين *S. sclerotiorum* و *A. alternata* هما الأكثر ضراوة/شراسة، إذ سجلتا شدة إصابة بلغت 80.73 و 73.76%، على التوالي. لدراسة عوامل المكافحة الحيوية المحتملة، تمّ تقييم 24 عامل مضاد معزول من التربة لتحري نشاطها المضاد للفطور ضدّ ستة مسببات مرضية باستخدام طريقة التلامس المباشر. أظهر الفطر *Trichoderma harzianum* أقوى تثبيط (48.99−85.42%) ضدّ مسببات أمراض مختلفة، يليه الفطران *Humicola grisea* (32.21−80.68%) و *Gliocladium catenulatum* (26.64−83.66%). أخيراً، تمّ اختبار فعالية أربعة مضادات في الجسم الحي حقلياً ضد مسببات الأمراض الستة في تصميم القطاعات كاملة العشوائية مع معاملات وقائية وعلاجية على أوراق البطيخ. قلّل كلٌّ من *T. harzianum* و *T. viride* حدوث المرض بشكل ملحوظ.

كلمات مفتاحية: الفطور المضادة، الفصيلة القرعية، المجتمعات الفطرية، الفطور المسببة للأمراض.

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