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Evaluation of the efficiency of *Trichoderma*, *Penicillium*, and *Aspergillus* species as biological control agents against four soil-borne fungi of melon and watermelon

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Abstract

Various experiments were carried out to promote biological control under semi-arid ecological conditions. In vitro assay, *Aspergillus flavus* seemed to be the most effective bioagent against *Fusarium oxysporum* f. sp. *niveum* and *Fusarium solani* f. sp. *cucurbitae* with mycelial inhibition rate above 50%. *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus terreus* exhibited an exceptional hyperparasitism against *F. oxysporum* f. sp. *melonis.* The mycelial growth of five *Macrophomina phaseolina* isolates decreased in the presence of *Trichoderma harzianum* (44.42%). In greenhouse experiments, both *A. flavus* and *A. fumigatus* used preventively of melon inoculated with FOM generated the highest damage reduction rate of top and root dry weights (40–42 and 51–52%, respectively) and the lowest disease severity index (DSI). *A. flavus* was also effective in improving the plant development with the highest shoot (SDW) and root (RDW) dry weight values. *Penicillium digitatum*, *Trichoderma harzianum*, and *Trichoderma viride* treated preventively on watermelon and melon plants inoculated with *M. phaseolina* decreased the damage of SDW and RDW and DSI. The development rate revealed the growth improvement potential of *T. harzianum* (watermelon, 15%) and *A. flavus* (melon, 12%). Watermelon plants inoculated with *F. solani* f. sp. *cucurbitae* and treated curatively with *Trichoderma erinaceum*, *T. viride*, and *A. flavus* and other inoculated by *F. oxysporum* f. sp. *niveum* and treated by *Trichoderma helicum* recorded the highest values of growth parameters, similarly for *T. erinaceum* on melon plants inoculated by *F. solani* f. sp. *cucurbitae*. Among all treatments for plants inoculated by *F. oxysporum* f. sp. *melonis*, those three bioagents *T. viride*, *T. erinaceum*, and *A. flavus* revealed efficiency in plant growth. *Trichoderma harzianum* is the best bioagents against cucurbit soil-borne pathogens. Preventive treatment represents an effective strategy. Dipping roots with bioagent fungi suspension improve a good interaction pathogen antagonist.

Keywords: Root pathogens, Biological control activity, *Fusarium* species, *Macrophomina phaseolina*, Melon, Watermelon

Introduction

Fusarium species are worldwide pathogenic fungi of many crop plants. *Fusarium oxysporum* Schltdl. is one of the most important phytopathogens causing *Fusarium* wilt disease in more than a hundred species of plants (Boughalleb & El Mahjoub 2006). The disease management of *Fusarium* wilt usually consists of soil fumigation, seed treatment, use of disease resistant varieties, and biological control bacteria to reduce infection and disease severity (Zhang et al. 2008). *Fusarium* root and stem rot are regarded as also one of the most devastating diseases in cucurbits (Pavlou & Vakalounakis 2005). Due to the persistent nature of these pathogens in soil, subsequent crops of susceptible melon and watermelon cultivars increase pathogen populations. The diseases are best managed with resistant cultivars. However, new virulent populations (physiological races) may develop in specific locations. Biological control of soil-borne pathogens by microorganisms has been considered a good environmentally alternative to the chemical treatment

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methods (Eziashi et al. 2007). Many antagonistic microorganisms have been proved to be active In vitro or in vivo. *Trichoderma* spp. Pers. (Shabir-U-Rehman, et al. 2013), *Aspergillus* species Micheli (Suárez-Estrella et al. 2007), and *Penicillium* spp. Link (De Cal et al. 2009) are the most known among the extensive lists. *Trichoderma* spp. are the most widely studied biological control agents (BCAs) for root and shoot pathogens (Hajieghrari et al. 2008), applied even in post-harvest (Woo et al. 2014). Gava and Menezes (2012) showed that selected isolates of *Trichoderma* spp. were efficient to control soil-borne pathogens of melon in field. Several microorganisms have been reported as plant pathogen antagonists, but only a small number were applied on a commercial scale (Fravel 2005).

The use of rhizophere *Trichoderma harzianum* Rifai, for controlling the spread of *Macrophomina phaseolina* in agronomical crops, has been suggested (Vasebi et al. 2013). Furthermore, different species of *Trichoderma* have been found to be effective in protecting the root system for some crops against other types or strains of pathogenic fungi, e.g., *Fusarium solani* (Mart.) Sacc. and *M. phaseolina* (Malik & Dawar 2003). Recently, Khalili et al. (2016) demonstrated that three T. *harzianum* isolates significantly inhibited the growth of M. phaseolina in vitro and field studies.

The aims of the present study were to evaluate the in vitro potential biological control of *Trichoderma* spp., *Penicillium* spp., and *Aspergillus* spp., against three species of *Fusarium*, and *M. phaseolina*, and to confirm their efficiency against the main cucurbit soil-borne pathogens in pot planted with melon and watermelon seedlings.

Material and Methods

Pathogen and antagonist strains

Twenty-two pathogens belonging to genus *Fusarium*, i.e., *F. oxysporum* f. sp. *niveum*, *F. oxysporum* f. sp. *melonis*, *F. solani* f. sp. *cucurbitae*, and *M. phaseolina* were used in vitro, and only eight were chosen for in vivo studies. Ten antagonist's isolates were tested: four *Trichoderma* sp. (*T. erinaceum* Bissett, C.P. Kubicek & Szakács (watermelon); *T. viride* Schumach.; *T. helicum* Bissett, C.P. Kubicek & Szakács; and *T. harzianum* (melon)), two *Penicillium* sp. (*P. digitatum* (Pers.) Sacc. and *P. italicum* Wehmer (watermelon)), and four *Aspergillus* sp. (*A. niger* (melon), *A. flavus* Link, *A. fumigatus* Fresen., and *A. terreus* Thom (grafted watermelon)). The colonial and microscopic characteristics of the fungal isolates were determined. The pathogen and potential bioagents used in the present research were obtained from the Culture Collection Unit of the Laboratory of Phytopathology (ISA Chott Meriem, Sousse, Tunisia), and they were also isolated from infected cucurbit and tomato plants collected from agricultural fields in Tunisia (Table 1).

In vitro experiment: antagonistic effect

Two disc plugs (0.5-cm diameter) of pathogen and antagonist (4 days old culture) were transferred respectively to a single potato dextrose agar (PDA) plate (9-cm diameter). The antagonist plug was placed on the one side of the plate (about 2 cm from the edge of the plate towards the center), while the pathogen plug was placed at the other side of the plate opposite to the antagonist plug leaving a distance of 5 cm between the two plugs. A plug of PDA medium was used as control treatment while the pathogen plug was placed at the other side. Three replications (two plates/replicate) for each individual treatment were made, and the plates were incubated at 28 ± 2 °C for 5 days. The inhibition percent of the radial growth was evaluated according to the formula of Hmouni et al. (1996): $I\ (\%) = (1 - C_n/C_0) \times 100$; where C_n is the radial growth of the pathogen in the presence of the antagonist and C_0 is the radial growth of control colonies.

In vivo experiment: evaluation of antagonist biological control activity

In vivo experiments were divided into two assays, the first one represented a preventive treatment of watermelon and melon against *F. oxysporum* f. sp. *melonis* and *M. phaseolina* root invasion, respectively. This assay was carried out by root-dipping watermelon and melon seedlings (15 days old) into flask containing a conidial suspension of the different antagonists for 30 min and 24 h before inoculation. For the curative assay, melon and watermelon seedlings were treated 24 h after inoculation with the pathogens by watering each plant with the antagonist suspension (10 ml) as mentioned in Table 2. Two cultivars of melon (cvs. Bonta and Anannas d'Amérique) and two of watermelon (cvs. Sirocco and Charleston Gray) were used in this assay. The seeds were sown in nursery seed trays with cells of volume 250 ml, with 15 plants per each treatment with 3 replicates (5 plants per replicate and treatment). The substrate used in the experiment consisted of a mixture of peat and vermiculite (1:1), which was autoclaved twice at 120 °C. The 2-l pots are then placed in a greenhouse for 30 days. Two positive controls were performed (one by inoculating the plants with only the pathogen and the other with the antagonist (10 ml)) and distilled water for the negative control. The experimental design was a randomized complete block design (RCBD), and the entire experiment was repeated twice. For each fungal species, one cucurbit plants randomly have been distributed in each treatment.

Inoculum preparation

For *Fusarium* species and bioagent fungi, the isolates were grown on PDA at 25 °C for 4 days until sporulation, and then, an Erlenmeyer flask containing 50 ml of

Table 1 Collection of 22 soil-borne pathogens isolates cucurbit plant host, regions, and date sampling. Four *Fusarium oxysporum* f. sp. *niveum* isolates, five *Fusarium oxysporum* f. sp. *melonis* isolates, eight *Fusarium solani* f. sp. *cucurbitae* isolates, and five *Macrophomina phaseolina* isolates

Pathogens	Code	Host	Regions	Sampling date
Fusarium oxysporum f. sp. *niveum*	FON1	Watermelon	Chebika	2009/2010
	FON2		Jbeniana	2009/2010
	FON3		Hajeb	2009/2010
	FON4		Chott Meriem	2010
Fusarium oxysporum f. sp. *melonis*	FOM 1	Melon	Monastir	2011
	FOM 3			2011
	FOM 4		Kairouan sud	2011
	FOM 6			2011
	FOM 8		Sejnen	2011
Fusarium solani f. sp. *cucurbitae*	FSC1	Watermelon	Jbeniana	2010
	FSC2		Hajeb	2010
	FSC3			2010
	FSC4		Beja	2010
	FSC5		Chebika	2010
	FSC6	Squash	Elkef	2010
	FSC7	Watermelon	Jbeniana	2010
	FSC8		Hajeb	2010
Macrophomina phaseolina	MP1	Melon	Chott Meriem	2011
	MP2	Grafted watermelon	Chott Meriem	2011
	MP3	Watermelon	Chott Meriem	2011
	MP4	Tomato	Chott Meriem	2011
	MP5	Melon	Chott Meriem	2011

Table 2 Different treatments applied on watermelon and melon seedlings in vivo biological control assay. Two type of treatments: preventive (application of fungal antagonist before 24 h of pathogen) and curative (application of fungal antagonist 24 h after the inoculation)

Seedlings	Treatments					
	Preventive		Curative			
	Melon	Watermelon and melon	Melon		Watermelon	
Pathogens	*F. o.* f. sp. *melonis*	*M. phaseolina*	*F. s.* f. sp. *cucurbitae*	*F. o.* f. sp. *melonis*	*F. s.* f. sp. *cucurbitae*	*F. o.* f. sp. *niveum*
Antagonists	FOM1/FOM6	MP1/MP2	FSC2/FSC5	FOM1/FOM6	FSC2/FSC5	FON1/FON2
Aspergillus flavus	+	+	+	+	+	+
A. fumigatus	+	+	–	–	–	–
A. niger	+	+	–	–	–	–
A. terreus	+	–	–	–	–	–
Penicillium italicum	+	+	–	–	–	–
P. digitatum	+	+	–	–	–	–
Trichoderma viride	+	+	+	+	+	+
T. harzianum	+	+	–	–	–	–
T. helicum	–	–	+	+	+	+
T. erinaceum	–	–	+	+	+	+

+ done, – not done

potato dextrose broth (PDB, 20 g/l) was inoculated with four pieces individually. Spore production was induced in an orbital shaker, and the spores were recovered from culture by filtration. A hemocytometer was used to determine the concentration of the spores (10^6 spores/ml). In the case of *M. phaseolina*, the isolate were grown on PDA. Thus, 20 plates mixed with 2500 g of autoclaved potting mix and placed in 20-cm pots.

Evaluation parameters

At the end of the experiment, the plants were carefully removed from the pots, and the root systems were gently washed in tap water. Each root system was rated for the disease severity index (DSI) according to each pathogen. For *F. oxysporum*, we adopted the scale described by Vakalounakis and Frangkiadakis (1999) (0 = no symptoms; 1 = light vascular discoloration in the stem with or without stunting; 2 = vascular discoloration in the stem, stunting, wilting with or without yellowing of cotyledons; and 3 = dead seedlings). For *F. solani* f. sp. *cucurbitae*, the DSI was described by Boughalleb et al. (2005) (0: healthy; 1: slight yellowing of leaves with slight rot pivot and lateral roots and crown rot; 2: significant yellowing in leaves with or without wilting, stunting of plants, severe rot at the pivot and lateral roots, significant rot and browning of vessels in the stem; 3: death of the plant). For *M. phaseolina*, the scale used was described by Ravf and Ahmad (1998) (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, and 5: more than 75% of shoot tissues infected). Other variables were measured to estimate the response of the cucurbits, such as the degree of inhibition exhibited by the antagonist: Damage reduction rate (R (%)) was calculated according to the two positive controls. Damage reduction of shoot and root dry weights: (R (%) = ((DWA − DWP) / DWA) × 100, which DWA is the dry weight (shoot and root) of inoculated plants with antagonist and DWP is the dry weight (shoot and root) of inoculated plant with only the pathogen.

The effect of the antagonist alone on the plants was also studied as the development rate of the dry shoot and root weights: D (%) = ((DWA − DW) / DW) × 100, where DWA is the dry weight (shoot and root) of the plants inoculated only by the antagonist and DW is the dry weight (shoot and root) of the healthy plants.

At the end of the curative treatment, agronomic parameters were determined including the shoot and root fresh (SFW and RFW) and dry weights (SDW and RDW) and the plant shoot and root height (SH and RH, respectively).

Statistical analysis

The data were analyzed by ANOVA using SPSS version 20.0 statistical software (SPSS, SAS Institute, USA) to evaluate parameter values differences. Differences between treatments were determined by Duncan's multiple range test at 5% of significance level.

Results and discussion

In vitro experiment: antagonism effect

Data presented in Tables 3 and 4 indicated clearly that there was a significant reduction in mycelia growth after confrontation of tested pathogens with all antagonists. As shown in Table 3, the different species exhibited a significant reduction of mycelium growth of *F. oxysporum* f. sp. *niveum* which varied from 7.22 (FON2/*Penicillium italicum)* to 74.68% (FON4/*Aspergillus flavus*). This potential antagonist seemed to be the most effective bioagent with inhibition rate above 50%.

A. flavus reduced also the development of all *F. solani* f. sp. *cucurbitae* isolates and obtained data ranging from 46.25 (FSC8) to 49.6% (FSC2). However, *F. solani* f. sp. *cucurbitae* isolates showed a good resistance against the three *Trichoderma* species with inhibition rate below 30%. Among the different potential bioagents, *Trichoderma erinaceum* and *Penicillium digitatum* succeed to decrease the mycelial growth of *F. oxysporum* f. sp. *niveum* (41%) and *F. solani* f. sp. *cucurbitae* (32%) (Table 3).

The results from the dual culture tests are shown in Table 4. It appears that the growth rates of the five isolates of *F. oxysporum* f. sp. *melonis* differed according to the used antagonists. Three *Aspergillus* species exhibited growth inhibition and showed hyperparasitism against the colonies of *F. oxysporum* f. sp. *melonis*. Evaluation of the inhibition zones surrounding the *A. flavus*, *A. niger*, and *A. terreus* revealed an inhibition of 25%, with values comprised between 16.56 (FOM1/*A. flavus*) and 36.59% (FOM8/*A. flavus*) and from 20.54 (FOM1/*A. niger*) to 30.46% (FOM6/ *A. niger*) and 22% (from 18.81 (FOM3/*A. terreus*) to 35.14% (FOM1/*A. terreus*), respectively.

For *M. phaseolina*, the two *Trichoderma* species were revealed to be effective. In fact, the mycelial growth of the five *M. phaseolina* isolates decreased in the presence of *Trichoderma harzianum* with an average of 44.42% (values recorded between 38.74 and 52.42%) and *Trichoderma viride* (values ranged between 33.27 and 42.43%). *P. italicum* was the most efficient one with values between 31.95 (MP5) and 46.63% (MP3). However, values increased in the case of direct confrontation of *M. phaseolina* and the four *Aspergillus* species (Table 4).

In vitro biological control activity experiment revealed that *A. flavus* seemed to be the most effective bioagent with mycelial inhibition rate above 50% of *F. oxysporum* f. sp. *niveum* and it was able to reduce the mycelial growth of all *F. solani* f. sp. *cucurbitae* isolates, followed by *T. erinaceum*. The three *Aspergillus* species (*A. flavus*, *A. niger*, and *A. terreus*) and *T. harzianum* exhibited an important growth inhibition against the colonies of *F. oxysporum* f. sp. *melonis.*

Table 3 Effect of direct dual confrontation, of two *Penicillium* spp. isolates, three *Trichoderma* spp. isolates, and *Aspergillus flavus*, on mycelia growth inhibition of four *F. oxysporum* f. sp. *niveum* isolates and eight *F. solani* f. sp. *cucurbitae* isolates after 5 days of incubation at 28 °C, means of six Petri plates (two plates per replicate)

Pathogens	Code	Mycelial growth inhibition percentage (%)[a]						
		P. digitatum	*P. italicum*	*T. erinaceum*	*T. viride*	*T. helicum*	*A. flavus*	*P* values[b]
F. oxysporum f. sp. *niveum*	FON1	40.56b AB	28.90c AB	46.08ab A	41.29b A	39.91b A	56.73a AB	> 0.05
	FON2	32.10c BC	7.22d D	34.30b B	29.31c BC	31.00c AB	52.08a AB	> 0.05
	FON3	32.98b BC	28.97b AB	40.92b AB	38.54b A	32.23b AB	64.71a A	0.0425
	FON4	39.55b AB	30.44b A	42.66b A	36.95b AB	40.00b A	74.68a A	0.0335
F. solani f. sp. *cucurbitae*	FSC1	27.48bc C	21.89c C	37.08b AB	30.94bc B	29.23bc B	49.26a B	> 0.05
	FSC2	38.02b B	24.92c BC	25.04c C	17.08c C	19.21c B	49.60a B	> 0.05
	FSC3	44.65a A	26.19c B	21.83c CD	26.90c BC	17.44c C	48.94a B	> 0.05
	FSC4	39.13b AB	28.57c AB	25.57c C	14.20c C	20.00c B	46.25a B	> 0.05
	FSC5	28.35b C	25.21b B	26.13b C	31.00b B	24.91b B	48.89a B	> 0.05
	FSC6	24.00b C	24.09b BC	19.60b CD	18.57b C	19.84b B	46.93a B	> 0.05
	FSC7	35.85b B	24.27b BC	17.08bc D	10.93c D	19.28bc B	46.93a B	> 0.05
	FSC8	19.49b D	17.74b CD	24.54b C	15.34b C	22.00b B	46.25a B	> 0.05
P values[c]		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	< 0.05	

[a]Mycelial growth inhibition percentage values; means of three replicates (two Petri plates per replicate). Duncan's multiple range test: values followed by different letters are significantly different at $P \leq 0.05$. Capital letters are for comparison of means in the same column. Small letters are for comparison of means in the same row
[b]Duncan's multiple range test is for comparison of means among fungal antagonists with the same pathogen on mycelial growth inhibition
[c]Duncan's multiple range test is for comparison of means among pathogens in the same fungal antagonist on mycelial growth inhibition

These results are in agreement with many reports. In fact, El-Sheshtawi et al. (2014) demonstrated that the presence of many biological control agents for *Fusarium* wilt which are able to exhibit high properties to inhibit conidial production over 90%, such as *T. harzianum*, *Penicillium oxalicum* Currie & Thom, and non-pathogenic *F. oxysporum*. Furthermore, Boughalleb et al. (2008) showed the good effect of three *T. harzianum* isolates against *F. oxysporum* f. sp. *niveum* and *F. solani* f. sp. *cucurbitae*, with a reduction of the colony diameter up to 50%. In the present

Table 4 Effect of direct confrontation of two *Penicillium* spp. isolates, two *Trichoderma* spp. isolates, and four *Aspergillus* sp. isolates on mycelial growth inhibition of *F. oxysporum* f. sp. *melonis* and *M. phaseolina* after 5 days of incubation at 28 °C, means of six Petri plates (two plates per replicate)

Pathogens	Code	Mycelial growth inhibition percentage (%)[a]								
		P. digitatum	*P. italicum*	*T. viride*	*T. harzianum*	*A. flavus*	*A. niger*	*A. fumigatus*	*A. terreus*	*P* value[b]
F. oxysporum f. sp. *melonis*	FOM1	19.31b C	11.43c D	11.61c C	16.75b D	16.56b C	20.54b B	11.75b D	35.14a A	> 0.05
	FOM3	20.38a B	19.73b B	10.24c D	19.8b B	25.95a AB	21.6a AB	26.35a A	18.81b C	> 0.05
	FOM4	17.34b C	16.12b C	26.43a A	21.88a BC	22.1a BC	24.42a AB	21.26a BC	20.6a C	> 0.05
	FOM6	19.91b B	23.27a AB	22.55a AB	16.6b C	25.71a AB	30.46a A	15.19b C	24.51a AB	> 0.05
	FOM8	13.29c D	25.6b AB	8.71d D	23.31b C	36.59a A	27.86b AB	11.07c D	12.4c D	> 0.05
P value[c]		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
M. phaseolina	MP1	48.01a A	36.22a A	42.43a A	42.38a A	23.77b B	29.33b B	35.97a A	21.84c B	> 0.05
	MP2	38.54a B	35.39a B	38.15a B	52.42a A	33.51ab B	29.78a B	44.81a A	44.92a A	> 0.05
	MP3	30.51ab A	46.63a A	37.90a B	46.21a A	42.56a A	30.63a B	24.54c C	24.64c C	> 0.05
	MP4	27.09b C	43.97a A	33.27ab B	38.74b B	37.34a B	31.79a B	20.79c D	20.29cd D	> 0.05
	MP5	35.14a B	31.95ab B	41.46a A	42.38a A	29.79b BC	27.29ab C	29.13bc BC	28.23b BC	> 0.05
P value[c]		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	

[a]Mycelial growth inhibition percentage values; means of three replicates (two Petri plates per replicate). Duncan's multiple range test: values followed by different letters are significantly different at $P \leq 0.05$. Capital letters are for comparison of means in the same row. Small letters are for comparison of means in the same column
[b]Duncan's multiple range test is for comparison of means among fungal antagonists with the same pathogen on mycelial growth inhibition
[c]Duncan's multiple range test is for comparison of means among pathogens in the same fungal antagonist on mycelial growth inhibition

research, among the tested potential bioagents, *T. erinaceum* and *P. digitatum* revealed to be able to decrease the mycelial growth of these two *Fusarium* species. In the same sense, Sreevidya and Gopalakrishnan (2016) found that the colony diameter of *F. solani* f. sp. *cucurbitae* was significantly decreased with *Penicillium* spp. used at higher concentration (75%). Dwivedi (2013) confirmed the fungi toxicity of four *Aspergillus* species (*A. niger*, *A. flavus*, *A. sulphureus* Desm., *A. luchuensis* Inui), two *Trichoderma* species *(T. viride*, *T. koningii* Oudem.), and two *Penicillium* species (*P. citrinum* Thom, *P. italicum)* against *F. solani*. This in vitro assay revealed that pathogenic fungi were significantly decreased even at low concentration of *Aspergillus* spp. Our findings for the two *Trichoderma* species against *M. phaseolina* were confirmed clearly. In fact, *M. phaseolina* mycelial growth decreased significantly in the presence of *T. harzianum* (44.42%). These results proved those of Khalili et al. (2016).

A microscopic study was conducted in order to determine the effects of some antagonists on the mycelial growth of *F. oxysporum* f. sp. *melonis*, *F. oxysporum* f. sp. *niveum*, and *M. phaseolina* (Fig. 1A, B). Compared to controls, treated *Fusarium* species mycelium showed strong lysis (Fig. 1B (a, f)), induction of mycelial cords via anastomosis between hyphal filaments (Fig. 1B (b)), mycelium winding (Fig. 1B (c, e)), and early chlamydospore formation (Fig. 1B (d, g)). The antagonistic effect is limited to not only the mycelial growth reduction but also the penetration, progression, colonization, and sporulation of the antagonist such as penetration sites of antagonist (Fig. 1B (i)) and lysis of *M. phaseolina* cells (Fig. 1B (j)).

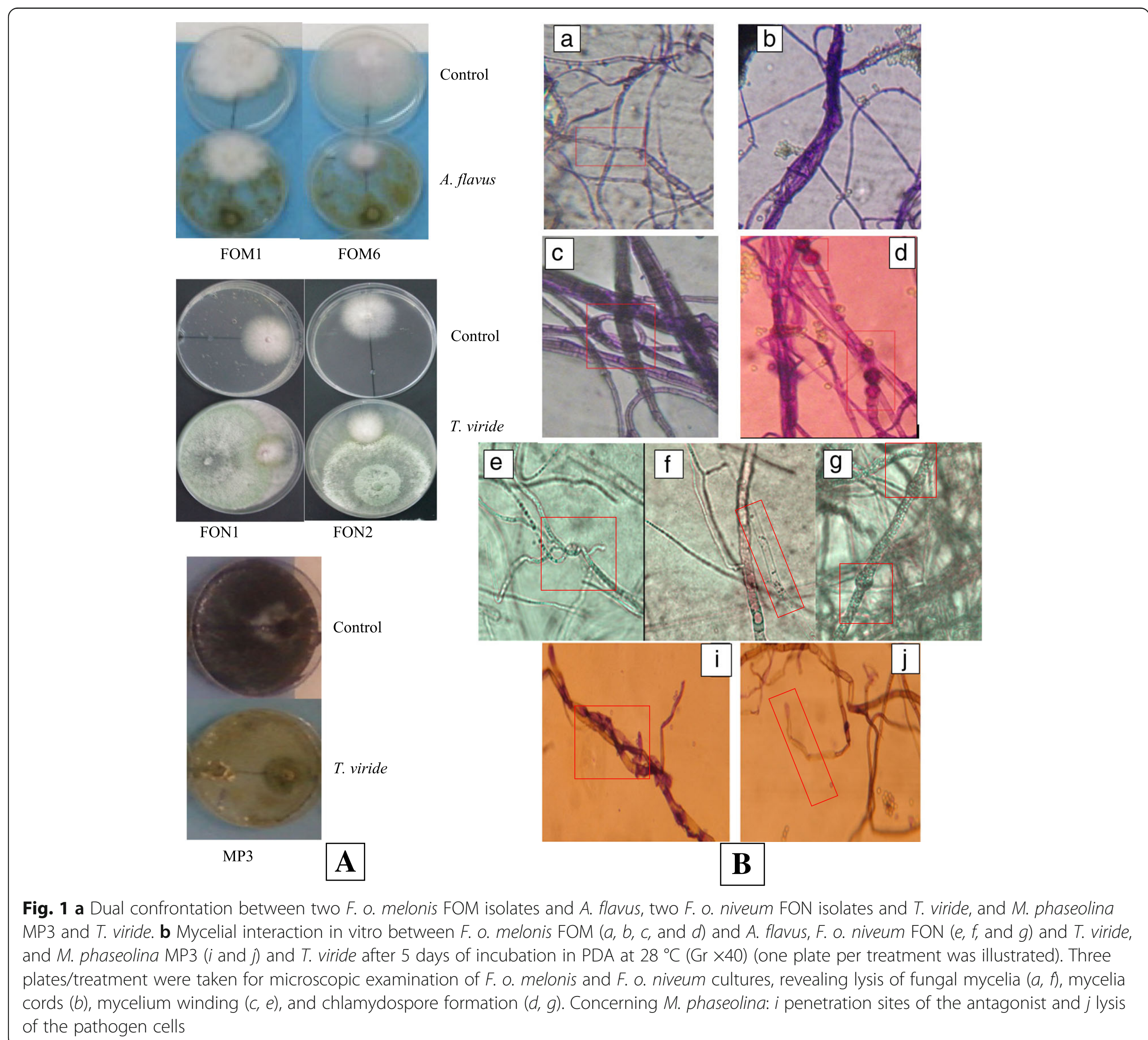

Fig. 1 **a** Dual confrontation between two *F. o. melonis* FOM isolates and *A. flavus*, two *F. o. niveum* FON isolates and *T. viride*, and *M. phaseolina* MP3 and *T. viride*. **b** Mycelial interaction in vitro between *F. o. melonis* FOM (*a, b, c,* and *d*) and *A. flavus*, *F. o. niveum* FON (*e, f,* and *g*) and *T. viride*, and *M. phaseolina* MP3 (*i* and *j*) and *T. viride* after 5 days of incubation in PDA at 28 °C (Gr ×40) (one plate per treatment was illustrated). Three plates/treatment were taken for microscopic examination of *F. o. melonis* and *F. o. niveum* cultures, revealing lysis of fungal mycelia (*a, f*), mycelia cords (*b*), mycelium winding (*c, e*), and chlamydospore formation (*d, g*). Concerning *M. phaseolina*: *i* penetration sites of the antagonist and *j* lysis of the pathogen cells

Table 5 Damage reduction rate of shoot and root dry weight (%) and disease severity index values recorded by melon seedlings inoculated with two *F. oxysporum* f. sp. *melonis* isolates and treated preventively by four *Aspergillus* spp. isolates, two *Penicillium* spp. isolates, and two *Trichoderma* spp. in vivo assay

Antagonists	Damage reduction rate of shoot dry weight %[a]		Damage reduction rate of root dry weight %[a]		Disease severity index[a]	
	FOM1	FOM6	FOM1	FOM6	FOM1	FOM6
A. flavus	40.61a[b]	42.15a	51.89a	53.2a	0.33b	0.5b
A. fumigatus	39.89a	42.25a	49.63a	52.55a	0.5b	0.67b
A. niger	21.73bc	18.28b	34.6b	21.27c	1.67a	1.83a
A. terreus	36.66ab	37.75a	46.86ab	42.19ab	0.83ab	0.67b
P. italicum	27.19abc	20.49b	38.04b	32.53abc	1ab	1b
P. digitatum	37.81ab	32.68a	47.17ab	32.53abc	0.83ab	0.67b
T. viride	17.61c	12.59b	23.92b	23.92c	1ab	1.33ab
T. harzianum	28.75abc	32.97a	40.87b	40.46ab	1ab	0.5b
P value[b]	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

[a]Damage reduction rate values present the mean of three replicates (5 plants/replicate); R (%) = ((DWA – DWP) / DWA) × 100, where DWA is the dry weight (shoot and root) of inoculated plants with antagonist and DWP is the dry weight (shoot and root) of inoculated plant with only the pathogen and disease severity index scale (Table 3), which present the mean of three replicates (5 plants/replicate)
[b]Duncan's multiple range test: values followed by different letters are significantly different at $P \leq 0.05$

In vitro antagonism by various antagonistic fungi on pathogenic organisms is a field of study in which reports are constantly thronging. High reduction of pathogen growth in vitro tests was observed by all antagonists. In this work, a microscopic observation of the interaction of *F. oxysporum* f. sp. *melonis*, *F. oxysporum* f. sp. *niveum*, and *M. phaseolina* and some antagonists confirmed the antibiosis such as penetration, progression, colonization, and sporulation. Similar results with other fungi have previously been reported by Benitez et al. (2004).

The possible mechanisms proposed to explain the antagonism were the competition. Therefore, one of the most interesting aspects of biological control is the study of the mechanisms employed by bioagents to reduce soil-borne disease incidence.

In vivo experiment: evaluation of antagonist biological control activity

The preventive and curative application of antagonist showed a good result with a disease incidence reduction in some agronomic traits in watermelon and melon reaching 50%.

Preventive treatment

Fusarium oxysporum f. sp. melonis

All tested antagonists for in vitro confrontation with *F. oxysporum* f. sp. *melonis* (FOM) were used for in vivo experiment. Both *A. flavus* and *A. fumigatus* have recorded the highest damage reduction rate of dry shoot and root weights of melon inoculated by FOM1 (41 and 52%) and FOM6 (42 and 53%). The in vivo effect of the biological agents were less noted for *T. viride*, and the values were ranged between 17.61 (FOM1) and 12.59% (FOM6), and 23.92% (FOM1 and FOM2), respectively. *A. flavus* and *A. fumigatus* significantly decreased the disease severity index (SDI) with values of 0.33 (FOM1) and 0.5 (FOM6) and with 0.5 (FOM1) and 0.67 (FOM6), respectively. The wilt was more apparent on inoculated melon plant treated with *A. niger* (1.67 (FOM1) and 1.83 (FOM6)) (Table 5). After 1 month of inoculation, the shoot and root dry weights of melon plants treated only with antagonists increased compared to non-treated plants (control). *A. flavus* induced the best results with an increase of shoot dry weights (11.45%) and of root dry weights (13%). T. *harzianum* (8.36 and 10.08%) and

Table 6 Beneficial effect of four *Aspergillus* spp. isolates, two *Penicillium* spp. isolates, and two *Trichoderma* spp. isolates on melon plants in vivo assay, revealed by development rate of shoot and root dry weights (%)

Antagonists	Development rate of shoot dry weight %[a]	Development rate of root dry weight %[a]
A. flavus	11.45a[b]	12.87a
A. fumigatus	8.03abc	10.16ab
A. niger	1.72d	4.55b
A. terreus	3.24cd	7.5ab
P. italicum	4.13bcd	7ab
P. digitatum	5.41bcd	6.14ab
T. viride	7.03abc	9.26ab
T. harzianum	8.36ab	10.08ab
P value	> 0.05	> 0.05

[a]Development rate presents the mean of three replicates (5 plants/replicate); D (%) = ((DWA – DW) / DW) × 100, where DWA is the dry weight (shoot and root) of the plants inoculated only by the antagonist and DW is the dry weight (shoot and root) of the healthy plants
[b]Duncan's multiple range test: means followed by different letters are significantly different at $P \leq 0.05$

Table 7 Damage reduction rate of top and root dry weight (%) and disease severity index values recorded by watermelon and melon seedlings inoculated with two *M. phaseolina* isolates and treated preventively by four *Aspergillus* spp. isolates, two *Penicillium* spp. isolates, and two *Trichoderma* spp. isolates in vivo assay

Cultivars	Preventive treatment	Damage reduction rate of shoot dry weight %[a]		Damage reduction rate of root dry weight %[a]		Disease severity index[a]	
		MP1	MP2	MP1	MP2	MP1	MP2
Watermelon	*A. flavus*	14.88b[b]	19.39c	11.73c	19.40c	2.33a	2.67a
	A. fumigatus	8.94c	22.19b	35.09b	50.18a	2.33a	1.83abc
	A. niger	31.97ab	27.70b	15.92c	27.70b	2.33a	1.50bc
	P. italicum	13.75b	20.87b	26.85bc	43.00ab	2.17b	2.17ab
	P. digitatum	43.36ab	33.56b	27.78bc	33.57abc	1.67b	1.50ab
	T. viride	40.42ab	44.22b	34.30b	44.22ab	1.33b	1.33abc
	T. harzianum	51.11a	52.71a	54.33a	52.71a	1.50b	1.50c
P value[c]		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Melon	*A. flavus*	3.69c	14.09c	18.31b	16.92d	1.33ab	2.00a
	A. fumigatus	12.64c	28.40b	30.58ab	26.99cd	2.67a	1.33ab
	A. niger	29.51ab	35.91b	26.29ab	37.35abc	1.50ab	1.33ab
	P. italicum	7.38c	23.44c	20.25ab	27.90cd	1.50ab	1.50ab
	P. digitatum	34.42a	39.51a	34.06a	33.30bcd	1.33ab	0.83b
	T. viride	30.56ab	29.09b	35.41a	48.18ab	1.50ab	1.33ab
	T. harzianum	27.80b	29.52b	37.23a	50.68a	1.33ab	0.50b
P value[c]		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

[a]Damage reduction rate values present the mean of three replicates (5 plants/replicate); *R* (%) = ((DWA – DWP) / DWA) × 100, where DWA is the dry weight (shoot and root) of inoculated plants with antagonist and DWP is the dry weight (shoot and root) of inoculated plant with only the pathogen and disease severity index scale, which present the mean of three replicates (5 plants/replicate)
[b]Duncan's multiple range test: means followed by different letters are significantly different at $P \leq 0.05$

A. fumigatus (8.03 and 10.16%) were also effective in improving the plant development (Table 6).

M. phaseolina

The two *Trichoderma* species (*T. harzianum* and *T. viride*) and *P. digitatum* exhibited the highest damage reduction of shoot and root dry weights (*R* (%)) for inoculated watermelon plants. The damage reduction rate values were ranged between 51.11 and 40.42% (MP1) and from 52.71 to 33.56% (MP2) and varied from 54.33 to 27.78% (MP1) and between 52.71 and 33.57% (MP2), for the two parameters, respectively. However, the three *Aspergillus* species revealed to be less efficient. Watermelon plants treated with *A. flavus*, *A. fumigatus*, and *A. niger* showed symptoms on roots with highest disease severity index (2.33). However, the lowest value was exhibited on plant

Table 8 Beneficial effect of four *Aspergillus* spp. isolates, two *Penicillium* spp. isolates, and two *Trichoderma* spp. isolates, on watermelon and melon plants in vivo assay, revealed by development rate of shoot and root dry weights (%)

Antagonists	Watermelon		Melon	
	Development rate of shoot dry weight %[a]	Development rate of root dry weight %[a]	Development rate of shoot dry weight %[a]	Development rate of root dry weight %[a]
A. flavus	5.98c[b]	4.99b	11.45a	12.87a
A. fumigatus	3.02c	3.44b	8.03ab	10.16ab
A. niger	3.57c	4.86b	1.72c	4.55b
P. italicum	11.41b	7.76ab	4.12bc	7.01ab
P. digitatum	13.93ab	14.64a	5.41bc	6.13ab
T. viride	11.91ab	10.32ab	7.03ab	9.26ab
T. harzianum	15.12a	15.17a	8.36ab	10.08ab
P value[b]	> 0.05	> 0.05	> 0.05	> 0.05

[a]Development rate presents the mean of three replicates (5 plants/replicate); *D* (%) = ((DWA – DW) / DW)× 100, where DWA is the dry weight (shoot and root) of the plants inoculated only by the antagonist and DW is the dry weight (shoot and root) of the healthy plants
[b]Duncan's multiple range test: means followed by different letters are significantly different at $P \leq 0.05$

treated with *T. viride* (1.33). Both *P. digitatum* and *T. viride* recorded the highest values of damage reduction of shoot and root dry weights of melon plants. The lowest values of disease severity index was registered on plants treated with *P. digitatum* (0.83 for MP2) and with *T. harzianum* (0.55 for MP2) (Table 7). For watermelon plants, *Trichoderma* and *Penicillium* species exhibited the highest development rate (*D* (%)) ranging from 11.41 to 15.12% and from 7.76 to 15.17%, for the shoot and the root dry weights, respectively. However, the best behavior of melon plants was observed when they are treated with *A. flavus*, *T. harzianum*, and *A. fumigatus* (Table 8).

Curative treatment

Watermelon

The efficiency of the three *Trichoderma* species and *A. flavus*, applied through plantation, on growth parameters was studied under greenhouse conditions. The results for *F. solani* f. sp. *cucurbitae* (FSC5) revealed that *T. harzianum* increased significantly the root height (16.6 cm), root fresh (0.47 g), and dry weight (0.23 g). In the same sense, the treatment with *A. flavus* produced the highest values of shoot height (49.6 cm) and dry weight (2.22 g). Watermelon plants inoculated with FSC2 and treated by *T. viride* exhibited a beneficial effect on shoot height (44.9 cm), fresh weight (6.14 g), and root dry weight (0.16). Plants inoculated by *F. oxysporum* f. sp. *niveum* and treated with the three *Trichoderma* species showed an improvement of the different growth parameters. The treatment with *T. helicum* generated the highest shoot height (TH) (61.3 cm) and increased also the shoot fresh weight (SFW) of watermelon plants inoculated with FON2 (12.6 g). *T. viride* and *T. erinaceum* improved the root fresh weight and the shoot and root dry weights with values of 0.75, 2.91, and 0.23 g, respectively (Table 9).

Melon

The best growth parameters on melon plants inoculated by FSC5 were recorded in the presence of *T. erinaceum* with values of 16.7 cm (RH), 7.31 (SFW), 0.46 g (RFW),

Table 9 Comparison of different growth parameter values: shoot and root heights (cm), shoot and root fresh weights (g), and shoot of root dry weights (g) recorded by watermelon seedlings inoculated by two *F. solani* f. sp. *cucurbitae* isolates (FSC 5 and FSC 2) and two *F. oxysporum* f. sp. *niveum* isolates (FON 1 and FON 2) and treated curatively by three *Trichoderma* spp. isolates and *A. flavus*

Pathogens	Treatments	Growth parameters					
		SH (cm)[a]	RH (cm)[a]	SFW (g)[a]	RFW (g)[a]	SDW (g)[a]	RDW (g)[a]
FSC5	*T. erinaceum*	46.80efgh[b]	16.40abcde	6.46def	0.21ef	1.55defghi	0.15defg
	T. viride	48.70defg	15.20cdefg	6.20efg	0.35cde	1.44fghij	0.17bcdef
	T. helicum	44.00fghij	16.60abcd	5.21fgh	0.47bcd	1.63defgh	0.23ab
	A. flavus	49.60def	16.40abcde	5.04fgh	0.27def	2.22b	0.18abcd
	FSC5	40.80hij	13.50fgh	4.20hi	0.14f	1.90bcd	0.10g
FSC2	*T. erinaceum*	38.60ij	14.30defgh	5.61fgh	0.33cdef	1.47fghij	0.16cdef
	T. viride	44.90efghi	16.90abc	6.14efg	0.31cdef	1.38ghij	0.16cdef
	T. helicum	41.10hij	17.90ab	5.88fgh	0.28def	1.71cdefg	0.14defg
	A. flavus	45.40efghi	18.60a	5.22fgh	0.29def	1.28hijk	0.15defg
	FSC2	27.50k	12.20h	2.91i	0.18ef	0.97k	0.10g
FON1	*T. erinaceum*	51.70cde	15.80bcdef	8.21cd	0.49bc	2.91a	0.23a
	T. viride	60.70ab	16.60abcd	10.77 b	0.75a	2.05bc	0.18bcde
	T. helicum	61.30a	15.80bcdef	8.73c	0.33cdef	1.51efghi	0.13defg
	A. flavus	57.00abc	14.00efgh	10.62 b	0.42cd	2.01bc	0.13defg
	FON1	42.00ghij	12.10h	5.98fgh	0.18ef	1.12jk	0.14defg
FON2	*T. erinaceum*	54.20bcd	16.60abcd	11.73ab	0.71a	1.78cdef	0.18bcde
	T. viride	50.30cdef	16.40abcde	11.21ab	0.65ab	1.46fghij	0.21abc
	T. helicum	54.00bcd	16.00bcde	12.60a	0.51bc	1.86bcde	0.16cdef
	A. flavus	46.10efgh	13.10gh	7.91cde	0.28def	1.79cdef	0.13defg
	FON2	27.80k	12.40h	4.42ghi	0.20ef	0.99k	0.12fg
Control		37.00j	12.60h	5.08fgh	0.21ef	1.18ijk	0.13defg
P value[b]		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

[a]Shoot and root height (cm), shoot and root fresh weight (g), shoot of root dry weight (g), mean of three replicates (5 plants/replicate)
[b]Duncan's multiple range test: means followed by different letters are significantly different at $P \leq 0.05$

Table 10 Comparison of different growth parameters values: shoot and root heights (cm), shoot and root fresh weights (g), and shoot of root dry weights (g) recorded by melon seedlings inoculated by two *F. solani* f. sp. *cucurbitae* isolates (FSC 5 and FSC 2) and two *F. oxysporum* f. sp. *melonis* isolates (FOM) and treated curatively by three *Trichoderma* spp. isolates and *A. flavus*

Pathogens	Treatments	Growth parameters					
		TH (cm)[a]	RH (cm)[a]	TFW (g)[a]	RFW (g)[a]	TDW (g)[a]	RDW (g)[a]
FSC5	*T. erinaceum*	37.00ab[b]	16.70a	7.31a	0.46a	1.36b	0.18a
	T. viride	39.50a	14.90abc	6.99a	0.38ab	1.34b	0.12cdef
	T. helicum	38.00a	11.20ef	5.14b	0.28bcde	1.00c	0.13cde
	A. flavus	37.80a	14.10bcd	4.85b	0.27bcde	1.23b	0.17ab
	FSC5	15.43g	11.00ef	3.78cde	0.21de	0.22g	0.08f0
FSC2	*T. erinaceum*	32.90c	16.70a	5.26b	0.34bc	1.39b	0.18a
	T. viride	31.60c	15.50ab	7.36a	0.34bc	1.65a	0.15abc
	T. helicum	30.90cd	14.80abc	5.23b	0.27bcde	1.36b	0.15abcd
	A. flavus	33.70bc	16.60a	4.56bc	0.29bcd	1.28b	0.13bcd
	FSC2	24.70ef	11.00ef	2.40f	0.17e	0.60de	0.09ef
FOM	*T. erinaceum*	22.50f	15.10ab	3.37def	0.23cde	0.51ef	0.09ef
	T. viride	25.80ef	12.90cde	4.66bc	0.24cde	0.35fg	0.12cdef
	T. helicum	25.00ef	13.60bcd	5.12b	0.26cde	0.53ef	0.13bcd
	A. flavus	23.00f	15.30ab	4.35bcd	0.26bcde	0.29g	0.11def
	FOM	15.30g	10.73f	3.28def	0.21de	0.23g	0.08f
Control		27.80de	12.50def	12.50def	0.22de	0.75d	0.17ab
P value[b]		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

[a]Shoot and root height (cm), shoot and root fresh weight (g), shoot of root dry weight (g), mean of three replicates (5 plants/replicate)
[b]Duncan's multiple range test: means followed by different letters are significantly different at $P \leq 0.05$

1.36 g (SDW), and 0.18 g (RDW), followed by *T. viride* treatments for plants inoculated by FSC2. In all the treatments for plants inoculated by *F. oxysporum* f. sp. *melonis*, significant reduction of disease incidence was noticed compared to control, especially for *T. viride*, *T. erinaceum*, and *A. flavus* (Table 10).

The present investigation revealed that the highest damage reduction rate of top and root dry weights was recorded on melon inoculated by *F. oxysporum* f. sp. *melonis* and treated preventively by *A. flavus* and *A. fumigatus*, also, decreased the disease severity index. *A. flavus* and *T. harzianum* were effective in improving the plant development. Among all curative treatments of inoculated melon plants by *F. oxysporum* f. sp. *melonis*, *T. viride*, *T. erinaceum*, and *A. flavus* were the most effective in reducing the disease incidence of this fungus. This stimulation results in greater axial growth and root mass compared to the control, which is consistent with the work of Mouria et al. (2007) who showed that all strains of *T. harzianum* stimulated the growth of tomato, including vegetative and root biomass. Several reports have previously demonstrated the successful use of biological control agents against *Fusarium* diseases of various crops. Bernal-Vicente et al. (2009) reported the specific biological control effect of *T. harzianum* against *F. oxysporum* f. sp. *melonis* under greenhouse nurseries. Watermelon and melon plants treated with *P. digitatum* and *T. viride* and inoculated with *M. phaseolina* recorded the highest damage reduction of shoot and root dry weights and the lowest disease severity index values. The development rate revealed the growth improvement induced by *T. harzianum* (watermelon, 15%) and *A. flavus* (melon, 12%). In fact, the application of *Trichoderma* to the soil as biological control agent in the greenhouse or under field conditions not only resulted in reduced disease severity of *M. phaseolina* but also enhanced plant growth (Srivastava et al. 2008). The efficacy of the three *Trichoderma* species and *A. flavus*, applied curatively of watermelon and melon, on growth parameters was studied under pot culture conditions. Watermelon plants inoculated with *F. solani* f. sp. *cucurbitae* and treated with *T. erinaceum*, *T. viride*, and *A. flavus* showed an improvement of growth parameters. *T. helicum* and *A. flavus* were effective on plants inoculated by *F. oxysporum* f. sp. *niveum*. The best growth parameters on melon plants inoculated by *F. solani* f. sp. *cucurbitae* were obtained in the case of *T. erinaceum*. Our results supported those of Harman et al. (2004) showing the use of *Trichoderma* spp. as plant growth enhancers, due to its production of growth hormones and enhanced transfer of minerals to the rhizosphere. The pathogen incidence and disease severity of plant inoculated only with pathogens were higher than the other treatments. Gava and Menezes (2012) revealed that *Trichoderma* spp. isolates have been

shown to be efficient colonizers of the melon root system; however, the field efficacy did not exceed 50%. For *M. phaseolina*, watermelon and melon plants treated preventively with *P. digitatum*, *T. harzianum*, and *T. viride* recorded the highest values of damage reduction of shoot and root dry weights and the lowest disease severity index. Vasebi et al. (2013) determined the direct interaction between antagonist isolates and *M. phaseolina* involving increased fresh and dry weights of root and foliar parts, which supports my argument. Similar studies have previously shown that antagonists increase seed germination and promote plant growth (Sreedevi et al. 2011). Many studies demonstrated the promising results for *Trichoderma* species in the biological control of plant diseases applying the mechanisms of competition, antibiosis, and mycoparasitism mediated by hydrolytic enzymes (Munir et al. 2014). *Trichoderma*-based *Trichoderma viride* species have been investigated for over 80 years. Numerous researches have been focused on searching and selecting antagonist microorganisms on diverse soil pathogens. Also, synergism between different forms of action modes occurs as the natural condition for the biological control of fungal pathogens. It is widely known that environmental parameters such as abiotic (soil type, soil temperature, soil pH, water potential, and such like) and biotic (plant species and variety, microbial activity of the soil) factors as well as other factors such as method and timing of applications may have influence on the biological control efficacy.

Conclusions

Dipping watermelon and melon root in antagonists' spore suspensions prior to inoculation of the culture substrate allowed not only the protection of the plants but also the improvement of the agronomic parameters, including better axial growth and greater root biomass. *Aspergillus* spp. were effective, applied preventively, in reducing *F. oxysporum* f. sp. *melonis* disease incidence. Furthermore, *Trichoderma* spp., applied preventively and curatively, showed a significant biological control activities on watermelon and melon plants inoculated with *M. phaseolina*, *F. solani* f. sp. *cucurbitae*, and *F. oxysporum* f. sp. *niveum* and could be recommended for biological control use. However, although *Aspergillus* spp. and *Penicillium* spp. were effective against the tested phytopathogens, fungi are not recommended for biological control assay due to their carcinogenic properties.

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Authors' contributions

All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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