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# Effectiveness of *Trichoderma* spp. obtained from re-used soilless substrates against *Pythium ultimum* on cucumber seedlings

## Wirksamkeit von *Trichoderma* spp. aus wiederverwendeten erdelosen Trägersubstraten gegenüber *Pythium ultimum* an Gurkensämlingen

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### Abstract

Thirty-nine *Trichoderma* strains isolated from effective re-used substrates used in soilless systems and two commercial formulations (*Trichoderma viride* TV1 and Remedier WP) were tested against *Pythium ultimum*, the causal agent of cucumber damping-off, under greenhouse and growth chamber conditions. *Trichoderma* was applied to the soil or by root dipping. Plant growth promotion activity of the *Trichoderma* strains was also evaluated in absence of the pathogen. The best and most consistent results were obtained by applying *Trichoderma* to the soil, 7 days before soil infestation with the pathogen. Twelve out of 39 *Trichoderma* strains (FC 1, 2, 6, 7, 12, 19, 24, 38, 39, 69, 72 and 80) showed the best activity against *P. ultimum* and four of them provided a 95% efficacy. The activity of such strains resulted slightly better than that of the commercial formulation Remedier. Some of the best strains also showed a good growth promoting ability, as demonstrated by a positive effect on biomass produced. Therefore, the good biocontrol ability of *Trichoderma* was confirmed in strains isolated from soilless systems. Such biocontrol agents may play a role in the suppressiveness of substrates used for soilless cultivation.

**Key words:** biological control, damping-off, soilless systems

### Zusammenfassung

Neunundreißig *Trichoderma*-Stämme wurden aus wiederverwendeten erdelosen Trägersubstraten isoliert und zusammen mit zwei kommerziellen Formulierungen (*Trichoderma viride* TV1 und Remedier WP) im Gewächshaus und in Phytotronen auf ihre Wirksamkeit gegenüber *Pythium ultimum*, dem Erreger der Umfallkrankheit der Gurke, untersucht. *Trichoderma* wurde zum Boden zugegeben oder als Wurzeltauchbehandlung appliziert. Die pflanzenwachstumsfördernde Aktivität der *Trichoderma*-Stämme wurde daneben in Abwesenheit des Erregers untersucht. Die besten und konsistentesten Ergebnisse wurden mit einer Bodenbehandlung 7 Tage vor der Inokulation des Bodens erzielt. Zwölf von 39 untersuchten *Trichoderma*-Stämmen (FC 1, 2, 6, 7, 12, 19, 24, 38, 39, 69, 72 and 80) zeigten die höchste Aktivität gegenüber *P. ultimum* und vier von ihnen besaßen eine 95%ige Wirksamkeit. Ihre Aktivität übertraf die der kommerziellen Formulierung Remedier geringfügig. Einige der wirksamsten Stämme zeigten ebenfalls einen positiven Einfluss auf die gebildete Biomasse und damit eine deutliche pflanzenwachstumsfördernde Aktivität. Das hohe antagonistisch Potential von *Trichoderma* konnte daher für Stämme aus erdelosen Trägersubstraten bestätigt werden. Diese Stämme könnten zur Suppressivität von Substraten beitragen, die für die erdelose Kultivierung verwendet werden.

**Stichwörter:** biologische Kontrolle, erdelose Aufzuchtsysteme, Umfallkrankheit

## 1 Introduction

*Pythium ultimum* is the causal agent of damping-off on many crops, including cucumber (Zitter et al. 1996; Koening et al. 1999; Martin and Loper 1999). This pathogen is particularly important in soilless systems, where, once introduced, it can easily and quickly spread to the whole cultural system (Stanghellini and Rasmussen 1994). Few measures are available to prevent *P. ultimum* infections (Fig. 1) and resistant cultivars are not available for most crops (Cuartero et al. 1999). Root-borne diseases can be reduced by soil disinfestation, a practice that is becoming very difficult to adopt due to the continuous loss of registered fumigants, and by various non-chemical strategies, including sanitation, cultural practices and biological control.

A recent development of biological control is represented by its application into soilless systems, which, being more microbiologically buffered systems than soil, permit an easy introduction and establishment of biocontrol agents (Paulitz and Bélanger 2001). At first, microbial optimization of soilless systems has been successfully attempted with the introduction of well known antagonists, such as selected *Trichoderma* strains (Van Os and Postma 2000; Garibaldi et al. 2003; Van Os et al. 2004). A further development has been represented by the exploitation of suppressiveness of soilless systems (Postma 2009). In several cases, suppressiveness of soilless systems towards certain rootborne and soilborne diseases proved the existence of beneficial microorganisms in the system (Postma et al. 2000; Minuto et al. 2007). Among the indigenous microflora responsible for the antagonistic activity, *Pseudomonas fluorescens*, *Achromobacter* sp., *Serratia* sp., *Rhodococcus*, *Streptomyces* spp., *Pythium oligandrum*, *Trichoderma harzianum*, among others, have been identified in different studies (Postma et al. 2005; Clematis et al. 2009).

These studies led to a new generation of biocontrol agents, isolated from soilless systems and a number of studies are now trying to understand their characteristics in terms of conditions of survival, ability to colonize the host, also in relation to their presence at different stages of crop growth (Calvo-Bado et al. 2003, 2006).

Recent researches showed the possibility to stimulate the suppressiveness of the cultivation system by applying biological control agents isolated from soilless cultivation systems (Postma 2009; Srinivasan et al. 2009).

Several microorganisms, belonging to *Pseudomonas fluorescens*, *Trichoderma* spp. and *Fusarium* spp. isolated from re-used substrates (rockwool, peat and perlite) were used in soilless systems in Italy (Clematis et al. 2009).

In the present study the ability of several *Trichoderma* spp. strains, isolated from recycled rockwool against *P. ultimum* on cucumber was evaluated.

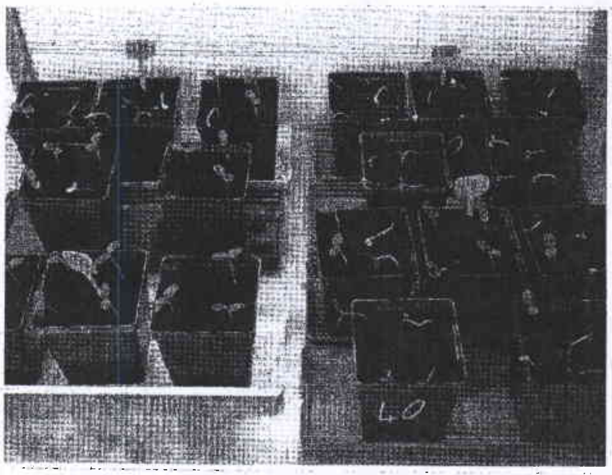


Fig. 1: Symptoms of damping off caused by *Pythium ultimum* on cucumber seedlings under growth chamber condition.

## 2 Materials and methods

### 2.1 Layout of experimental trials, plant material and substrates

Eighteen trials were carried out during the period November 2007 – February 2009 under greenhouse (14 trials) or growth chamber (four trials) conditions, at the Center for the Innovation in the Agro-Environmental Center AGROINNOVA of the University of Torino, located at Grugliasco. Cucumber plants (cv Marketmore, Furia Sementi, Monticelli Terme, Parma) were grown in 160-plug-trays filled with Brill Type 5 substrate (Brill Substrate, Georgsdorf, Germany) with a fine structure for nursery production with 15% of blond peat, 85% of black peat, pH 5.5-6, disinfested at 80°C for 30 min. The substrate was fertilized with 1,100 g $m^{-3}$ of NPK and traces of molybdenum. The same substrate and the same fertilization were used for all trials in all experiments.

Plants were maintained in greenhouse at 25°C. Nine-day-old seedlings at two cotyledons stage of development were used in the different trials.

Seven different types of experiments were performed, with the main differences being based on the length of the period from the treatment with *Trichoderma* and the transplant, and the method of treatment with *Trichoderma* (Table 1).

### 2.2 Biocontrol agents (Trichoderma spp. strains)

Thirty-nine *Trichoderma* spp. strains isolated from recycled rockwool (Clematis et al. 2009), maintained at 8°C on potato dextrose agar, were used throughout the work and compared with two commercial formulations, based respectively on *Trichodermaviride* (*T. viride* TV1, Agribiotec srl, Cavezzo, Italy), and *T. harzianum* strain ICC012 + *Trichoderma viride* strain ICC080 (Remedier WP, Isagro Ricerca rl, Milano, Italy).

For soil application, *Trichoderma* spp. strains were grown into 1000-ml-flask containing a wheat kernel medium (300 g wheat kernels and 320 ml water, sterilized at 121°C for 30 min) at 25°C in a growth chamber under a 12-h fluorescent photoperiod. Fifteen-day old cultures of *Trichoderma* spp. were applied as inoculum at 5 g of fresh biomass per liter of soil (corresponding to $5 \times 10^8$CFU $g^{-1}$).

For root dipping, *Trichoderma* spp. was grown into 1000-ml-flask containing 250 ml liquid casein hydrolysate medium and maintained under static culture conditions at 25°C. After 15 days, the mycelium produced was transferred into 200 ml sterile distilled water and homogenized with a rotary hand. The conidia suspension obtained for each *Trichoderma* spp. isolate was standardized to $1 \times 10^8$CFU $ml^{-1}$.

The two commercial formulations *T. viride* TV1 and Remedier WP were applied in all trials at the label rates of respectively 3 and 0.25 g $l^{-1}$ of soil.

### 2.3 Inoculation with the pathogen

A highly virulent strain of *Pythium ultimum* (coded as Protector 1), isolated from diseased cucumbers, was grown into 1000-ml-flasks containing a wheat-hemp seed medium (200 g wheat kernels, 100 g hemp seeds and 320 ml water, sterilized at 121°C for 30 min) and maintained at 20°C in a growth chamber under a 12-h fluorescent photoperiod. After 15 days of incubation, soil infestation was carried out by mixing into per liter steamed soil with 1 g of fresh biomass of *P. ultimum* (Table 1).

### 2.4 Evaluation of plant growth promoting activity

In experiment VI, carried out under glasshouse, no inoculation with the pathogen was performed. The different *Trichoderma* spp. strains were applied as soil mix.

### 2.5 Evaluation of the biocontrol activity

In the trials carried out with the soil mix application method (Experiment I, II, III, IV, and VII), *Trichoderma* strains (5 g $l^{-1}$

Table 1: Conditions of the different types of experiments carried out

| Type of experiment | Number of trials carried out | Application of *Trichoderma* spp. at day | Soil infestation with *P. ultimum* at day | Days between *Trichoderma* spp. treatment and transplantation | Method of *Trichoderma* application and experimental conditions |
|---|---|---|---|---|---|
| I | 2 | 0 | 0 | 0 | Soil mix, growth chamber |
| II | 3 | 0 | 0 | 0 | Soil mix, glasshouse |
| III | 2 | 0 | 7 | 14 | Soil mix, growth chamber |
| IV | 3 | 0 | 7 | 14 | Soil mix, glasshouse |
| V | 3 | 0 | 0 | 0 | Root dipping, glasshouse |
| VI | 2 | 0 | –* | 7 | Soil mix, greenhouse |
| VII | 3 | 0 | 7 | 14 | Soil mix, glasshouse |

* In this type of experiment, the soil was not infested with the pathogen.

of soil) and the commercial bioproducts at the recommended dosages were applied in individual soil bags containing 8 l of disinfested organic soil. The pathogen (1 g $l^{-1}$ of soil) was introduced into the soil and mixed thoroughly, at 0 or seven days after the treatment with the antagonists (Table 1). Cucumber transplant occurred immediately or after 7 days allowing different periods of incubation of the soil mix containing the antagonists and the pathogen (Table 1). The soil mix of each bag was then transferred in four pots (2 l each). A total of 20 plants (5 plants/pot) were used for each treatment in a completely randomized block design. Plants were maintained under glasshouse (20–23 °C) or growth chamber (18–20 °C), as indicated in Table 1.

In the root dipping application method (Experiment V), nine-day-old seedlings were carefully removed form trays and their roots were dipped in 100 ml of *Trichoderma* suspension ($1 \times 10^8$CFU $ml^{-1}$) for 10 min followed by transplanting in the pots. *P. ultimum* (1 g $l^{-1}$ of soil) was mixed into bags (8 l soil) and the content of each bag was distributed to four pots (2 l each). The commercial formulations of *Trichoderma* TV 1 and Remedier were applied according to manufacturer's instructions, respectively.

The twelve strains of *Trichoderma* showing the best activity were tested again (Experiment VII), by adopting soil mix application under greenhouse conditions. Inoculated and not inoculated controls were maintained for each trial.

### *2.6 Disease assessment*

Each trial lasted two weeks, disease severity was assessed by counting the dead plants (DP) at the end of the trials. Each experiment type was repeated two or three times (Table 1). Data are expressed as average value of the different trials. The efficacy of different treatment with *Trichoderma* in controlling damping off (CE) was calculated as:

(1) $CE_{dp} = (nDP_i - nDP_t)/nDP_i \times 100$

Where,
dp = dead plants.
n = number of dead plant.
i = inoculated control (soil infested with the pathogen without *Trichoderma*).
t = treatment with *Trichoderma*.

### *2.7 Measurement of plant growth parameters*

At the end of each trial, the total biomass was weighed in order to evaluate the effect of the different strains of *Trichoderma* spp. on plant growth. The fresh weight obtained was expressed as relative value (RW) calculated as:

(2) $RW_{fs} = (FS_t - FS_i)/(FS_0 - FS_i) \times 100$

Where,
fs = fresh shoot.
t = treatment with *Trichoderma*.
i = inoculated control (soil infested with the pathogen without *Trichoderma*).
0 = not inoculated control (without *Trichoderma* and pathogen).

The plant growth promoting ability in the trials without the pathogen inoculation (Experiment VI) was calculated as:

(3) $RW_{fs} = FS_t/FS_0 \times 100$

Where,
fs = fresh shoot.
t = treatment with *Trichoderma*.
0 = not inoculated control (without *Trichoderma* and pathogen).

### *2.8 Statistical analysis*

Each trial was performed with four replicates per treatment, and included appropriate inoculated and non inoculated controls. The data were statistically analyzed as average value of different trials for each experiment type. All data were analyzed for significant differences by analysis of variance (ANOVA) and with Duncan's multiple range test at ($P< 0.05$) by using SPSS Software 13.0. The influence of disease severity on biomass production at the end of the trials was analysed by calculating the Pearson's correlation coefficient.

## 3 Results

The method used for infesting the soil with *P. ultimum* resulted in all trials in very high damping-off incidence, ranging from 37.5 to 75% dead plants in the inoculated controls (Table 2 and 5). The different trials carried out for the different experiment types provided consistent results.

When the different *Trichoderma* strains were applied at the same time with the pathogen, under growth chamber conditions (Experiment I, Table 2), the percent of dead plant of the control was 37.5%. A high number of strains provided a good disease control. The best control (87% efficacy) was provided by strains FC 25, 69, 71, 84 and the formulation Remedier. An efficacy ranging from 60 to 80% was provided by strains FC 1, 5, 6, 12, 16, 17, 24, 26, 27, 30, 35, 39, 40, 72, 79, 80 and 83, as calculated with formula 1. Twenty eight out of 41 tested *Trichoderma* provided a disease control of at least 50%. The formulation *Trichoderma viride* TV1 did not provide a satisfactory control (Table 2).

When the same experimental approach was used under greenhouse conditions, with a higher disease incidence in the control plots (48.3% diseased plants), only six strains out of 41 provided a disease control equal or higher than 50%, as calculated with formula 1. The best control was provided by strains FC 12 and FC 37, with a 69% reduction of disease incidence, followed by FC 1, FC 35, FC 83 and the formulation Remedier, which provided more than 50% disease reduction. The other commercial formulation of *Trichoderma* (TV 1) tested did not provide a satisfactory disease control (Table 2).

When the treatment with *Trichoderma* was carried out 7 days before soil infestation with *P. ultimum*, under growth chamber conditions, with 40% disease incidence in the inoculated control, 31 out of 41 strains showed an efficacy equal or higher than 50%, as calculated with formula 1. A complete disease control was provided by strains FC 26, 27, 37 and 69. The strains FC 18, 25, 30, 79, 80, 84 and the formulation Remedier provided an efficacy ranging between 80 and 94%. The formulation TV 1 provided a 63% control efficacy (Table 2). When the same experimental approach was adopted under greenhouse conditions, disease incidence was 45% in the inoculated control and 11 out of 41 *Trichoderma* strains provided an efficacy equal or higher than 50%, as calculated with formula 1. The best results, with a 72% efficacy, were provided by the strains FC 2, 12, 38, 39 and 72. Remedier provided only 44% disease control, while TV 1 was again less effective (Table 2).

In experiment V, carried out under greenhouse conditions, by applying *Trichoderma* by root dipping, disease incidence was very high, with 75% of dead plants in the inoculated control. Under these experimental conditions, only Remedier provided a satisfactory disease control, with 56% of efficacy as calculated with formula 1. All other strains were less effective (Table 2).

Biomass production was always significantly affected by the disease. In all trials, biomass was reduced as a consequence of damping-off (Table 3). A negative correlation between disease severity and biomass production was always observed (Table 4). When the plant growth promoting ability of the tested *Trichoderma* strains was tested in the absence of

Table 2: Effect of different *Trichoderma* spp. on damping-off of cucumber caused by *P. ultimum* in different experiments, expressed as percent of dead plants

| *Trichoderma* strain | *Trichoderma* co-applied with *P. ultimum* | | | | *Trichoderma* pre-applied with *P. ultimum* | | | | Root dipping | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Experiment I (growth chamber) | | Experiment II (glasshouse) | | Experiment III (growth chamber) | | Experiment IV (glasshouse) | | Experiment V (glasshouse) | |
| | Percent of dead plants (100%) | CE* | Percent of dead plants (100%) | CE | Percent of dead plants (100%) | CE | Percent of dead plants (100%) | CE | Percent of dead plants (100%) | CE |
| Not inoculated control | 0.0 ± 0.0 a ** | 100 | 0.0 ± 0.0 a | 100 | 0.0 ± 0.0 a | 100 | 0.0 ± 0.0 a | 100 | 0.0 ± 0.0 a | 100 |
| Inoculated control | 37.5 ± 0.4 g | 0 | 48.3 ± 0.5 d | 0 | 40.0 ± 0.5 e | 0 | 45.0 ± 0.5 c | 0 | 75.0 ± 0.9 ef | 0 |
| FC1 | 15.0 ± 0.5 abcde | 60 | 20.0 ± 0.9 abc | 59 | 20.0 ± 0.8 abcd | 50 | 27.5 ± 1.5 abc | 39 | 55.0 ± 1.1 bcde | 27 |
| FC2 | 17.5 ± 0.6 bcdef | 53 | 28.3 ± 1.1 bcd | 41 | 17.5 ± 0.8 abcd | 56 | 12.5 ± 0.7 ab | 72 | 55.0 ± 0.9 bcde | 27 |
| FC4 | 25.0 ± 1.3 defg | 33 | 38.3 ± 1.3 bcd | 21 | 20.0 ± 0.5 abcd | 50 | 27.5 ± 1.1 abc | 39 | 66.7 ± 1.2 def | 11 |
| FC5 | 12.5 ± 0.7 abcde | 67 | 43.3 ± 1.1 cd | 10 | 25.0 ± 0.9 bcde | 38 | 25.0 ± 0.9 abc | 44 | 50.0 ± 1.4 bcde | 33 |
| FC6 | 10.0 ± 0.8 abcd | 73 | 31.7 ± 1.1 bcd | 34 | 12.5 ± 0.7 abcd | 69 | 27.5 ± 1.5 abc | 39 | 80.0 ± 1.0 f | −7 |
| FC7 | 17.5 ± 0.6 bcdef | 53 | 30.0 ± 1.7 bcd | 38 | 25.0 ± 1.0 bcde | 38 | 22.5 ± 0.6 abc | 50 | 50.0 ± 1.0 bcde | 33 |
| FC8 | 22.5 ± 0.8 cdefg | 40 | 30.0 ± 0.6 bcd | 38 | 25.0 ± 0.7 bcde | 38 | 37.5 ± 1.1 bc | 17 | 46.7 ± 1.1 bcd | 38 |
| FC9 | 22.5 ± 0.4 cdefg | 40 | 48.3 ± 1.4 d | 0 | 27.5 ± 0.7 cde | 31 | 32.5 ± 0.9 bc | 28 | 50.0 ± 1.4 bcde | 33 |
| FC10 | 22.5 ± 0.4 cdefg | 40 | 48.3 ± 0.5 d | 0 | 25.0 ± 0.5 bcde | 38 | 40.0 ± 0.9 bc | 11 | 43.3 ± 1.0 bcd | 42 |
| FC12 | 10.0 ± 0.5 abcd | 73 | 15.0 ± 1.0 ab | 69 | 10.0 ± 0.8 abcd | 75 | 12.5 ± 0.7 ab | 72 | 46.7 ± 1.2 bcd | 38 |
| FC15 | 20.0 ± 0.9 bcdef | 47 | 30.0 ± 1.2 bcd | 38 | 10.0 ± 1.1 abcd | 75 | 40.0 ± 1.5 bc | 11 | 53.3 ± 1.3 bcde | 29 |
| FC16 | 10.0 ± 0.8 abcd | 73 | 41.7 ± 1.2 bcd | 14 | 15.0 ± 1.2 abcd | 63 | 30.0 ± 0.8 bc | 33 | 58.3 ± 1.4 cdef | 22 |
| FC17 | 10.0 ± 0.5 abcd | 73 | 31.7 ± 1.5 bcd | 34 | 20.0 ± 1.4 abcd | 50 | 22.5 ± 1.4 abc | 50 | 55.0 ± 1.0 bcde | 27 |
| FC18 | 17.5 ± 1.0 bcdef | 53 | 31.7 ± 1.2 bcd | 34 | 7.5 ± 0.5 abc | 81 | 30.0 ± 1.5 bc | 33 | 46.7 ± 1.2 bcd | 38 |
| FC19 | 20.0 ± 1.1 bcdef | 47 | 36.7 ± 1.3 bcd | 24 | 17.5 ± 1.2 abcd | 56 | 30.0 ± 1.2 bc | 33 | 40.0 ± 1.0 bc | 47 |
| FC23 | 22.5 ± 0.6 cdefg | 40 | 48.3 ± 0.9 d | 0 | 25.0 ± 0.5 bcde | 38 | 40.0 ± 1.4 bc | 11 | 60.0 ± 1.3 cdef | 20 |
| FC24 | 12.5 ± 0.5 abcde | 67 | 25.0 ± 1.1 abcd | 48 | 10.0 ± 1.1 abcd | 75 | 17.5 ± 1.2 abc | 61 | 61.7 ± 1.2 cdef | 18 |
| FC25 | 5.0 ± 0.5 ab | 87 | 31.7 ± 1.0 bcd | 34 | 7.5 ± 0.7 abc | 81 | 20.0 ± 1.4 abc | 56 | 53.3 ± 0.9 bcde | 29 |
| FC26 | 7.5 ± 0.5 abc | 80 | 48.3 ± 1.1 d | 0 | 0.0 ± 0.0 a | 100 | 30.0 ± 1.2 bc | 33 | 56.7 ± 1.6 cdef | 24 |
| FC27 | 15.0 ± 0.7 abcde | 60 | 25.0 ± 1.5 abcd | 48 | 0.0 ± 0.0 a | 100 | 30.0 ± 0.9 bc | 33 | 51.7 ± 1.4 bcde | 31 |
| FC30 | 12.5 ± 0.7 abcde | 67 | 36.7 ± 0.6 bcd | 24 | 7.5 ± 0.5 abc | 81 | 27.5 ± 1.1 abc | 39 | 58.3 ± 1.2 cdef | 22 |
| FC31 | 17.5 ± 0.6 bcdef | 53 | 25.0 ± 0.6 abcd | 48 | 12.5 ± 1.1 abcd | 69 | 25.0 ± 1.0 abc | 44 | 60.0 ± 1.3 cdef | 20 |
| FC35 | 10.0 ± 0.5 abcd | 73 | 20.0 ± 1.2 abc | 59 | 20.0 ± 1.1 abcd | 50 | 27.5 ± 1.1 abc | 39 | 55.0 ± 1.1 bcde | 27 |
| FC36 | 20.0 ± 0.8 bcdef | 47 | 33.3 ± 1.2 bcd | 31 | 20.0 ± 1.4 abcd | 50 | 40.0 ± 1.3 bc | 11 | 36.7 ± 1.3 bc | 51 |
| FC37 | 17.5 ± 0.6 bcdef | 53 | 15.0 ± 1.5 ab | 69 | 0.0 ± 0.0 a | 100 | 27.5 ± 0.9 abc | 39 | 48.3 ± 1.6 bcd | 36 |
| FC38 | 17.5 ± 0.6 bcdef | 53 | 33.3 ± 1.7 bcd | 31 | 12.5 ± 1.2 abcd | 69 | 12.5 ± 0.7 ab | 72 | 66.7 ± 1.2 def | 11 |
| FC39 | 12.5 ± 0.5 abcde | 67 | 35.0 ± 0.8 bcd | 28 | 17.5 ± 1.2 abcd | 56 | 12.5 ± 0.5 ab | 72 | 48.3 ± 1.6 bcd | 36 |
| FC40 | 12.5 ± 0.7 abcde | 67 | 35.0 ± 0.9 bcd | 28 | 10.0 ± 0.5 abcd | 75 | 40.0 ± 1.9 bc | 11 | 40.0 ± 1.4 bc | 47 |
| FC59 | 20.0 ± 0.8 bcdef | 47 | 26.7 ± 1.1 bcd | 45 | 25.0 ± 1.0 bcde | 38 | 37.5 ± 0.8 bc | 17 | 51.7 ± 1.1 bcde | 31 |
| FC67 | 25.0 ± 0.9 defg | 33 | 30.0 ± 1.2 bcd | 38 | 27.5 ± 0.5 cde | 31 | 25.0 ± 1.4 abc | 44 | 46.7 ± 1.4 bcd | 38 |
| FC68 | 32.5 ± 0.5 fg | 13 | 38.3 ± 1.2 bcd | 21 | 25.0 ± 0.5 bcde | 38 | 37.5 ± 1.4 bc | 17 | 50.0 ± 1.3 bcde | 33 |
| FC69 | 5.0 ± 0.5 ab | 87 | 30.0 ± 1.4 bcd | 38 | 0.0 ± 0.0 a | 100 | 17.5 ± 0.8 abc | 61 | 50.0 ± 1.3 bcde | 33 |
| FC70 | 27.5 ± 0.5 efg | 27 | 33.3 ± 1.4 bcd | 31 | 30.0 ± 0.5 de | 25 | 27.5 ± 0.9 abc | 39 | 51.7 ± 1.2 bcde | 31 |
| FC71 | 5.0 ± 0.5 ab | 87 | 35.0 ± 1.5 bcd | 28 | 10.0 ± 1.1 abcd | 75 | 40.0 ± 1.3 bc | 11 | 55.0 ± 1.5 bcde | 27 |
| FC72 | 12.5 ± 0.5 abcde | 67 | 25.0 ± 0.5 abcd | 48 | 12.5 ± 0.9 abcd | 69 | 12.5 ± 0.7 ab | 72 | 56.7 ± 1.3 cdef | 24 |
| FC79 | 15.0 ± 0.7 abcde | 60 | 25.0 ± 1.0 abcd | 48 | 7.5 ± 0.5 abc | 81 | 42.5 ± 1.5 c | 6 | 58.3 ± 1.1 cdef | 22 |
| FC80 | 12.5 ± 0.5 abcde | 67 | 31.7 ± 1.2 bcd | 34 | 2.5 ± 0.4 a | 94 | 22.5 ± 1.0 abc | 50 | 48.3 ± 1.6 bcd | 36 |
| FC83 | 15.0 ± 0.7 abcde | 60 | 23.3 ± 0.9 abcd | 52 | 10.0 ± 0.8 abcd | 75 | 32.5 ± 1.6 bc | 28 | 60.0 ± 1.3 cdef | 20 |
| FC84 | 5.0 ± 0.5 ab | 87 | 36.7 ± 1.3 bcd | 24 | 2.5 ± 0.4 a | 94 | 37.5 ± 1.4 bc | 17 | 56.7 ± 1.1 cdef | 24 |
| T.TV1 | 25.0 ± 0.7 defg | 33 | 38.3 ± 1.4 bcd | 21 | 15.0 ± 0.7 abcd | 63 | 32.5 ± 0.5 bc | 28 | 46.7 ± 1.4 bcd | 38 |
| Remedier | 5.0 ± 0.5 ab | 87 | 21.7 ± 1.1 abcd | 55 | 5.0 ± 0.7 ab | 88 | 25.0 ± 1.0 abc | 44 | 33.3 ± 1.2 b | 56 |

* Efficacy of *Trichoderma* in controlling damping-off calculated as shown in formula 1 (see the text).
** Values followed by different letters within a column differ significantly (Duncan's test $P < 0.05$).

Table 3: Biomass of cucumber, expressed as fresh weight, at the end of the different experiments

| *Trichoderma* strain | Biomass | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Trichoderma* co-applied with *P. ultimum* | | | | *Trichoderma* pre-applied with *P. ultimum* | | | | Root dipping | |
| | Experiment I (growth chamber) | | Experiment II (glasshouse) | | Experiment III (growth chamber) | | Experiment IV (glasshouse) | | Experiment V (glasshouse) | |
| | g | RW* | g | RW | g | RW | g | RW | g | RW |
| Not inoculated control | 6.1 ± 1.6 a** | 100 | 39.3 ± 7.6 a | 100 | 12.6 ± 0.7 a | 100 | 19.8 ± 7.7 a | 100 | 4.6 ± 0.7 a | 100 |
| Inoculated control | 3.1 ± 0.7 e | 0 | 21.7 ± 5.6 efg | 0 | 5.9 ± 1.6 i | 0 | 7.7 ± 1.3 f | 0 | 1.5 ± 1.6 fg | 0 |
| FC1 | 3.9 ± 0.9 bcde | 28 | 27.1 ± 4.4 bcde | 31 | 8.5 ± 2.7 bcdefghi | 39 | 10.8 ± 5.2 cdef | 26 | 2.6 ± 1.4 cdefg | 18 |
| FC2 | 4.1 ± 1.5 bcde | 34 | 25.0 ± 3.9 bcdefg | 19 | 8.2 ± 1.2 bcdefghi | 35 | 13.1 ± 2.6 bcdef | 44 | 2.3 ± 1.6 cdefg | 17 |
| FC4 | 3.2 ± 1.3 de | 5 | 26.4 ± 3.1 bcdef | 26 | 7.8 ± 1.6 cdefghi | 28 | 9.4 ± 4.7 def | 14 | 1.8 ± 1.6 efg | 6 |
| FC5 | 3.9 ± 0.8 bcde | 27 | 24.7 ± 6.6bcdefg | 17 | 7.3 ± 1.3 defghi | 21 | 13.2 ± 4.5 bcdef | 45 | 2.4 ± 1.5 bcdefg | 28 |
| FC6 | 4.8 ± 1.5 abc | 58 | 27.7 ± 4.4 bcd | 34 | 9.5 ± 2.1 bcdef | 54 | 11.6 ± 4.1 bcdef | 32 | 1.4 ± 1.2 g | –9 |
| FC7 | 4.0 ± 1.0 bcde | 32 | 26.6 ± 4.0 bcdef | 28 | 8.6 ± 2.7 bcdefghi | 41 | 12.2 ± 3.7 bcdef | 37 | 2.2 ± 0.9 bcdefg | 27 |
| FC8 | 3.3 ± 1.6 cde | 8 | 24.6 ± 5.6 bcdefg | 16 | 6.3 ± 2.1 hi | 6 | 9.4 ± 4.0 def | 14 | 2.5 ± 1.2 bcdefg | 31 |
| FC9 | 3.2 ± 1.3 de | 5 | 27.4 ± 5.2 bcd | 33 | 6.4 ± 1.5 hi | 7 | 13.1 ± 4.4 bcdef | 45 | 2.7 ± 1.9 bcdefg | 34 |
| FC10 | 3.3 ± 1.4 cde | 9 | 24.7 ± 5.3 bcdefg | 17 | 6.2 ± 2.1 hi | 4 | 10.7 ± 4.0 cdef | 24 | 2.6 ± 1.3 bcde | 36 |
| FC12 | 4.8 ± 1.3 abc | 57 | 23.3 ± 2.9 bcdefg | 9 | 9.7 ± 3.2 bcde | 56 | 12.9 ± 3.9 bcdef | 43 | 2.3 ± 1.2 bcdefg | 32 |
| FC15 | 3.9 ± 1.4 bcde | 29 | 26.9 ± 5.9 bcde | 29 | 9.9 ± 3.6 abcd | 60 | 11.6 ± 4.6 bcdef | 32 | 3.1 ± 2.1 bcdefg | 27 |
| FC16 | 4.3 ± 1.1 bcde | 41 | 25.8 ± 3.6 bcdefg | 23 | 7.3 ± 3.0 defghi | 20 | 10.8 ± 2.6 cdef | 25 | 1.8 ± 1.1 cdefg | 19 |
| FC17 | 4.5 ± 0.9 bcde | 46 | 30.3 ± 5.4 b | 49 | 7.9 ± 3.4 bcdefghi | 30 | 15.3 ± 4.0 abc | 63 | 2.6 ± 2.1 bcdefg | 27 |
| FC18 | 4.1 ± 1.2 bcde | 35 | 29.5 ± 2.6 b | 44 | 8.1 ± 1.8 bcdefghi | 34 | 13.8 ± 6.6 bcde | 50 | 2.6 ± 1.4 bcde | 37 |
| FC19 | 3.9 ± 1.5 bcde | 26 | 25.1 ± 6.0 bcdefg | 19 | 8.8 ± 3.1 bcdefghi | 43 | 11.1 ± 4.4 bcdef | 28 | 2.8 ± 1.1 bcd | 41 |
| FC23 | 3.2 ± 1.0 de | 5 | 28.6 ± 4.6 bc | 39 | 6.5 ± 2.2 ghi | 8 | 8.4 ± 3.8 ef | 5 | 2.4 ± 2.1 cdefg | 16 |
| FC24 | 4.8 ± 1.5 abc | 58 | 26.7 ± 5.4 bcdef | 28 | 8.4 ± 2.3 bcdefghi | 38 | 13.0 ± 5.0 bcdef | 44 | 1.8 ± 0.9 dfg | 13 |
| FC25 | 5.0 ± 1.1 ab | 63 | 26.2 ± 7.2 bcdefg | 25 | 9.0 ± 2.1 bcdefgh | 47 | 9.5 ± 4.0 def | 14 | 2.1 ± 0.7 bcdefg | 23 |
| FC26 | 4.6 ± 0.8 bcde | 49 | 20.8 ± 5.6 g | –5 | 10.2 ± 1.6 abcd | 65 | 11.1 ± 6.2 bcdef | 28 | 2.4 ± 1.5 cdefg | 20 |
| FC27 | 4.6 ± 2.0 bcde | 49 | 21.3 ± 2.7 fg | –2. | 9.9 ± 2.7 abcd | 60 | 13.0 ± 3.1 bcdef | 43 | 2.4 ± 1.6 bcdefg | 28 |
| FC30 | 4.4 ± 1.5 bcde | 43 | 26.6 ± 6.8 bcdef | 28 | 9.6 ± 2.8 bcdef | 55 | 9.7 ± 3.7 def | 16 | 2.3 ± 2.1 cdefg | 16 |
| FC31 | 4.1 ± 1.1 bcde | 35 | 27.3 ± 3.6 bcd | 32 | 8.4 ± 3.2 bcdefghi | 38 | 14.3 ± 3.3 bcd | 54 | 1.7 ± 1.1 defg | 15 |
| FC35 | 5.2 ± 1.8 ab | 70 | 29.9 ± 3.8 b | 47 | 6.6 ± 1.8 efghi | 11 | 12.4 ± 3.5 bcdef | 39 | 2.5 ± 1.4 bcde | 35 |
| FC36 | 3.7 ± 1.6 bcde | 22 | 26.8 ± 5.9 bcdef | 29 | 7.9 ± 3.6 bcdefghi | 30 | 9.6 ± 4.9 def | 16 | 2.6 ± 1.3 bcde | 40 |
| FC37 | 4.5 ± 1.2 bcde | 48 | 27.2 ± 4.2 bcd | 31 | 10.4 ± 1.9 abc | 68 | 11.6 ± 3.8 bcdef | 32 | 2.8 ± 2.0 bcde | 39 |
| FC38 | 4.1 ± 1.3 bcde | 36 | 23.7 ± 3.4 cdefg | 11 | 8.4 ± 2.4 bcdefghi | 38 | 14.2 ± 4.2 bcd | 54 | 2.0 ± 1.8 defg | 12 |
| FC39 | 4.6 ± 0.7 bcde | 51 | 25.4 ± 4.9 bcdefg | 21 | 7.8 ± 2.7 defghi | 29 | 16.3 ± 5.0 ab | 71 | 2.6 ± 1.9 bcde | 35 |
| FC40 | 4.1 ± 1.0 bcde | 33 | 24.9 ± 6.6 bcdefg | 18 | 8.3 ± 1.3 bcdefghi | 37 | 10.3 ± 5.8 cdef | 21 | 2.8 ± 1.5 bc | 50 |
| FC59 | 3.2 ± 1.0 de | 5 | 25.8 ± 4.7 bcdefg | 23 | 7.3 ± 2.4 cdefghi | 21 | 12.8 ± 5.3 bcdef | 42 | 2.6 ± 1.4 bcdefg | 25 |
| FC67 | 3.2 ± 0.6 de | 5 | 27.0 ± 3.9 bcde | 30 | 6.4 ± 2.2 ghi | 7 | 11.4 ± 3.8 bcdef | 31 | 2.7 ± 1.4 bcdefg | 32 |
| FC68 | 3.2 ± 0.6 de | 5 | 25.9 ± 7.2 bcdefg | 24 | 6.5 ± 2.1 fghi | 10 | 8.2 ± 3.8 f | 4 | 1.9 ± 1.3 bcdefg | 23 |
| FC69 | 5.3 ± 1.6 ab | 73 | 26.3 ± 4.6 bcdefg | 26 | 10.9 ± 2.5 ab | 75 | 12.3 ± 2.2 bcdef | 38 | 1.9 ± 1.2 bcdefg | 24 |
| FC70 | 3.2 ± 0.5 de | 5 | 29.7 ± 5.4 b | 45 | 6.4 ± 2.2 ghi | 7 | 10.5 ± 1.3 cdef | 23 | 1.9 ± 0.9 cdefg | 20 |
| FC71 | 4.9 ± 1.6 abc | 59 | 24.8 ± 4.5 bcdefg | 18 | 9.1 ± 3.2 bcdefgh | 48 | 9.5 ± 4.9 def | 14 | 2.3 ± 1.8 bcdefg | 29 |
| FC72 | 4.7 ± 1.8 abcd | 54 | 20.8 ± 5.0 g | –5 | 9.4 ± 2.8 bcdefg | 53 | 12.6 ± 4.6 bcdef | 40 | 1.6 ± 0.8 defg | 13 |
| FC79 | 4.6 ± 1.4 bcde | 51 | 27.5 ± 6.1 bcd | 33 | 9.9 ± 4. 2 abcd | 60 | 10.2 ± 3.8 cdef | 21 | 2.2 ± 1.5 cdefg | 18 |
| FC80 | 4.3 ± 0.8 bcde | 42 | 29.8 ± 4.3 b | 46 | 10.3 ± 2.1 abcd | 66 | 15.6 ± 3.3 abc | 65 | 2.1 ± 1.3 bcdefg | 31 |
| FC83 | 4.2 ± 1.3 bcde | 38 | 28.7 ± 7.0 bc | 40 | 9.5 ± 1.9 bcdef | 55 | 9.0 ± 4.9 def | 11 | 1.9 ± 1.3 cdefg | 18 |
| FC84 | 4.6 ± 1.1 bcde | 51 | 23.7 ± 4.4 cdefg | 11 | 10.5 ± 1.3 abc | 69 | 8.7 ± 5.7 ef | 8 | 2.2 ± 1.3 cdefg | 20 |
| T.TV1 | 3.8 ± 0.8 bcde | 24 | 22.4 ± 3.4 defg | 4 | 7.7 ± 2.2 cdefghi | 27 | 11.1 ± 2.7 bcdef | 28 | 2.6 ± 1.5 bcdefg | 33 |
| Remedier | 4.6 ± 1.0 bcde | 51 | 27.1 ± 2.5 bcde | 31 | 10.2 ± 2.8 abcd | 65 | 9.8 ± 3.2 def | 17 | 3.2 ± 1.5 b | 54 |

* Relative value of the weight calculated as shown in formula 2 (see the text).
** Values followed by different letters within a column differ significantly (Duncan's test $P < 0.01$).

Table 4: Correlation between disease index and corresponding biomass in the different experiments

| Type of experiment | Pearson's coefficients | Significance of correlation |
|---|---|---|
| I | −0.411 | * |
| II | −0.185 | * |
| III | −0.750 | * |
| IV | −0.577 | * |
| V | −0.717 | * |
| VII | −0.322 | * |

* Significant correlation ($P \leq 0.01$) with Pearson's Test.

the pathogen (Experiment VI, Table 5), five strains out of 41, including the commercial formulations Remedier, produced an increase of biomass higher than 130%, as calculated with formula 3 (Table 5).

When the twelve strains of *Trichoderma* providing the best and more consistent results in the different trials were tested under greenhouse conditions in comparison with the commercial formulation Remedier (Experiment VII, Table 6), all tested strains provided a disease biocontrol efficacy higher than 50%. Four of them (FC 7, 38, 69 and 80) provided a 95% disease control, followed by FC 39 (91% efficacy). They all were slightly more effective than the formulation Remedier (Table 6).

*Trichoderma* strains coded FC12 showed significant and consistent biocontrol activity under all application methods (soil mixing and root dipping) in the presence of pathogen and manifested plant growth promotion ability in the absence of the pathogen.

## 4 Discussion

The results obtained in this study confirm the good antagonistic attitude of *Trichoderma* spp., a fungus very widely studied as biocontrol agent (Papavizas 1985; Harman 2000). *Trichoderma* spp. plays a major role as biocontrol agent, owing to its capabilities of ameliorating crop-yields by multiple roles, being able to control several pathogens and also to promote plant growth (Harman et al. 2004; Verma et al. 2007). *Trichoderma* spp. share almost 50% of fungal BCAs market, mostly as soil treatment for growth enhancement and this makes them interesting candidates to investigate (Whipps et al. 2001). The strains tested in this work, isolated from a soilless system, confirmed in general a good antagonistic activity.

More strains were active when tested under growth chamber conditions, while under the most stringent greenhouse conditions a reduced number of strains showed an efficacy higher than 50%. The best strains provided a consistent activity. Biocontrol activity was much higher when *Trichoderma* was applied to the soil a week before soil infestation with the pathogen. This interval permit to the antagonist to colonise the soil before the pathogen is introduced, thus resulting in a better ability to reduce the disease. This confirms previous observations on the need for *Trichoderma* to establish in the soil or in the planting mix (Lewis and Lumsden 2001). The level of disease control provided by *Trichoderma* was much lower when the antagonistic strains were applied by root dipping, probably because in this case the antagonist had no time to get established before infection started.

The best strains performed even slightly better than one of the commercial formulation of Remedier (*T. harzianum* strain ICC012 + *Trichoderma viride* strain ICC080), thus confirming that *Trichoderma* does not need very much complex formulations in order to be effective. It was reported that *T. hamatum* and *T. virens* were effective in preventing *Rhizoctonia solani* (> 80%) and pathogen reduction (> 75%) under greenhouse studies when applied with bran flakes or alginate pellets (Lewis et al. 1990). In general, conidia without any amendments were ineffective (Verma et al. 2007) and the biological

Table 5: Evaluation of the plant growth promoting ability of different strains of *Trichoderma*, applied to the soil in the absence of the pathogen (Experiment VI)

| *Trichoderma* strain | Biomass | |
|---|---|---|
| | g | RW* |
| Not inoculated control | 9.9 ± 1.4 defg** | 100 |
| FC1 | 11.7 ± 2.5 abcdef | 119 |
| FC2 | 11.5 ± 2.3 abcdefg | 117 |
| FC4 | 9.7 ± 1.4 efg | 99 |
| FC5 | 12.6 ± 1.7 abcd | 128 |
| FC6 | 12.1 ± 2.0 abcde | 123 |
| FC7 | 11.0 ± 1.6 abcdefg | 111 |
| FC8 | 11.5 ± 1.9 abcdefg | 117 |
| FC9 | 11.5 ± 2.3 abcdefg | 117 |
| FC10 | 11.5 ± 2.2 abcdefg | 116 |
| FC12 | 12.3 ± 2.6 abcde | 124 |
| FC15 | 12.2 ± 1.4 abcde | 123 |
| FC16 | 11.0 ± 1.9 abcdefg | 111 |
| FC17 | 12.0 ± 2.1 abcdef | 121 |
| FC18 | 11.0 ± 2.0 abcdefg | 111 |
| FC19 | 8.9 ± 2.8 g | 90 |
| FC23 | 11.5 ± 1.1 abcdefg | 116 |
| FC24 | 11.7 ± 1.7 abcdef | 118 |
| FC25 | 9.3 ± 3.9 fg | 95 |
| FC26 | 12.0 ± 2.1 abcdef | 122 |
| FC27 | 13.6 ± 1.4 a | 138 |
| FC30 | 11.5 ± 3.0 abcdefg | 117 |
| FC31 | 10.8 ± 1.3 bcdefg | 109 |
| FC35 | 10.0 ± 1.8 defg | 102 |
| FC36 | 11.7 ± 2.5 abcdef | 119 |
| FC37 | 11.0 ± 2.1 abcdefg | 112 |
| FC38 | 12.4 ± 2.3 abcde | 126 |
| FC39 | 10.8 ± 1.9 bcdefg | 110 |
| FC40 | 11.8 ± 2.2 abcdef | 119 |
| FC59 | 13.1 ± 1.1 ab | 132 |
| FC67 | 10.6 ± 0.9 bcdefg | 108 |
| FC68 | 10.3 ± 1.8 cdefg | 104 |
| FC69 | 12.1 ± 2.1 abcde | 122 |
| FC70 | 11.4 ± 3.3 abcdefg | 116 |
| FC71 | 12.3 ± 2.0 abcde | 125 |
| FC72 | 9.3 ± 1.9 fg | 95 |
| FC79 | 11.5 ± 0.9 abcdefg | 117 |
| FC80 | 13.2 ± 2.3 ab | 134 |
| FC83 | 11.1 ± 2.1 abcdefg | 113 |
| FC84 | 12.9 ± 1.8 abc | 131 |
| T.TV1 | 12.3 ± 1.6 abcde | 125 |
| Remedier | 12.9 ± 1.0 abc | 131 |

* Relative value of the weight calculated as shown in formula 3 (see the text).
** Values followed by different letters within a column differ significantly (Duncan's test $P < 0.05$).

Table 6: Effect of twelve selected strains of *Trichoderma* and the commercial formulation Remedier on damping-off of cucumber caused by *Pythium ultimum*

| *Trichoderma* strain | Experiment VII | | | |
|---|---|---|---|---|
| | Percent of dead plants (100%) | CE* | Biomass (g) | RW** |
| Not inoculated control | 0.0 ± 0.0 a*** | 100 | 15.5 ± 7.9 a | 100 |
| Inoculated control | 36.7 ± 0.7 e | 0 | 8.3 ± 4.7 b | 0 |
| FC1 | 15.0 ± 0.6 cd | 59 | 10.9 ± 4.6 ab | 36 |
| FC2 | 8.3 ± 0.5 abcd | 77 | 12.5 ± 7.2 ab | 59 |
| FC6 | 11.7 ± 0.7 bcd | 68 | 12.1 ± 7.6 ab | 52 |
| FC7 | 1.7 ± 0.3 ab | 95 | 13.6 ± 6. 2 ab | 74 |
| FC12 | 11.7 ± 0.7 bcd | 68 | 10.7 ± 5.2 ab | 33 |
| FC19 | 5.0 ± 0.5 ab | 86 | 11.0 ± 3.7 ab | 38 |
| FC24 | 16.7 ± 0.6 d | 55 | 11.1 ± 5.6 ab | 39 |
| FC38 | 1.7 ± 0.3 ab | 95 | 12.8 ± 7.1 ab | 62 |
| FC39 | 3.3 ± 0.4 ab | 91 | 13.1 ± 6.4 ab | 67 |
| FC69 | 1.7 ± 0.3 ab | 95 | 13.6 ± 6.1 ab | 74 |
| FC72 | 8.3 ± 0.8 abcd | 77 | 10.8 ± 4.8 ab | 35 |
| FC80 | 1.7 ± 0.3 ab | 95 | 13.5 ± 7.1 ab | 73 |
| Remedier | 6.7 ± 0.7 abc | 82 | 11.9 ± 5.8 ab | 49 |

* Efficacy of *Trichoderma* in controlling damping-off calculated as shown in formula 1(see the text).
** RW relative value of the weight calculated as shown in formula 2 (see the text).
*** Values followed by different letters within a column differ significantly (Duncan's test $P < 0.05$).

control activity is formulation dependent (Bae and Knudsen 2005). Also with a simple food base, mycelium used in our study for soil treatment showed better biocontrol activity than conidia applied by root dipping.

The plant growth promoting activity of many *Trichoderma* spp. is well known (Naseby et al. 2001; Whipps 2001; Harman et al. 2004). In our study, 95.1% (37 out of 41 strains, with the exception of strains FC4, FC19, FC25 and FC72) of the *Trichoderma* strains significantly promoted the biomass of the cucumber seedlings. The strains coded FC27, FC59, FC80, FC84 and the formulation Remedier significantly increased the fresh shoot weight compared with the control. The capability of *Trichoderma* spp. to colonize roots is very well known. This root colonization also increases the growth of roots and of the entire plant, thereby increasing plant productivity and yields. They also help plants to overcome abiotic stresses and improve nutrient uptake (Harman et al. 2004).

In conclusion, the thirty-nine strains from the recycled substrates of soilless crops grown showed different biocontrol and plant growth promotion degree on cucumber under different application methods. Thirty-five *Trichoderma* strains promoted the growth of the cucumber seedlings compared with the control in the absence of *Pythium ultimum*. In particular, application of antagonists by soil mixing one week before soil infestation with the pathogen provided better biocontrol activity in comparison with others methods of application (root dipping, and antagonists co-applied with pathogen). Antagonists showed better biocontrol capability under growth chamber conditions than greenhouse conditions. Twelve selected strains provided more than satisfactory disease control and plant growth promotion under greenhouse conditions.

For the crops that are produced in greenhouses and later transplanted into the field, a window of opportunity is available during greenhouse cultivation to colonize roots with *Trichoderma* spp. prior to move plants into the cultivation systems.

Our study support the selected use of biocontrol agents to reduce Pythium root rot and show that one commercial formulation is effective. The concept of combining a biological control agent in a planting mix at seeding is an efficient, low cost means to provide to short-cycle crops disease protection. Our study also indicates that effective *Trichoderma* spp. strains can be obtained from re-used substrates, thus providing a new generation of biocontrol agents, adapted to the new cultivation methods. Their good activity explains the suppressiveness reported in soilless systems (Postma et al. 2005; Clematis et al. 2009).

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## References

Bae, Y.S., G.R. Knudsen, 2005: Soil microbial biomass influence on growth and biocontrol efficacy of *Trichoderma harzianum*. Biol. Control **96**, 236-242.

Calvo-Bado, L.A., T.R. Petitt, N.R. Parsons, G.M. Petch, J.A.W. Morganorgan, J.M. Whipps, 2003: Spatial and temporal analysis of the microbial community in slow sand filters used for treating horticultural irrigation water. Appl. Environ. Microbiol. **69**, 2116-2125.

Calvo-Bado, L. A., G. Petch, N.R. Parsons, J.A.W. Morgan, T.R. Petitt, J.M. Whipps, 2006: Microbial community responses associated with the development of oomycete plant pathogens on tomato roots in soilless growing systems. J. Appl. Microbiol. **100**, 1194-1207.

Cuartero, J., H. Laterrot, J.C. Van Lenteren, 1999: Host-plant resistance to pathogens and arthropod pests. In: R. Albajes, M.L. Gullino, J.C. Van Lentere, Y. Elad (eds.): Integrated Pest Management in Greenhouse Crops, Vol. 14, pp. 124-138. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Garibaldi, A., F. Clematis, M.L. Gullino, 2009: Microrganismi isolate da substrati di coltura riciclati per il fuori suolo possono contenere il marciume del colletto del pomodoro. Protezione delle colture **2** (2), 63.

Clematis, F., A. Minuto, M.L. Gullino, A. Garibaldi, 2009: Suppressiveness to *Fusarium oxysporum* f. sp. *radicis-lycopersici* in re-used perlite and perlite-peat substrates in soilless tomatoes. Biolo. Control **48**, 108-114.

Garibaldi, A., A. Minuto, V. Grasso, M.L. Gullino, 2003: Application of selected antagonistic strains against *Phytophthora cryptogea* on gerbera in closed soilless systems with disinfestation by slow sand filtration. Crop Prot. **22**, 1053-1061.

Harman, G.E., 2000: Myths and dogmas of biocontrol changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Dis. **84**, 377-393.

Harman, G.E., C.R. Howell, A. Viterbo, I. Chet, M. Lorito, 2004: *Trichoderma* species – opportunistic, avirulent plant symbionts. Nature Rev. Microbiol. **2**, 43-56.

Koenning, S.C., C. Overstreet, J.S. Noling, P.A. Donald, J.O. Becker, B.A. Fortnum, 1999: Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994: J. Nematol. **31** (4 s), 587-618.

Levis, J.A., T.H. Barksdale, G.C. Papavizas, 1990: Greenhouse and field studies on the biological control of tomato fruit rot caused by *Rhizoctonia solani*. Crop Prot. **9**, 8-14.

Levis, J.A., R.D. Lumsden, 2001: Biocontrol of damping-off of greenhouse-grown crops caused by *Rhizoctonia solani* with a formulation of *Trichoderma* spp. Crop Prot. **20**, 49-56

Martin, F.N., J.E. Loper, 1999: Soilborne plant diseases caused by *Pythium* spp.: ecology, epidemiology, and prospects for biological control. Crit. Rev. Plant Sci. **18**, 11-181.

Minuto, A., F. Clematis, M.L. Gullino, A. Garibaldi, 2007: Induced suppressiveness to *Fusarium oxysporum* f.sp. *radicis-lycopersici* in rockwool substrate used in closed soilless systems. Phytoparasitica **35**, 77-85.

Naseby, D.C., J.A. Pascual, J.M. Lynch, 1999: Effect of biocontrol strains of *Trichoderma* on plant growth, *Pythium ultimum* populations, soil microbial communities and soil enzyme activities. J. Appl. Microbiol. **88**, 161-169.

Papavizas, G.C., 1985: *Trichoderma* and *Gliocladium*: Biology, ecology and the potential for biocontrol. Annu. Rev. Phytopathol. **23**, 23-54.

Paulitz, T.C., R.R. Bélanger, 2001: Biological control in greenhouse systems. Annu. Rev. Phytopathol. **39**, 103-133.

Postma, J., 2009: The status of biological control of plant diseases in soilless cultivation. In: U. Gisi, I. Chet, M.L. Gullino (eds.): Recent Developments in Management of Plant Diseases, Springer Netherlands, Dordrecht, The Netherlands, in press.

Postma, J., M.J.E.I.M. Willemsen-De Klein, J.D. Van Elsas, 2000: Effect of the indigenous microflora on the development of root and crown rot caused by *Pythium aphanidermatum* in cucumber grown in rockwool. Phytopathology **90**, 125-133.

Postma, J., B.P.J. Geraas, R. Pastoor, D. Van Elsas, 2005: Characterization of the microbial community involved in the suppression of *Pythium aphanidermatum* in cucumber grown on rockwool. Phytopathology **95**, 808-818.

Srinivasan, K., G. Gilardi, A. Garibaldi, M.L. Gullino, 2009: Bacterial antagonists from used rockwool soilless substrates suppress Fusarium wilt of tomato. J. Plant Pathol. **91**, 145-152.

Stanghellini, M.E., S.L. Rasmussen, 1994: Hydroponics: a solution for zoosporic pathogens. Plant Dis. **78**, 1129-1138.

Van Os, E.A., J. Postma, 2000: Prevention of root diseases in closed soilless growing systems by microbial optimization and slow sand filtration. Acta Hortic. **532**, 97-102.

Van Os, E.A., J. Postma, T.R. Pettit, W. Wohanka, 2004: Microbial optimization in soilless cultivation: a replacement for methyl bromide. Acta Hortic. **635**, 47-58.

Verma, M., S. Brar, R.D. Tyagi, R.Y. Surampalli, J.R. Valero, 2007: Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control. Biochem. Eng. J. **37**, 1-20.

Whipps, J.M., 2001: Microbial interactions and biocontrol in the rhizosphere. J. Exp. Bot. **52**, 487-511.

Zitter, T.A., D.L. Hopkins, C.E. Thomas, 1996: Compendium of Cucurbit Disease. APS Press, St. Paul, MN, USA.