

EVALUATION OF *Trichoderma* spp. AS A BIOCONTROL AGENT AGAINST *Phytophthora parasitica*

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Abstract

The genus *Phytophthora* causes great damages to agricultural production, especially to potatoes and tomatoes cultures. To face these losses, it is of interest to reduce or inhibit the activity of this aggressive pathogen. Some species of *Trichoderma* have great potential for the biological control of several plant pathogens, including diseases caused by *Phytophthora parasitica*, *Rhizoctonia solani*, *Pythium ultimum*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum* etc. The purpose of this study was to assess the biocontrol efficacy of three *Trichoderma* strains (*T. asperellum* T36, *T. asperellum* T50, *T. harzianum* T78) against *Phytophthora parasitica*. In vitro tests were carried out using dual culture technique. In vivo tests were carried out with pepper seedlings (*Capsicum annuum* cv. Lamuyo) and conidial standard suspension of *Trichoderma* isolates as biocontrol agents. Of the three *Trichoderma* isolates tested for their effectiveness against mycelial growth of pathogen, *T. asperellum* T36 (81.2%) exhibited maximum inhibition of *P. parasitica*, compared with the control, followed by *T. asperellum* T50 (79.6%) and *T. harzianum* T78 (77.7%). Likewise for the in vivo test, the inoculation of the *Trichoderma* biocontrol agent showed that the percentage of dead plants seedlings was significant reduced. *T. asperellum* T36 is a useful biological alternative to pesticides for the control of *P. parasitica* in pepper seedlings.

Key words: biocontrol, *Phytophthora parasitica*, *Trichoderma*.

INTRODUCTION

The genus *Phytophthora* is classified as oomycetes and is an important plants pathogen. *Phytophthora* spp. has a severe economic impact on agriculture, the induced economic losses being approximately 170 billion US dollars (Haverkort et al., 2009; Wu et al., 2012; Fatima et al., 2015). *Phytophthora* spp. grows through the root and the stem system of plant, destroying it by, causing root and stem rot. The plants diseases were primarily managed with fungicide applications but the phytopathogens have developed resistance.

For the management of fungal crop diseases another effective way is the biological control. Some microorganisms have the ability to antagonize pathogens (Fatima et al., 2015). The genus *Trichoderma* is known as a biocontrol agent (BCAs) and can suppress diseases via several mechanisms including antibiosis, competence, mycoparasitism, enzyme activity,

induced plant defence, (Papavizas and Lumsden, 1980; Howell, 2003).

In this study we evaluated the biocontrol potential of three *Trichoderma* strains, *T. asperellum* T36, *T. asperellum* T50, *T. harzianum* T78 against *Phytophthora parasitica*.

MATERIALS AND METHODS

Fungal isolates

The fungal virulent pathogen *Phytophthora parasitica* belongs to Culture Collections of CEBAS-CSIC Institute, Murcia, Spain. The strain was isolated from pepper plants showing disease symptoms and was maintained on pea agar medium at 28°C for 7 days.

The biological control agents (BCAs), *Trichoderma asperellum* T36, *Trichoderma asperellum* T50 belong to Culture Collections of ICECHIM Institute, Romania. Both strains were isolated from soil. *Trichoderma harzianum* T78 was obtained from Culture Collections of CEBAS-CSIC Institute.

The antagonistic strains were grown and maintained on potato dextrose agar medium (PDA) at 26 to 28°C for 7 days.

Dual culture technique

The antagonistic activity of *Trichoderma* strains against *P. parasitica* was evaluated by dual culture method described by Edington et al., 1971.

Plates of rye agar medium were inoculated with a 5 mm disc from five-day-old cultures of each *Trichoderma* strains which was placed 2 cm away from the periphery of the plate. Same size agar disc of *P. parasitica* was placed at the opposite side of *Trichoderma* sample, 2 cm away from the periphery of the plate. The control contains only the fungal phytopathogen. The cultures were incubated at 28 °C in darkness. After 5 days of incubation the growth of the fungi was recorded by measuring the radial growth of the pathogens. The inhibition percentage was calculated in relation with the control by using the formula:

$$I (\%) = (C-T) / C \times 100$$

where,

I = percent of inhibition; C = diameter of radial growth of *P. parasitica* in control; T = diameter of radial growth of *P. parasitica* in the presence of antagonistic strains.

Greenhouse experiment

In this study four treatments were formulated: T1 - compost, black peat and *T. harzianum* T78 (10^6 ufc g⁻¹); T2 - compost, black peat and *T. asperellum* T36 (10^6 ufc g⁻¹); T3 - Compost and black peat; T4 - Black peat.

Seeds of pepper (*Capsicum annum* cv. Lamuyo) were sterilized in 10% NaClO for 3 minutes and rinsed for three times in sterile water. The pepper seeds were planted in 150 pots, with one seed per pot (Figure 1).



Figure 1. The pepper seedlings in growth chamber

For each treatment forty-eight pots were used: 18 pots were used as control (without pathogen) and thirty pots were inoculated with *P. parasitica*. All plants were incubated in growth chamber under daylight conditions. After 14 days of sowing the seedlings were inoculated with *P. parasitica* (10^5 ufc g⁻¹, in all treatments). The seedlings were harvested four weeks after sowing. The number of infected plants was recorded every day.

RESULTS AND DISCUSSIONS

In vitro growth inhibition of *P. parasitica* by *Trichoderma* strains

The antagonism of *Trichoderma* strains against *P. parasitica* was observed in dual culture.

Figure 2 and Table 1 show the inhibition of *P. parasitica* by the *Trichoderma* strains. The results of dual culture demonstrated that *T. asperellum* T36 determined the maximum growth inhibition of *P. parasitica* (81.2%), followed by *T. asperellum* T50 (79.6%) and *T. harzianum* T78 (77.7%) (Table 1).

Table 1. Inhibitory effects of *Trichoderma* isolates against *P. parasitica*

Antagonistic isolates	Inhibition in dual culture (%)
<i>T. asperellum</i> T36	81.2
<i>T. asperellum</i> T50	79.6
<i>T. harzianum</i> T78	77.7

Inhibition of *Phytophthora* growth by *Trichoderma* spp. was also reported previously by (Fatima et al., 2015; Jiang et al., 2016).

Greenhouse trial

The results of the greenhouse experiment showed that the inoculation of *Trichoderma* strains in the growing media was an effective treatment to control *P. parasitica* in pepper seedlings.

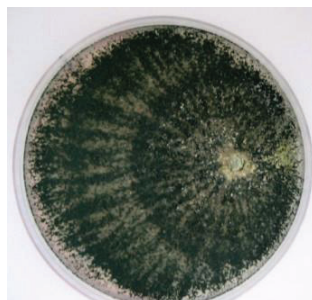
Figure 3 demonstrated that the treatment T2 (fortified with *T. asperellum* T36) showed the lowest percentage of dead pepper seedlings induced by *P. parasitica* (54%), followed by treatment T1, T3 and T4 (66%, 80% and 92.66%, respectively).



Control - *P. parasitica* alone



Dual plate culture of *T. asperellum* T36 and *P. parasitica*



T. asperellum T36 (control)



Dual plate culture of *T. asperellum* T50 and *P. parasitica*



T. asperellum T50 (control)



Dual plate culture of *T. harzianum* T78 and *P. parasitica*



T. harzianum T78 (control)

Figure 2. Antagonistic test between *Trichoderma* strains and *Phytophthora parasitica*

T. asperellum T36 (treatment T2) was more effective than *T. harzianum* T78 (treatment T1) in reducing the percentage of dead pepper seedlings.

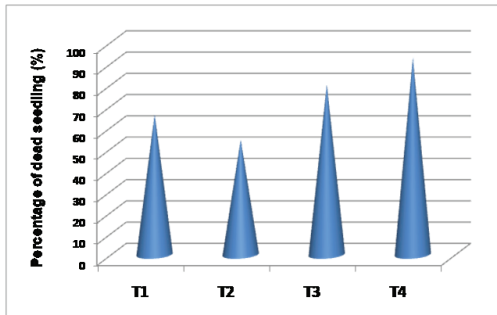


Figure 3. Percentage of dead pepper seedlings by *P. parasitica*

Similar results were reported previously by (Mpika et al., 2009; Segarra et al., 2013). Figure 4 shows the survival of a pepper seedling after harvesting (a) and a dead pepper seedling with stem rot (b).



a) Pepper seedling survival



b) Dead pepper seedling (stem destroyed)

Figure 4. Pepper seedling after harvesting

CONCLUSIONS

All three isolates of *Trichoderma* demonstrated biological control activity against *P. parasitica*. In the growth chamber experiment, the disease severity was reduced by *T. asperellum* T36 and

T. harzianum T78. Treatment T2 with *T. asperellum* T36 was the most effective treatment to control *P. parasitica* in pepper seedlings.

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