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Short communication. Selection of *Trichoderma* spp. isolates against *Rhizoctonia solani*

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Abstract

The aim of this work was to select regional isolates of *Trichoderma* spp. active in the antagonism against *Rhizoctonia solani*, fungal causal agent of seedling death in vegetable crops of northeast Argentina. The antagonistic activity of nine monosporic isolates of *Trichoderma* spp. was evaluated in dual culture and the production of non-volatile metabolites in eight of them was evaluated using the cellophane test. All isolates significantly diminished the mycelial growth of *R. solani* in dual culture. Isolates 1, 3, 4 and 7 were more efficient at producing non-volatile metabolites. These isolates have been selected to evaluate their antagonistic potential to reduce melon seedling death in greenhouse and brought about a significant reduction of the disease caused by *R. solani*. The results showed the possibility of using regional *Trichoderma* spp. isolates to reduce melon seedling death by *R. solani* in warm and wet climates in the northwest of the Corrientes province.

Key words: antagonism, *in vitro*, greenhouse, melon, soilborne pathogens, biological control.

Resumen

Nota corta. Selección de aislamientos de *Trichoderma* spp. contra *Rhizoctonia solani*

El objetivo de este trabajo fue seleccionar en Corrientes (Argentina) aislamientos regionales de *Trichoderma* spp. eficaces contra *Rhizoctonia solani*, agente fúngico causal de la muerte de plántulas en cultivos hortícolas. Se evaluó la actividad antagónica de nueve aislamientos monospóricos de *Trichoderma* spp. en cultivos duales y de ocho por producción de metabolitos no volátiles, utilizando la técnica de papel celofán. Todos los aislamientos disminuyeron significativamente el crecimiento micelial de *R. solani* en cultivos duales. Los aislamientos 1, 3, 4 y 7 fueron más eficaces en la inhibición de *R. solani* por producción de metabolitos no volátiles. Dichos aislamientos fueron seleccionados a fin de evaluar su potencial antagónico en la reducción de muerte de plántulas de melón en invernáculo. Los cuatro aislamientos manifestaron reducción significativa de la enfermedad causada por *R. solani*. Los resultados muestran la posibilidad de utilizar aislamientos regionales de *Trichoderma* spp. para reducir la muerte producida por *R. solani* en plántulas de melón en los climas cálidos y húmedos del noroeste de la provincia de Corrientes.

Palabras clave: antagonismo, *in vitro*, invernáculo, melón, hongos patógenos de suelo, control biológico.

Rhizoctonia solani Kühn is a soil pathogen that causes diseases in a wide range of hosts of agricultural, horticultural and flower crops (Van de Booger, 1999). It can cause severe damage specially during the seedlings pre-emergence and post-emergence stages, causing rotten roots and stems, plant wilting and death, and is a limiting factor in the production of horticultural plants in all crop growing areas (Mitidieri, 1988; Bucki *et al.*, 1998).

Measures currently used to control *R. solani* correspond to chemical products and cultivation practices. However, this type of control is restricted for financial and ecological reasons (Mitidieri, 1988; Van der Booger, 1999).

Possibilities of replacing or reducing the amount of chemical products for control of diseases caused by soil fungi, by alternative techniques such as biological control, have been studied over the last few decades. The efficacy of this control method has mainly been demonstrated for pathogenic soil fungi such as *R. solani*, *Sclerotium rolfsii* and *Pythium* spp. (Hadar *et al.*, 1979; Elad *et al.*, 1980; Jackisch-Matssura and Menezes, 1999). The antagonistic potential of *Trichoderma* spp. against *R. solani* has been demonstrated in seedlings of bean (*Phaseolus vulgaris* L.) (Elad *et al.*,

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1980), tomato (*Lycopersicon esculentum* Mill.), eggplant (*Solanum melongena* L.) (Hadar *et al.*, 1979) and radish (*Raphanus sativus* L.) (Harman *et al.*, 1981; Patricio *et al.*, 2001), among others. In Argentina, isolates of *Trichoderma* spp. were studied *in vitro* (Mitidieri, 1988; Cortese *et al.*, 1992; Bucki *et al.*, 1998; Durman *et al.*, 1999) and in greenhouse, and it was found that some of them were efficient biocontrollers of *R. solani* in eggplant and tomato seedlings in wet temperate regions (Bucki *et al.*, 1998; Durman *et al.*, 1999). The changes observed in the antagonistic potential of the different isolates of *Trichoderma* spp. make it necessary for the selected organisms to be adapted to a specific ecological or geographical region (Silveira *et al.*, 1994).

Owing to the importance of seedling death and the difficulties of controlling it by conventional practices, the aim of this work was to select regional isolates of *Trichoderma* spp., adapted to warm and wet climatic conditions and to the protected horticultural crop system in the northwest of the Corrientes province.

Three isolates from the hypha tip of *R. solani*, anastomosis group AG4, were used as phytopathogens, obtained from melon (*Cucumis melo* L.) (Rhm), pepper (*Capsicum annuum* L.) (Rhp) and tomato seedlings (Rht) with stem and root rot symptoms, from the Department Capital of Corrientes province. As potential biocontrollers, nine monosporic isolates of *Trichoderma* spp. were studied, isolated from the rhizosphere of healthy eggplant, pepper and tomato. To perform the tests, isolates of *R. solani* and *Trichoderma* spp. were grown in 1.5% potato glucose agar (PGA), pH 6.5 incubated in the dark at 24-25°C for 7-10 days.

Dual cultures (Bell *et al.*, 1982) were set up with the nine *Trichoderma* spp. isolates to measure their effect on the mycelial growth of *R. solani*. A randomized block design with four repeats was used. Each of the nine isolates was grown with the three indicator pathogens making a total of 27 treatments. Cultures were also carried out for the three pathogens.

The percentage of mycelial growth inhibition (% MGI) of *R. solani* was determined at 72 h, using the modified formula of Edgington *et al.* (1971) cited in Jackisch-Matssura and Menezes (1999).

Microscopic observations (400 ×) were done of the dual cultures at 72 h.

Using the cellophane technique (Dennis and Webster, 1971), the isolate Rhm of *R. solani* and eight monosporic *Trichoderma* spp. isolates were tested. Eight *Trichoderma* spp. isolates out of the nine original ones were selected on the basis of their behavior in dual cultures, low growth (isolate 5), intermediate growth (isolates 4, 8 and 9) and high growth (isolates 1, 3, 6 and 7). A completely randomized block experimental design was applied with eight repeats and nine treatments (eight isolates and one control). The variable studied was % MGI of Rhm at 72 h.

Isolates 1, 3, 4 and 7 of *Trichoderma* spp. were selected because their antagonistic activity were significantly higher than the other isolates reflected by the production of non volatile metabolites on Rhm mycelial growth. The effect on the reduction of seedling death in melon cv Rocio de Miel was studied.

An Argiudol Vertex soil was used, brought from upland (*albardones*) of the Parana river -Corrientes, at 17 cm depth (OM: 1.7%; clay: 11.6%; lime: 21.5%; fine sand: 64.7%; coarse sand: 2.2%; pH 5.6), unsterilised, in 20 cm diameter and 18 cm deep pots, artificially infected with 5 Rhm discs of 1.5 cm diameter mycelium, seven days before sowing.

Two antagonist applications were made to seeds and soil. Seeds of melon cv. Rocio de Miel were treated by 30 min immersion in an individual suspension of *Trichoderma* spp. isolates. The concentration was adjusted to 10^6 conidia ml^{-1} using a Neubauer chamber. Immediately after, 15 seeds per pot were sown. The untreated control consisted in non-inoculated seeds, submerged for 30 min in sterile distilled water.

To apply the antagonists directly to the soil, five 1.5 cm diameter discs corresponding to each *Trichoderma* spp. isolate were placed in the soil seven days before sowing. A total of 15 seeds were sown per pot. Untreated controls corresponded to pots with soil infected with Rhm, not inoculated with *Trichoderma* spp. Pots were kept in a greenhouse throughout the experiment, at 23-32°C. A completely randomized block design was used with four repeats. The comparative study of the isolates antagonist activity in reducing seedling death consisted in calculating the percentage of live seedlings 20 days after sowing.

Analysis of variance and Tukey's test were applied in the three experiments.

In the dual culture test, the three isolates of *R. solan*i presented a behavior similar to the studied *Trichoderma* spp. isolates and the antagonist-pathogen interaction was not significant (P = 0.99). For this reason, the behavior of the nine *Trichoderma* spp. isolates was studied for the *R. solani* group (Table 1), revealing that isolate 5 produced less % MGI than the others.

Table 1. Mycelial growth inhibition (% MGI) of *Rhizoctonia solani* together with the nine isolates of *Trichoderma* spp. in dual cultures

Isolate	% MGI
7	59 a
6	58 a
3	57 a
1	56 a
2	53 a
9	52 a
8	51 a
4	51 a
5	38 b

Same letters in columns indicate non-significant differences between means, according to Tukey's test ($\alpha = 0.05$).

Table 2. Mycelial growth inhibition (% MGI) of *Rhizoctonia solani* with eight isolates of *Trichoderma* spp. using the cellophane technique

Isolate	% MGI
4	85 a
1	83 ab
3	79 ab
7	69 bc
8	54 bc
5	46 c
6	39 c
9	0.3 d

Same letters in columns indicate no significant differences between means, according to Tukey's test ($\alpha = 0.05$).

In microscope observations made at 72 h in dual cultures, growth of the three *R. solani* isolates was stopped when they came into contact with *Trichoderma* isolates 1, 3, 6 and 7. There was a prominent contact line at the meeting point of the paired microorganisms. These isolates not only inhibited *R. solani* growth but also, after making contact, covered the whole surface of the Petri dish growing over the phytopathogen mycelia. This shows how the *Trichoderma* spp. is more effective in the fight for the colonized area, winning the competition for space and nutrition (Sousa Rocha and Oliveira, 1998). The contact lines formed by isolates 2, 4, 8 and 9 were less notorious. After this, the *Trichoderma* spp. continued growing slowly until they covered the whole phytopathogen surface. However, the opposite occurred against antagonist 5, which stopped growing when it came into contact with and was covered by *R. solani*, possibly because of its slow growth.

All isolates of *Trichoderma* spp. caused morphological changes such as plasmolysis or cellular shortening in the *R. solani* hyphae. Moreover, curling of the phytopathogen on the hyphae of isolates 1, 3 and 7 was observed.

The results obtained in the cellophane method showed the greatest antagonistic effect in isolates 1, 3 and 4, with an inhibition of Rhm mycelial growth higher than 78% (Table 2). Isolate 9 was not effective; however, it was able to reduce mycelial growth of the pathogen in dual cultures, suggesting that it does not act by producing non-volatile metabolites but by other mechanisms of competition or parasitism instead. The remaining isolates showed an intermediate behavior.

These data coincide with those obtained by Dennis and Webster (1971), and Michereff *et al*. (1993) who, using different *Trichoderma* species, observed reduced mycelial growth of several pathogenic fungi, including *R. solani*, showing a change in the ability to produce metabolites, not only between species but also between different isolates of the same species.

According to Bell *et al.* (1982), the *in vitro* results do not necessarily express the degree of antagonism and biological control in natural crop conditions but reflect the genetic potential and variability of the antagonist, and the phytopathogen resistence to the antagonism. Therefore, *Trichoderma* spp. isolates 1, 3, 4 and 7 were selected for the greenhouse experiment because of their good behavior *in vitro.* These isolates significantly reduced ($\alpha = 0.05$) the disease produced by *R. solani* compared to the untreated control in melon seedlings grown in pots (Fig. 1).

There were no significant differences among the isolates either when they were applied directly to the se-

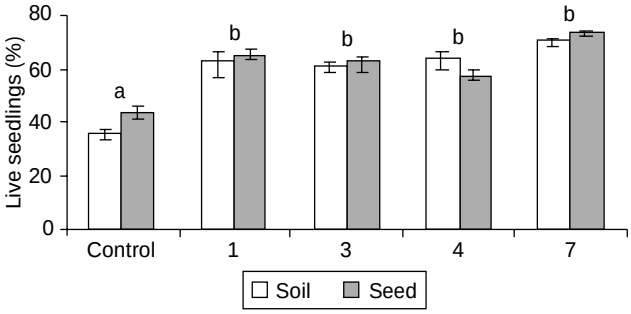

Figure 1. Percentage of live melon plants 20 days after treatment with four different isolates of *Trichoderma* spp. (1, 3, 4, and 7) and inoculation with *R. solani*, in greenhouse pots, with their corresponding standard deviations. *Trichoderma* spp. were applied in two ways: to the soil and to the seeds. The number of plants corresponds to the mean of 4 repeats of 90 plants each.

eds or to the soil. However, as Hadar *et al*. (1979) and Elad *et al*. (1980) pointed out, *Trichoderma harzianum* can be applied in a wheat bran nutritional product (*Triticum aestivum* L.) since it improves the colonization of the soil and increases the antagonistic effect. Similar results were obtained by Patricio *et al*. (2001) using wheat seeds colonized by *Trichoderma* spp., and Bucki *et al*. (1998) obtained good results in eggplant seedlings applying *Trichoderma* spp. as a conidial suspension.

The results showed the possibility of using regional *Trichoderma* spp. isolates to reduce melon seedling death by *R. solani* in warm and wet climates in the northwest of the Corrientes province. We, therefore, believe it is necessary to perform trials in crops grown under greenhouse conditions to improve the selection of antagonists against *R. solani*.

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