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Antagonist capacity of native paraguayan isolates of *Trichoderma* spp. against *Macrophomina phaseolina* isolated from soybean (*Glycine max*) and sesame (*Sesamum indicum* L.)

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Introduction

Macrophomina phaseolina, a global devastating necrotrophic fungal pathogen, infects more than 500 plant hosts (Wyllie 1988). Diseases caused by *M. phaseolina* (e.g., seedling blight, charcoal rot, stem rot, and root rot) are favored with higher temperatures (30-35°C) and low soil moisture (Sandhu et al. 1999).

To confront this problem, chemicals are commonly used, but their repeated and excessive use can generate environmental and health problems for humans and animals (Vinale et al. 2008). The fungus of the genus *Trichoderma* has been used as a biocontrol agent, especially against soil phytopathogenic fungi (Infante et al. 2011).

In this work we evaluate the antagonistic capacity of 25 native Paraguayan isolates of *Trichoderma* spp. against *Macrophomina phaseolina* isolated from soybean and sesame, by means of confrontation and antibiosis assay.

Methodology

Twenty-five isolates of *Trichoderma* from the Department of Phytochemistry of the Facultad de Ciencias Químicas of the Universidad Nacional de Asunción were previously isolated from different agricultural areas of the Eastern Region of Paraguay and kept in 80% glycerol at 4°C. Two isolates of the phytopathogenic fungus *M. phaseolina* were also used, one of them isolated from stubble of a soybean crop (*Glycine max*) from the Department of Itapúa and the other one isolated from sesame seeds (*Sesamun indicum* L.) from the department of San Pedro, Paraguay.

The direct confrontation assay was performed using the dual culture technique in sterile Petri dishes (7 cm in diameter) containing 15 ml of PDA (Papa-Dextrose-Agar, Liofilchem®). The inoculum was obtained from active growth plates of both *Trichoderma* spp. and the phytopathogen *M. phaseolina*, taking 5 mm diameter discs. A mycelial disk of the phytopathogen was placed at the edge of the Petri dish, and at the opposite edgethe mycelial disk of *Trichoderma* spp. was placed, keeping separated by a distance of 65 mm. On the other hand, control were placed on separate plates, one with a mycelial disk of each *Trichoderma*spp. isolate and another with a phytopathogen disc. Cultures were kept in the dark at 25-30°C for 4 days, measurements were made of the mycelial growth of the fungal colony every 24 hours. All experiments were performed in triplicate.

To evaluate the antibiosis produced by high and low molecular weight molecules secreted by *Trichoderma* from plates containing *Trichoderma* spp. in active growth, 5 mm diameter mycelial discs were cut and subsequently cultured in the Petri dish center containing PDA culture medium on which a cellophane or dialysis membrane 10 kDa (Sigma-Aldrich D9402-100FT), with a diameter of 7 cm and previously sterilized at 120°C for 15 minutes wrapped in individual filter paper sachets, the pre-sterilization dialysis membranes were washed 3 times with distilled water at 90°C.

This was incubated for 30 hours in the dark and at temperatures ranging from 25 to 30°C. After the isolates of *Trichoderma* spp. reached the total coverage of the membrane, proceeded to remove

it, taking out the mycelium of the fungus and preserving in the agar the molecules secreted by *Trichoderma* spp. Phytopathogenic fungi were cultured on the same plates containing the substances secreted by the antagonist. This assay was performed in triplicate and plaques with culture of the phytopathogens were used in PDA medium using the membranes without prior culture of the *Trichoderma* spp.

The percent growth inhibition (PIC) of *M. phaseolina* for both the Confrontation assay and the Antibiosis assay was determined using the following equation: $PIC = \frac{C-T}{T} \times 100$

Where, C is the measure of phytopathogen growth in the control plaque, T is the measure of phytopathogen growth on the assay plate (confrontation or antibiosis).

The software Prisma Graphic was used, using descriptive statistics and analysis of variance was performed through ANOVA and post HocTukey or in case of comparison of means of two groups the Student's t-test was used. A significance of 95% was considered.

Results and discussion

In the confrontation assay all *Trichoderma* isolates shown inhibition on the growth of *M. phaseolina* FCQ26 and FCQ39. For the selection of the best antagonists, inhibition at or above 75% is considered in the plaques in a comparison assay (Bell et al. 1982). *Trichoderma* FCQ36, FCQ37 and FCQ45 isolates were the best antagonists against *M. phaseolina* FCQ26 (Table 1).

Table 1. Percentage of Inhibition of radial growth of *M. phaseolina* FCQ26 affected by *Trichoderma* in the dual culture technique.

Trichoderma spp.	**Percent Growth Inhibition**		
	48 hs	**72 hs**	**96 hs**
FCQ36	25 ± 4	66 ± 2	75 ± 1
FCQ38	29 ± 4	66 ± 3	77 ± 3
FCQ47	27 ± 2	62 ± 2	77 ± 2

In contrast for *M. phaseolina* FCQ39 seven *Trichoderma* isolates were the best antagonists (Table 2).

When comparing the inhibition of radial growth exerted by the different *Trichoderma* spp. on *M. phaseolina* FCQ26 and FCQ39 at 96 hours incubation, in the confrontation assay, it is observed that in general they have similar antagonistic behavior for both isolates of the

Table 2. Percentage of Inhibition of radial growth of *M. phaseolina* FCQ39 affected by *Trichoderma* in the dual culture technique.

Trichoderma spp.	**Percent Growth Inhibition**		
	48 hs	**72 hs**	**96 hs**
FCQ22	17 ± 2	50 ± 4	74 ± 3
FCQ28	15 ± 5	61 ± 3	76 ± 4
FCQ32	19 ± 3	62 ± 3	77 ± 3
FCQ36	25 ± 5	63 ± 4	75 ± 3
FCQ37	33 ± 2	66 ± 0	78 ± 1
FCQ45	16 ± 0	66 ± 0	80 ± 2
FCQ47	31 ± 7	69 ± 5	81 ± 4

pathogen. However, it can be seen that the *Trichoderma* isolates FCQ14, FCQ28, FCQ30 and FCQ45 exert a greater inhibition on *M. phaseolina* FCQ39, and these differences are significant (Figure 1).

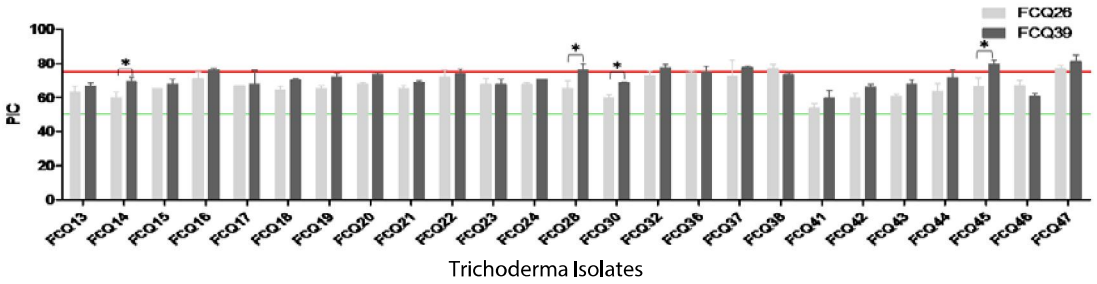

Figure 1. Percentage inhibition of growth of *M. phaseolina* FCQ26 and FCQ39 against different isolates of *Trichoderma* spp.

In the antibiosis assay it was observed that the low molecular weight molecules exert inhibition on the growth of both phytopathogen isolates (Figure 2).

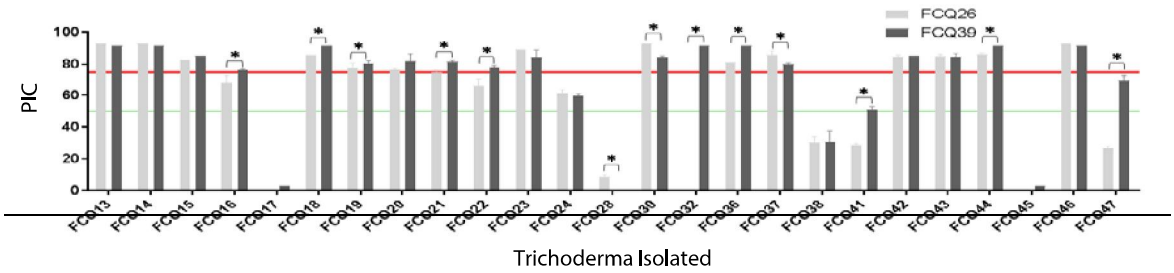

Figure 2. Percentage inhibition of growth of *M. phaseolina* FCQ26 and FCQ39 due to the antibiosis generated by low molecular weight molecules secreted by *Trichoderma* spp.

When the molecules of low molecular weight are in the presence of molecules of high molecular weight, in some cases the antibiosis is potentiated, in others it remains the same and others it decreases. (Figure 3).

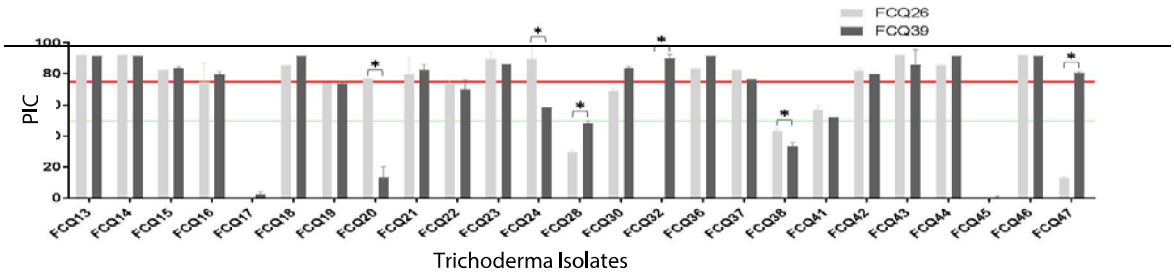

Figure 3. Percentage inhibition of the growth of *M. phaseolina* FCQ26 and FCQ39 due to the antibiosis generated by molecules of high and low molecular weight secreted by the *Trichoderma* spp.

Conclusion

In this study, it was found that the 25 native *Trichoderma* isolates are potential biocontrol agents of the phytopathogen *M. phaseolina* isolated from soy or sesame, among which there are isolates of the antagonist that would be most effective, which can be selected for use.

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