IV CONGRESO NACIONAL DE CIENCIAS AGRARIAS

"Conocimiento e innovación para el desarrollo sostenible"

LIBRO DE RESÚMENES

19, 20 y 21 / Abril / 2017
Campus UNA
San Lorenzo, Paraguay

Universidad Nacional de Asunción
Facultad de Ciencias Agrarias

Congreso Nacional de Ciencias Agrarias. (4a. : 2017 abr. 19-21; San Lorenzo, Paraguay).
Libro de resúmenes / editores Enrique Asterio Benítez León, Guillermina Macchi Leite, Alba Liz González. – San Lorenzo, Paraguay: FCA/UNA, 2017.
1 pendrive ; capacidad 116 Mb.

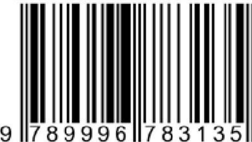
Incluye tablas, figuras, bibliografías.

1. Producción agrícola. 2. Producción animal. 3. Producción forestal. 4. Economía. 5. Desarrollo rural. 6. Bosques. 7. Medio ambiente. 8. Suelos. 9. Ingeniería agrícola. 10. Protección Vegetal. 11. Biotecnología. I. Benítez León, Enrique Asterio, ed. II. Macchi Leite, Guillermina, ed. III. González, Alba Liz, ed. IV. Titulo.

CDD : 630

Diseño editorial e interactividad: ERVAZ Arte Digital - Tel. 021 420 844
Colaboración: Lourdes Monserrat Mora Insfrán

Fotos de tapa: Lourdes María González Soria, Jorge Daniel Caballero Mascheroni, Gloria Arminda Resquín Romero, Enrique Asterio Benítez León, Luis Dario Macchi Leite.



Characterization of species of *Trichoderma* spp. in its efficacy for the biological control of native isolates of *Macrophomina phaseolina*

Dani Daniel Ruiz-Díaz[1], María Eugenia Flores-Giubi[1], Javier E. Barúa[1*]

[1]Facultad de Ciencias Químicas, Universidad Nacional de Asunción. San Lorenzo, Paraguay.
*Author for correspondence: javierbarua@qui.una.py

Introduction

Agricultural crops are affected in yield and quality due to the action of phytopathogenic organisms, mainly fungi, generating significant economic losses (Nina et al. 2011). Among the traditional methods for the control of phytopathogens the use of chemical products is the most popular. Although the indiscriminate use of these products has led to the appearance of mechanisms of resistance of phytopathogenic fungi to fungicides, and also contributes to environmental pollution (Guédez et al. 2012), which has led to the need to generate alternative methods that are ecologically friendly. The main strategy has been the identification of soil microorganisms that operate as effective antagonists and that their biological use is safe. Among native soil microorganisms, *Trichoderma* spp. (Hypocreales, Ascomycota) has been extensively studied and has been proposed as a biocontrol agent (Guigón et al. 2010).

Methodology

Test of direct confrontation of *Trichoderma* spp. against phytopathogenic fungi

At the edge of a Petri dish with PDA medium was placed a 5 mm diameter agar disc with pathogen mycelium *Macrophomina phaseolina* (Tassi) Goid. FCQ6 from soy stubble and FCQ9 from infected sesame crops) and at the opposite edge another 5 mm disc with mycelium of the antagonist (*T. harzianum* T34 (CECT2413), *T. brevicompactum* IBT40841, *T. arundinaceum* IBT40837). All were incubated at 30°C in the dark for 5 days, measurements were made of the radial growth of colony mycelium every 24 hours. The percentages of inhibition obtained in each confrontation were compared by an analysis of variance (ANOVA) and the comparison between the means were estimated the by Tukey ranks test. ($p=0.05$). Significant differences were considered with 95% confidence $p<0.05$ for ANOVA and for the Tukey Test.

Analysis of organic extracts

Two mycelium disks of 5 mm diameter were inoculated from each *Trichoderma* strain per quadrupled in 100 ml of PDB liquid culture medium (Potato dextrose broth). They were incubated for 7 days at room temperature in the dark and in static culture. The mycelium was separated by filtration through Nytal filters and the filtrate was extracted with ethyl acetate three times (Filtrate: organic solvent 1:1, 1:½, 1:½). They were then evaporated to dryness by a rotary evaporator. The extracts were resuspended in ethyl acetate and were chromatographed using silica gel F_{254} TLC (Macherey-Nagel, 0.20 mm, 4x6 cm) as the stationary phase and Hexane: Ethyl acetate (1: 1) as the mobile phase. The plates were visualized under ultraviolet light at 254 nm and 366 nm, and were exposed to chemical developers, oleum, vanillin and anisaldehyde.

For a more exhaustive analysis, the *Trichoderma* organic extracts were dissolved in acetonitrile (ACN), prepared at a concentration of 5 mg mL^{-1} and filtered with 0.2 µm PTFE filters and analyzed on an Ultra High Performance Liquid Chromatograph (UPLC). As a mobile phase were prepared a mixture of water (with 0.1% formic acid and 0.5% ammonia) and methanol in a concentration gradient of 0 to 100% metanol,

with a running time of 5 minutes and flow of 0.4 mL min^{-1}.

Results and discussion

The confrontation between the reference strains of *Trichoderma* and the phytopathogenic *M. phaseolina* FCQ6 y FCQ9, observing the mycelial growth of black color corresponding to *M. phaseolina* and of green-yellow color to those of *Trichoderma.* Measurements were performed at 48 hours, 72 hours and 96 hours.

The inhibition of growth of *M. phaseolina* was determined by measuring the radial growth zone of the *M. phaseolina* isolates in the absence of *T. harzianum* (control) and in the presence of the fungus (confrontation), getting the measures observed in Figure 1, for strain FCQ6 and Figure 2 for strain FCQ9, the percentage of growth inhibition (PIC) of phytopathogenic fungus was 55.6% for FCQ6 and 52.8% for FCQ9.

Trichoderma brevicompactum presented a PIC of 46.8% for FCQ6 and 47.3% for FCQ 9. In the confrontation between *T. arundinaceum* and the isolate of *M. phaseolina* FCQ6 a PIC of 35% was observed at 96 h, lower than the PIC values obtained in the experiments with *T. harzianum* and *T. brevicompactum*. For the FCQ9 strain, a PIC of *Macrophomina* of 49.5% was observed at 96 h. The inhibition may be due to the production of hydrolytic enzymes or toxins secreted by *Trichoderma*, which could degrade the cellular structures of phytopathogens.

Data analysis of percent inhibition among the three species of *Trichoderma*and *M. phaseolina* FCQ6 showed that *T.harzianum* and *T. brevicompactum* produce significantly greater inhibition than *T. arundinaceum*at 72 h and 96 h from the start of the confrontation, no significant differences were observed between *T. harzianum* and *T. brevicompactum* at any of the evaluated times (Figure 1).

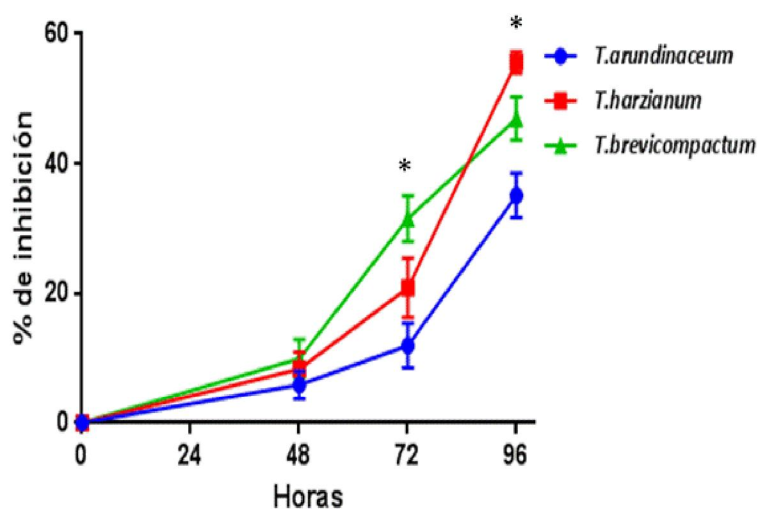


Figure 1. Inhibition of growth of *Macrophomina phaseolina* FCQ6.

The lines indicate the percentage of inhibition exerted by the three Trichoderma species, which were evaluated at 48, 72 and 96 hours, blue line: T. arundinaceum, red line: T. harzianum and green line: T. brevicompactum. Groups marked with an asterisk indicate significant differences between groups ($p<0.05$, ANOVA and posthoc $p<0.05$ Tukey).

PÓSTER

In the confrontations between species of *Trichoderma* and *Macrophomina phaseolina* FCQ9 no significant differences were observed in the percentages of inhibition between the three species of *Trichoderma* used.

These results demonstrated that the different species of *Trichoderma* exert inhibition of the growth of *M. phaseolina*. In addition, the ability of inhibition of a particular *Trichoderma* strain depends on which microorganism it is facing.

When comparing the inhibition capacity against the two isolates of *Macrophominaphaseolina,T. arundinaceum* showed significant differences at 72 hs and 96 hs of confrontation, with greater inhibition of FCQ9 than FCQ6 from *Macrophomina phaseolina*. (Figure 2), *T. harzianum* only presented difference at 72 h presenting greater inhibition of strain FCQ9*, T. brevicompactum* did not present significant differences in its capacity of inhibition of the strains.

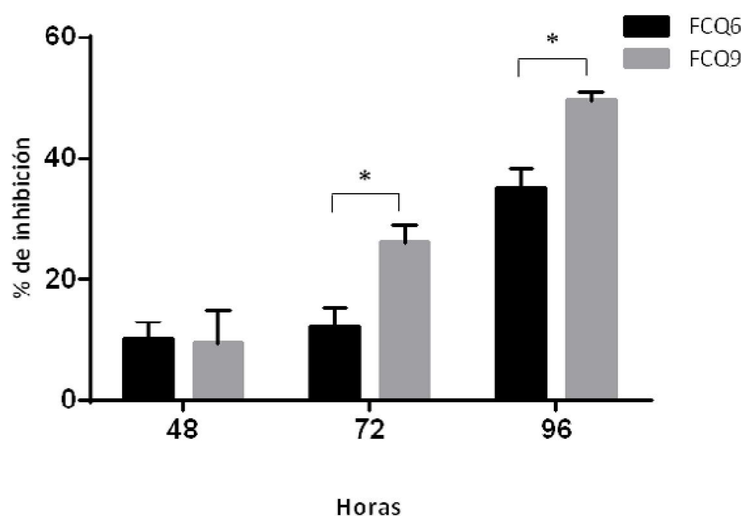


Figure 2. Percentage of growth inhibitionexerted by *T. arundinaceum* on *Macrophomina phaseolina* FCQ6 and FCQ9. Groups with an asterisk indicate significant differences between groups ($p<0.05$ ANOVA and $p<0.05$ Tukey).nd posthoc $p<0.05$ Tukey).

In this way, the different origin of isolation of plant pathogens from the same species allowed to observe the different response of these to the same biocontrol agent during the interaction.

Chromatographic profile

Thin Layer Chromatography (TLC)

Signals observed in TLCunder UV light showed that they are compounds containing extended conjugations or could be aromatic compounds. Chemical developers, oleum, anisaldehyde and vanillin were used to observe spots of different colorations depending on the chemical nature of the compounds (Figure 3). The results showed that the organic extracts obtained presented an important chemical complexity since, regardless of the developer used, several spots were observed, suggesting the presence of several secondary metabolites secreted into the culture medium.

AL ÍNDICE

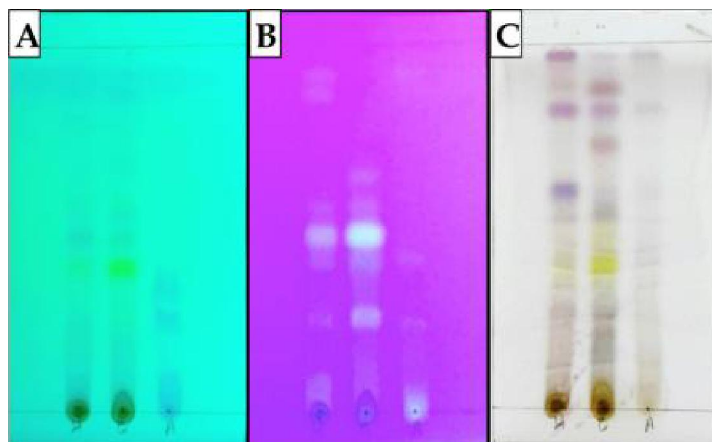


Figure 3. Thin layer chromatography of the organic extracts obtained. UV 256 nm (A) and UV366nm (B), Anisaldehyde (C)The order of sowing the sample, from left to right: *T. harzianum, T. brevicompactum and T. arundinaceum.*

Ultra High Performance Liquid Chromatography (UPLC)

The chromatograms obtained from the different extracts showed remarkable differences between them, in the organic extract of *T. harzianum*, detected the presence of 2-phenylethanol with m/z 123 ($M+H^+$), β-sitosterol with m/z 415 ($M + H^+$), 6-pentyl-2H-pyran-2-one with m/z 167 ($M + H^+$), and 1,8-dihydroxy-3-methylanthraquinone with m/z 255 ($M + H^+$). All these compounds have been previously evaluated in their antifungal activity against several phytopathogens, including *M. phaseolina*, being 1.8-dihydroxy-3-methyl anthraquinone the one that has shown a greater capacity of inhibition of the growth of these microorganisms (Ahluwalia et al. 2015). In the organic extract of *T. arundinaceum*, was detected the presence of 2-phenylethanol with m/z 123 ($M+H^+$),Tyrosol with m/z 139 ($M + H^+$), and toxin harzianum A with m/z 401 ($M+H^+$).

Harzianum A has been previously described in this fungus and is described as a potent mycotoxinbelonging to the family of trichotecenes (Degenkolb et al. 2008).

The analysis of the organic extract of *T. brevicompactum* give not yielded conclusive results.

In *T. brevicompactum* it is characteristic the production of the mycotoxintrichodermin, (Degenkolb et al. 2008), although in our growing conditions we have not been able to verify its presence.

These results demonstrate that the *Trichoderma* strains evaluated in this work are able to synthesize a wide diversity of secondary metabolites and these could be implicated in the antagonistic effect observed against *M. phaseolina* FCQ6 and FCQ9.

Conclusion

T. harzianum and *T. brevicompactum* showed the highest growth inhibition values of the two phytopathogen isolates, *T. arundinaceum* showed an important inhibition of the growth of the strain FCQ6, although inferior to those obtained with *T. harzianum*and *T. brevicompactum*.

From the chromatographic analysis performed on the organic extracts, it was determined that there is differential production of metabolites among the *Trichoderma* species evaluated.

More thorough chemical analyzes should be performed to confirm the identity and biological activity of the metabolites described in this paper.

Bibliographic references

Nina, R; Smeltekop, H; Almanza, JC; Loza, M. 2011. Evaluación de la capacidad biocontroladora de cepas nativas de *Trichoderma*spp. sobre *Rhizoctonia*sp. y *Fusarium* sp. en café (*Coffea arabica*) en condiciones experimentales. Journal of the Selva Andina Research Society 2(1):43-52.

Guédez, C; Cañizaleza, L; Castillo, C; Olivar, R. 2012. Evaluación *in vitro* de aislamientos de *Trichoderma harzianum* para el control de *Rhizoctonia solani*, *Sclerotium rolfsii*y *Fusarium oxysporum* en plantas de tomate. Revista de la Sociedad Venezolana de Microbiología 32(1):44-49.

Guigón-López, C; Guerrero-Prieto, V; Vargas-Albores, F; Carvajal-Millán, E; Ávila-Quezada, GD; Bravo-Luna, L; Ruocco, M; Lanzuise, S; Woo, S; Lorito, M. 2010. Identificación molecular de cepas nativas de *Trichoderma* spp., su tasa de crecimiento *in vitro* y antagonismo contra hongos fitopatógenos. Revista Mexicana de Fitopatología 28(2):87-96.

Ahluwalia, V; Kumar J; Rana, V; Sati, O; Walia, S. 2015. Comparative evaluation of two *Trichoderma harzianum* strains for major secondary metabolite production and antifungal activity. Natural Product Research 29(10):914–920.

Degenkolb, T; Dieckmann, R; Nielsen, K; Gräfenhan, T; Theis, C; Zafari D; Chaverri, P; Ismaiel, A; Brückner, H; von Döhren, H; Thrane, U; Petrini, O; Samuels, GJ. 2008. The *Trichoderma brevicompactum* clade: a separate lineage with new species, new peptaibiotics, and mycotoxins. Mycological Progress 7:177–219.