
**IN VITRO AND IN VIVO ANTAGONISM OF *SCLEROTIUM ROLFSSII*
SACC BY STRAINS OF *TRICHODERMA* SPP.**

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ABSTRACT

The aim of this study was to evaluate in vivo and in vitro antagonistic potential of five strains of *Trichoderma* spp. against phytopathogenic fungus *Sclerotium rolfsii*. Two in vitro tests were carried out, which was rated the pathogen pairing and antagonist and the inhibition of pathogen growth by volatile metabolites method. The pairing test consisted in inoculating of both pathogen and antagonist in the same petri dish and rating mycelial growth of the pathogen. In volatile metabolites test methodology was applied to overlapping plates, where we evaluated the inhibition of mycelial growth of the pathogen. In vivo test consisted of planting cowpea seeds and inoculating spores of the pathogen and antagonist, and then made biometrics. The data obtained by the pairing method showed that there was only 36% of mycelial growth of the pathogen paired with TC001, TC002 and TC003 strains compared to the control. The technique of overlapping plates showed that the TC003 and TC005 strains were the most effective in inhibiting the mycelial growth with 93.77 and 90.44% inhibition consecutively. The in vivo test showed that strains of *Trichoderma* spp. They were able not only to combat the pathogen, but also conferred growth promoting bean plants.

Keywords: biological control, *Vigna unguiculata*, mycoparasitism, plant growth promotion.

INTRODUCTION

The fungus *Sclerotium rolfsii* Sacc is known to cause rot of the colon and is distributed in tropical and subtropical regions. Cultivated crops such as bean species and wild plants are hosts of this fungus (Bianchini *et al*, 2005). This group of diseases provoke significant losses especially due to premature death of seedlings, it also affect the crop in all growth stages.

Biological control is seen as the natural control of living organisms using other living organisms (Azevedo *et al*, 2000). The use of biological control agents in agriculture is growing because it is necessary the increasing of sustainable farming practices and reducing the use of agrochemicals.

Melo (1996) points out that *Trichoderma* fungi are antagonistic to other microorganisms, especially pathogens. Due to hyperparasite ability of these fungi, *Trichoderma* spp. has important use in the biological control because the ability to parasitise structures of resistance such as sclerotios produced by the plant pathogen *S. rolfsii*.

Studies have noted that *Trichoderma* exerts significant inhibition in the growth and spores germination of plant pathogens fungi such as *Fusarium solani* [16] and liquid culture isolates metabolites extracted from this genus exhibit inhibitory activity against *Phytophthora* isolates (Bae *et al*, 2016) thus proving its usefulness as a biological control agent.

The goal of this study was to evaluate antagonistic potential *in vitro* and *in vivo* from five strains of *Trichoderma* spp. against phytopathogenic fungus *S. rolfsii*.

Material and methods

Fungi strains

Five *Trichoderma* strains TC001, TC002, TV003, TC004, TC005 were obtained from a microorganism collection that belongs to the Agricultural Microbiology Laboratory of the Center for Agricultural Sciences, Federal University of Alagoas. The isolated *Sclerotium rolfsii* used in the study was provided by Phytopathology Laboratory of Agricultural Sciences Center of the Federal University of Alagoas.

Antagonism assay for direct confrontation and interaction between hyphae

The method consisted of inoculating pathogen and antagonist in the same petri dish containing malt extract medium culture having a sterile cover slip in the center of the plate for check interaction between the hyphae, a 5mm disc containing mycelium and conidia of each isolate inoculated opposite poles in the plate and was incubated at a temperature of $25 \pm 2^{\circ}\text{C}$ for seven days in the absence of light. The evaluation was made by rating scales proposed by Rodrigues (2010) with some modifications where scales were expressed as percentage of pathogen growth.

After the incubation period, coverslips were removed and superimposed on microscope slides with dye Bromophenol Blue to verify the interaction between the hyphae. The experimental design was completely randomised with six treatments and five replications, and five strains of *Trichoderma* spp. and control corresponding to a board containing only the pathogen. The data were submitted to analysis of statistics through Sisvar software (Ferreira, 1998).

Metabolites volatile Assay

This test consisted on inoculating both antagonist and pathogen in overlapping Petri dishes, which dishes containing conidia and mycelia antagonist was inoculated on the bottom plate and the pathogen in the top plate and lately incubated at $25^{\circ}\text{C} \pm 2$ for seven days, and then the pathogen colonies were measured with the aid of a digital calliper. The experimental design was completely randomised with six treatments and five replications. Data were analysed using the software Sisvar (Ferreira, 1998).

Antagonism test in vivo

For testing *in vivo* antagonism the cowpea planting (*Vigna unguilata* L.) was made in pots of 500 mL containing vermiculite sterile substrate by two cycles of autoclaving (1 atm), without fertilisation in order to evaluate only the effect inoculation of the pathogen and antagonistic fungi. In each pot was inoculated 10^{-4} suspension of spores of the pathogen and antagonist used being two controls: one corresponding to the absence of antagonist or pathogen inoculation (CONTROL 1) and a corresponding inoculation of the pathogen and the antagonist (CONTROL 2). The experimental design was completely randomized with seven treatments and four replications.

The planting was conducted for 35 days in a greenhouse at room temperature and daily irrigation. Three seeds were planted per pot, and thinned performed seven days after the planting, to one plant per pot. At the end of 35 days, the plants were taken to the Agricultural Microbiology Laboratory for biometrics. It was evaluated plant height, biomass, root length and dry weight. Data were analyzed using the software Sisvar (Ferreira, 1998).

Results and discussion

Direct pairing test

Through the initial test pairing was noted that the TC001, TC002 and TC003 strains were able to inhibit the growth of the pathogen, an increase of 36% compared to control (Fig 1).

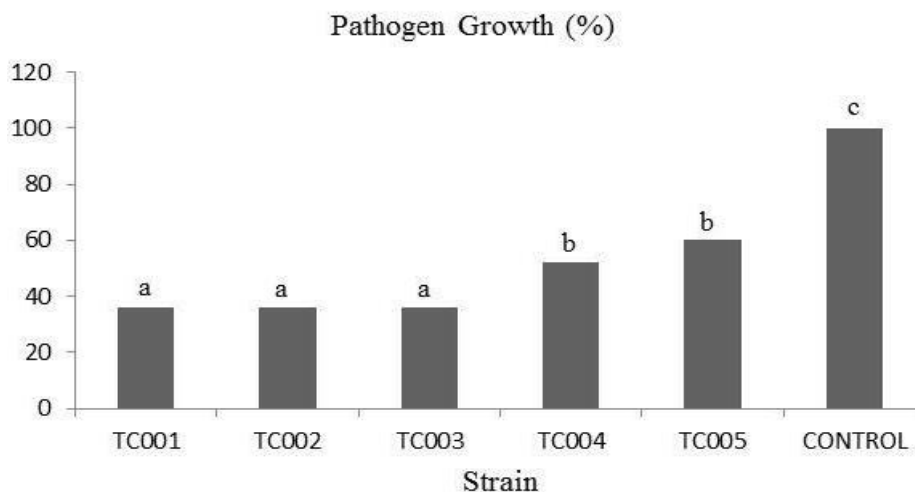


Fig. 1. Mycelial growth of plant pathogenic fungus *S. rolfsii* in the presence of strains of *Trichoderma* spp.. Means followed by same letter do not differ statistically from each other ($p \leq 0.05$) by Scott-Knott test.

In vitro antagonism tests are important because they are able to select potentially skilled fungal strains for use in agriculture as biological control agents of plant pathogens.

The fungus *Trichoderma* spp. has been reported by several authors as a biological control agent like *Fusarium oxysporum* (Dolatabad *et al*, 2012), *Rhizoctonia solani* (Hajieghari *et al*, 2008), *Fusarium solani* (Abeysinghe, 2007) among others.

This ability *Trichoderma* species have as biological control agents for soil borne plant pathogens is intrinsically related to their natural habitat, since this is a fungus that lives naturally in soil as saprophytes.

Assay volatile metabolites

The test of volatile metabolites revealed that all strains used in this study were efficient in the control of *S. rolfsii*, however, the TC003 and TC005 strains were the most efficient, showing respectively 93.77 and 90.44%, potential inhibition of the pathogen's mycelial growth as shown in Fig. 2.

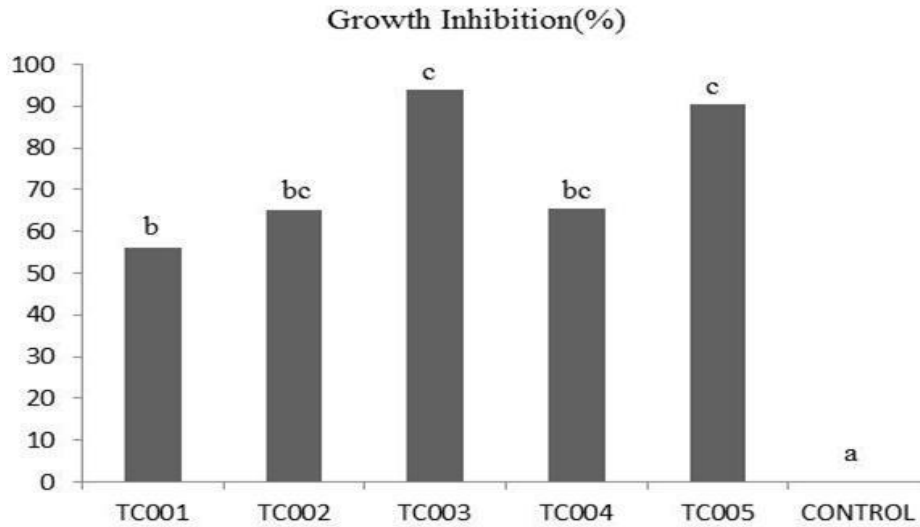


Fig. 2. Inhibition of mycelial growth of *S. rolfsii* by *Trichoderma* spp. the volatile metabolites method of superposed plates. Means followed by the same letter are not statistically different from each other ($p \leq 0.05$) by Skott-Knott test.

Several studies have shown that, in addition to direct parasitism (Benhamare and Chet, 1996) the presence of secondary metabolites are involved in the antagonism of *Trichoderma* against *S. rolfsii* including antibiotics (Dennis and Webster, 1971) the enzyme chitinase (Lima *et al*, 1997), beta-1,3-glucanase (El-Katatny *et al*, 2001), among other routes. Within this information, we can confirm that micoparasitism activity of *Trichoderma* spp. It may be related to different mechanisms of action or production of secondary metabolites.

Antagonism test in vivo

The antagonism test *in vivo* showed that presence of antagonistic fungi, not only conferred health of plants but also acted as growth promoters as shown in Table 1.

Table 1 - Biometrics string bean plants (*V. unguiculata* L.) inoculated with *Trichoderma* spp. and *S. rolfsii*.

Strain	Plant height (cm)	Biomass (g)	Root lenght (cm)	Plant dry wheight (g) ns*
TC001	4 a	0.399 ab	8.4 a	0.0486 a**

TC002	10.1 b	0.811 b	13.7 b	0.145 a
TC003	9.1 b	0.658 ab	11.1 b	0.133 a
TC004	6.2 a	0.244 ab	11 b	0.084 a
TC005	11.3 b	0.399 ab	10.44 b	0.136 a
CONTROL 1	7.2 a	0.186 ab	10.4 b	0.125 a
CONTROL 2	5.6 a	0.03 a	1.9 a	0.40 a

* Not significant ($p \geq 0.05$). ** Means followed by the same letter are not statistically different from each other ($p \leq 0.05$) by Skott-Knott test.

Recent studies show that *Trichoderma* spp. species are capable of promoting growth in plants. Contreras-Carnejo et al. (2009) study reports that these fungi are related to the production of hormones and growth factors or the efficient use of nutrients and the ability of giving the plant better absorption of nutrients from the soil. *Trichoderma* spp. may have affinity to the rhizosphere and work in symbiosis with the plant once the soil is its natural habitat.

According to the results, we found that the TC002, TC003 and TC005 strains were the most efficient, showing the greatest plant height values, biomass, root growth and dry matter. Considering the extension of the plant, biomass production and root growth stimulation can be associated with phenotypic characteristics related to the presence of auxin.

Aguiar et al. (2012) reported that the inoculation of *T. viride* was effective in the control of *S. sclerotiorum*, but also has increased the percentage of survival of bean plants in vivo tests.

According to the data obtained from this study, we can clearly see that the presence of the antagonist fungus conferred growth promoting to bean seedlings in relation to the CONTROL 2, which received inoculation of the pathogen and was noted the presence of small and stunted seedlings, as well as its damping off.

Conclusions

The use of *Trichoderma* spp. strains was efficient in both *in vitro* and *in vivo* control of *S. rolfsii* and growth promotion in cowpea.

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