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Effects of Trichoderma harzianum fungus on the incidence of the charcoal rot disease on sesame

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Abstract

The effect of the *Trichoderma harzianum* fungus on the incidence of the charcoal rot disease in sesame was studied. The ways of using *Trichoderma harzianum* fungus, the application of the seed; application in clay granules and application in rice grains were evaluated. The evaluation was carried out for three years, on a plot infested naturally, located at the experimental field Turén, and for a year on infection beds located at INEA, Portuguesa and artificially inoculated with 400 sclerotium of *M. phaseolina* / g of soil. The variance analysis combined for the three years of the essay did not show statistical differences between years neither between treatments, but it did between the interaction year x treatment, subsequently evaluating each year separately obtaining an inconsistency in results by the absence of statistical differences between treatments for each year. The same result was observed in the analysis of the information obtained on the infection beds. These results do not show any difference of *T. Harzianum* on the incidence of the charcoal rot, also evidenced the necessity of evaluating cultural practices that might improve the environmental conditions that allows the fungus *T. Harzianum* to develop all its antagonist capacity.

Key words: Biologic control, *Sesamum indicum, Macrophomina phaseolina*

Introduction

In Portuguesa state, sesame crop (*Sesamum indicum* L.) constitutes one of the main agriculture products, sowing here the highest quantity of land in Venezuela. Sesame crops are normally affected by the disease known as charcoal rot, which causal agent is the fungus that lives in the soil *Macrophomina phaseolina* (Tassi) Goid., which can cause considerable losts in the crop yields (1, 11, 13).

The efficient eradication of *M. phaseolina* is quite difficult, observing that treatments based on the use of herbicides and fungicides have not been too effective and have caused poor results (12). However, one

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alternative for the control of diseases caused by fungi living in the soil, such as *Sclerotium rolfsii* Sacc., *Rhizoctonia solani* Kühn and *Pytium* spp., is the use of antagonist fungi as for example *Trichoderma harzianum* Rifai, (4, 6, 8, 10). Therefore, the aim of this research was to evaluate, for three years in the field and one year on infected beds, the application effect of *T. Harzianum* on the incidence of charcoal rot on sesame crop.

Materials and methods

The strain used belonging*T. Harzianum* was isolated from infected plants with charcoal rot, collected from sesame crops located at La Colonia Agrícola Turén, Portuguesa state. Once isolated, the strain was kept on essay tubes with acidified potato dextrose agar (APDA) until its usage.

The multiplication of *T. Harzianum* was done on polished rice grains, which were put on a can with 500 mL by means of 200 g of rice/100 mL of sterile distilled water and then sterilize them on a device for 15 minutes at 121ºC and 15 psi, then were inoculated and incubated for 15 d on lab conditions, after this time were let on air. Dry granules were mashed with a homogenizer and sieved on a sieve machine of 250 mm. The sieved material was used for the inoculation of the seed and clay granules, and the material that was left on the sieve was used to do the treatment on the soil.

In all essays the certified sesame seed var. Píritu was used by means of 4 kg/ha of seed, quantifying the number of plants per plot 35 days after emerged the seedlings and at the end of the flowering season, in order to determine the percentage of healthy plants.

Field essays: During periods of limited precipitation (december-april) on 1993, 1994 and 1995, three essays were done with the purpose of determining the application effect of *T. Harzianum* on the incidence of charcoal rot.

For this purpose, a plot infected naturally with *M. Phaseolina* was selected at the Experimental Field Turén (CET), located at la Colonia Agrícola Turén, soils with a loamy texture, pH of 8.2 and with adequate inoculum levels of *M. Phaseolina* to produce the epiphyte of the disease (1).

The experimental design used was randomized plots with 4 treatments and 4 replications. The experimental units of 14.4 m^2 (3.6 m X 4 m) were sowed with 6 rows of sesame and with 0.6 m of separation. Between plots the separation was of 1 m, being the total area of the essay 285 m^2. On each experimental unit 4 central rows were evaluated.

The evaluated treatments were the following: 1) Seed treatment with spores of *T. harzianum* (ST); 2) Spores application of *T. Harzianum* on clay granules, [(obtained from clayey mashed soil and passed through a sieve of 3 mm diameter, and later sterilise in autoclave twice for 1 h at 121ºC and 15 psi). The seed as well as

clay granules were impregnated with a sterile solution of sacharose at 5% (nutritive substrate for *T. harzianum*) and then were put on a can with the spores of the fungus to impregnate the seed and clay granule]. 3) Grains of polished rice colonized by *T. harzianum* (GR) and 4) (absolute witness) sesame seed without being treated. GA and GR treatments were put in the furrow of the crop with the sesame seed, by means of 140 kg (9). Treatments were applied every year.

Essays on plots: In 1996, in INIA-Portuguesa from december to april (a season with few precipitations), a similar essay to those done at CET was carried out, but on plots artificially infected with esclerotium of *M. phaseolina*. Irrigation was applied weekly to keep humidity low.

The essay was carried out in three plots of 16 m x 1.2 m (19.2 m^2). Plots were divided every 4 m of length creating 4 experimental units of 4 m x 1.2 m, sowing 4 rows with sesame divided by 0.3 m, evaluating the two central rows. The bed soil was previously disinfected using formalin 5% and inoculated by means of 400 sclerotium of *M. Phaseolina* by gram of soil (11).

The strain of the inoculated fungus was obtained from sick sesame plants collected at CET.

To increase the inoculum, the isolated strain was multiplied on acidified PDA for 15 days under lab conditions, subsequently the sclerotium and mycelium mass was mixed with distilled water and sieved twice. The washed mass was extended on filtered paper and let dry for 48 h at 30ºC aseptically, to then being ground on a mortar, obtaining *ca* $6x10^5$ units that cause colonies per gram.

To do the inoculation on the plots, 2.5 g of sclerotium were mixed twice for 1 h, on 4 kg of sterilized soil (substrate), in autoclave per plot. The evaluated treatments were the same mentioned on the field test.

The data was analyzed statistically on a randomized plot design and a mean test was done according to Dunca's multiple range tests.

Results and discussion

Field essays. The variance analysis for the three evaluated years at CET did not show statistical differences between treatments (P= 0.5955; CV= 9.74%) (table 1).

The variance analysis for each year showed for the first year of evaluation significant statistical differences between treatments (P= 0.018; CV= 6.61%), and doing Duncan's multiple range test (P<0.05), GA was determined as the best treatment (97.23% of healthy plants), with a trustable level of 95%. For the second year, the variance analysis did not determine statistical differences between treatments (P= 0.7767; CV= 8.39%). Meanwhile, in the last years differences between the evaluated treatments were not found

Table 1. Mean test of the three years of evaluation of the application ways of *Trichoderma harzianum* to determine the effect of the fungus on the incidence of charcoal rot disease in the Experimental Field Turén, Portuguesa state.

Treatment[1]	Porcentage of healthy plants[2]
ST	92.912[a]
GR	93.332[a]
GA	91.551[a]
T	90.252[a]

[1]SR= Spores application of *T. Harzianum* to the seed. GR= grains of polished rice where *T. Harzianum* developed. T= absolute witness. GA= spores application of *T. Harzianum* to clay granules.
[2]Values with the same letter are statistically the same according to Duncan's múltiple range test (P<0.05).

(P= 0.4891; CV= 11.99%).

Test on plots. The statistical analysis of the obtained data in this test, did not registered significant differences among treatments (CV= 6.81%) (table 2). On the other hand, strains of *M. phaseolina* obtained at CET have an excellent capacity of producing sclerotium on PDA caldo and a very good virulence (14), thus garanteeing a good artificial inoculation in the plots.

Mean test for the interaction between treatments and the years of evaluation determined that GA treatment (first year) obtained the highest percentage of healthy leaves, and GA treatment (third year) the lowest percentage of healthy leaves. The latter results and the lack of statistic differences between the averages of each treatment indicate that the effect of treatments is inconsistent.

On the other hand, analyzing the information separately obtained during the three years of evaluation in CET, is observed the same tendency, since there are significant differences for the first year between treatment, which was GA.

According to the results obtained in the essay carried in the plots, the same response to the one of essays in the field was obtained, by the lack of statistical differences among treatments.

Results show that there was not any effect of *T. Harzianum* on the incidence of charcoal rot disease. Likewise, Pineda and Tortolero (13) report the same tendency to the results obtained in this essay, where statistical differences are found with the application of *T. Harzianum* spores to sesame seed and polinazed rice grains by the fungus, but there are not significant differences in the following year among the evaluated treatments.

From all application ways of *T. Harzianum* reported, it is said that the spores application of this fungus to the crop seeds is the one with more

Table 2. Mean test of treatment of the essay named application ways of *Trichoderma harzianum* to determine its effect on the incidence of charcoal rot in infection beds.

Treatment[1]	Porcentage of healthy plants[2]
T	89.333[a]
GR	85.045[a]
ST	79.038[a]
GA	74.730[a]

[1]ST= spores application of *T. Harzianum* to the seed. GA= spores application of *T. Harzianum* to clay granules. GR= grains of polished rice where *T. Harzianum* develops. T= absolute witness.
[2]Values with the same letter are statistically the same according to Duncan's múltiple range test ($P<0.05$).

benefits, since collonizing roots protects them against diseases (2, 3, 7, 15), however, it was not the best treatment in this research.

It can be inferred that the observed results can be explained analyzing factors that favor the development of the charcoal rot disease. In sorghum (*Sorghum bicolor* [L.] Moench), the incidence of the illness is favoured by the combination of different environmental conditions that cause stress in the plant, such as high temperatures and hydric deficit (5). In sesame, this disease reaches the highest incidence in plants of 60 d old ca (11), which according to the sow season of this crop in Portuguesa state (periods with scarce precipitation from december to april), agrees to high temperatures and hydric deficit that causes stress in the plants. These mentioned conditions predispose sesame plants towards the disease (11), and at the same time are abiotic factors that disfavour the stablishemnt in the soil of the antagonist *T. harzianum* (7).

Conclusions

The results obtained in this research show that *T. Harzianum* does not prove any reduction on the incidence of the charcoal rot disease in the field conditions where sesame is sowed. Likewise, it is shown the necessity of repeating this kind of test in order to reconmend some antagonist proposed as an alternative for the control of the illness. Thus, the environmental conditions where sesame is sowed at Portuguesa state, as well as the new cultural practices, must be studied with the aim of obtaining a reduction on the incidence of charcoal rot.

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