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In Vitro Inhibition of *Curvularia eragrostidis* by the Antagonistic Fungi *Trichoderma* spp. and *Acremonium cephalosporium*

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Abstract **– Yam (*Dioscorea cayennensis* Lam.) Plantations in the Brazilian Northeast are severely attacked by the disease known as foliar burning or black-spotted, whose etiological agent is the fungus *Curvularia eragrostidis*. Under favorable epidemiological conditions, this disease, characterized mainly by necrotic spots on the leaves, can seriously compromise production. The alternative control of plant diseases aims at the use of alternative and effective means that minimize the costs with the use of fungicides. Within this type of control is associated the use of antagonistic microorganisms with deleterious action in some types of pathogens. The objective of this work was to evaluate the efficiency of the in vitro control of *Curvularia eragrostidis*, which causes the black pin of the yam, through the use of the antagonists *Trichoderma* spp. and *Acremonium cephalosporium*. The experiment was conducted at the Center of Agricultural Sciences of the Federal University of Alagoas. The experimental design was completely randomized using 05 treatments and 04 replicates. The treatments analyzed were followed by pairing of an isolate of *Curvularia eragrostidis* with 04 isolates of *Trichoderma* spp. and 01 isolated from *Acremonium cephalosporium*. For comparison of means the Scott-Knott's test was applied at a 5% probability level. It can be verified that the isolates of *Trichoderma* spp. were more efficient in inhibiting in vitro growth of the pathogen analyzed when compared to the other treatments.**

Keywords **– Alternative Control, Inhibition, Antagonistic Fungi, Biocontrol, Pairing.**

I. Introduction

Yam (*Dioscorea* spp.), Belonging to the Dioscoraceae family, is the base ocean of the chains of many tropical countries in Africa, especially Nigeria and Côte d'Ivoire. African countries produced 55.020.008 t of tubers, corresponding to 96% of world production (57.293.948 t), giving them a prominent position. Among the South American countries, Brazil is in first place, with 25 thousand hectares harvested and production of 225 thousand tons [1].

Yam plantations (*Dioscorea cayennensis* Lam.) In the Brazilian Northeast are severely attacked by the disease known as foliage burn or black-spotted, whose etiological agent is the fungus *Curvularia eragrostidis*. Under favorable epidemiological conditions, such as nights 20 and 22 with a relative base of 100%, and diurnal days between 25 and 28 ° with a corresponding proportion of 65%, aggravated by the presence of winds, the disease, characterized mainly by necrotic spots in the leaves, can seriously compromise production [2].

The primary symptom of leaf-blight is a dark brown necrotic spot, often surrounded by a yellow halo. These spots, which tend to have a circular shape, reach, on average, 2 to 3 cm in diameter, being partially limited by the veins of the leaf blade. It is common coalescence of spots, forming large necrotic areas. The secondary or reflex symptom is the small size of the commercial tufts and seeds. In extreme cases of incidence and severity, large areas of burned and dead plants are formed, with loss rates that may reach 100% [3].

The alternative control of plant diseases aims at the use of alternative and effective means that minimize the costs with the use of fungicides. Within this type of control is associated the use of antagonistic microorganisms with deleterious action in some types of pathogens. The objective of this work was to evaluate the efficiency of the in vitro control of *Curvulária eragrostidis*, which causes the black pin of the yam, through the use of the antagonists *Trichoderma* spp. and *Acremonium cephalosporium*.

II. Materials and Methods

A. Description of the Experimental Area

The experiment was conducted at the Laboratory of Agricultural Microbiology of the Center of Agricultural Sciences of the Federal University of Alagoas in the city of Rio Largo-AL located at 9° and 29'45 "south latitude, 35° and 49'54" longitude and 165 m altitude. The experimental design was completely randomized using 05 treatments and 04 replicates. The treatments were followed by pairing of a fragment of *Curvulária eragrostidis* with 04 isolates of *Trichoderma* spp. and 01 case of *Acremoniun cephalosporium*.

B. Obtaining micro-organisms

The isolate of *Curvulária eragrostidis* was obtained from the tissues presenting symptom. Isolation was done by plating the transition region in PDA (potato-dextrose-agar) culture medium. Subsequently, the confirmation of the *Curvulária eragrostidis* isolates was performed by optical microscopy observation of the pathogen reproduction structures.

The isolates of *Trichoderma* spp. and *Acremonium cephalosporium* were obtained from Atlantic Forest soil. Isolation was carried out using the soil suspension subjected

to plaqueming in Martin culture medium and purification of the fungal isolates in PDA culture medium. The confirmation of the isolates was made by observing the reproductive structures under an optical microscope.

The pairing between pathogenic fungi and antagonistic fungi was done by transferring a 10 mm disc of each isolate (pathogenic x antagonistic) to the borders of the Petri dish containing PDA culture. The pairing was evaluated in a period of 5 days after inoculation and the evaluation was done through the grading scale [4].

The treatments were described with the captions followed with the letters "T" for the pairing of *Curvularia eragrostidis* with *Trichoderma* spp. and "AC" for the pairing of *Curvularia eragrostidis* with *Acremonium cephalosporium*.

C. Data analisis

For comparison of means the Scott-Knott's test was applied at a 5% probability level. For this analysis, statistical software ASSISTAT was used [5].

III. Results and Discussion

An analysis of variance of the results showed that the growth of *Trichoderma* spp. on the fungus *Curvularia eragrostidis*. The results can be seen in Table 1.

Table 1. Analysis of variance corresponding to the means of the treatments evaluated in the maturation of *Curvularia eragrostidis* with antagonistic fungi.

Treatments	Mean of treatments	F
T1	2.75 c	
T2	4.0 b	
T3	2.5 c	
T4	3.0 c	
AC1	6.0 a	
CV %	9.36	70.3**

** Significant at the 1% probability level ($p \leq 0.05$). Averages followed by the same letter do not differ statistically.

According to the scale of notes used, the isolates of *Trichoderma* spp. (T1 and T3) were the most aggressive in the growth control of *Curvularia eragrostidis*. Following these, the T4 isolates inhibit the growth of the pathogen growing to 3/4 of the plaque. The T2 isolate is the least efficient in controlling the pathogen in relation to the other isolates of Trichoderma spp. and the AC1 isolate that corresponds to the fungus *Acremonium cephalosporium* didn't inhibit the growth of the pathogen. These results can be seen in Figure 1.

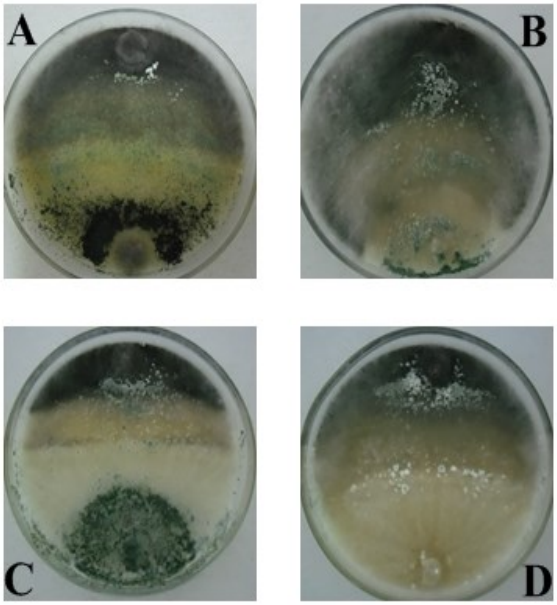

Fig. 1 - Observation of the pairing between isolates of *Trichoderma* spp. and *Curvulária eragrostidis*. A: T1; B: T2; C: T3; D: T4.

The variability in the control efficacy of the different *Trichoderma* spp. isolates evaluated may be associated with their intrinsic characteristics such as the ability to overcome pathogen defense mechanisms, compete with microorganisms [6]. The interface occurs when microorganisms, biocontrol agents, secondary metabolites are toxic that inhibit the development of other microorganisms. The ability to compete for the occupation of pathogen infection sites is a necessary feature for a biocontrol agent, since the antagonist is expected to grow more effectively than the pathogen at the site of infection [7]. In this process of mycoparasitism, the antagonists act directly on phytopathogens by the excretion of enzymes that degrade the cell wall, such as: chitinases, β-1,3-glucanases, β-1,6-glucanases, proteases, and antibiotics, such as gliotoxin , and viridine [8]-[9].

Studies with different isolates of *Trichoderma* spp. in the control of *Rhizopus stolonifer*, concluded that on the third day, all the isolates of *Trichoderma* spp. inhibited the growth of *Rhizopus stolonifer* [10]. Corroborating with this work, and showing the efficiency of the use of *Trichoderma* spp. on the biological control of phytopathogens of importance for large agricultural crops, such as *Curvularia* spp. in Yam culture. The greater antagonistic action in these treatments may have occurred due to less competition for the nutrients of the culture medium, favoring the fungus *Trichoderma* spp., or a greater production of protease and cysteine, enzymes produced by *Trichoderma* spp. which inactivate the enzymatic capacity of the phytopathogen [10].

IV. Conclusion

The treatment 3 (T3), with *Trichoderma* spp. was more efficient in inhibiting the in vitro growth of *Curvularia eragrostidis* when compared to other treatments,

characterizing it as an efficient alternative for phytopathogen control, and consequently reducing the use of pesticides.

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