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Alternatives for the Biocontrol of *Fusarium oxysporum* f. sp. Cubense, Causal Agent of Fusarium wilt or Panama Disease in Guineo (Musa balbisiana ABB) Under Field Conditions

Trinidad Castillo-Arévalo[1*]

[1]National Agrarian University, Department of Agricultural and Forestry Protection, Faculty of Agronomy, Km 12.5, northern highway, Managua, Nicaragua

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***Corresponding author:** Trinidad Castillo-Arévalo
National Agrarian University, Department of Agricultural and Forestry Protection, Faculty of Agronomy, Km 12.5, northern highway, Managua, Nicaragua

Abstract **Original Research Article**

The objective of this research was to evaluate the performance of Trichoderma spp, *Bacillus subtilis*, a mixture of both and the chemical Mertect® 50 SC for the management of fusarium disease in the municipality of La Chocolata, Rivas, Nicaragua. Panama disease, caused by *Fusarium oxysporum* f. sp. cubense (Foc), is one of the most damaging diseases of musaceae. Biological control techniques have been implemented to avoid contamination of water sources and adverse health effects due to the use of chemicals. In relation to the control of the disease, the bioformulation that presented better results was the base mixture of Trichoderma spp plus Bacillus subtilis with 3.24 % of severity, followed by the formulation of Trichoderma spp, with 4.44 % and the chemical control Mertect® 50 SC with 6.03% of severity, without presenting significant differences with the chemical and biological controls. The variables evaluated in this study were percentage of severity and Area Under the Disease Progress Curve of Fusarium damage per plant. *Fusarium oxysporum* f. sp. Cubense was identified as the main causal agent of guineo wilt (Musa balbisiana ABB) associated with this crop. Disease prevalence and growth were found to occur during the dry season. The use of biological organisms to control the disease is shown to be as effective as chemicals and safer for the environment and public health.

Keywords: Mal de Panama, biological control, *Bacillus subtilis*, Trichoderma sp., Musa Spp.

INTRODUCTION

Banana production is among the most important crops in tropical and subtropical countries. They rank fourth in importance worldwide after rice, wheat, and maize (Castillo-Arévalo, 2022). However, the production of these crops is threatened by the attack of diseases such as Panama Disease or Fusarium wilt. This disease caused by *Fusarium oxysporum* f. sp. cubense (FOC) represents one of the most destructive and economically important diseases in the Musa genus (Lara, 2009).

Currently, the Fusariosis disease that affects species of the Musa genus continues to be a problem because it can remain in infected banana soils for more than 30 years, destroying cultivars susceptible to race 1 such as the cultivar Gros Michel (AAA), established by small and medium producers in Nicaragua and Costa Rica (Caballero *et al*., 2011).

Initially, chlorosis is observed on older leaves, yellowing begins along the leaf margins, as it progresses it reaches the central vein, the leaf petiole collapses. The internal symptoms are characterized by reddish-brown spots in the vascular system of the rhizomes, corms, and pseudo stem, as the disease progresses the youngest leaves are affected and die, forming a skirt of dead leaves around the stem (Alvarado and Diaz, 2007 and Shew and Lucas, 1991).

The pathogen is a soil-dwelling organism that survives among crops in infected plant debris lying in the soil in the form of mycelium and in any of its spore forms, but most frequently in the form of chlamydospores, especially in cold temperate regions. It spreads over short distances through water and

contaminated farm equipment, and over long distances (Castillo-Arévalo, 2022), mainly in infected transplants or in the soil that goes into them. It is common that once an area has been infected by fusarium it remains so for an indefinite period (Ochoa *et al*., 2004).

Ploetz and Correll (1988) state that regardless of its origin, water is an excellent means of transporting spores and therefore an excellent route of infection.

The results of some research on the efficacy of biological microorganisms in the control of Foc are contradictory, and most refer to the efficacy of in vitro or greenhouse trials under controlled conditions (Ploetz, 2015).

Mitov and Oliva (1975) reported inhibitory activity of Trichoderma spp. strains in treatments prior to inoculations with the pathogen on susceptible plants.

Zhang *et al*., (2004) studied the inhibitory effect of 150 isolates of Trichoderma spp. from 40 different soils and other materials, of which 39 showed efficacies in inhibiting Foc in in vitro dual culture experiments. Efficacy was related to mycoparasitism, enzymatic inhibition and lysis.

Nel *et al*., (2006) reported slight suppression using Trichoderma isolates in the control of Foc in greenhouse trials on Cavendish bananas and referred the best efficacy as pathogen antagonists to non-pathogenic *Fusarium oxysporum* strains.

MATERIAL AND METHODS

Description of the study developed

The work was carried out at the Santa María farm, located in the community of La Chocolata de Rivas, Nicaragua, from January to June 2015, located between the coordinates 11º 41' North, 85º 83' West longitude, 120 km from Managua, Panamerican Highway South, and 54 km from Peñas Blancas (border with Costa Rica).

Study area. The research work was carried out in a Taungya system, in an area of 1.0 ha, in which the research on "Evaluation of growth, sequestration potential and carbon fixation of six forest species in association with Mussa balbisiana ABB" was developed.

Five treatments were evaluated in the experiment: Trichoderma sp and Bacillus subtilis, a mixture of the two, the chemical Mertect® 50 SC, and the control without any application. The doses evaluated were by spraying at 15 cm from the base of the pseudo stem every fifteen days for five months.

Table 1: Study treatments

Commercial Product	Active Ingredient	Biological organism	Dosage / hectare	# Study treatments
Trichoecol® 5 WP	*Trichoderma sp*	Biological	250 grams	T1
Serenade® SC1,34	*Bacillus subtilis*	Biological	8 liters	T2
Mertect® 50 SC	Tiabendazol - 50%	Pesticide	1.5 liters	T3
Serenade® + Trichoecol® 5 WPmn	*Trichoderma sp + Bacillus subtilis*	Biological	8 liters + 250 grams	T4
Absolute Control	No Application			T0

Laboratory identification and description of fusarium

Pathological analysis of vegetative material

Prior to identification and description, the pathological analysis of vegetative material was carried out in the phytopathology laboratory of the Instituto de Protección y Sanidad Agropecuaria, using fungal growth induction techniques, from diseased plant tissue that was subsequently sown in culture media such as: Agar-Water (AA) and Papa Dextrose Agar (PDA) to induce the sporulation of reproductive structures of the pathogen.

Severity

With the severity values, the disease index described by Castillo-Arévalo (2022) was calculated using the following formula and scale:

$$IXE = \frac{\sum(\text{number of diseased plants x each grade of the scale})}{(\text{Total number of plants observed})x(\text{major degree of the scale})} \text{ x } 100$$

Table 2: Severity scale of Fusarium (Panama disease) described by Castillo-Arévalo (2022)

Level of involvement	Symptom Description
 1	Healthy plant (asymptomatic)
 2	Plants with initial symptoms, lower leaves with chlorosis, cessation of leaf emission.
 3	Plants with advanced disease symptoms, cracking of the pseudo stem, yellow leaves on top and hang down
 4	Plant totally dead due to disease

Calculation of Area under the Disease Progress Curve (ABCPE) for Fusarium Disease

With the severity records by treatment, the Area Under the Disease Progress Curve (ABCPE) was calculated. The formula used was the one proposed by Castillo-Arévalo, 2023).

$$ABCPE = \sum_{i=1}^{n-1} \left[\frac{X_{i+1} + X_i}{2}\right](t_{i+1} - t_i)$$

Meaning of letters:

xi = Percentage of affected tissue.
t = Time (days)
n = Number of evaluations

Abbott efficacy index

The percentage of severity per experimental treatment was calculated, and with these data the index of efficacy of the treatments in the reduction of the severity of the pathogenic microorganism was calculated using the Abbott formula modified by (Castillo-Arévalo, 2023) for this study using the Abbott formula modified by (Castillo-Arévalo, 2023) for this study:

[1] $eficacia = \left[1 - \left(\frac{Ni}{NMax}\right)\right] x100$

Meaning of the letters:

Ni = is the percent severity per treatment at the end of the trial.

N max = is the maximum number of severities per treatment

per treatment obtained among all treatments and replicates at the end of the experiment.

Statistical analysis of the data

After the data were collected, they were arranged by variables in a data table in Excel, then each variable was compared between treatments, Shapiro-Wilk and Levyn normality assumptions test was performed to measure constant variances, performing an analysis of variance, using the InfoStat program (2020). The significance level used in the analysis was (p = 0.05).

RESULTS

Table 2: Evaluation of the effect of the bioformulations on Fusarium

Active Ingredient	% Initial severity	% severity
	Media ± ES	Media ± ES
Trichoderma sp	25.52±1.83 a	4.44±2.32 a
Bacillus subtilis	30.25±1.83 a	8.33±2.32 a b
Tiabendazol - 50%	25.31±1.83 a	6.03±.32 ab
Trichoderma sp + Bacillus subtilis	30.25±1.83 a	3.24±2.32 a
Absolute Control	32.37±1.83 b	52.21±2.26 c
C.V.	25.37	27.45
p-valor	0.0001	0.0001
F; df; n	15.23; 19; 20	12.23; 19; 20

ES=Standard error; SD=Significant Difference; C.V.=Coefficient of Variation; p=Probability; F=Fisher calculated; df=Degrees of freedom of the error; n=Number of data used in the analysis. *Means with different letters: significant differences exist.

Comparison of the average severity of Fusarium disease

In the analysis of variance, a significant difference was found (p=≤0.05). In general, it is observed that the Trichoderma sp + Bacillus subtilis treatment had better control over the disease with (3.24%), followed by the Trichoderma treatment (4.44%); then the chemical treatment (6.03%), followed by *Bacillus subtilis* (8.33%) and the absolute control (52.21%) of severity.

Table 2 shows the data on the effect of biological treatments on the severity of Foc wilt in artificially inoculated plants by direct spraying at the base of the soil. The antagonists had a marked inhibitory effect on the disease, especially when it colonized the soil and root system prior to infection.

Figure 1 indicates us that the combination of the bioformulated based on *Trichoderma* sp + *Bacillus subtilis* realized better parasitism on the microorganism in study, but in the separation of means indicates us that between only the bioformulated based on Trichoderma sp shows us that it is equal the effectiveness when applying only this last one, being a benefit for the producer because it diminishes the costs for application in field, at the same time was obtained a (R^2 = 0. 79) of pathogen growth per day with the application of these microorganisms under study, while without the application of bioformulated the pathogen growth per day is 13.05% per day, bringing destructive consequences for the root system and would cause total death of the plants.

Figure 2 indicates us that the biological treatment of the mixture of *Trichoderma sp + Bacillus subtilis* is very effective, followed by Trichoderma sp not finding significant difference between these two biological treatments.

Figure 3 allows us to determine probabilities of occurrence of (R^2 = 0.31) per day on leaf area of the affected plants in the department of Rivas, Nicaragua.

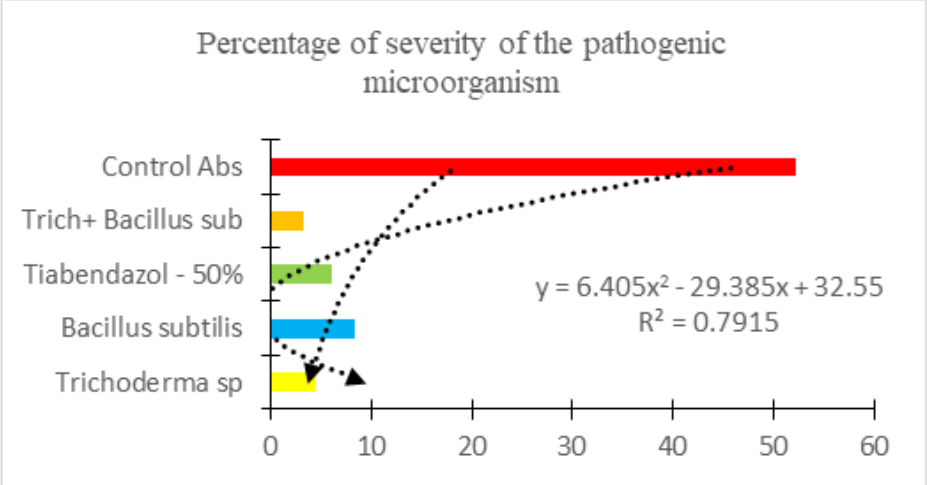

Figure 1: % Percentage of severity of the pathogenic microorganism

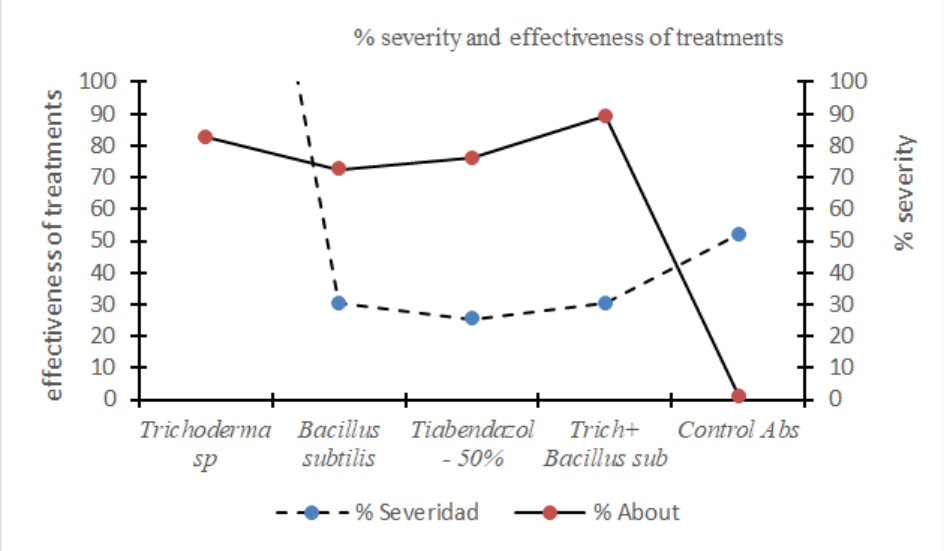

Figure 2: % Percentage severity and effectiveness of treatments

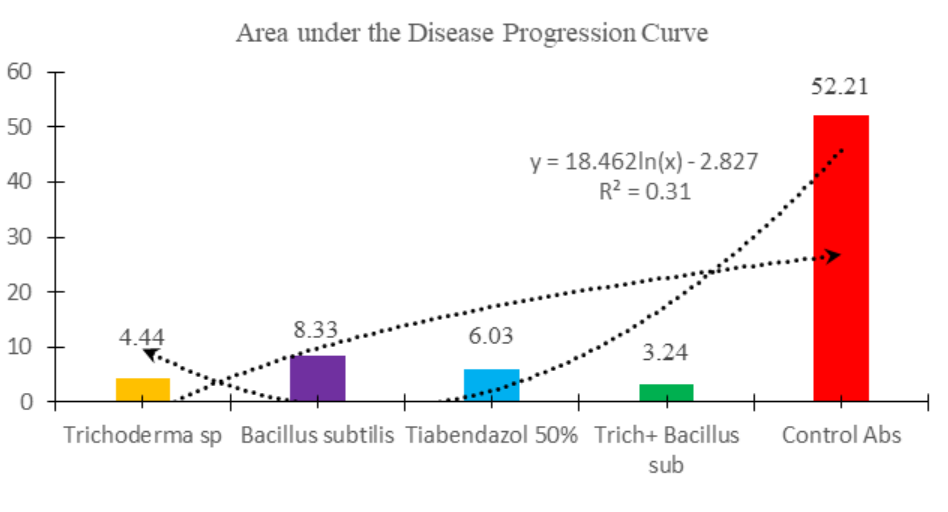

Figure 3: % Area under the Disease Progression Curve

DISCUSSION

Studies conducted by Gutiérrez (2011) in his research obtained the same results as this study with (36.3%). Sundin and Jacobs (1999), in their study the formulation of B. subtilis, presented the best performance in the test, being the same as in this research. These results coincide with those previously obtained by Mitov and Oliva (1975), (Alvarez, 2004) and (Hwang and Ko, 2004).

According to studies conducted by Parets (2002), in different crops including tobacco (Nicotiana tabacum L.) and potato (Solanum tuberosum L.) found satisfactory results in stimulating growth, with the application of Trichoderma sp. According to studies conducted by Lara (2009), and Kejela *et al*., (2016) in research developed with Bacillus sp reduces the prevalence of *Colletotrichum gloeosporoides* and *F. oxysporum* in coffee (Coffea arabica L.) nursery plantations up to 100% effectiveness with severity up to 90%.

According to Tejera *et al*., (2011), the genus Bacillus generates advantages for its use in agricultural biotechnology such as the presence of endospores, the motility that facilitates the colonization of the plant, the ability to produce plant growth promoting substances, and substances responsible for its activity. Acosta *et al*., (2013) found an antagonistic action on the fungus under study, as in the results of this research.

Grice and Peterson (2003) indicate that, in treatments without fungicide application, the percentage increase of the disease coincides with the results of this research.

Fonseca (2020) in his study found that the disease progressed faster compared to the other treatments where a disease increase rate of r = 0.02 (2% day^{-1}) was observed in the same way as this research. Similarly, Castillo-Arévalo (2022 a, b), indicates that the use of biological controllers reduced the growth rate of epiphyte (R2 = 0.16), which is the same as the result of this research.

Studies carried out by Castillo-Arévalo (2022), when comparing the effectiveness of using biological treatments in comparison with the use of pesticides to stop the advance of the attack by phytopathogenic microorganisms, shows that, as in this study, no significant difference is found between them, being less risky for public health and not contaminating the environment.

CONCLUSION

Fusarium oxysporum f. sp. Cubense was identified as the main causal agent of fusarium wilt or Panama disease on Guineo (*Musa balbisiana* ABB) associated with this crop in Rivas, Nicaragua.

It was found that the prevalence and growth of the disease occurred during the dry season in the department of Rivas.

The results of this study demonstrate that the use of biological organisms to control the disease is as effective as chemical ones and safer for the environment and public health.

It is demonstrated that the pathogen growth presents an advance of 13.05% (day^{-1}), in the department of Rivas, during the dry season.

Of the biological treatments, the bioformulated *Trichoderma* sp + *Bacillus subtilis* is the best management option to control the epiphytotic, followed by the entomopathogenic fungus Trichoderma.

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