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Trichoderma spp. condiospores production and its application with *Streptomyces* spp. for the management of *Mycosphaerella fijiensis* in banana

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ABSTRACT

Background/Objective. Black Sigatoka (BS) is one of the main phytopathologies that reduces banana production in Mexico. Developing biological products based on antagonists is a predominant study activity. The production of conidiospores of *Trichoderma* spp. strains was evaluated in solid state fermentation using whole rice grains and cracked corn, and the effect of foliar applications of conidiospores against the BS epidemiology in banana cv. Great dwarf.

Materials and methods. In solid state fermentation, the yield of four strains of *Trichoderma* spp. (T-82, T-85, T-94 and T-99) in whole rice (WR) and cracked corn (CC) was evaluated, an A×B factorial design was used (A=strains and B=substrate). The two strains with the best yield (T-99 and T-85) and a *Streptomyces* spp. based product was applied in the field to evaluate the epidemiology of BS through the severity, weighted average of infection (WAI) and area under the disease progress curve (AUDPC), through a randomized block design.

Results. CC increased the yield of *Trichoderma* spp. in 71%, strains T-94 (1.41×10^8 conidiospores g^{-1}) and T-85 (1.20×10^8 conidiospores g^{-1}) achieved the highest yields. The T-85 strain reduced the severity, WAI and AUDPC of BS compared to applications of the chemical control "Mancozeb".

Conclusion. CC was the best substrate to obtain greater yield in *Trichoderma* spp. T-94 and T-85. The weekly application of conidiosporas of *Trichoderma* T-85 reduces the severity, WAI and AUDPC of the SN in banana cv. Great dwarf.

Keywords*:* epidemiology, sporulation, fermentation, yield, severity, Mancozeb.

Introduction

Currently, banana (*Musa* spp.) is one of the most important crops in agriculture, ranking second in tropical fruit production in Mexico. In Michoacán was cultivated 5,803 ha in 2023, yielding 177,760.46 t with a productivity of 31.64 t ha^{-1} (SIAP, 2024). Bananas have high nutritional value, being rich in carbohydrates, fiber, protein, minerals, and vitamins, making them a staple fruit in human nutrition (Fernández *et al*., 2021).

The "*Gran Enano*" cultivar has been grown for over 30 years in the border region between Michoacan and Colima states (Álvarez *et al*., 2011). It is susceptible to several diseases, the most significant being Black Sigatoka (BS), caused by the fungus *Mycosphaerella fijiensis* (anamorph = *Pseudocercospora fijiensis*, Cruz *et al*., 2020). *M. fijiensis* spreads and reproduces through ascospores and conidia, which serve as the initial inoculum (Churchill, 2011). It has both sexual and asexual reproductive stages. In the asexual stage, the first lesions appear as streaks on the underside of leaves, caused by asexual spores (conidiospores). In the sexual stage, ascospores develop inside of asci within pseudothecia, which appear as black spots or grayish lesions on infected leaves in the final stage of the disease (Muimba, 2018). Leaves with BS symptoms experience reduced photosynthesis, leading to economic losses of 33 to 69% in production (Kablan *et al.,* 2012).

Chemical control with fungicides is the most commonly used strategy by banana producers. In Mexico, Mancozeb (ethylene bisdithiocarbamate) is the most widely used fungicide for controlling BS (Mena and Couoh, 2015) due to its broad biological spectrum and low cost. However, studies have shown that ethylenethiourea, a metabolite formed when the compound dissociates in water in the presence of oxygen, is highly mobile in soils due to its high water solubility and is responsible for its long-term toxicity to humans and the environment (Alza *et al.*, 2016). For this reason, biological control of BS is a viable option as it is environmentally friendly.

The *Trichoderma* genus has a broad range of control over phytopathogens (Kumar *et al.*, 2023) due to mechanisms such as competition, antibiosis, parasitism, and endophytism (Saeed, 2022). These traits make *Trichoderma* a promising alternative for controlling phytopathogenic agents, a feature that chemical fungicides lack (Acosta *et al.*, 2013).

Additionally, *Trichoderma* spp. offers advantages for large-scale production, as it can be cultivated through liquid, solid, and biphasic fermentation. Solid-state fermentation (SSF) for producing *Trichoderma* spp. conidiospores is carried out

using grains, cereals, or agro-industrial residues (López *et al.*, 2022). Whole and broken rice grains have been among the most commonly used substrates; however, they are costly due to their high economic value compared to maize (Rini *et al.*, 2018; Hernández *et al.*, 2019).

On the other hand, *in vitro* biological control of *P. fijiensis* has involved the use of bacteria (Chávez *et al.*, 2020) and actinobacteria (Moreno *et al.*, 2016), one of which is *Streptomyces galilaeus*, known for its effectiveness in inhibiting the *in vitro* growth of *P. fijiensis* (Castillo *et al.*, 2007; Moreno *et al.*, 2016). However, no *in situ* studies have been conducted to evaluate *Streptomyces* in the epidemiology of BS.

Therefore, the objective was to assess the performance of *Trichoderma* spp. strains produced on solid substrates and to determine the epidemiology of BS through applications of *Trichoderma* sp. conidiospores and *Streptomyces* sp. (Blitefree®) in the cultivation of "*Gran Enano"* banana.

Materials and Methods

Large-Scale Production of *Trichoderma* spp. conidiospores

Isolation and identification of *Trichoderma* spp. strains. The strains were isolated from the rhizosphere soil of banana (*Musa* spp.) plants in an organic orchard in Tecoman, Colima, Mexico (18°50'16.8" N, 103°50'06.4" W), with no history of *Trichoderma*-based biological product applications. Sampling was conducted across four hectares within a 40-ha farm. Soil samples were collected at a depth of 15–20 cm, with a composite sample (2 kg) prepared per hectare from five subsamples (0.4 kg each). The soil was placed in ziplock bags, transported, and stored in a cooler and refrigerator (5°C) for processing within 24 hours of collection.

Isolation was performed by washing soil particles, using 1 g of soil rinsed with 3 L of sterile distilled water through a stack of three sieves (Alcon®, Mexico) with decreasing mesh sizes of 500, 250, and 150 µm. The washed particles (150 µm) were placed on sterile filter paper discs for 5 hours to remove excess moisture. Ten particles were inoculated onto potato dextrose agar (PDA) supplemented with chloramphenicol (150 ppm, Sigma-Aldrich®, USA) (Bills *et al.*, 2004). Five Petri dishes were incubated at 25±3°C, 75±5% relative humidity (RH), with a 12-hour light/dark cycle. Colonies exhibiting morphological characteristics typical of the *Trichoderma* genus were purified through subculturing on PDA.

The *Trichoderma* spp. isolates were identified at the genus level using the taxonomic keys proposed by Rifai (1969), Barnett and Hunter (1972), and Bissett

et al. (2015). The primary macroscopic morphological characteristics considered included mycelium color, mycelium texture, and the formation of concentric rings. Microscopic characteristics were determined using an inverted microscope (Axio Vert A1, Zeiss®, Germany) and included conidia shape, phialides, and the presence of chlamydospores.

The isolates were deposited in the culture collection of the Faculty of Biological and Agricultural Sciences at the University of Colima. Four native *Trichoderma* spp. strains (T-82, T-85, T-94, and T-99) were selected for the experiments and maintained in slanted PDA tubes under controlled conditions (25±3°C, 75±5% RH, and a 12-hour light/dark cycle). Ten-day-old colonies from these tubes were used for large-scale conidiospore production through solid-state fermentation (SSF).

Inoculum preparation. Two slanted tubes with 10-day-old *Trichoderma* spp. colonies were used per strain to prepare 500 mL of spore suspension for each strain. Each tube was supplemented with 10 mL of sterile distilled water containing Tween 80 (0.05%). The colony surface was scraped with a sterile bacteriological loop and transferred to a flask containing 480 mL of sterile distilled water with Tween 80 (0.05%). Two tubes with *Trichoderma* colonies were used per strain. If the suspension did not reach 500 mL, it was adjusted with sterile distilled water. The spore suspension concentration was quantified using a Neubauer chamber in quadruplicate and adjusted to 1.0×10^7 conidiospores mL^{-1}.

Preparation of substrates for SSF. For the SSF experiment, two substrates were evaluated: whole rice (WR) and broken yellow corn (BYC). WR was washed three times with potable water to remove dust, then spread on trays and sun-dried until its moisture content was below 15%. Once dried, 120 g of WR was packed into 2 kg high-density polyethylene bags and sterilized in an autoclave at 120 °C (1.5 psi) for 20 minutes. Each bag was inoculated with 5 mL of inoculum (1.2×10^7 conidiospores mL^{-1}) and incubated for 18 days at 24.7–29.6 °C with a 12-hour light/dark cycle.

The yellow corn grains were broken using a dry grain mill (Molino del Rey 0.5 HP, Mexico) and then sieved to remove dust. The broken grains underwent four washing steps: first, with potable water to remove the corn husk and empty grains; second, with a *Fullgrow*® solution (Agroscience, Mexico) at 1.5 mL L^{-1} of water; third, with hot water (80 °C) for 10 minutes; and finally, with room-temperature water as a rinse. The BYC was then dried until its moisture content was below 15%. The dried BYC was packed into 2 kg high-density polyethylene bags (120 g per bag) and sterilized as previously described. Each bag was inoculated with 5 mL of conidiospore suspension (1.2×10^7 conidiospores mL^{-1}) and incubated for 18 days at 24.7–29.6 °C, 75±5% RH, with a 12-hour light/dark cycle. The incubation process

included 12 days for substrate colonization, mycelial growth, and sporulation. During the remaining six days, the bags were opened in the incubation room, and a dehumidifier (*Delonghi*®, USA) was used for eight hours per day to remove excess moisture from the substrate.

Response variable. Yield was determined (conidiospores g^{-1} of colonized substrate) 18 days after inoculation and incubation of the substrates. A 4 g sample of colonized substrate (BYC or WR) was placed in a 50 mL conical tube containing 36 mL of sterile distilled water with 0.05% Tween 80. The mixture was vortexed for 1 minute. Conidiospore concentration was quantified using a Neubauer chamber under a microscope (Axio Vert A1, Zeiss®, Germany) at 40× magnification. Serial dilutions were used when spore counts exceeded 250 conidiospores per counting field in the Neubauer chamber.

Experimental design and data analysis. A completely randomized design with a factorial A×B arrangement was used. Factor A corresponded to the *Trichoderma* spp. strain (T-82, T-85, T-94, and T-99), while Factor B was the substrate (BYC and WR), resulting in eight treatment combinations. Each treatment had 10 replicates, with each replicate consisting of a 2 kg bag containing 120 g of inoculated substrate. Data were analyzed using a two-factor analysis of variance (ANOVA) with a 95% confidence level (α=0.05). A multiple range comparison was then performed using Tukey's test (α=0.05). Statistical analyses were conducted using *Statgraphics Plus*® for Windows®.

Epidemiology of Black Sigatoka in the field

Experimental site. The study was conducted from January to April 2019 in a 30-year-old "*Gran Enan*" banana orchard (18°67′57.73" N, 103°67′12.89" W, Google Earth, 2018) located in Coahuayana de Hidalgo, Coahuayana Municipality, Michoacán. The orchard covered an area of two hectares with a planting density of 1,900 plants ha^{-1}. The soil type was fluvisol (INEGI, 2025), at an elevation of 30 meters above sea level. The predominant climate in the region is warm subhumid A(w), with an average temperature of 26–30 °C and an annual precipitation of 1,558 mm.

Establishment of the *in situ* experiment. The experiment consisted of four treatments: two *Trichoderma* spp. strains (T-84 and T-94), selected for their highest conidiospore production in the SSF experiment; a biorational product, *Blitefree*® (an extract of *Streptomyces* sp. from Altus Biopharm S.A. de C.V.) at a dose of 1.5 L ha^{-1}; and a chemical fungicide, *Mancozeb*®, at a dose of 2 kg ha^{-1}. Both

products were purchased from *Proveedora Agrícola* in Tecomán, Colima. The *in situ* experiment was conducted in randomly assigned blocks (500 m²) measuring 20 m in width and 25 m in length. Treatments were applied weekly for three months, and sampling data were collected every week after the first application. The experimental plot exhibited symptoms of BS, with small white-yellowish to brown spots, approximately 1 mm in length, known as "pizcas," barely visible on the underside of the leaves (Torrado and Zapata, 2008).

Treatment application. Treatments were applied using a motorized sprayer with a 15 L capacity (*STIHL*®, Mexico). For *Trichoderma* sp. and *Blitefree*®, soybean oil (1 mL L^{-1}) was used as a natural adhesive, while *Innex*® (1 mL L^{-1}) was used as an adhesive for *Mancozeb*. The application rate for each treatment and chemical control was 100 L ha^{-1}. The doses for *Blitefree*® and *Mancozeb* were 2.5 g L^{-1} and 2.5 mL L^{-1}, respectively. For field applications of *Trichoderma* spp. strains, a concentration of 1×10^{7} conidiospores mL^{-1} (2×10^{12} conidiospores ha^{-1}) was used. Treatments were applied weekly between 16:00 and 20:00 hours during the winter (January 2018) – spring (April 2019) period.

Epidemiological response variables

Severity of BS. Severity was assessed at seven-day intervals on all leaves of the selected plants using the six-category Stover scale (1970), where: (1) leaves showed no disease symptoms or had up to 10 spots; (2) leaves had less than 5% of the leaf area affected; (3) leaves had 6–15% of the leaf area affected; (4) leaves had 16–33% of the leaf area affected; (5) leaves had 34–50% of the leaf area affected; and (6) leaves had more than 50% of the leaf area affected.

Weighted infection average (WIA). WIA was assessed by dividing the leaf into two equal parts. A leaf with half of the lamina affected received a score of 5 (34–50%), and if the other half was also affected, it received a score of 6 (>50%). To assign scores of 3 and 4, each half of the lamina was further divided into two equal parts, representing one-quarter of the leaf. If up to half of this quarter was affected, the leaf received a score of 3 (6–15%); if more than a quarter was affected, it received a score of 4 (16–33%).

To obtain a score of 2, one-quarter of the lamina was further divided into two equal parts. If up to half of this section was affected, the leaf received a score of 2 (<5%). A score of 1 was assigned when the leaf had up to 10 spots. This procedure was repeated for each experimental unit (Martins *et al.*, 2007). WIA was calculated using the formula: WIA = (sum of % of leaves in each grade × respective grade) / 100.

Area under the disease progress curve (AUDPC). AUDPC is a useful quantitative summary of disease intensity over time, locations, or management strategies. The AUDPC for each treatment was calculated using the equation described by Shaner and Finney (1977).

$$ABCPE = \sum_{i=1} \frac{Y\,i+1+\ Y\,i}{2} * (t\,i+1\ -ti)$$

Where: *Yi* represents disease intensity, and *t* is the evaluation period in days.

Experimental design and data analysis. The experiment was conducted using a randomized block design with four treatments and three replicates. The treatments included two *Trichoderma* spp. strains (T-94 and T-85), *Blitefree*®, and the chemical control (*Mancozeb*) as a reference. Data on severity, WIA, and AUDPC were analyzed using analysis of variance (ANOVA) and Tukey's test ($\alpha = 0.05$). Statistical analyses were performed using *StatGraphics Plus* for Windows®.

Results

Yield of *Trichoderma* spp. strains in solid-state fermentation. *Trichoderma* T-94 (1.41×10^8 conidiospores g^{-1}) produced significantly more conidiospores (p=0.0050) than *Trichoderma* T-99 (7.13×10^7 conidiospores g^{-1}) and T-82 (8.25×10^7 conidiospores g^{-1}), but statistically the same amount as *Trichoderma* T-85 (1.20×10^8 conidiospores g^{-1}) (Table 1). BYC was the best substrate (p=0.0060) for *Trichoderma* spp. strains (Table 1), producing 71% more conidiospores (1.31×10^8 conidiospores g^{-1}) than WR (7.66×10^7 conidiospores g^{-1}). The *Trichoderma* T-94 – BYC (1.55×10^8 conidiospores g^{-1}) and *Trichoderma* T-85 – BYC (1.49×10^8 conidiospores g^{-1}) interactions showed the highest sporulation (p=0.0015) compared to *Trichoderma* T-99 – WR (4.17×10^7 conidiospores g^{-1}) and *Trichoderma* T-82 – WR (4.57×10^7 conidiospores g^{-1}); however, they were statistically similar to the rest of the interactions (Table 1).

Epidemiology of black Sigatoka in the field

Severity. In the samplings at 7 (p=0.587), 14 (p=0.150), and 21 (p=0.440) days, no significant differences were observed between treatments. At 28 days, *Blitefree*® showed the highest severity (1.43) (p=0.026), while the *Trichoderma* T-85 and *Trichoderma* T-94 strains had severities of 1.10 and 1.12, respectively. At 35 days (F=5.89, p=0.0014), *Blitefree*® and *Mancozeb*® resulted in the highest severity

Table 1. Yield of *Trichoderma* spp. strains on solid substrates.

Factor Strain	Sporulation Conidiospores/g of colonized grain
Trichoderma T-85	$1.20\times10^{8} \pm 1.7\times10^{7}$ ab
Trichoderma T-94	$1.41\times10^{8} \pm 2.2\times10^{7}$ a
Trichoderma T-99	$7.13\times10^{7} \pm 1.2\times10^{7}$ b
*Trichoderma*T-82	$8.25\times10^{7} \pm 1.3\times10^{7}$ b
Substrate	Conidiospores/g of colonized grain
Whole rice (WR)	$7.66\times10^{7} \pm 1.0\times10^{7}$ b
Broken yellow corn (BYC)	$1.31\times10^{8} \pm 1.3\times10^{7}$ a
Interactions	Conidiospores/g of colonized grain
Trichoderma T-85 - WR	$9.20\times10^{7} \pm 1.9\times10^{7}$ ab
Trichoderma T-85 - BYC	$1.49\times10^{8} \pm 2.4\times10^{7}$ a
Trichoderma T-94 - WR	$1.32\times10^{8} \pm 1.6\times10^{7}$ ab
Trichoderma T-94 - BYC	$1.55\times10^{8} \pm 4.1\times10^{7}$ a
Trichoderma T-99 - WR	$4.17\times10^{7} \pm 5.3\times10^{6}$ b
Trichoderma T-99 - BYC	$1.02\times10^{8} \pm 1.1\times10^{7}$ ab
Trichoderma T-82 - WR	$4.57\times10^{7} \pm 3.1\times10^{6}$ b
Trichoderma T-82 - BYC	$1.20\times10^{8} \pm 1.1\times10^{7}$ ab
Significance	*p*-value
Strain	**
Substrate	**
Interactions	**

Means (± standard error) within rows with different letters are significantly different according to Tukey's multiple range test (p=0.05).

values of 1.45 and 1.39, respectively, whereas strains T-85 and T-94 showed the lowest values, with 1.03 and 1.28, respectively (Table 2). In the samplings at 42 (F=8.68, p=0.0001), 49 (F=16.55, p=0.00001), and 56 (F=15.31, p=0.00001) days, *Mancozeb*® exhibited the highest severity values of 1.48, .61, and 1.58, respectively, while *Trichoderma* T-85 had the lowest severity values, with 1.09, 0.87, and 0.93, respectively (Table 2).

At 63 days after application (daa) (F=3.36, p=0.0248), *Trichoderma* sp. T-94 had the highest average severity (1.58). *Blitefree*® (1.53) and *Mancozeb*® (1.54) also showed higher severity compared to *Trichoderma* T-85 (1.31). In the samplings at 70 (F=5.01, p=0.0038), 77 (F=4.97, p=0.0040), and 84 (F=4.69, p=0.0054) days, *Trichoderma* sp. T-85 had the lowest severity compared to the other treatments. Conversely, *Blitefree*® and *Trichoderma* T-94 exhibited the highest severity values in those samplings (Table 2).

Weighted infection average (WIA). Table 3 presents the WIA values. In the samplings at 35 and 42 days, *Blitefree*® and *Mancozeb*® showed the highest WIA

Table 2. Severity of black Sigatoka in "*Gran Enano"* banana through applications of *Trichoderma* spp.

Treatments	Sampling days											
	7	14	21	28	35	42	49	56	63	70	77	84
Trichoderma T-94	0.93	1.03	1.02	1.12 b	1.28 b	1.24 c	1.37 b	1.23 c	1.58 a	1.80 a	1.72 a	1.70 a
Trichoderma T-85	1.10	1.15	1.18	1.10 c	1.03 c	1.09 d	0.87 d	0.93 d	1.31 c	1.39 c	1.34 c	1.35 c
Blitefree®	1.12	1.26	1.18	1.43 a	1.45 a	1.45 b	1.32 c	1.45 b	1.53 b	1.72 a	1.74 a	1.72 a
Mancozeb	0.98	1.01	1.17	1.23 b	1.39 a	1.48 a	1.61 a	1.58 a	1.54 b	1.60 b	1.44 b	1.53 b
p=	ns	ns	ns	*	**	**	**	**	*	**	**	**

Means within rows with different letters are significantly different (Tukey, *p*=0.05). ns = not significant, * = significant, ** = highly significant.

Table 3. Weighted infection average (WIA) of black Sigatoka in "*Gran Enano"* banana through applications of *Trichoderma* spp.

Treatments	Sampling days											
	7	14	21	28	35	42	49	56	63	70	77	84
Trichoderma T-94	0.49	0.53	0.49	0.52	0.57 b	0.53 b	0.53 a	0.43 b	0.54	0.61	0.56	0.52
Trichoderma T-85	0.57	0.57	0.54	0.52	0.45 c	0.49 c	0.38 b	0.37 b	0.52	0.53	0.49	0.47
Blitefree®	0.54	0.60	0.54	0.64	0.65 a	0.62 a	0.53 a	0.59 a	0.54	0.59	0.56	0.49
Mancozeb	0.46	0.44	0.53	0.52	0.6 1b	0.61 b	0.60 a	0.56 a	0.55	0.54	0.47	0.54
p=	ns	ns	ns	ns	*	*	**	**	ns	ns	ns	ns

Means within rows with different letters are significantly different (Tukey, *p*=0.05). ns = not significant, * = significant, ** = highly significant.

values (*F*=6.08, *p*=0.0185; *F*=5.40, *p*=0.0252) with 0.65 and 0.62, respectively. Conversely, *Trichoderma* T-85 had the lowest WIA values, with 0.45 (35 days) and 0.49 (42 days). In the subsequent samplings at 49 (0.38) and 56 (0.37) days, *Trichoderma* T-85 again showed the lowest WIA values compared to *Blitefree*® and *Mancozeb*®, which ranged between 0.53 and 0.60. For the remaining samplings (63, 70, 77, and 84 days), no significant differences were found (*p*>0.05, Table 3).

Area under the disease progress curve. Figure 1 presents the total AUDPC for black Sigatoka in "*Gran Enano"* banana over 12 samplings (84 days). *Trichoderma* T-85 had the lowest AUDPC value (11.76) (*F*=153.01, *p*=0.0001), significantly lower than the other treatments, which showed values of 13.32 (*Blitefree*®), 13.03 (*Mancozeb*®), and 20.49 (*Trichoderma* T-94). Figure 2 confirms these results, showing that *Trichoderma* T-85 had the lowest AUDPC starting from the fifth sampling (35 days), remaining well below the chemical treatment. In contrast, the *Trichoderma* T-94 treatment had the highest AUDPC, distinguishing itself from

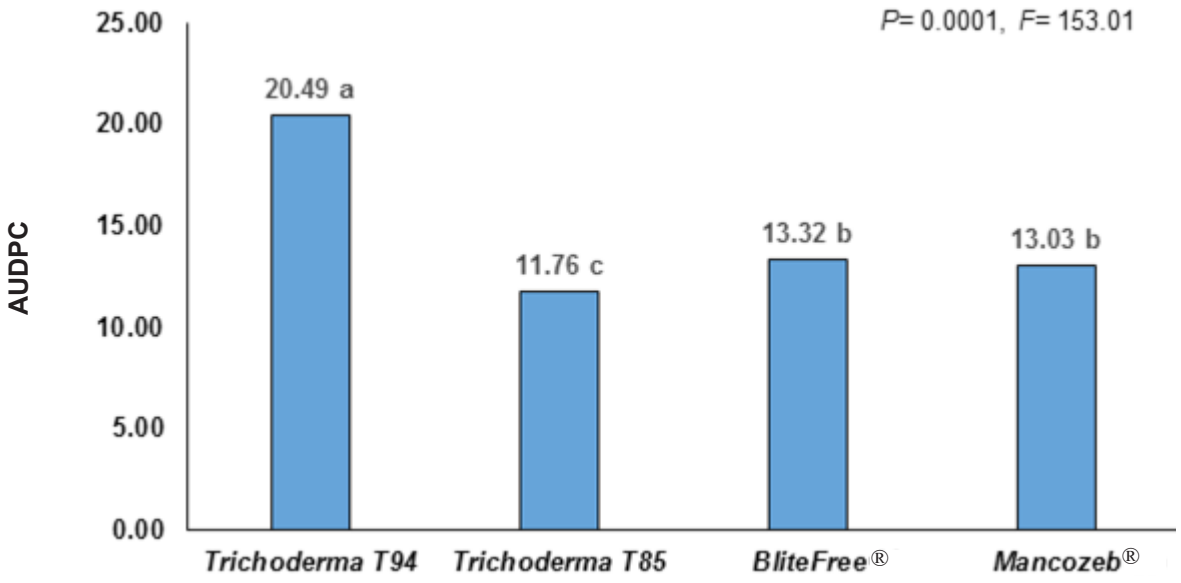

Figure 1. Total area under the disease progress curve (AUDPC) of black Sigatoka in "*Gran Enano"* banana through applications of *Trichoderma* spp.

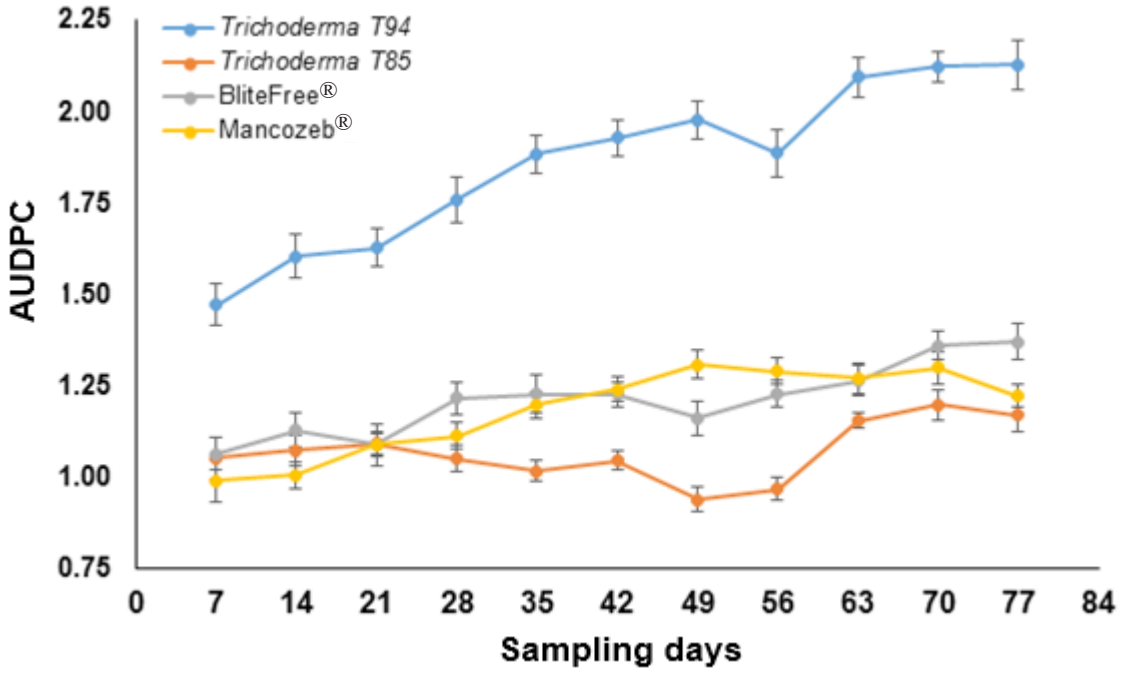

Figure 2. Behavior of the area under the disease progress curve (AUDPC) of black Sigatoka in *Gran Enano* banana through applications of *Trichoderma* spp.

the first monitoring at 7 days. *Mancozeb*® had lower AUDPC values (F=153.01, p=0.0001) compared to *Blitefree*® in the samplings at 14, 28, 49 and 77 days.

Discussion

Trichoderma spp. is widely recognized as a biological control agent against phytopathogenic fungi due to its multiple mechanisms of action. Large-scale production of *Trichoderma* spp. has commercial potential, and despite the availability of formulated products on the market, it is essential to explore new strains targeting specific phytopathogens. The micropropagules of *Trichoderma* spp. in the form of conidiospores are preferred over chlamydospores and mycelial biomass due to their viability and stability in field applications (Cavalcante *et al.*, 2008). In Mexico, the most commonly used substrates for *Trichoderma* sp. conidiospore production are whole and broken rice grains, with current costs of $30.0 and $18.0 MXN, respectively, according to the *Sistema Nacional de Información e Integración de Mercados* (*SNIIM*, 2024). The present study demonstrates that in solid-state fermentation, broken corn grains are a superior substrate for *Trichoderma* spp. conidiospore production. Broken corn can replace rice grains due to its higher yield (71% more) and lower price ($9.0 MXN per kg, according to *SNIIM*, 2024).

The yield of *Trichoderma* in SSF depends on both biotic and abiotic factors. Biotic factors include the *Trichoderma* species and strains, while abiotic factors involve substrate characteristics such as type, moisture content, particle size, bed structure, fermentation volume, and incubation conditions, including light, temperature, humidity, and fermentation time (Kumar *et al.*, 2023).

Some studies report higher yields than those obtained with *Trichoderma* T-85 and T-94 grown on BYC. These differences could be attributed to substrate temperature and humidity. Arévalo *et al.* (2017) reported yields of 3.63×10^9 and 1.45×10^9 conidiospores g^{-1} on whole and broken rice, respectively. Similarly, Cavalcante *et al.* (2008) recorded high *Trichoderma* spp. yields on both whole rice (1.45 to 5.83×10^8 conidiospores g^{-1}) and broken corn (0.18 to 7.45×10^8 conidiospores g^{-1}) using *Trichoderma harzianum*, *T. viride*, *T. koningii*, and *T. polysporum*.

Conversely, other studies suggest that *Trichoderma* spp. has low yields when grown on whole rice or broken corn. López *et al.* (2022) reported yields ranging from 0.33 to 9.20×10^7 conidiospores g^{-1} on whole rice and 0.14 to 3.70×10^7 conidiospores g^{-1} on BYC for *T. harzianum*, *T. longibrachiatum*, and *Trichoderma* sp. Likewise, Gorotiza *et al.* (2023) observed yields between 5.4×10^5 and 1.2×10^7 conidiospores g^{-1} for *Trichoderma* spp. when combining different proportions of rice and BYC.

The nutritional content of corn may be the key to improving *Trichoderma* yield, as corn has a higher starch content than rice (Mex *et al.*, 2016). Starch consists of

two polysaccharides, amylose and amylopectin, both of which are linear polymers of glucose units. *Trichoderma* sp., as a filamentous microfungus, prefers to grow in media or substrates with a high carbon content, as carbon is one of its primary nutrients (Colina *et al.*, 2009).

Regarding the epidemiology of BS, *Trichoderma* T-85 was the most effective strain in controlling disease severity and AUDPC. *Trichoderma* may be interacting with BS through its mechanisms of action, such as mycoparasitism and antibiosis. In previous studies, Sagratzki *et al.* (2015) evaluated BS severity index using four *Trichoderma* strains and a chemical fungicide (*Azoxystrobin*). Their results showed that *Azoxystrobin* reduced BS severity by 82%, followed by *Trichoderma viride* with 66%.

This trend was not observed with *Mancozeb*®, which resulted in higher severity, WIA, and AUDPC compared to *Trichoderma* T-85. *P. fijiensis*, the causal agent of BS, may have developed resistance. In this regard, Aguilar *et al.* (2014) reported that *Mancozeb*® has a high LC_{50} value (ranging from 49.21 to 112.25 mg L^{-1}) compared to other fungicides such as *Azoxystrobin* (13.25 to 51.8 mg L^{-1}) and *Propiconazole* (1.22 to 10.01 mg L^{-1}). These findings suggest that *P. fijiensis* has developed resistance to *Mancozeb*®, including isolated populations from the state of Colima.

In another study, Arzate (2006) evaluated the WIA of *P. fijiensis* in banana and used *Trichoderma* sp. strains for *in situ* control of BS. Two *Trichoderma* sp. strains were the most effective, achieving the lowest WIA at 42 days after application (daa). These results align with the present study, as *Trichoderma* sp. T-85 showed the lowest WIA in the samplings at 35, 42, and 49 daa, even when compared to *Mancozeb*®.

Conversely, Agamez *et al.* (2012) studied a native *Trichoderma* sp. strain, a *Trichoderma*-based bioformulation, and *Propiconazole* as a chemical control for BS in *Hartón* banana. They measured WIA but found no significant differences in BS control among treatments. It is possible that after three or four applications of *Trichoderma* sp. on plants with BS, the treatment becomes effective in reducing WIA and severity, with noticeable results between 35 and 49 days. Biological products require time to establish and control the phytopathogen or disease. Additionally, environmental conditions are crucial, as the timing of application (rainy or dry seasons) plays a significant role in achieving effective BS management in the field (Becker *et al.*, 2021).

On the other hand, *Blitefree*® (*Streptomyces* spp.) resulted in similar severity levels and AUDPC values to *Mancozeb*®. This product contains an extract from actinomycetes, which may explain why it was not more effective than *Trichoderma* sp. Conidiospores may interact more easily with *M. fijiensis* through mycoparasitism compared to *Streptomyces* extract, which could be more effective at higher doses.

This aspect should be studied further under *in situ* conditions, as *in vitro* studies suggest that some species, such as *Streptomyces galilaeus* CFFSUR-B12, exhibit antagonism against *M. fijiensis* through antibiosis due to chitinase production (Moreno *et al.*, 2016).

Conclusions

Broken yellow corn is a better substrate for producing *Trichoderma* spp. conidiospores, yielding 71% more than other substrates. The strains with the highest conidiospore production were *Trichoderma* T-94 and *Trichoderma* T-85. However, in weekly *in situ* foliar applications, only *Trichoderma* T-85 effectively reduced severity, WIA, and AUDPC of black Sigatoka in *Gran Enano* banana, starting 28 days after application. *Mancozeb*® and *Blitefree*® were less effective.

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