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Trichoderma species have potential in suppressing *Fusarium oxysporum* f.sp *cubense* infecting banana in Kenya

Samuel Musime Malaka ^{1, 2*}, David Mwongera Thuranira ³, Maina Mwangi ¹, Shem Bonuke Nchore ⁴, Hudson Alumiro Lubabali ², Sylvia Kuria ³, Daniel Omingo Omari ² and Charity Wangari Gathambiri ³

¹Kenyatta University, Department of Agricultural Science and Technology, P.O. Box 43844-00100, Nairobi, Kenya
²Kenya Agricultural and Livestock Research Organization-Coffee Research Institute, P.O. Box 4-00232, Ruiru, Kenya
³Kenya Agricultural and Livestock Research Organization- Horticulture Research Institute, P.O. Box 220-0100, Thika, Kenya
⁴Kenyatta University, Department of Plant Sciences, P.O. Box 43844-00100, Nairobi, Kenya

⁴Kenyatta University, Department of Plant Sciences, P.O. Box 43844-00100, Nairobi, Kenya **Corresponding author** - <u>samuelmalaka540@gmail.com</u>

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ABSTRACT

Objectives: This study examined the effectiveness of carbendazim, *Trichoderma harzianum*, *T. asperellum*, *T. viride*, and a combination of the carbendazim and the bio-fungicides for the management of Foc both under *in vitro* and greenhouse conditions.

Methodology and Results: The poisoned food technique was used in the *in vitro* tests. Fungal radial growth was recorded daily for seven days. In the greenhouse, the test products and Foc were applied directly into the soil, disease severity data was recorded at seven day intervals for 98 days using a scale of 1-5. Data on plant biomass was recorded at the end of the experiment (98 days). ANOVA was used to analyze data on fungal radial growth, disease severity, and plant biomass. The Student-Newman-Keuls (SNK) test was used to compare the results at $P \le 0.05$. For the *in vitro* test, all treatments significantly (P<0.05) suppressed the growth of Foc compared to the untreated control. In the greenhouse trials a significant difference (P<0.05) in external/yellowing symptoms was observed but vascular discoloration was not different.

Conclusions and application of findings: This study provides insights into the performance of bio fungicides and carbendazim in managing Panama disease.

Keywords: Banana, Fusarium oxysporum f.sp. cubense, , Panama disease, Trichoderma sp.

INTRODUCTION

Banana is one of the primary food crops cultivated in Kenya for both domestic consumption and sale (Mbaka *et al.*, 2008). During times of drought, the foliage and pseudo-stems of the banana plant are utilized as cattle feed. Banana leaves are also utilized for packing agroproduce and roofing. The crop has high potential to improve food and nutritional security while also being a source of revenue for smallholder farmers. Most

banana producers cultivate dual-purpose cultivars that can be used for both cooking and dessert (Wahome et al., 2021). Panama disease is present in all banana growing regions in Kenya, with up to 80% of plantations developing symptoms (Momanyi, 2019). Race 1 and race 2 of the Foc pathogen have been reported in Kenya, but race 4 has not been reported. Wang'ae, Muraru, Bluggoe, Gros Michel, and Apple banana are some of the local banana varieties that have been reported to be susceptible to Foc races in Kenya (Kung'u & Jeffries, 2001). Currently, there are no effective long-term Foc management techniques (Jones, 2018). The current methods of preventing Panama disease include early disease detection and removal of infected plants, strict hygiene standards, and the use of resistant banana varieties, such as the Dwarf Cavendish, Giant Cavendish, FHIA 17, Williams and FHIA 23, which are resistant to Foc races 1 and 2. However, Cavendish varieties lack the flavor of Gros Michel variety

MATERIALS AND METHODS

Study area: *In vitro* test was conducted at the Kenya Agricultural and Livestock Research Organisation (KALRO) -Horticulture Research Institute, Thika while greenhouse experiments were carried out at KALRO Coffee Research Institute, Ruiru.

Isolation and confirmation of pathogenicicty of Fusarium oxysporum fsp.cubense race 1 for use in in vitro and greenhouse trials: Samples of infected banana and plantain plants were obtained from 57 farms in Central and Eastern Kenya. Banana root samples were obtained from three sampling points of the banana mat using a soil auger, a Jembe/hoe, and a panga at 0-20 cm depth as described by Guimaraes et al. (2021). The tools used for sample collection were cleaned and sterilized with 70 % ethanol to prevent contamination between sampling points in the farms. The banana roots collected were packed in nylon bags, well labeled, and

(Dita et al., 2018). Due to the nature of the disease, chemical fungicides may not be able to effectively control Panama disease once it invades the plants. Soil fumigants may not eradicate it from the farm since it produces survival structures (chlamydospores) that remain dormant in the soil for long periods of time. To prevent Foc infection, it is recommended to dip banana seedlings in Carbendazim (10g in 10 L of water) or in Propamocarb (50ml in 20 L of water). Several other synthetic fungicides and biopesticides have been tried for Foc control (Ringera et al., 2013). For example, Rhizatech® from Dudutech reduced Foc inoculum by 55% (Mukhongo et al., 2015). This study aimed to determine the *in vitro* and greenhouse efficacy of Trichoderma harzianum, Trichoderma asperellum, and Trichoderma viride in managing F. oxysporum f.sp. cubense affecting bananas in Central and Eastern Kenya.

kept in a cooler box containing ice cubes before transporting them to the Horticulture Research Institute Mycology laboratory for fungal isolation. Banana roots were cleaned using running water. The root tissues were cut into small pieces measuring about 5 mm long. Each piece was then surface-sanitized by dipping in 70% ethanol for 10 seconds, 1% sodium hypochlorite for 1 minute, and rinsed twice in sterile distilled water. The root tissues were air-dried in a laminar flow hood on a sterile paper towel. The air-dried roots were then plated on Agar Agar that had been amended with 2.5 mg/mL chloramphenicol to inhibit bacterial growth (Perez et al., 2014). The Petri plates were incubated for three days in the dark at 22.5 °C. After 72 hours, fungal growth was observed on the plated tissues. Pure fungal colonies were transferred to new plates with PDA. Pure colonies were obtained using the single-spore subculture procedure

(Perez *et al.*, 2014; Magdama *et al.*, 2019; Kalman *et al.*, 2020). Isolates suspected to be *Fusarium* strains were identified as *Fusarium oxysporum* f.sp *cubense* using morphological features and molecular characterization using specific primers for Foc races 1, 2, and 4. All positive isolates were amplified by a Foc race 1 specific primer. The isolate used in subsequent experiments in the study was a confirmed pathogenic Foc strain that was virulent to the Gros Michel banana variety during the pathogenicity test experiment.

In vitro efficacy of bio fungicides against Fusarium oxysporum f.sp. cubense Commercial fungicides including bio Trichoderma viride (Osho Chemical Industries Limited), T. harzianum (Koppert Biological Sciences), and T. asperellum (Real IPM, Thika, Kenya), and the commercial fungicide Carbendazim 500 SC (control), were tested for anti-fungal activity against Foc according to the procedure described by Adhikari et al. (2018). PDA was prepared and sterilized in an autoclave for 15 minutes at 121°C and 2.5mg/ml chloramphenicol was added to inhibit bacterial growth. Test commercial bio fungicides were added at a ratio of 0.6, 0.1 and 0.1% for T. viride, T. harzianum, and T. asperellum, respectively, as recommended for of fungal pathogens control bv the manufacturers. Carbendazim 500 SC alone

Table 1: Treatments and	their	descriptions
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was used at a concentration of 0.5% and at a concentration of 0.25% when in combination the biofungicides. The with PDA supplemented with test fungicides was dispensed into sterile Petri dishes under aseptic conditions and allowed to cool and solidify. After that, a 10mm disc of a seven-day-old Foc culture was cut aseptically with a sterile cork borer and transferred upside down in the sterile plated media. The treatments were replicated four times. The plates were incubated at 24 \pm 1°C for seven days, with the fungal colony growth monitored every 24 hours. The colony diameter was measured in millimeters using a digital Vernier caliper. The percentage control was calculated using the formula:

% control = $\frac{C-T}{C} \times 100$ where C is the untreated (control) colony diameter and T is the treatment colony diameter (Gakuubi *et al.*, 2017).

Efficacy of bio fungicides against *Fusarium oxysporum* f.sp. *cubense* in the greenhouse

The greenhouse experiment was carried out using sterile soil and tissue-cultured banana seedlings (cv. Gros Michel) that were two months old. Before inoculation, soil was removed from the banana plantlets by gently washing the roots with sterile distilled water to expose the roots. The treatments that were used in the study are as shown in table 1.

S.No.	Treatment	Application rate (%)
1	Trichoderma viride	0.60
2	T. harzianum	0.10
3	T. asperellum	0.10
4	Carbendazim 500 SC	0.50
5	Trichoderma viride + Carbendazim	0.25
6	T. harzianum + Carbendazim	0.25
7	T. asperellum + Carbendazim	0.25

There were two control treatments, one with Foc alone and one with untreated plants. The experiment was set up in a Complete Randomized Design (CRD) with four replications. Each bio fungicide treatment was applied in the rhizosphere in a volume of 20 mls two weeks before pathogen inoculation. Since the bio fungicides used were

commercial, they were applied according to the manufacturers' recommendation. The biofungicide treatments were re-applied during pathogen application. Except for the untreated control, each treatment received a volume of 20mls of 1 x 10⁷ CFU's/ml Foc spore density. External disease severity (based on chlorosis) was recorded at seven day intervals for 98 days using a scale of 1 to 5 as described by Perez *et al.* (2014), where 1 indicates no symptoms observed, 2 = initial yellowing on lower leaves, 3 = all lower leaves have turned yellow, and some of the younger leaves have also become discolored, 4 = intense yellowing on all leaves, and 5 = plants dead/complete wilting.

Internal symptoms were assessed once at 98 days after inoculation by assessing the extent of vascular discoloration, using a scale of 1 - 5 where: 1 indicates no symptoms, 2 = initial rhizome discolorations, 3 = slight rhizome discolorations throughout the vascular system, 4 = rhizomes with necrosis in the majority of

RESULTS

In vitro efficacy of bio fungicides against *Fusarium oxysporum* f.sp. *cubense*: There was a significant (P<0.05) difference in suppression of the growth of Foc by all treatments when compared to the control

the interior tissues, and 5 = rhizomes completely necrotic as described by Perez *et al.* (2014). At the end of the experiment, the plant root and shoot biomass were assessed after drying them in an oven at 80°C for 72 hrs. Pathogen re-isolation was also done at the end of the experiment from the plant root and vascular tissues to confirm whether the pathogen responsible for the observed symptoms was *Fusarium oxysporum* f.sp *cubense*.

Data analysis:The data for *in vitro* trials, external and internal symptoms, as well as plant biomass, were organized in MS Excel spreadsheets and subjected to ANOVA. Correlation analysis on disease severity and plant biomass was also performed using R statistical software to determine relationship between disease severity and banana yield. Significantly different means were separated using the Student-Newman-Keuls (SNK) test at P \leq 0.05.

(Table 1 & 2, Plate 1 & 2). *T. asperellum*, carbendazim, and the three bio fungicide/carbendazim combinations were more effective in suppressing Foc.



Figure 1: Mean colony diameter (mm) of Foc at 168 hrs in the *in vitro* testing of fungicides. Key: T.harz = *T. harzianum*, T.vir = *T.viride*, T.asper = *T.asperellum*, Carben = Cabendazim.



Plate 1: Foc colony diameter in different treatments at 168 hrs of growth, plate A = Control, B = Trichoderma viride, C = Trichoderma harzianum, and D = Trichoderma asperellum.

Note the fast-growing *Trichoderma* mycelia in plate B, C and D. A loopful of the growing fungus was picked at the end of the experiment and confirmed under the microscope to be *Trichoderma* spp. No growth occurred in treatments with Carbendazim. All treatments differed significantly (P<0.05) in suppressing Foc (Table 3), of the treatments, Carbendazim 500SC alone or combined with *T. viride*, T.

harzianum, and *T. asperellum* suppressed Foc by over 75%. *Trichoderma asperellum* on its own had 70-73% control, while *T. harzianum* achieved 61-67% suppression of Foc compared to the control, *T.viridae*, *T. asperellum*, and T. *harzianum* (Figure 2). Disease symptoms were observed on all the plants in each treatment except in the untreated control.



Figure 2: Suppression of Foc mycelial growth by *T. asperellum* (T.asper), *T. harzianum* (T. harz), *T. viride* (T. vir) and Carbendazim 500 SC (Carben).

Efficacy of bio fungicides against *Fusarium* oxysporum f.sp. cubense in the greenhouse: Panama disease severity was significantly higher (P<0.05) on banana plants treated with Foc alone, when compared to all other treatments (*Trichoderma viride*, *T. harzianum*, *T. asperellum*, Carbendazim 500 SC, Carbendazim + *T. viride*, Carbendazim + *T. harzianum*, Carbendazim + *T. asperellum*, and untreated control). For the internal symptoms, the treatments had no significant (P>0.05) difference in disease severity compared to Foc alone (Figure 3).



Figure 3: Panama disease severity based on internal and external symptoms under different management treatments in the greenhouse (Disease score 1-5). Key: T.harz = T. harzianum, T.vir = *T.viride*, T.asper = *T.asperellum*, Carben = Cabendazim. Data is a mean of four replicates.

The root biomass varied significantly (P<0.05) between bananas treated with *T. asperellum* and those treated with Foc alone. Bananas treated with *T.asperellum* also had a significantly (P<0.05) higher root biomass than the untreated control. There was no significant (P>0.05) difference in terms of banana root biomass between the plants treated with *T. viride*, *T. harzianum*, *T. asperellum* + Carbendazim, *T. harzianum* + Carbendazim, *T. viride* + Carbendazim, Carbendazim alone, the untreated control, and the bananas treated with

Foc alone (Figure 4). The shoot biomass differed significantly (P<0.05) between the plants treated with *Trichoderma viride* and *T. asperellum*, compared to the other treatments. There was no significant (P>0.05) difference in terms of banana shoot biomass between the bananas treated with *T. harzianum*, *T. asperellum* + Carbendazim, *T. harzianum* + Carbendazim, *T. viride* + Carbendazim, Carbendazim alone, the untreated control, and the bananas treated with Foc alone (Figure 4).

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Figure 4: Effect of Foc on banana plant biomass under different management treatments. Key: T.harz = T. harzianum, T.vir = T.viride, T.asper = T.asperellum, Carben = Cabendazim.

Association between disease severity and plant biomass: Bananas that recorded the lowest disease severity had the highest plant biomass and vise-versa. The untreated control recorded no infection with Foc. Banana plants treated with *Trichoderma* bio controls recorded a lower disease severity compared to the untreated control but at the same time recorded the highest shoot biomass (Figure 5).





Figure 5: Correlation of disease severity internal symptoms to plant biomass

DISCUSSION

The bio fungicides (T. asperellum, T. viride and T. harzianum) suppressed the growth of Foc under in vitro conditions and resulted in higher root and shoot biomass under greenhouse conditions. Results show that Trichoderma species suppressed disease while also promoting plant growth. Trichoderma species produce hormone-like secondary metabolites, auxin-like compounds, and peptides in the rhizosphere (Vinale et al., 2014) that help in plant growth. The production of secondary metabolites could also contribute to Trichoderma spp suppressing Foc foliar symptoms. However Trichoderma spp did not suppress vascular discoloration, unlike Carbendazim. Furthermore. Trichoderma spp outcompetes and antagonizes other fungal microbes, such as the pathogenic Foc, allowing the plant to thrive without experiencing pathological stress. Trichoderma spp coils around fungal hyphae, it produces lytic enzymes that degrade the cell wall of fungal pathogens and cause loss of cell content (Elad et al., 1983; Ousley et al., 1994). Halifu et al. (2019) reported that seedling biomass, root structure index, soil nutrients, and soil enzyme activity of *Pinus sylvestris* var. mongolica plant increased significantly after Trichoderma spp inoculation. The reduced plant biomass under Carbendazim treatment could be attributed to the fact that the chemical is a broad-spectrum fungicide that inhibits the growth of some fungi, including those that can act as bio control agents, such as Trichoderma spp. This could also explain the inhibition of Trichoderma spp in vitro when combined with

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food and nutrition security through development and dissemination of technologies"

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