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EVALUATION OF BIOCONTROL EFFICACY OF TRICHODERMA HARZIANUM AGAINST FUSARIUM OXYSPORIUM IN TOMATOES (SOLANUM ESCULENTUM L.)

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ABSTRACT

Utilizing biological techniques to manage plant diseases has demonstrated efficacy in fostering ecosystem sustainability and augmenting agricultural output and quality. A study was undertaken at the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria to evaluate the suppressive impact of T. harzianum on the proliferation of F. oxysporusm f. sp. lycopersici, and the advancement of F. oxysporum infection in tomato plants. The trial comprised culturing only T. harzianum in the Petri dish, culturing T. harzianum and F. oxysporum in the Petri dish (dual culture), and culturing only F. oxysporum in the petri dish, and with Mancozeb. The influence of T. harzianum on the advancement of F. oxysporum infection in tomato plants comprises of four distinct concentrations (1g, 2g, 3g, and 4g) of T. harzianum extract. The results revealed that the dual culture of T. harzianum and F. oxysporum successfully suppressed the mycelial growth of F. oxysporum. On the seventh day, the level of antagonistic activity exhibited by T. harzianum against F. oxysporum peaked at 9.05mm. Leaf yellowing and severe wilting, indicative of Fusarium wilt, were seen during the monitoring period. Tomato disease incidence and severity exhibited a consistent linear decline with increasing concentrations of T. harzianum. The disease severity reached its maximum level, (31.6%) on week four. Mancozeb treatment compared favorably with 4g T. harzianum in decreasing the mycelial growth of F. oxysporium, and reducing wilt incidence and severity. Therefore, T. harzianum can function as a biocontrol agent, providing a sustainable substitute for synthetic fungicides in the control ofFusariumoxysporium wilt disease.

Keywords: Biofungicides, fungal pathogens, Fusarium, Trichoderma, biocontrol

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INTRODUCTION

Tomato plant, (*Solanum esculentum* L.), is widely recognized for its strong market demand, economic significance (Barari, 2016), medicinal properties, and abundance of essential elements such as vitamin B, vitamin C, phosphorus, potassium, and magnesium (Ali *et al*., 2020).*Fusarium oxysporium* is a significant soil-borne disease that has economic implications as it diminishes both the quality and quantity of tomatoes (Singh and Kamal, 2012). *F. oxysporum* f. sp. lycopersici is the primary cause of fusarium wilt disease in tomatoes. This disease poses a significant danger as it can lead to substantial economic losses, ranging from

10 to 90%, depending on environmental conditions (Singh and Kamal, 2012). The signs of the disease caused by *F. oxysporum* include leaf yellowing; stem browning, wilting, root browning, reduced root system, and necrosis. These symptoms lead to a decrease in photosynthesis in infected plants (Tomar *et al*.,2017). *F. oxysporum* spores (chlamydospores) in the soil make cultural and chemical control methods less successful in combating the

Traditional control methods are ineffective, and synthetic fungicides are toxic, expensive, and polluting (Senthilkumaret al., 2011; Sundaramoorthy and Balabaskar, 2013).

Biological control methods, such as Trichoderma, a mycoparasitic fungus, are cost-effective and risk-free alternatives to chemical pesticides for plant diseases (Zin and Badaluddin 2020; Ferreira and Musumeci 2021).Pathogenic antagonists in the biological control of diseases are environmentally and economically beneficial because they do not affect humans or animals (Alwathnani et al., 2012; Ghazalibiglar et al. 2016).

T. harzianum reproduces rapidly and outcompetes fungal pathogens for resources and space.

It exhibits antibiosis, fungistasis, and myco-parasitism (Howell, 2003; Ramezani, 2009; Soodet al., 2020). Trichoderma inhibits plant pathogenic fungi such Rhizoctonia, Fusarium, and Pythium, with varying genetics (Zin and Badaluddin 2020; Barbosa et al., 2022).Trichoderma directly affects pathogenic fungi by producing antibiotics and lytic enzymes like cellulases, hemicellulases, xylases, and chitinases (Qin et al.,2021). T. harzianum and T. virens produce Trichodermamine B and viridin, virone, and trichosetin, which may block the polygalacturonase enzyme released by F. oxysporum f. sp. lycopersici (Howell, 2003; Singh et al., 2022; Harman et al., 2004). The study aimed at evaluating the inhibitory effects of T. harzianum against F. oxysporium f. sp. lycopersici mycelial growth, and its wilt incidence and severity reduction for long- term sustainable disease management in Nigeria.

MATERIALS AND METHODS

Location of the study area

The study was conducted at the botanical garden of Imo State University, Owerri situated in the humid rainforest ecosystem of Nigeria, during the 2020/2021 cropping seasons. The latitude and longitude coordinates of the Imo State University, Owerri are:5.4891° N, 7.0177° E. This region is predominantly inhabited by farmers, and the ecosystem is known to contribute to the higher occurrence and intensity of tomato wilt (Terna et al., 2017). The laboratory experiment was conducted in the Department of Plant Science and Biotechnology, Imo State University Owerri.

Soil analysis

The top 0- 3cm soil profile of the study area, at the start of the experiment, was processed and analyzed for some physical and chemical properties following standard procedures (Page, 1983; White, 1988) (See Table 1).

Experimental Design

The impact of *T. harzianum* on the mycelium growth of *F.oxysporium* was examined using a completely randomized block design (CRBD) consisting of four blocks and four treatments replicated three times. The treatments consisted of the following: *T. harzianum* in the Petri dish, culturing *T. harzianum* and *F. oxysporum* in the Petri dish (dual culture), and culturing only *F. oxysporum* in the Petri dish, supplemented with the positive control,(Mancozeb). Each treatment was replicated three times. The influence of *T. harzianum* on the advancement of *F. oxysporum* infection in tomato plants comprises of four distinct concentrations (1g, 2g, 3g, and 4g) of *T. harzianum* extract.

Isolation and identification of *Trichoderma harzianum*

The *T. harzianum* was isolated using the plate dilution method as described by Mendoza-Mendoza et al. (2016) and Brito-Vega (2020). Ten grammes of soil material, was individually suspended in 500 mL conical flasks containing 100 mL of sterilised distilled water. The mixture was then agitated for a duration of 5 minutes. Subsequently, one-milliliter portions were extracted using a graded micropipette (100-1000 μL) and transferred to test tubes containing 4 mL of sterile distilled water. Each sample was subjected to serial dilutions (1/10 w/v), and 0.5 mL portions from each dilution were evenly spread on Petri dishes containing PDA culture medium. The culture medium was supplemented with ampicillin and amoxicillin (1 mg mL-1 of each). A glass was used to uniformly distribute the sample material on the surface of the culture medium. Each dilution was replicated three times. The petri dishes were placed in an incubator set at a temperature of 25 °C ± 1 for a duration of eight days. Subsequently, the T. harzianum was isolated again to obtain pure culture. After a duration of eight days, samples of mycelium were extracted from the colonies using a platinum loop and applied onto PDA culture media using streaking. *T. harzianum* was identified by its quick and extensive development and its white-to-green coloration. A compound microscope was used to observe the morphological features of conidia and conidiophores.

Isolation and identification of *Fusariumoxysporium*

Following the methods outlined by Suwandi *et al.* (2022), *F.oxysporium* obtained from tomato roots exhibiting wilt symptoms in the field and from soil contaminated with *F. oxysporium* were transported to the laboratory. The diseased tomato root samples were cut into one-centimeter-long with a steriled sharp knife, and then washed under running water

and their surfaces were sterilised by immersing them in a solution containing 1% sodium hypochlorite for 2 minutes. Afterward, the segments were rinsed three times with distilled water and dried on filter paper in a laminar airflow. Subsequently, the samples were placed on a Petri dish containing 2% (w/v) agar and 0.1% streptomycin sulphate, which served to suppress bacterial growth. The samples were then kept in an incubator for a period of 48 hours. The mycelium was transferred to the Potato Dextrose Agar (PDA) medium using the single hyphae technique. The obtained isolation results were utilised for subsequent research. The fungus was subsequently identified by examining its culture characteristics and microscopic traits, as described by Leslie and Summerell (2006) and Jidda (2017).

Seed collection, sowing, and experimental procedures

Certified tomato seeds were obtained from the Imo State Agricultural Development Programme, Owerri in October 2022. The seeds were sun-dried and stored in a fabric bag, where they were maintained at an ambient temperature of 25°C. Prior to sowing, tomato seed were primed with sterile water for a duration of 10 hours. Aseptic technique was employed to gather spores from 14-day old cultures by scraping the surface of the Potato Dextrose Agar (PDA) using a sterilised spatula. The spore count was determined using a hemocytometer. The spores were diluted in sterile distilled water to achieve a final spore concentration of 1.0 x 106 colony-forming units per millilitre (CFU/ml). *T.harzianum* was measured at various concentrations of 1g, 2g, 3g, and 4g, derived from cultures that were 14 days old.

Pots with a top diameter of 24 cm, bottom diameter of 18 cm, and height of 20.5 cm were sterilised using a 10% solution of sodium hypochlorite in order to minimise the presence of unwanted germs. The pots were filled to a three-quarter capacity with loam soil, and was sterilised in the oven at a temperature of 80°C for a duration of 48 hours. Three tomato seeds were planted at a depth of 0.5 cm in each pot. Each pot received an identical volume of 300 ml of sterilised water, which had been treated in an autoclave. The tomato seedlings were reduced to one seedling per pot 10 days after they first appeared. *T. harzianum* was given at concentrations of 1g, 2g, 3g, and 4g, along with Mancozeb treatments, on the 14th day after emergence (DAE). Fourteen days after applying Trichoderma and Mancozeb treatments, ten millilitres of Fusarium oxysporium was administered to the pots. Each pot received a weekly application of 250 cc of sterile water, repeated four times.

Preparation of crude extracts of *Trichoderma harzianum*

The *T. harzianum*were cultivated on PDA medium and subjected to incubation at a temperature of 25 ± 2°C for a duration of 7 days. Subsequently, the fungal growth was carefully removed from the agar surface in order to get a mycelial sample weighing 5 g. Next, the mycelial growth was introduced into a solution of 200 mL of 80% ethanol containing 0.2 M HCl. The mixture was then placed on a rotating shaker and incubated at a temperature of 25 ± 2°C for a duration of 24 hours. The specimens were subsequently subjected to centrifugation at a speed of 9000 rpm in order to isolate the solid constituents. The solid components were subjected to a repeated extraction operation using ethyl acetate as the solvent. Ultimately, the extracts were amalgamated and subjected to partial evaporation utilising a rotatory evaporator.

The inhibitory effect of *T. harzianum*on *F. oxysporum f. Spplycospesici*

Following the procedure (Takudzwa*et al*.,2022), two distinct media were formulated, Potato dextrose agar (PDA) and PDA enriched with Mancozeb (75% WP). The two media were prepared individually in accordance with the manufacturer's instructions. The purified strains of *T. harzianum* and *F. oxysporum* were cultivated in large quantities using aseptic techniques on potato dextrose agar (PDA) in Petri dishes with a diameter of 90 mm. Aseptic cork-borer was utilised to excise a mycelial disc with a diameter of 5 mm from pure cultures of *T. harzianum and F. oxysporum.* Subsequently, the two fungal discs were positioned 45 mm apart in dual cultures. The control experiments include 5 mm mycelial disc from pure

cultures of *T. harzianum and F. oxysporum* cultivated alone, and *F. oxysporum* cultivated in PDA supplemented with Mancozeb (positive control). The Petri dishes were arranged in a Completely Randomised Block Design (CRBD) consisting of four blocks, with each treatment being reproduced three times. The plates were placed in an incubator set at a temperature of 25 ± 2°C for a duration of seven days. Sterile conditions were maintained throughout the whole procedure.

The mycelial growth inhibition

The formula for calculating mycelial growth inhibition is (C - T) / C, where C represents the growth of mycelium in the control group and T represents the growth of mycelium in the treatment group.

Effects of T. harzianum on Tomato wilt development

The frequency of disease occurrence was assessed on a weekly basis by enumerating the number of plants exhibiting signs of *F. oxysporum* within a period of 1 to 4 weeks following inoculation. The calculation was performed via the formula provided below:

$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \times 100$$

The frequency of disease occurrence was assessed on a weekly basis by enumerating the quantity of tomato plants exhibiting wilt symptoms during the time frame of 1 to 4 weeks after inoculation.

The disease severity

The severity of the disease was evaluated on a weekly basis, beginning from 2 weeks after inoculation (WAI), using a grading system ranging from 1 to 5. The rating was determined based on observable symptoms such as yellowing, wilting, and necrosis on both the leaves and stem. The rating scale ranged from 0 to 5, with 0 indicating healthy plants with no symptoms. A rating of 1 indicated 1-2 leaves showing yellowing, wilting, or necrosis, accounting for 1-25% of the plant. A rating of 2 indicated 3-5 leaves showing these symptoms, accounting for 26-50% of the plant. A rating of 3 indicated 6-8 leaves showing symptoms, accounting for 51-75% of the plant. A rating of 4 indicated 9-11 leaves showing symptoms, accounting for 76-100% of the plant. Finally, a rating of 5 indicated that the plant was dead.

DATA ANALYSIS

All data collected underwent statistical analysis using analysis of variance (ANOVA). The significance of differences between means was assessed using Tukey's HSD test ($p < 0.05$) with the use of MINITAB 19 software.

RESULTS

Effect of *T. harzianum* on mycelium growth of *F. oxysporum*.

Notable disparities were observed in the growth of *F. oxysporum* in pure culture, dual culture, and mancozeb on the third and seventh days following inoculation. In a dual culture setting,

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the presence of *T.harzianum* resulted in a significant ($p < 0.05$) decrease in the growth of *F. oxysporum* mycelium between 3 and 7 days after inoculation (DAI). A significant difference ($p < 0.05$) was seen in the inhibition of *F.oxysporium* mycelial growth between different days of inoculation of *T. harzianum* and control treatments (Table 2). *T. harzianum* exhibited the most pronounced growth inhibitory activity (9.05mm) against *F. oxysporum* on the 7th day of incubation, whereas day 3 demonstrated the least inhibitory activity (17.2mm), (Table 2). The use of Mancozeb resulted in the most significant decrease in mycelial development during the observation periods.

The mycelial diameter exhibited a negative correlation with the inhibition percentage in the dual culture, (Table 2).Our findings indicate that the mycelial growth of *F. oxysporium* differed among the various treatments and observation days.

Field Disease incidence

The symptoms of Fusarium wilt disease showed substantial variation across all treatments during the study periods. During the monitoring period, we saw typical indications of Fusarium wilt, such as leaf yellowing and severe wilting. The onset of yellowing symptoms were observed on the older leaves, subsequently leading to chlorosis and wilting of the foliage. The seedlings from the sterile water exhibited no signs of disease throughout the whole assessment period.

The plants that were treated with Mancozeb had the lowest percentage of disease incidence, (15%). On the other hand, the treatment with sterile water had the greatest percentage of disease incidence (95%),(Fig.1).The impact of varying doses of *T. harzianum* on the rate of tomato wilt disease incidence was statistically significant ($P \leq 0.05$). The occurrence of tomato wilt disease decreased in a linear manner as the concentrations of *T. harzianum* increased (Fig.1).

Efficacy of *Trichoderma harzianum* against *Fusarium oxysporum* wilt disease severity in tomato plants from week 1 to 4 after inoculation.

The severity of the disease progressively escalated from week 1 to week 4. Week 4 exhibited the most notable disease severity, (31.6%) (Table 3). The negative control (sterile water) exhibited the highest disease severity (38.08%), while 4g of *T. harzianum*recorded 6.03%. In contrast, mancozeb had the lowest disease severity at 3.64% across the monitoring periods (Table 3).

The impact of varying concentrations of *T. harzianum* on the severity of tomato wilt disease was statistically significant ($P \leq 0.05$). The severity of tomato wilt disease showed a linear decline as the concentrations of *T. harzianum* increased, (Table 3).

DISCUSSIONS

The result of the study showed that *Trichoderma harzianum* is a suitable biocontrol agent for the *F. oxysporium* wilt disease due to its effectiveness in inhibiting the growth of *F. oxysporium* and decreasing wilt incidence and severity of tomato.

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The inhibition of *F. oxysporium* mycelial growth by *T. harzianum* observed in this study was similar to previous findings of Lakhdari et al.,(2018) who demonstrated the the mycelial growth inhibition of *T. harzianum* against *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *Alternaria solani*, and Carvalho et al.,(2014), against *Fusarium oxysporum f. sp. Phaseoli.* In addition, the findings are supported by Alwathnani *et al*.,2012; Sundaramoorthy and Balabaskar,2013) who documented the suppression of *Fusarium oxysporum* spp by Trichoderma spp.

The suppression of *F. oxysporium* f spp lycospesici mycelial growth by *T. harzianum* may be attributed to the synthesis of antibiotics (Bhardwaj and Kumar, 2017), the release of cell wall degrading enzymes leading to cellular damage (Zhao *et al*., 2014), and the high colonisation rates of *T. harzianum*, which suppress the growth of competing microorganisms (Bizos *et al*., 2020).

The decreased tomato wilt disease incidence by the application of *T. harzianum* observed in the present study was consistent with the report by (Harman, 2005; Moosa *et al*.,2017) who documented a decrease in the occurrence of tomato wilt disease incidence due to the application of *T. harzianum*.

The decreased tomato wilt disease severity by *T. harzianum* application observed in the present study collaborates with Khalil, and Shimaa, (2020) who reported decreased foliar yellowing and wilt or vascular browning of tomato, and Haque et al.,2023, who reported drastic reduction of tomato wilt severity and improved tomato growth and biomass.Inaddition, application of *T. harzianum* on peanut seeds was reported to reduce both the incidence and the severity of peanut brown root rot (Erazo et al., 2021).The decrease in wilt disease severity by *T. harzianum* may be attributed to the synthesis of secondary metabolites, increased enzymatic activity, and changes in the pathogen hyphae (Erazo et al., 2021).

CONCLUSION

The strong antagonistic activity of *T. harzianum* against *F. oxysporium* growth, and reduction of the wilt disease incidence and severity, therefore qualify *T. harzianum* to be exploited in the development of natural fungicides to avoid the negative effects of synthetic fungicides.

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APPENDICES

Table 1. Physical and chemical properties of the soil profile of the study area

Sand	Silt	Clay	pH	Organic matter	Organic carbon	Nitrate	PO_4	SO_4	Ca^2+	Mg^2+	K2+	Na^2+	CEC
830 kg–1	330 kg–1	90g kg–1	6.01	20 g kg–1	0.96 g kg–1	12.6 mg/kg	4.21 mg/kg	121.73 mg/kg	7.62mg /kg	7.41 mg/kg	6.69 mg/kg	1.59mg /kg	23.32 Ca/mg

Table 2. Effect of *T. harzianum* on mycelium growth inhibition of *F. oxysporum* (mm)

	Mycelium growth (mm) on different days			F- value	P- value
Treatments	Day 3	Day 5	Day 7		
Trichoderma only	17.48(0.47)c	78.54(0.41)b	86.76(0.23)a	29722.68	0.002
Fusarium only	32.14(0.18)c	53.91(0.33)b	69.00(0.31)a	13144.66	0.001
Dual culture	17.32(0.11)a	12.999(0.28)b	9.047(0.27)c	930.90	0.003
Mancozeb (400 ppm)	12.31(0.58)a	6.51(0.48)b	5.36(0.31)b	187.08	0.001

Means that do not share a letter within a column in a treatment are significantly different (Turkey's HSD test ($p < 0.05$).Numbers in brackets are ± standard deviation.

Figure1: Effects of *Trichodermaharzianum* at different concentrations on percentage disease incidence.

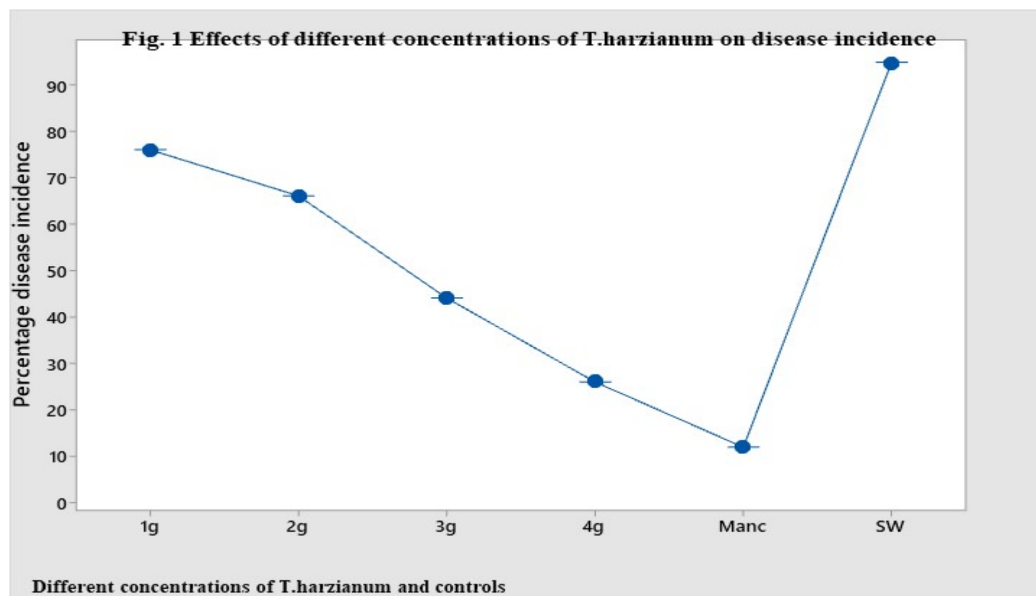

Table 3: Effects of *Trichoderma harzianum* at different concentrations on percentage disease severity from week 1 to 4 after inoculation

Treatments	WK1	WK 2	WK 3	WK 4	F-value	P-value
SW	0.51(0.09)d	20.40(0.53)c	50.60(0.36)b	80.82(0.42)a	24929.38	0.001
1g	0.403(0.025)d	10.40(0.10)c	23.43(0.38)b	36.23(0.25)a	13415.43	0.002
2g	0.33(0.083)d	10.40(0.10)c	30.07(0.12)b	54.3(0.40)a	11691.62	0.000
3g	0.23(0.064)d	3.04(0.05)c	14.40(0.20)b	23.37(0.32)a	9096.37	0.000
4g	0.17(0.12)c	1.27(0.21)c	12.97(1.42)b	15.50(0.10)a	356.96	0.001
Manc.	0.03(0.06)c	0.83(0.47)c	5.43(0.32)b	8.70(0.36)a	431.80	0.000

Means that do not share a letter within a column in a treatment are significantly different (Turkey's HSD test ($p < 0.05$). Numbers in brackets are ± standard deviation.