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ANTAGONISTIC POTENTIAL OF *Trichoderma harzianum* AGAINST *F. oxysporum* f. sp. *lycopersici* ISOLATES CAUSING FUSARIUM WILT DISEASE OF TOMATO (*Solanum lycopersicum* L.)

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ABSTRACT

Antagonistic effect of *T. harzianum* was carried out on *F. oxysporum* f. sp. *lycopersici*. isolates of tomato variety (UC 82B) in a screen house located at the Teaching and Research Farm of Federal University of Agriculture, Makurdi during 2015 cropping season to determine the ability of the antagonistic in controlling Fusarium wilt diseases of tomato. The *F. oxysporum* isolates tested were coded as: FoAs1, FoAs2, FoAg, FoNb, FoSb, FoAm, FoAk, FoOr, FoAd and FoUAM together with an uninoculated control. The experiment was a 2 x 11 factorial laid out in Completely Randomized Design (CRD) and replicated three times. *T. harzianum* was introduced at three different times (Two days before, same time and two days after the inoculation of *F. oxysporum*). *In vitro* tests results revealed antagonistic effects of *T. harzianum* on *F. oxysporum* isolates. Growth inhibition was significantly higher ($P \leq 0.05$) when *T. harzianum* was introduced two days before inoculation of *F. oxysporum*. Interaction shows that *T. harzianum* introduced two days before inoculation of *F. oxysporum* gave better inhibition of all the *Fusarium* isolates tested except isolates FoAd and FoAg compared with when the antagonist was introduced at the same time and when it was introduced two days after inoculation of *F. oxysporum*. Also, *T. harzianum* ($P \leq 0.05$) totally inhibited the growth of isolates FoAg, FoAs1, FoNb, FoOr and FoUAM but not those of FoAd, FoAk, FoAm, FoAs2 and FoSb. It is therefore recommended that *T. harzianum* be used in the management of fusarium wilt disease of tomato

Key Words: Antagonistic; *Fusarim oxysporum*; Inhibition, Isolates; *T. harzianum*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) originated from South American Andes (Naika *et al*., 2005), one of the most popular and widely cultivated vegetables in the world with an estimated yield of 180,766,329 metric tonnes (FAO, 2019). Nigeria is ranked 14th among the fifteen top countries in the world and second largest producer in Africa after Egypt (FAO, 2020).

Fusarium oxysporum is a major fungi pathogen causing rots in a wide variety of hosts, some of the most susceptible crops are tomato, potatoes, bananas, tobacco, peas, cowpeas, lentils, muskmelon, water melon, sweet potatoes, groundnuts, peppers, egg plants, cotton and cabbage (Egel and Martyn, 2013; Sani and Gwa, 2018; Mamkaa and Gwa, 2018). There are over 100 formae speciales of *Fusarium oxysporum* within the species which are host-specific. For example; *F. oxysporum* f. sp. *batatas* affects sweet potato; *F. oxysporum* f. sp. *cubense* causes Panama disease on banana. *F. oxysporum* f. sp. *lycopersici* causes vascular wilt in tomato. *F. melonis* attacks muskmelon and cantaloupe.

Deterioration of tomato fruits has been reported to constitute up to as high as 60% of losses in Nigeria (Kutama *et al*., 2007). A lot of diseases are known to limit production of tomato, of which *Fusarium solani* f. sp. *Eumartii* and *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) (*Fusarium* wilt) is one of the most important (Akrami and Yousefi, 2015). *Fusarium* wilt alone causes over 30 to 40% yield loss in tomatoes (Anita and Rabeeth, 2009). Fusarium stem and root rot (FSRR) is a soil-borne tomato disease caused by *Fusarium oxysporum* Schlect f. sp. radicis-lycopersici roots and quite frequent in greenhouse tomato production (Pavlou and Vakalounakis, 2005). *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) is a soil borne fungal pathogen that infects plants from the roots at all stages of growth and causes major economic losses by making plants to wilt and become necrotic (Cotxarrera *et al.*, 2002).

Management of *Fusarium* wilt has been primarily through development of resistant cultivars as part of an integrated management approach (Akrami and Yousefi, 2015) as well as fungicides treatment (Nwankiti and Gwa, 2018). However, fungicides cause hazard to human health, increase environmental pollution and are expensive and not readily available to small scale farmers. Alternative eco-friendly approach for control of plant diseases is advocated (Rojo *et al.*, 2007; Gwa *et al.*, 2021). Use of antagonistic micro-organisms is one of the alternatives advocated especially against soil borne pathogens (Akrami and Yousefi, 2015, Gwa *et al.*,

2019; Gwa and Ekefan 2021). Among the various antagonists, *Trichoderma* spp. has been widely used for the management of plant diseases. Studies have proved the potential of *Trichoderma* spp. as biological agents antagonistic to several plant pathogens (Ekefan *et al.,* 2009; Houssien *et al.,* 2010; Gwa *et al.,* 2019).

In view of the fact that there are few varieties of tomato reported to be resistant to *F. oxysporum* f. sp. *lycopersici* (Sacc.) and the negative impact of fungicidal treatments on the environment, it is important to explore biocontrol measures for management of Fusarium wilt caused by *F. oxysporum.* The present research is therefore, designed to explore the potential of *T. harzianum* for the management of Fusarium wilt disease of tomato.

Materials and Methods

Experimental Site

Laboratory experiments were conducted during 2015 cropping season at the Federal University of Agriculture, Makurdi to determine the biocontrol potential of *Trichoderma harzianum* for the management of Fusarium wilt of tomato (*Solanum lycopersicum*) in Makurdi. The location falls within Latitude 7º 45' North and Longitude 8º 37' East with an average altitude of 97m above sea level in the Southern Guinea Savannah Agro-ecological zone of Nigeria.

Sources of plant material and *Trichoderma harzianum*

Naturally infected tomato plants were obtained from farmers' field during 2015 cropping season at flowering stage in Tarka, Gboko and Makurdi Local Government Areas of Benue State, Nigeria. *Trichoderma harzianum* was obtained from Advanced Plant Pathology Laboratory, University of Agriculture Makurdi, Benue State, Nigeria.

Preparation of Culture Medium

Potato Dextrose Agar (PDA) medium was prepared according to manufacturer's recommendation by dissolving 39g of PDA in 1 liter of distilled water. The mixtures were sterilized in an autoclave at 33Kg P.S.I and 121ºC for 15 minutes (Ritchie, 1991). The molten medium was allowed to cool to about 40ºC, after which 0.16g/L of Streptomycin sulphate was added to prevent growth of bacteria and shaken gently in order to mix well before pouring 20ml into 9cm Petri dishes and allowed to solidify.

Isolation of *Fusarium oxysporum*

Infected tomato plant materials were collected and washed under running tap water to remove dirt. Samples were cut into small pieces of about 2mm × 2mm and further sterilized in 5% Sodium hypochlorite for 2 minutes. Sterilized pieces were raised in four successive changes of sterile distilled water to remove Clorox and bloated dried on sterile filter paper for about 2 minutes (Gwa, *et al.,* 2021). Four pieces were plated on each Petri dish containing solidified Potato Dextrose Agar (PDA) on a laminar air flow chamber. The plates were incubated at ambient condition for six days. After six (6) days, fungal growth was examined and the fungi were aseptically sub-cultured with a flamed inoculation needle onto newly prepared solidified PDA plates. Plates were incubated at ambient condition for six (6) days. Identification of the pure cultures was done with the aid of a compound microscope using an identification guide (Agrios, 2005). Fungal isolates identified were coded based on the location from which they were collected as follows: Asase1 (FoAs1), Asase2 (FoAs2), Agromiller (FoAg), Northbank (FoNb), Southbank (FoSb), Amih (FoAm), Orduen (FoOr), Adudu (FoAd) and University of Agriculture Makurdi (FoUAM).

In-vitro Interaction of *Trichoderma harzianum* with *Fusarium oxysporum* in dual culture

The method of Gwa and Nwankiti (2017) was adopted for this experiment. *Trichoderma harzianum* were tested *in-vitro* for antagonistic effects against *Fusarium oxysporum* isolates. Mycelial plug (5mm) was taken from the edge of 5 days old culture of each *F. oxysporum* isolates using a cork borer and placed at the periphery of 9cm Petri dishes containing PDA. Similar sized discs of *Trichoderma harzianum* (5 days old) were placed on the same plates; 6cm apart. The treatments consisted of inoculation of *T. harzianum* and *F. oxysporum* at same time (Th x Fo), 2days before inoculation of *Fusarium oxysporum* (Th2dbiFo), 2day after inoculation of pathogens (Th2daiFo) and a control (Fo alone). Each treatment was replicated three times in a completely randomized design. Plates were incubated at ambient temperature (normal laboratory temperature) and examined daily for three days.

Data collection

The data Collected were:

(a). Measurement of daily radial growth of *T. harzianum* and *F. oxysporum* was carried out in millimeter using using metric rule

(b). Percent Inhibition over control was calculated using the formula developed by Dissanayake (2014).

$$PI = \frac{C - T}{C} x\ 100$$

Where:
PI = Percent Inhibition over control (%)
C = the distance (measured in mm) from the point of inoculation to the colony margin in control plate.
T = the distance of fungal growth from the point of inoculation to the colony margin in treated plate in the direction of the antagonist.

Assessment of the effect of *Trichoderma harzianum* for control of Tomato wilt Caused by *F. oxysporum*

Seeds of UC 82B tomato varieties were sown in 5kg potted sterilized sandy loam soil which was artificially concomitantly inoculated with 5ml of *F. oxysporum* and *T. harzianum* containing $1x10^6$spores/ml using 20ml syringe needle at two weeks after planting. The treatments consisted of ten isolates of *F. oxysporum* isolates + *T. harzianum* and control for each isolate of *F. oxysporum* (without *T. harzianum*). The treatments were replicated three times in Randomized Complete Block Design.

Data collection

(a) Disease incidence.
Fusarium wilts incidences were taken at weekly intervals, starting from three Weeks After Planting (WAP) to 13 WAP. Data collected at harvest time on the 13th week after planting (13WAP) were analyzed accordingly.

Statistical analysis for experiment 2

All data collected were subjected to analysis of variance using Genstat. Statistical Package (Discovery Edition 12) and significantly different means were separated at 5% level of probability using Fisher's Least Significant Difference (FLSD).

RESULTS

In-vitro* Interaction of *Trichoderma harzianum* with *Fusarium oxysporum

Effect of time of introduction of *T. harzianum* on percentage growth inhibition of *F. oxysporum*

Percentage growth inhibition of *F. oxysporum* at different time of introduction of *T. harzianum* is presented in Table 1. Growth inhibition was significantly higher (P≤0.05) when *T. harzianum* was introduced two days before inoculation of *F. oxysporum* compared with when the antagonist was introduced at the same time and when it was introduced two days after inoculation of *F. oxysporum.*

The Fusarium isolates tested responded differently to *T. harzianum.* Percentage radial growth of Fusarium wilt was significantly higher (P≤0.05) in isolates FoAk, FoAd, FoAs2, FoUAM, FoAm and FoOr compared with the others. The interaction shows that *T. harzianum* introduced two days before inoculation of *F. oxysporum* gave better inhibition of all the isolates tested except isolates FoAs1 and FoAs2 compared with when the antagonist was introduced at the same time and when it was introduced two days after inoculation of *F. oxysporum*. The growth inhibition was least when *T. harzanianum* was introduced two days after inoculation of *F. oxysporium* isolates

Table 1: Effect of Time of Introduction of *T. harzianum* on Percentage Growth Inhibition of *F. oxysporum* isolates

	Mycelial Growth Inhibition *(%)*			
Isolate	***Th x Fo.***	***Th*2dbi *Fo***	***Th*2dai *Fo***	**Isolate Mean**
FoAd	50.71	48.95	05.87	35.17
FoAg	37.34	23.75	08.32	23.14
FoAk	50.08	56.94	18.52	41.84
FoAm	35.90	48.50	12.47	32.29
FoAs1	15.54	28.83	07.45	17.27
FoAs2	28.70	63.43	13.66	35.27
FoNb	38.38	46.41	06.15	30.31
FoOr	31.59	56.14	08.71	32.15
FoSb	38.33	42.51	11.36	30.74
FoUAM	41.48	45.79	11.24	32.84
TOI Mean	**36.80**	**46.12**	**10.38**	**31.10**

FLSD (P≤0.05)
Isolates = 3.97, TOI = 2.18, Isolates x TOI = 6.88

KEY:
TOI – Time of Introduction, *Th* x *Fo.* – Introduction of *Trichoderma harzanium* the same time with *Fusarium oxysporum*, *Th*2dbi *Fo* – *Trichoderma harzanium* inoculated two days before introduction of *Fusarium oxysporum*, *Th*2dai *Fo.* – *Trichoderma harzanium* inoculated two days after introduction of *Fusarium oxysporum*, FoAd – *Fusarium oxysporum* from Adudu, FoAg – *Fusarium oxysporum* from

Agromiller, FoAk – *Fusarium oxysporum* from Akor, FoAm – *Fusarium oxysporum* from Amih, FoAs1 – *Fusarium oxysporum* from Asase1, FoAs2 – *Fusarium oxysporum* from Asase2, FoNb – *Fusarium oxysporum* from Northbank, FoOr – *Fusarium oxysporum* from Orduen, FoSb – *Fusarim oxysporum* from Southbank, FoUAM – *Fusarium oxysporum* from University of Agriculture Makurdi, FLSD – Fisher's Least Significant Difference at 5% level of Probability.

Effect of *T. harzianum* on incidence of Fusarium wilt of Tomato Concomitantly Inoculated with *F. oxysporum* and *T. harzianum* in Makurdi, Nigeria.

Effect of *T. harzianum* on incidence of Fusarium wilt of Tomato Concomitantly inoculated with *F. oxysporum* and *T. harzianum* in Makurdi, Nigeria are presented in Table 2. The incidence of Fusarium wilt in plants concomitantly inoculated with *F. oxysporum* and *T. harzianum* in the screen house was significantly lower (P≤0.05) compared with the control. Fusarium isolates tested responded differently to *T. harzianum* as a biocontrol agent in the screen house experiment. The incidence of Fusarium wilt was significantly (P≤0.05) lower in isolates FoAg, FoAs1, FoNb, FoOr and FoUAM compared with the other isolates. The interaction shows that *T. harzianum* inhibited the growth of isolates FoAg, FoAs1, FoNb, FoOr and FoUAM and not those of FoAd FoAk, FoAm, FoAs2 and FoSb.

Table 2: Effect of *T. harzianum* on Incidence of Fusarium wilt (%) of Tomato concomitantly inoculated with *F. oxysporum* and *T. harzianum* in Makurdi, Nigeria.

	Treatment		
Isolate	**Control(*F. oxy*)**	***F.oxy + T.harz***	**Isolates Mean**
FoAd	86.70	60.00	73.30
FoAg	93.30	0.00	46.70
FoAk	80.00	53.30	66.70
FoAm	93.30	46.70	70.00
FoAs1	80.00	0.00	40.00
FoAs2	80.00	53.00	66.70
FoNb	80.00	0.00	40.00
FoOr	86.70	0.00	43.30
FoSb	86.70	53.30	70.00
FoUAM	93.30	0.00	46.70
Treatment Mean	**86.00**	**26.70**	

FLSD (P≤0.05)
Isolates = 9.47
Treatment = 4.23
Isolates × Treatment = 13.39

KEY:
FoAd – *Fusarium oxysporum* from Adudu; FoAg – *Fusarium oxysporum* from Agromiller.
FoAk – *Fusarium oxysporum* from Akor; FoAm – *Fusarium oxysporum* from Amih; FoAs1 – *Fusarium oxysporum* from Asase1; FoAs2 – *Fusarium oxysporum* from Asase2; FoNb – *Fusarium oxysporum* from Northbank; FoOr – *Fusarium oxysporum* from Orduen; FoSb – *Fusarim oxysporum* from Southbank; FoUAM – *Fusarium oxysporum* from University of Agriculture Makurdi; FLSD – Fishers Least Significant Difference at 5% level of Probability.

DISCUSSION

Tomato is a fruit vegetable crop that is eating raw as salad or cooked. It's a crop that is attacked by different kinds of pathogens. Fungi are the most devastating kinds of pathogens both in the field and in store. Findings from our study of percentage growth inhibitions of *F. oxysporum* isolates at different times of introduction of *T. harzianum* revealed that *T. harzianum* has inhibitory effects on the mycelial growth of *F. oxysporum* in all the treatments in the dual culture.

The findings revealed that *T. harzianum* inhibited the growth of *F. oxysporum* when inoculated in to the pots containing tomato plants. This may be due to the fast growth of the antagonist and competition for nutrient and space with the pathogen resulting in colonization of the pathogen by the antagonist. Hermosa *et al*. (2012) noted that the mechanism of action by *T. harzianum* was by competition with *F. oxysporum* for nutrients and space, as well as mycoparasitism on the pathogen and probably secretion of antibiotics. The rapid growth and competition for nutrient and space by the antagonist inhibited the growth of *Fusarium oxysporum* infecting the seedlings of tomato. Similar study was carried out by Midhun and Sobita (2017) that revealed the efficacy of Trichoderma spp. against *Fusarium oxysporum* f. sp. lycopersici (FOL)

infecting pre-and post-seedling of tomato. Studies conducted by Lubaina and Murugan, (2015) observed enhancement of plant growth of tomato after application of Trichoderma in field and greenhouse. Similarly, research carried out by Aydi Ben Abdallah *et al.* (2016) found that fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. lycopersici is one of the economically most important disease in major tomato growing regions worldwide. Ghazalibiglar *et al.* (2016) found that *Fusarium oxysporum* is a devastating pathogen which caused between 10% and 50% yield losses in many tomato production areas.

Siameto *et al*. (2011) and Nwankiti and Gwa, (2018) showed that *T. harzianum* must have acted by possible production of chitinolytic and/or glucanases enzymes. These enzymes function by breaking down the polysaccharides, chitin, and glucans that are responsible for the rigidity of *F. oxysporum* cell walls, thereby destroying the cell wall integrity and limiting the growth of the pathogen. In the same vein, Lubaina and Murugan (2015) showed that *T. harzianum* has biological effect on Alternaria leaf spot diseases of sesame.

Trichoderma species have been successfully used as biological control agents due to their high reproductive capacity, efficient utilization of nutrients, strong aggressiveness against other pathogens as well as rapid and effective colonization of wound sites against the invading pathogens (Arya, 2010; Suprapta, 2012; Gwa and Ekefan, 2021). The fast growth rate of this antagonist was also noticed by Ekefan *et al*. (2009) who showed that growth of *Colletotrichum capsici* was suppressed by *T. harzianum* isolates which eventually overgrew it within seven days of incubation.

Dual culture combinations of *T. harzianum* with *F. oxysporum* showed the inhibition of the pathogen in all the treatments with the highest percentage growth inhibition in the treatment where the antagonist was introduced 2 days before inoculation of the pathogen. According to Okigbo (2005) and Gwa and Abdulkardir (2017) there are no biological control agents that have enough competitive ability to displace an already established pathogen. Therefore, the time lapse between inoculation of *T. harzianum* and *F. oxysporum* might have contributed to the success recorded by the antagonist against *F. oxysporum* isolates. Introducing the antagonist 2 days before the pathogen gives the antagonist advantage in competitiveness for nutrient and space, mycoparasitism as well as production of antibiotics which is responsible for degradation of the fungus cell wall. Biological control of soil-borne plant pathogens with antagonistic *T. harzianum* has been found to be environmentally safe and cost effective compared to synthetic pesticides (Aydi Ben Abdallah *et al.,* 2016).

CONCLUSION

T. harzianum which was used as a biological agent inhibited the growth of *F. oxysporum* isolates tested *in-vitro* and reduced infection in the screen house. The antagonist has therefore proved effective in the inhibition of *F. oxysporum* isolates and can be used as alternative way of management of Fusarium wilt disease of tomato by farmers instead of using the synthetic chemicals that have been found to be harmful to soil, human, plants, animals and the entire ecosystem. It is also recommended that breeders should explore the possibility of incorporating the genes of this antagonist in new varieties of tomato to reduce fusarium wilt disease of tomato.

CONFLICT OF INTEREST DISCLOSURE

The authors declared that there is no conflict of interest regarding the publication of this paper.

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