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ANTAGONISTIC POTENTIAL OF *TRICHODERMA HARZIANUM* AGAINST *FUSARIUM OXYSPORUM* F. SP. *CUBENSE* ASSOCIATED WITH PANAMA WILT OF BANANA

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A B S T R A C T

Fusarium oxysporum f. sp. *cubense* is a soil loving pathogen of banana that can cause distortion of vascular system. It is responsible for the disease Fusarium wilt of bananas also renowned as panama wilt disease that has responsible of immense losses in the banana industry worldwide. In this study, diseased samples were collected from rhizosphere of banana plants in the research area of National Agriculture Research Centre (NARC), Islamabad and cultured on PDA to isolate pathogenic strains of *F. oxysporium* f. sp. *cubense*. Antagonistic potential of *Trichoderma harzianum* against *F. oxysporum* f. sp. *cubense* was evaluated under controlled conditions. *T. harzianum* produced up to 75.5% inhibition of colony growth of the pathogen followed by incubation for 72 h at 28+2°C *in vitro*. In pot culture *T. harzianum* considerably reduced disease severity. It proves that it is a potential biological control agent against banana wilt pathogen.

Keywords: Biological control, Fusarium wilts, Inhibition, Pot culture

INTRODUCTION

Banana (*Musa* sp.) is an important fruit crop of Pakistan. It is cultivated on 34, 8000 hectares with 154, 8000 tons annual production (FAO, 2011). Due to favorable soil and climatic conditions, it is successfully cultivated in Sindh province. Its 87 percent production from Sindh and 13 % from the whole country (Roberts *et al.,* 2012). Banana is the earliest crop cultivated by man and still world's most main cash crop (Molina and Valmayor, 1999). It is cultivated more than 120 countries in subtropical and tropical regions of the world and now more than 400 million people use as staple food (Molina and Valmayor, 1999). It is a perennial monocotyledon plant belongs to the family Musaceae. It is mostly cultivated in areas situated between 30ºN and 30ºS latitudes (Bentley *et al*., 1998). Export of dessert banana is the main fruit in the international fruit trade and the most popular fruit in the world (Anania, 2006). Banana production is a major source of income and is an essential source of food for more than 70 million people in Africa (Rutherford and Viljoen, 2003).

Fusarium wilts also known as panama wilt caused by *Fusarium oxysporum* f. sp. *cubense* is a major constraint to banana production worldwide. It is active saprophyte in organic matter, soil and several forms are pathogenic to plants (Smith *et al.,* 1988). This pathogen survives and spread through infested soil and infected plants. It can infect fruits and seeds but dispersal through seed is very rare (Agrios, 2005). Characteristic symptoms of *F. oxysporum* f. sp. *cubense* infection are discoloration or browning of foliar plant tissues. However, symptoms are more obvious on old plant during flowering stage and fruit maturity stages (Smith *et al.,* 1988). The fungus causes root rot, vascular wilt and damping off in a number of plant species (Saremi *et al.,* 1996).

F. oxysporum f. sp. *cubense* cause vascular wilt syndrome in banana and during 1960s it demolished whole banana industry (Leslie *et al.,* 2006). This fungus has many famous plant pathogenic strains and *F. oxysporum* the cause of panama wilt have four different races. Control of soil borne phytopathogens through biological control

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agents is effective way (Rakh *et al*., 2011). Only one application of a biological control agent as seed treatment reduces the seed decay and damping off of seedlings while controlling such, diseases mainly depend on fungicides treatments (Ahmad *et al.,* 2009). Application of fungicides hazardous for environment and human health, therefore, environment friendly approach against pathogens required for safety (Rojo *et al.,* 2010). Many studies have proved the potential of *Trichoderma* spp. as biological agent antagonistic behavior to several plant pathogens (Sivan and Chet, 1993).

Among different bio control agents *Trichoderma* spp. are common free-living fungi present in roots and soil. *Trichoderma* species is very interactive to root, soil and plant foliar environments. Due to release of different compounds it triggers the plant immune system and resistance in the form of local or systemic. *Trichoderma* is well recognized as biological weapon against different plant pathogens and used as growth regulator for roots, yield increase, flexibility towards stress and nutrients uptake from soil. *Trichoderma* spores are more tolerant against unfavorable environmental conditions when used in the form of product formulation and field applications (Amsellem *et al.,* 1999). Presence of mycelia mass plays a key role in antagonistic metabolites (Benhamou and Chet, 1993; Yedidia *et al.,* 2000). The objective of this study was to evaluate the efficacy of *T. harzianum* against *F. oxysporum* f. sp. *cubense* and to detect and quantify the reduction in pathogen population.

MATERIALS AND METHODS

Isolation and Multiplication of Pathogen: Pathogen was isolated from infected banana plants in fungal pathology laboratory of Crop Disease Research Institute of National Agriculture Research Centre (NARC) Islamabad. Small pieces of infected tissues were surface sterilized with 1% sodium hypochlorite and rinsed within distilled water thrice then dried on blotter paper to remove excess moisture. After that 4-5, infected pieces were placed on petri plates in a laminar flow chamber containing PDA media and incubated at 25°C for one week. After two days of incubation, bud tip of fungal mycelium was isolated and transfer to the new petri plates for purification. Pathogen identified was confirmed microscopically through illustrated genera of imperfect fungi (Barnett and Hunter, 1972) while *T. harzianum* culture was obtained from culture bank of mycology lab of NARC.

Dual Culture Technique: Colony interaction of *F. oxysporum* and *T. harzianum* was studied on PDA containing plates by using dual culture method (Skidmore and Dickinson, 1976). The growth inhibition in the colony of the test pathogen and the antagonistic fungi was calculated and interaction grade have been determined as proposed by formula (Porter, 1924).

$$\text{Percentage growth inhinition} = \frac{r - r1}{r} \times 100$$

r = radius of fungal colony without antagonist towards the center of the plate

r_1 = radius of the fungus colony from centre towards the antagonistic

Culture filtrate assay: Potato dextrose broth 100ml was inoculated with antagonist culture blocks obtained from the actively growing margins followed by incubation at 28±2°C for 15 days, after which the hyphal mat of each fungus was filtered first through whatman filter paper 1 and finally through millipore filter paper. Culture filtrate was evaluated against pathogen at four different concentrations 5, 10, 15 and 20% in petri plates. Pathogen 5 mm culture blocks were inoculated at the center of the plates. The plates were incubated at 28±2°C for five days and the radial growth was recorded periodically.

The percentage inhibition of growth was calculated as follows:

$$Percent\ growth\ inhibition = \frac{g1 - g2}{g1} \times 100$$

g1= Growth in control

g2= Growth under treatment

Pot Culture Assay: Soil taken from field was autoclaved at 121°C for 20 minutes at 15psi to kill bacteria and other microorganisms. The inoculum was prepared by growing a culture of *F. oxysporum* in a modified liquid broth czapek's Dox (CD) and mixed with autoclaved soil (Esposito and Fletcher, 1961). The mixture was incubated eight weeks at room temperature to allow the fungus to colonize the medium extensively. Banana seedlings of the variety Dwarf Cavendish were taken from tissue culture lab of NARC and transplanted in pots for evaluation. The both fungal spore suspension was made in 1 liter distilled water for inoculation separately. Spore was counted by using heamocytometer. Only freshly prepared inoculums were used. The following treatments were used to evaluate the effectiveness of antagonist:

T1= Seedlings were soaked in the water then transplanted into the pots treated with *F. oxysporum* inoculums

T2= Seedling roots were soaked in water then in *T. harzianum* suspension and after transplanted into the pots treated with *F. oxysporum* inoculums

T3= Seedlings soaked 1st with *F. oxysporum* then were inoculated after one week of transplantation with antagonist fungi

T4= Seedlings roots were soaked 1st with antagonist suspension and then inoculated with *F. oxysporum* after one week of transplantation

T5=Seedlings roots were soaked in sterilized water and then transplanted into the pots containing sterilized soil.

Data was recorded after 4-6 weeks of transplantations when symptoms start and compared with control

Disease rating scale: The disease development in controlled conditions was evaluated 4–6 weeks after inoculation using the disease incidence scale 0-5 which is 0- immune 1- healthy, 2- slight chlorosis and wilting with no petiole buckling, 3- moderate chlorosis and wilting with some petiole buckling and or splitting of leaf bases, 4- severe chlorosis, severe wilting, petiole buckling and dwarfing of newly emerged leaf, 5- completely dead (Ploetz *et al.*, 1999). Percentage disease incidence for pot culture assay was calculated using the formula of Sherwood and Hagedorn (1958):

$$\% \text{ disease incidence} = \frac{Number\ of\ diseased\ plants}{Total\ number\ of\ Plants} \times 100$$

STATISTICAL ANALYSIS

Experimental design Complete Randomized Design (CRD) was used. Experimental results were analyzed by using statistix ver. 8.1. Software. The results were compared through Least significant difference (LSD) between means at P≤ 0.05 (Steel *et al.,* 1997).

RESULTS

Evaluation of *T. harzianum* against *F. oxysporum* by using dual culture technique: The maximum colony inhibition of *F. oxysporum* due to *T. harzianum* was 75%. The mycelium of *T. harzianum* was found growing over the pathogen (Figure 1). *T. harzianum* showed best results against *F. oxysporum* in dual culture technique.

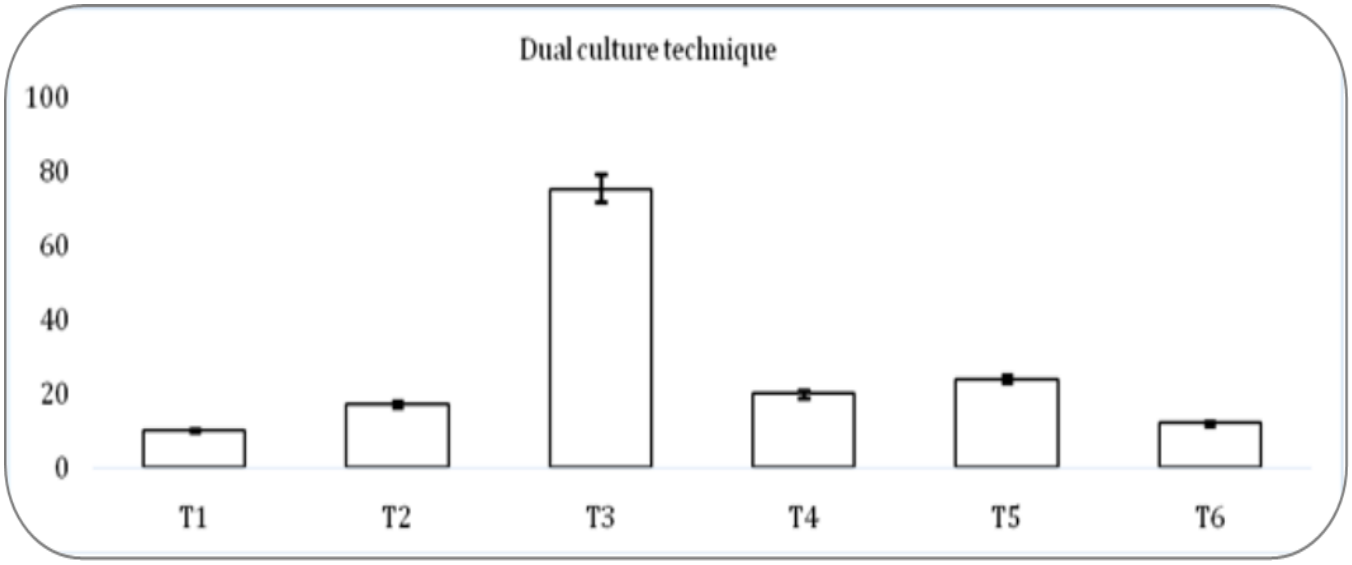

Figure 1. Colony interaction between *F. oxysporum* and *T. harzianum* in dual culture experiment. T1= Colony growth of pathogen towards antagonist (mm), T2= Colony growth of pathogen away from the antagonist, T3= growth inhibition of the pathogen in the zone of interaction (mm), T4= Colony growth of antagonist towards the center pathogen (mm), T5= Colony growth of antagonist away from the pathogen (mm) and T6= % of growth inhibition in the zone of interaction.

Evaluation of *T. harzianum* against *F. oxysporum* by using culture filtrate assay: The percentage inhibition of *F. oxysporum* in culture filtrate method was evaluated. Results indicate that the concentration of culture filtrate at 20% was more effective as compare to 5, 10 and 15% and inhibit the growth of *F. oxysporum* 75.5%, 19.33%, 23.33% and 31.33% respectively. The maximum inhibition of *F. oxysporum* was recorded in 20% culture filtrate of *T. harzianum* 75.5% (Figure 2).. The results showed that increasing level of inoculums of antagonist increase the inhibition of pathogen.

Figure 2. Effect of culture filtrate of *T. harzianum* towards growth of *F. oxysporum.* T1= 5%, T2= 10%, T3= 15% and T4= 20% concentration of *T. harzianum* against *F. oxysporum.*

Evaluation of *T. harzianum* against *F. oxysporum* in pot culture assay: The maximum disease incidence was recorded 83.33% in the *F. oxysporum* alone while performing pot culture assay and the minimum disease incidence 16.66% was recorded in seedlings which first inoculated with *T. harzianum* showed in (Figure 3).

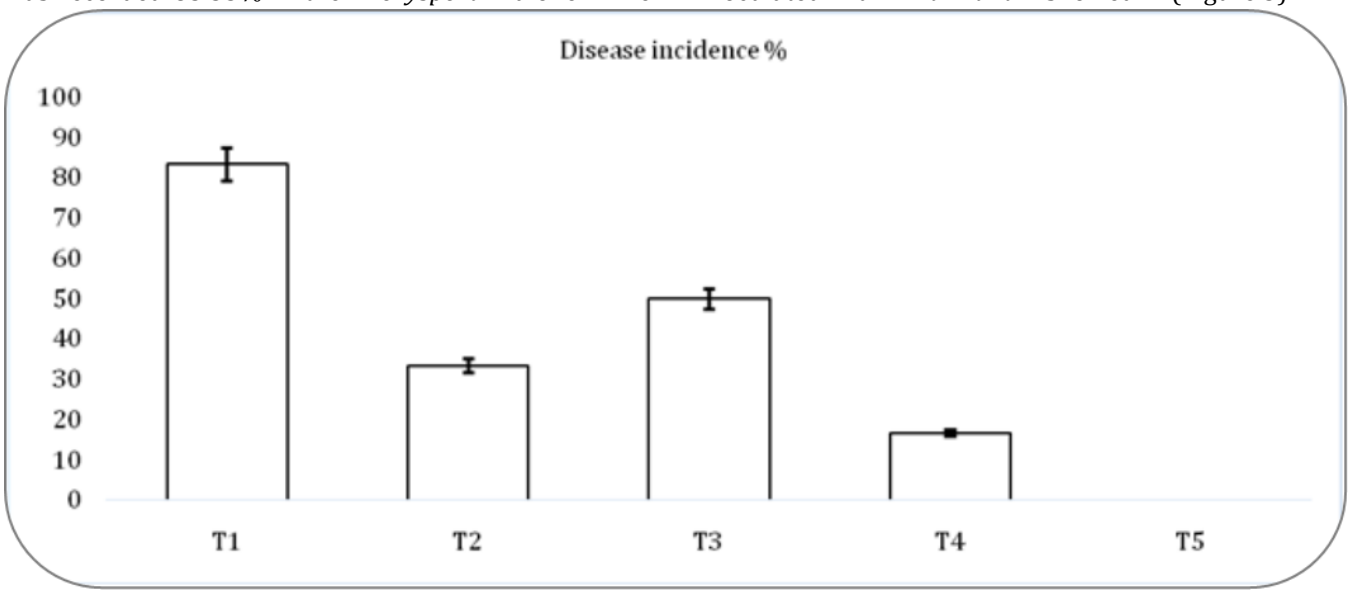

Figure 3. Evaluation of percentage disease incidence by using pot culture assay. T1= *F. oxysporum*, T2= *F. oxysporum*+ *T. harzianum* culture, T3= First *F. oxysporum* culture + after 1 week interval *T. harzianum* culture, T4= First *T. harzianum* culture + after 1 week interval *F. oxysporum* culture and T5= control

DISCUSSION

Due to residual effects of chemicals there is an increased trend of prevention through resistance development with the help of utilization of biological products particularly *Trichoderma* spp. which is recognized as potential bio control agent as a commercial bio-fungicides around the world (Harman *et al.,* 2004; Lorito *et al.,* 2010). *Trichoderma* are free living fungi and usually found in a diverse soil types such as forest, agriculture, prairie, desert soil and salt marsh (Albert *et al*., 2011). Aggregate species of *Trichoderma* can be distinguished based on macro and microscopic features. The antagonistic properties of *Trichoderma* spp. against different pathogens have also been reported

(Panneerselvam and Saravanamuthu, 1994; Ambikapathy *et al*., 2000).

In dual culture technique results revealed that *Trichoderma* spp. exhibited the maximum reticence of pathogen mycelial growth. During inhibition zone in dual culture distance between hyphae of both fungi suggests that some secretion which are diffusible non-volatile inhibitory material produced by the *Trichoderma* isolate. *Trichoderma* specie interacts and produces small quantities of extracellular exochitinases (Kullnig *et al.,* 2000; Brunner *et al.,* 2003). *T. harzianum* inhibits the growth of *F. oxysporum* (61.4%) in the dual culture technique described by (Muthukumar *et al*., 2006).

The maximum inhibition of *F. oxysporum* was recorded in 20% culture filtrate of *T. harzianum* 75.5% showed in (Figure 2). *T. harzianum* significantly ($P<0.05$) reduce the growth of *F. oxysporum*, on PDA after incubation for 72 h at 30°C reported (Ullah *et al*., 2011). Researchers reported that *Trichoderma* release different antibiotics which are trichodernin, trichodermol, harzianum A and harzianolide (Howell, 1998; Kucuk and Kivanc, 2004). Some cell wall degrading enzymes which are chitinases, glucanases that break down polysaccharides, chitins and glucanase, there by destroying cell wall reliability (Lorito *et al*., 1996; Harman and Kubicek, 1998; Woo *et al*., 2006). The maximum disease incidence was recorded 83.33% while performing pot culture assay showed in (Figure 3). In addition, the soil application of *Trichoderma* isolate increased the plant growth significantly ($P\leq 0.05$) compared to *F. oxysporum* alone inoculated. The pot culture evaluation carried out for rhizospheric *Trichoderma* against Fusarium wilt showed that the *T. harzianum* significantly ($P\leq 0.05$) reduced the Fusarium wilt disease severity (Srivastava *et al.,* 2010).

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