UMYU Scientifica, Vol. 1 NO. 1, September 2022, Pp 122 – 126
https://doi.org/10.56919/usci.1122.016
ISSN: 2955 – 1145 (print); 2955 – 1153 (online)
A periodical of the Faculty of Natural and Applied Sciences, UMYU, Katsina

ORIGINAL RESEARCH ARTICLE

In vitro Inhibitory Potential of *Trichoderma Species* on *Fusarium oxysporum* f.sp *vasinfectum* the Causal Organism of vascular wilt of Cotton (*Gossypium hirsutum* l.) in the Nigerian Sudan Savanna

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ABSTRACT

The purpose of this study was to determine the inhibitory potential of three species of *Trichoderma* namely; *Trichoderma asperelum, Trichoderma viride* and *Trichoderma harzianum* on *Fusarium oxysporum* f.sp *vasinfectum* (fov), the causal organism of vascular wilt in cotton. The experiment consisted of dual culture incubated at $25^0C \pm 1^0C$ on PDA, for 9 days. During the experiment, the three trichoderma species were obtained from the soil while the fusarium oxysporum isolate was obtained from IAR, Zaria, Nigeria. Antagonistic activity testing was determined using percentage inhibition of *Fusarium oxysporum* radial growth. The results of the study revealed that all the three *Trichoderma* species tested in this experiment had significantly inhibited the mycelial growth of fov at different degrees compared with untreated control. The percentage inhibition ranged from 54% for *Trichoderma. asperelum,* 62.8% by *Trichoderma viride* to the highest being 75.6% and a mean of 45.50% due to *Trichoderma harzianum.* These results showed that *Trichoderma harzianum* was the most effective followed by *Trichoderma viride* and lastly *Trichoderma asperellum.* This suggest that there are some similarities between the three isolates of *Trichoderma* as all the three species could inhibit the growth of *Fusarium oxysporum* f.sp *vasinfectum* but the best to be used as bio-control agent for vascular wilt of cotton caused by fov was *Trichoderma harzianum* pending further research.

ARTICLE HISTORY

Received August 1, 2022
Accepted September 17, 2022
Published September 30, 2022

KEYWORDS

Vascular wilt, Cotton, *Trichoderma* sp., *Fusarium oxysporum* f.sp. *vasinfectum, in vitro*

INTRODUCTION

Of the different species of cotton known to mankind, *Gossypium hirsutum, Gossypium barbadense, Gossypium arboreum* and *Gossypium herbaceum* are the most commonly grown cotton species in most parts of the world (Khadi *et al.,* 2010; Kutama *et al.*, 2015). Of these four widely grown species, the most commercially cultivated cotton is derived from two species; G. *hirsutum* (upland cotton) accounting for about 90% of world plantings and *G. barbadense (*long staple cotton) usually cultivated as shrubby annual in temperate and few tropical areas of the world (Brubaker *et al.,* 1999). In nature, the crop is a perennial shrub that grows to about 1.5meters in height but it is commercially cultivated as an annual with destruction of plant after harvesting the fruit for seed and fiber. In short, the economic importance of cotton production could favorably be compared with that of livestock farming in Northern Nigeria (Kutama *et al.*, 2007).

Prior to oil boom, cotton was one of the main sources of foreign exchange in Nigeria, Cotton industry was the second largest employer of labor after the public sector (Kutama *et al.*,2007). Cotton is the most important of all other crops cultivated and its cultivation is not restricted to the northern savanna zones of Nigeria but has spread to the derived savanna areas of Kwara, Osun, Oyo, Ondo and Edo State (Finelib, 2017). Cotton is one of the highly demanded natural materials due to its use in the production of fiber materials. As long as people continue to wear clothes there would always be demand for cotton. Additionally, oil can be extracted from cotton, it can be use to make plastic margarine, plastics exipient rubber and cosmetics, also linters which remain on the cotton fiber after ginning can be use to make bandage, swabs and cotton buds. Cotton is healthy source of vitamin E and vitamin K and important antioxidant. Cotton is an important cash crop in Nigeria which

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How to cite: Aliyu, U.; Kutama, A.S.; Zafar, S; Bashir, A. A. and Hadiza, M.M. (2022). *In vitro* Inhibitory Potential of *Trichoderma Species* on *Fusarium oxysporum* f.sp *vasinfectum* the Causal Organism of vascular wilt of Cotton (*Gossypium hirsutum* l.) in the Nigerian Sudan Savanna. UMYU Scientifica, 1(1), 122 – 126. https://doi.org/10.47430/usci.1122.016

produces lint and seed that serve as raw materials for the local textiles and seed crushing industries (Adeniji, 2007). Several physical and biological factors constrain the growth and development of cotton plant. Like any tropical crop, cotton is a host to more than twenty fungal pathogens and pests thus causing several damages of the crop annually and this affects its commercial production. Of these fungal pathogens, *Fusarium oxysporum,* the causal organism of vascular wilt, is one of the most important pathogen that causes drastic yield loss every year (Kareem *et al.,* 2016; Kutama *et al.*, 2016). *Fusarium oxysporum* f.sp *vasinfectum* is the particular strain that causes vascular wilt in cotton. First discovered in Australian cotton crop in brook/leci plain of the Darling down in March 1993 (Queensland, 2018), *Fusarium oxysporum* f.sp *vasinfectum* can be disseminated by infected seed and plant materials and by contaminated tools (Wang *et al.,* 2006). Control of *Fusarium oxysporum* f.sp *vasinfectum* may be achieved by applying tremendous volume of pesticide during the cotton plant culture, however the continued use of this fungicide created serious problem to the environment and human health (Tanoh *et al.,* 2015*).* To overcome these problems researchers look for alternative option such as biocontrol agents (BCA) for disease control either alone or in combination with other chemicals which are ecofriendly and sustainable method of disease control (Naher *et al.,* 2014).

Several studies have shown that some strains of *Trichoderma* have been powerful biocontrol agents against plant pathogen, which are applied to Agricultural land to achieve plant growth, promotion and biocontrol (Savazziniand Longa, 2009). Yahaya *et al.* (2013) have reported that *Trichoderma* species are among the most frequently isolated fungi and present in plant root system, that is to say there are several species of *Trichoderma* found in the root of many plants and are often isolated. These fungi are opportunistic avirulent plant symbionts and function as parasite and antagonist of many phytopathogenic fungi (Kareem *et al.,* 2016). This study therefore aimed at determining the inhibitory capacity of three *Trichorderma* species on cotton wilt pathogen; *Fusarium oxysporum* f.sp *vasinfectum.*

MATERIALS AND METHODS

Study area

The present study was carried out at the Plant Pathology laboratory, Department of Plant science Bayero University Kano located on the coordinates;…11° 98' 32.59' N; 8° 42' 43.97'E

Sourcing of fungal wilt pathogen of cotton

Fusarium oxysporum f.sp *vasinfectum* sample of isolate was obtained from plant pathology laboratory, Institute of Agricultural Research, (IAR) Zaria. Stock culture of the isolate was maintained on slants of PDA on McCartney bottle for subsequent study.

Isolation and identification of antagonist fungi (*Trichoderma* species)

Trichoderma species was isolated from farm soil by weighing 10g of the soil sample into conical flask containing 90ml of sterile distilled water. The suspension was shaken vigorously and then serially diluted. Aliquot of 1ml each from the serial dilution were then place on sterile Petri dishes and molten Potato dextrose agar (PDA) was poured on them as recommended by Kannangara *et al.* (2017). The plate was swirled to obtain the homogenous mixture of inocula and PDA. The plates were incubated at 25°C for 72 hours. The plates were then observed for the appearance of different species of *Trichoderma* in the mixed culture. All of the grown *Trichoderma* spp were then grown on another PDA medium to obtain pure cultures.

Colonial characteristics based on morphology of the various isolate were counted each and recorded. The cultural features such as color, texture, margin, form, elevation and aerial hyphae were all noted using Methuen handbook (Kornerup and Wanscher, 1978) and and a pictorial atlas for identification of fungi by Watanabe (2002). Sticky tape method (Flegel, 1980) was adopted for the microscopic identification using calibrated phase contrast microscope; spore shape, size and mycelial width of each isolate were measured. Each species of the Trichoderma was identified separately as described by Yahaya *et al.* (2013) and Kutama *et al.* (2013).

Antagonistic activity testing

The inhibitory potentials of each of the three species of *Trichoderma* (namely; *Trichoderma asperelum, Trichoderma viride* and *Trichoderma harzianum*) against *Fusarium oxysporum* f.sp *vasinfectum* (fov) were determined through dual culture technique as described by Kucuk and Kivanc (2003). Petri dishes (90mm) containing PDA were inoculated with 5mm plug of 7 days old pure culture of *Trichoderma* fungi and *Fusarium oxysporum* f.sp *vasinfectum*. One mycelial plug of each fungi was placed at opposite side of PDA and incubated at 25°C ± 1°C with the radial growth (cm) of fov being measured 3, 5, 7 and 9 days after incubation. Control Petri dishes were inoculated with fov on a sterile agar plug. A number of three replication were assigned to each treatment and plates were arranged in randomized complete design. Percentage inhibition of fov radial growth was determine using the formula

$$I = \frac{R2}{R1} \times 100$$

Where, I = percentage inhibition of radial growth of pathogen (%)
R1 = Radial growth of pathogen (cm) in control
R2 = Radial growth of pathogen (cm) in treatment

The ability of the *Trichorderma* sp. to over grow and inhibit the growth of the pathogen by giving them a score as per modified Sangayomi (2004) scale was modified for the percentage inhibition as follows;

0% = inhibition not effective,
>0.5 to19% = inhibition slightly effective,
20 to 39% = inhibition moderately effective,
40 to 59% = inhibition effective
60- 100% = inhibition very effective

Mycelial growth was measured using thread and rules as described by Kutama *et al.* (2016).

Data Analysis

The data were subjected to analysis of variance and treatment means were compared using Newman- Keuls test on a GENSTAT software Release 17.1 (PC/Windows 8), Copyright, 2014.

RESULTS AND DISCUSSION

Isolation and identification of antagonist fungi (*Trichoderma* species)

Table 1 shows the morphological characteristics of the three trichoderma species isolated and used in this research, namely; *T. harzianum, T. viride* and *T. asperullum.*

Table 1: Morphological Feature of Three *Trichoderma* species Isolated and Used in this Experiment

S/No	Morphological features	Species of Trichoderma identified
1	It forms white colony on most media, conidia are spherical, round or globose, slow growth to produce thick hyphae	*T. harzianum*
2	Globose, sub-globose or ellipsoidal and warted conidia with the longest L/W ratio	*T. viride*
3	Slightly ovoid conidia (in the form of chlamydospores and not teliospore), faster growth and sporulation, paired branched and coarse hyphae(mycelium),	*T. asperullum*

Effect of three *Trichoderma* species on the mycelial growth of *Fusarium oxysporum* f. sp. *vasinfectum*

Table 2 showed the results of the *in - vitro* effect of the three *Trichoderma* species on the mycelial growth of *Fusarium oxysporum* f. sp. *vasinfectum.* All the three tested *Trichoderma* species significantly ($p \leq 0.05$) inhibited the mycelial growth of the pathogen compared with the control (T1) at 3, 5, 7, and 9 days after incubation. However, of all the three species of trichoderma tested, *Trichoderma asperullum* (T2) was found to be the most effective in reducing the radial growth of the pathogen (4.2, 4.1, 4.0, and 3.9cm) at 3, 5,7, and 9 days after incubation, respectively. While *Trichoderma harzanium* (T3) was found to be the next effective in reducing the radial growth of the pathogen (4.1, 3.4, 2.9, and 2.1cm) at 3, 5,7, and 9 days after incubation, respectively. This was followed by *Trichoderma viride (*T4*)* with 4.1, 3.8, 3.7 and 3.4 cm (at 3,5, 7 and 9 days) after incubation, respectively been the least effective. The control plate (T1) has the highest mycelial growth of 4.6, 5.4, 7.1 and 8.6 at 3, 5, 7 and 9 days after incubation, respectively indicating that there was no any inhibition.

The present result shows that all the three species of *Trichoderma* suppressed the growth of *Fusarium oxysporum* f.sp *vasinfectum* at different magnitude. This result is at par with that of Dhodi *et al.* (2018) where twenty five promising isolates of *Trichoderma* including *Trichoderma harzianum, Trichoderma asperellum* and *Trichoderma viride* were reported to have suppressed the growth of *Fusarium oxysporum* f.sp *vasinfectum* nine days after incubation. The mode of activity of Trichoderma fungi on the tested pathogen; *Fusarium oxysporum* f.sp *vasinfectum* has been shown to be due to antibiosis and competition. This is evident in the report of Dhodi *et al,* (2018) and earlier on by Yahaya *et al.* (2013). The reason for the suppression of the pathogen is starvation and scarcity of limiting nutrients derived by the biocontrol agent at the expense of *Fusarium oxysporum* f.sp *vasinfectum* (Waghunde *et al.,* 2016). Studies suggested that during the *Trichoderma - Fusarium* interaction, some metabolites are synthesized by *Trichoderma* wall to degrade or inhibit the radial growth of the *Fusarium* (Vinale *et al.,* 2008). According to many workers, these compounds are volatile antibiotics such as β- phenyl- α-pyrone (6pp) and isocyanides derivatives or compounds which are soluble in water as the koningic acid, heptelidic acid and finally peptabols (Fogliano *et al.,* 2002). It is also reported that these metabolites have quite long distance of influence of the host (Oman and Zeilinger, 2010) and that a lytic activity trigger cascades (chitinase, glucanase and proteases) to degrade fungal cell wall (Fogliano *et al.,* 2002).

In this research, *Trichoderma harzianum (*T3*)* was most effective in reducing the radial growth of *Fusarium oxysporum* f.sp *vasinfectum* (3.4cm), followed by *Trichoderma viride* with 4.70cm and lastly *Trichoderma asperellum* (4.10). This result is in line with the finding of Kshirsagar and Todkar (2005), which stated that the isolate of *Trichoderma harzianum* was superior over other isolates in arresting the mycelia growth of *Fov* in which *Trichoderma viride* and *Trichoderma asperellum* were included. Shama *et al.* (2014) reported that *T. harzianum* and *T. viride* are the two most commonly used species and have been found effective when applied to 87 different crops.

Table 2: Effect of three *Trichoderma* species on the mycelia growth (cm) of *Fusarium oxysporum* f. sp. *vasinfectum* at 3, 5, 7and 9 days after inoculation

Days after incubation	Treatments (mycelium growth (cm)			
	T1	T2	T3	T4
3	4.60[a]	4.20[b]	4.10[b]	4.10[b]
5	5.40[a]	4.10[b]	3.40[d]	3.80[c]
7	7.10[a]	4.00[b]	2.90[d]	3.71[c]
9	8.60[a]	3.90[b]	2.10[d]	3.20[c]
Means	6.43	4.05	3.12	3.70
SE±				

Means followed by different letters are significantly different at (P≤ 0.05) using Newman-Keuls test

Key: T1= *Fusarium oxysporum* f. sp. *vasinfectum*
T2= *Trichoderma asperellum*
T3=*Trichoderma harzianum;*
T4 =*Trichoderma viride*

Percentage inhibition of *Trichoderma on Fusarium oxysporum* f. sp. *vasinfectum*

Table 3 shows the percentage (%) inhibition of three *Trichoderma* species on *Fusarium oxysporum* f. sp. *vasinfectum.* From the results, it was evident that highest percent inhibition was recorded with the use of *Trichoderma harzanium* (T3*)* which inhibited growth of the fungus by 10, 37, 59.2 and 75.6 percent at 3, 5, 7 and 9 days after incubation. This is followed by *T. viride* (T4) (10,29.7,47.9, 62.9)% at 3, 5, 7 and 9 days after incubation, respectively and finally *Trichoderma asperellum* (T2) which retarded the growth of *Fusarium oxysporum* f. sp. *vasinfectum* by 8.6, 24, 43.7 and 54 % at 3, 5, 7 and 9 days after incubation, respectively. Mycelial growth of the pathogen varied from 31% to 50.5% in different species of *Trichoderma.*

The result also showed that all the three spp of *Trichoderma* inhibited the growth of *Fusarium oxysporum* f. sp. *vasinfectum* at varying degrees. The result of the present research has tallied with the findings of Akarami (2011) who evaluated *T. harzianum*, T. *asperellum* and *T. virede* against *Fusarium oxysporum* of Lentil and found all of them able to inhibit the growth of the fungus in the laboratory. The result is also the in tandem with that of Manjur and Afiya (2019) which stated that *Trichoderma* isolates inhibits and control the growth of *Fusarium oxysporum* with *T. harzianum* being the most effective. Another study conducted by Arya *et al.* (2017) who studied the interaction between the pathogen; Fusarium *moniliforme varsubglutinans* and bioagents. The result in dual culture revealed that among the different bioagents tested, *T. harzianum* strains showed the maximum percentage inhibition of about 73%. Inhibition of growth of fov by *Trichoderma* may be as a result of the release of metabolites by *Trichoderma* (Kouakou *et al.,* 2020). This is also at par with Raza *et al.* (2014) who evaluated the antifungal potential of volatile and non volatile compounds of *Trichoderma harzianum* against *Fusarium oxysporum in-vitro* and demonstrated that strain of *T. harzianum* produced volatile compounds that can inhibit the growth of *Fusarium oxysporum* f.sp *neveum* , the causal agent of *Fusarium* wilt of water melon. *Trichoderma* isolates differentially limited the growth of pathogen, overgrew the pathogen colony and produced yellow pigment (Dolatobadi *et al.,* 2012)

Table 3: Percentage retardation of *Fusarium oxysporum* f. sp. *vasinfectum* mycelia growth by three *Trichoderma* sp.

Days after incubation	Inhibition %		
	T2	T3	T4
3	8.6[a]	10.0[b]	10.0[b]
5	24.0[c]	37.0[e]	29.7[d]
7	43.7[e]	59.2[f]	47.9[e]
9	54.0[f]	75.6[h]	62.8[g]
Means SE±	32.65	45.45	37.60

Means followed by different letters along rows are significantly different at (P≤ 0.05) using Newman-Keuls Test

Key: T2= *Trichoderma asperellum:*
T3= Trichoderma harzianum:
T4=*Trichoderma viride*

CONCLUSION

From the findings of this study, it is concluded that all the three species of *Trichoderma* inhibited the mycelia growth of *Fusarium oxysporum* f. sp. *vasinfectum* in dual culture *at* varying degree with *Trichoderma harzianum* being the most effective followed by *Trichoderma viride* and lastly *Trichoderma asperellum.* Therefore, *Trichoderma harzianum* is recommended as a biocontrol agent of *Fusarium oxysporum* f. sp. *Vasinfectum.*

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