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Efficacy of *Trichoderma* Species on some fungal Pathogens of Tomato (*Solanum lycopersicum* L.) Grown in Kashere and its Environs

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Abstracts
The effect of *Trichoderma* species as biocontrol agents on some fungal pathogens was investigated. Random sample of both soil and tomato (*Solanum lycopersicum* L.) were collected around Kashere environs, and was used for the isolation of *Trichoderma* species and fungal pathogens of tomato (*Solanum lycopersicum*) using standard plates count. Both *Trichoderma* species and fungal pathogens isolated, i.e *Fusarium oxysporum, Aspergillus niger, Aspergillus fumigatus,* and *Rhizoctonia solani* were identified macroscopically based on their cultural characteristics and microscopically with the addition of lactophenol cotton blue as stain. *Trichoderma* species were used for the inhibition of the fungal pathogens isolated, using dual culture techniques, filter paper disc of 8mm was soaked in 0.00 mg (control), 5.00 mg and 10 mg of *Trichoderma* species concentration were used to test for the antifungal activity on *Fusarium oxysporum, Aspergillus niger, Aspergillus fumigatus,* and *Rhizoctonia solani* in a cultured plate arranged in complete randomized design (CRD) and carried out in duplicates. *Trichoderma* species were found to inhibits significantly at $P \leq 0.05$ *Fusarium oxysporum, Aspergillus niger, Aspergillus fumigatus,* and *Rhizoctonia solani.* The highest zone of inhibition was recorded in *Aspergillus fumigatus* (37.00 mm) followed by *Rhizoctonia solani* (35.50 mm) and then *Aspergillus niger* (19.00 mm) with the least zone of inhibition observed in *Fusarium oxysporum* (15.00 mm); Hence, *Trichoderma* species can be considered as biocontrol agents for the control of some fungal pathogens.

Keywords: *Trichoderma* sp., biocontrol agents, fungal pathogens, tomato

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Introduction

Tomato (*Solanum lycopersicum* L.) are important cash crops of Kashere town, sub-location of Pindiga District. Most farmers in this area depend on it as a means of livelihood. As important as this crop is to the farmers, it is observed that they are faced with a problem of low yield associated mostly with fungal pathogens hampering vitality and high yield of the crop. *Trichoderma sp*. are agriculturally important for their beneficial effects on plant growth, development and for their capability to induce plant defense responses against pathogens, damage provoked by insects and abiotic stress (Harman *et al.,* 2004; Woo *et al.*, 2006).

Healthy fruits with good growth and vigor contribute to disease resistance and improved yield. It is important to identify strategies for

improved growth, vigor and disease resistance. *Trichoderma sp*. are known to promote growth and resistance to diseases in plants. There is immediate potential for the farmer to use microbial inoculants such as *Trichoderma sp*. especially when the seedlings are raised in the nursery beds and are later transplanted in the field. Adding microbial growth and biocontrol agents to the roots of transplants is an effective and inexpensive means to provide a more vigorous transplant with disease protection when it is transplanted into the field (Lo and Lin, 2002; Harman *et al*., 2004).

Furthermore, the use of these microorganisms as biocontrol agents and bio-fertilizers is a major step towards moving away from dependency on chemical inputs to enhance growth and control plant diseases. The demand for alternatives to chemical control is becoming stronger owing to concerns about safety and environmental impact of chemicals (Tucci *et al.,* 2011; Benıtez *et al.,* 2004). It is therefore, justified to use *Trichoderma* sp. as biocontrol agents.

Trichoderma is one of the most common filamentous imperfect saprophytic fungi present in all sort of agricultural soil and in other habitats such as decaying substrates and rhizosphere ecosystem. It is a fast growing, light to dark green coloured, potent biocontrol agent and used against plant diseases of various crops (Agrios, 2005). There are several different types of species that have been isolated and identified from different substrates worldwide (Harman, 2000).

Several workers have reported that species of *Trichoderma* can be utilized for multipurpose in the areas of crop production viz., promoting plant growth (Harman *et al.,* 2004; Ozbay and Newman, 2004), improving nutrient uptake and as well as heightened plant defense levels against biotic and abiotic stress (Howell, 2003) and protection viz., controlling soil-borne (Ozbay and Newman, 2004; Montealegre *et al.,* 2005; Ngo *et al.,* 2006), air-borne (Elad, 2000) and post-harvest diseases in a wide range of crops by different mechanisms.

Tomato (*Solanum lycopersicum* L.) belongs to the *Solanaceae* family and it is relatively short duration crop which gives a high yield, and is economically attractive. It is grown extensively in many parts of Nigeria and almost all the year around. Nigeria ranks as the 13th largest tomato producing country in the world and second highest producer in Africa (FAO, 2011).

Tomatoes are one of the most widely cultivated vegetable crops in Africa. Its fruit is rich in vitamins and is therefore used in salads, cooked as a vegetable or made into tomato paste and tomato sauce. They are grown for home consumption and also as an important cash crop for both smallholders and medium scale commercial farmers. (Varela *et al.,* 2003).

The major constraints in tomato production in this country are due to inadequate supply of good quality seeds, inadequate storage facilities, lack of proper diseases and pests' management and lack of sufficient processing facilities (Ugonna *et al.,* 2015). Among all these constraints, lack of poor managements of pests and diseases are major concern leading to low production of this vegetable crop in the Northern part of the country.

Tomato is incited by taxonomic diverse groups of phytopathogens among which are fungi, bacteria, viruses and parasitic nematodes (Agrios, 2005). There are about 200 known diseases of tomato, of which 30 are economically important. Among the diseases of this crop, fungal diseases are economically important and the most common diseases in vegetable production throughout the world (Kutama *et al.,* 2007; Shehu *et al.,* 2014; Sobia *et al.,* 2016).

Biological control is a safe method to reduce plant disease incidence without collateral damages to the environment and to human health induced by synthetic chemicals (Tucci *et al*., 2011). Some microorganisms, such as fungi belonging to the genus *Trichoderma*, are used as biocontrol agents (BCAs) to antagonize plant pathogens through a series of mechanisms including competition for nutrients and space, fungistatic, antibiosis and modification of the rhizosphere (Benıtez

et al., 2004). *Trichoderma* sp. are agriculturally important for their beneficial effects on plant growth, development and for their capability to induce plant defense responses against pathogens, damage provoked by insects and abiotic stress (Harman *et al.,* 2004; Woo *et al.*, 2006). For this reason, they are a major source of many biofungicides and biofertilizers (Verma *et al.,* 2007; Kaewchai *et al.,* 2009).

Chemical control is one of the most common methods and frequently used by farmers for the management of plant diseases. However, synthetic chemicals are highly toxic, hazardous and have negative impacts on the ecosystem including non-target organisms (Agrios, 2005). The massive and ever-increasing application of synthetic chemicals for plant pest control causes serious problems to both human health and the environment (Budnik and Baur, 2009; Debenest *et al.,* 2010). In this context, the use of biological control agents (BCAs) as an alternative to conventional practices for disease management is a declared objective of agricultural politics throughout the world. Many biopesticide and biofertilizer products are now available in the market, the majority of which are based on beneficial symbionts of the genus *Trichoderma* (Woo *et al.,* 2006). These fungi are well known for their ability to kill plant pathogens (Benitez *et al.,* 2004; Verma *et al.,* 2007), as well as to promote plant growth and resistance against biotic and abiotic stresses (Shoresh *et al.,* 2010).

The excess use of chemical pesticides not only polluted the environment but also deteriorated the overall fertility of the soil. There can be benefits using pesticides at initial level but later use can counterproductively increase pest resistance and kill the natural enemies of pests and also adversely affect the fertility of soil. In turn, bio-pesticides not only increase the fertility of soil, but also are eco-friendly and do not affect the other beneficial microorganisms. The pathogenic fungi are considered natural mortality agents and environmentally safe bio-pesticides. Bio-pesticides are more effective than chemical pesticides in long term use, and also cost effective (Mehta *et al.,* 2012). This research aims to determine the efficacy of *Trichoderma* sp. on fungal pathogens of tomato (*Solanum lycopersicum* L.*)* grown around Kashere and Environs.

Materials and Methods

Study Area

The area of this study is Kashere, a town in Akko Local Government Area, Gombe State. It is located on Latitude 9^0 46' 0" N and longitude 10^0 57' 0" E. Elevation of about 431 m above sea level. It has an estimated area of 427 km^2 (158 sq. km) and a population of 7,715 as at 2006 census (NPC, 2006). The population and activities of Kashere town increased in the last 12 years which may be due to the establishment of the new Federal University. The people are predominantly farmers and traders (Ntekim and Oraulike, 2004). The experiment was carried out in Biological Science Laboratory, Federal University of Kashere, Gombe State.

Samples Collection

Collection of Tomato Samples

Twenty (20) Samples of infected tomatoes were collected from farm field around Kashere and transported to the Biological Science Laboratory, Federal University of Kashere in a sterile polythene bag for fungal pathogens isolation.

Collection of Soil Samples

Ten (10) soil samples were collected around the rhizosphere of ten (10) different plants randomly within the campus of Federal University of Kashere. The soil samples were taken from a depth of 15 cm with a horizontal distance of 120 cm using a soil augur of the length of 2.5 m and diameter of 1.9 cm. Approximately 400 g of the soil samples were collected around each plant and then placed into sterile polythene bags, labeled with the information of the collection site, date and place then transported to the Biological Science Laboratory, Federal University of Kashere. The samples were then homogenized and spread on paper to remove plant materials then stored at 4^0C in a refrigerator before further analysis (O'Donovan *et al.,* 2013).

Materials Sterilization

Different laboratory apparatuses were used for this research; the apparatus was washed with detergent, rinsed with clean water and then dried. This was then followed with a proper sterilization in a hot air oven at high temperature of about 160^0C for 1 hour. This apparatus includes petri dishes, test tubes, pipettes, conical flask and universal bottles.

Serial Dilution and Sample Preparation

Serial Dilution of Tomato Samples

Ethanol (70%) was used to disinfect the surface of the work bench. About 1 g of the infected tomato fruit was cut using a sterile razor blade from each of the samples and it was transferred separately into a sterile motor and pestle, where distilled water was added and the sample was then crushed. 1 mL of each sample was added into a sterile test tube containing 9 mL of distilled water to make a suspension, each of the sample was then shaken vigorously and allowed for 15 min. 1 mL from each of the mixture in the test tubes was transferred to 9 mL of distilled water in another test tube with the help of a sterile pipette to make 10^{-2} and this procedure was repeated until 10^{-5} was made (Oprin *et al.*, 2017).

Serial Dilution of Soil Samples

Ethanol (70%) was used to disinfect the surface of the workbench. Ten grams (10 g) of each of the soil samples collected was weighed out and placed into a sterile conical flask then 100 mL of distilled water was added into the conical flask to have a mixture, then 1 mL of the soil mixture was transferred into a test tube containing 9 mL of distilled water using a sterilized pipette, creating 10^{-3} of each of the sample. The sample solution in the test tube was homogeneously mixed and labeled properly with an appropriate dilution (O'Donovan *et al.*, 2013).

Media Preparation

Media Preparation for Tomato Fungal Isolates

The media used for the isolation and characterization of fungal pathogens of tomato was Potatoes Dextrose Agar (PDA) where 39 g was dissolved in 1000 mL of distilled water and autoclaved at 121^0C for 15 minutes as describe by the manufacture company.

Media Preparation for Soil Sample Isolates

The media used for the isolation and characterization of *Trichoderma sp*. was Potatoes Dextrose Agar where 39 g was dissolved in 1000 mL of distilled water and 0.02 g/L of Rose Bengal was added to the Agar and autoclaved at 121^0C for 15 minutes. After autoclaving, the media was allowed to cool for some minutes, then 0.3 g/L of chloramphenicol and 0.1 g/L of streptomycin were added and mixed thoroughly. Rose Bengal was chosen due to its antifungal and antibacterial action, Streptomycin was used for rose Bengal resisting bacteria and Chlorempheniсol for its broad-spectrum of antibacterial action (Vargas, 2006).

Inoculation and Incubation

Inoculation and Incubation of Tomato Sample

One (1) mL of the aliquot from each of the serial dilution of the Tomato samples were added in to bottles containing PDA media. Each of the inoculum was then poured in to 9 cm diameter sterile petri dishes at 10 mL per plate in two duplicates. The plates were allowed to solidify and then incubated at 27^0C for 7 days (Oprin *et al.*, 2017).

Inoculation and Incubation of Soil Sample

One (1) mL of the aliquot from each of the serial dilution of the soil samples were added in to bottles containing the prepared media (PDA) in which Chloramphenicol, Streptomycin and Rose Bengal were added. Each of the inoculum was then poured in to 9 cm diameter sterile petri dish at 10 mL per plate in triplicate plates and allowed to solidify and later incubated at 27^0 C for 7 days (Vargas, 2006).

Identifications of The Isolates

Identifications of Tomato Fungal Isolate

Characterization and identification of the tomato fungal isolates was achieved by morphological examination of the colonies on the plate (macroscopic) for colonial appearance, size, elevation, form, edge, consistency, color, opacity and pigmentation, the isolates were identified and characterized based on their cultural characteristics. Microscopic identification of the tomato

fungal isolates was also carried out where a little portion of the fungal growth was picked using sterile wire loop and placed on a clean glass slide, a drop of Lactophenol cotton blue was added, the speck was emulsified on the slide and viewed under microscope at X10 and X40 0bjective lens (Oprin *et al.,* 2017).

Identifications of the Soil Fungal Isolates

Characterization and identification of the soil fungal isolates was also achieved by morphological examination of the colonies on the plates (macroscopic) for colonial appearance, size, elevation, form, edge, consistency, color, opacity and pigmentation, the isolates were identified and characterized based on their cultural characteristics. Microscopic identification of the soil isolates was carried out using lactophenol blue staining. A little portion of the fungal growth was picked and placed on a clean glass slide, and a drop of Lactophenol cotton blue was added, the speck was emulsified on the slide and viewed under microscope at X10 and X40 0bjective lens (O'Donovan *et al.,* 2013).

Antagonistic effect of the Trichorderma* sp. *on the fungal pathogens

Dual culture technique was used for this study where eight (8) mm disc of *Trichoderma sp*. were prepared and placed at the center of the plate culture medium streaked with the different fungal pathogens isolated from the tomato samples. Duplicated plates of this method containing two different concentrations of *the Trichoderma sp*. (5 mg and 10 mg) and a control (0 mg) for each of the differently isolated fungi were maintained and incubated at 27^0C for 7 days (Oprin *et al.,* 2017).

Measurement of Zones of Inhibition

After seven days of incubation, the cultures were brought out and different zone of inhibitions were noticed and a ruler was used to measure the diameter of the zone of inhibition both horizontally and vertically and the results in millimeter was recorded for all the test plates (Oprin *et al.,* 2017).

Results

The selective medium used in this work proved to be effective for the detection and identification of *Trichoderma sp*. The selective effect of this medium is based on the fact that *Trichoderma sp*. are relatively tolerant to high levels of rose-bengal, chloramphenicol and streptomycin and also has the capacity to grow and sporulate on media containing a low concentration of glucose. Their typical green color, which aided in their identification among other soil-borne fungi clearly manifested. Satisfactory bacterial suppression was obtained by the addition of rose-bengal, chloramphenicol and streptomycin and also inhibition of the growth of other plant pathogenic fungi with the exception of some few *Rhizopus sp*. and *Mucor sp*. which naturally and usually spread over other fungal colonies.

Table 1: Isolated Fungal species from Tomato Samples.

S/N	Tomato Samples	Fungal species
1	TSKF 1	*Fusarium* sp.**,** *Aspergillus* sp.
2	TSKF 2	*Sacchromycetes* sp
3	TSKF 3	*Rhizoctonia* sp., *Fusarium* sp.
4	TSKF 4	*Sacchromycetes* sp.
5	TSKF 5	*Aspergillus* sp.
6	TSKF 6	*Aspergillus* sp.
7	TSKF 7	*Fusarium* sp.
8	TSKF 8	*Rhizoctonia* sp.
9	TSKF 9	*Fusarium* sp.
10	TSKF 10	*Aspergillus* sp.
11	TSKF 11	*Sacchromycetes* sp.
12	TSKF 12	*Sacchromycetes* sp.
13	TSKF 13	*Fusarium* sp.
14	TSKF 14	*Fusarium* sp.

15	TSKF 15	*Fusarium* sp.
16	TSKF 16	*Fusarium* sp.
17	TSKF 17	*Fusarium* sp.
18	TSKF 18	*Fusarium* sp.
19	TSKF 19	*Sacchromycetes* sp.
20	TSKF 20	*Sacchromycetes* sp.

Key: TSKF=Tomato Sample Kashere Farm.

Table 2: Macroscopic and Microscopic Identification of Fungal Pathogens of Tomato.

S/No	Macroscopic	Stain used	Microscopic	Organisms Identified
1	The colony was pink with white patch on the surface and on the reverse side was brown in coloration on PDA.	Lactophenol blue	The macroconidia are canoe shape multiseptate which contain 3-6 septations and slightly pointed at the end.	*Fusarium oxysporum*
2	The colony was white with cottony surface and on the reverse side is brown in coloration on PDA	Lactophenol blue	Dark mycelium hyaline, long mycelium cell and branched at the upper part, no septation of branches set off from the main hyphae.	*Rhizoctonia solani*
3	The colonies were widely spread, black, with smooth white edges and spongy surface densely packed and brown on the river side on PDA.	Lactophenol blue	The conidiophore was long, erected from base to the vesicle, smooth walled, hyaline with globes conodial head.	*Aspergillus niger*
4	The colony was widely spread, dark green with white smooth edges and spongy and brown on the reverse side on PDA.	Lactophenol blue	The conidiophores were long, narrow at the base and broad near the vesicle, smooth wall hyaline.	*Aspergillus fumigatus*

Table 3: Collection and Identification of *Trichoderma* sp.

S/N	Site of Collection	Common Name	Colony Colour	Organisms Identified
1	*Mangifera indica*	Mango	Dark green	*Trichoderma sp.*
2	*Chamaerops humilis*	Fan palm	Dark green	*Trichoderma sp.*
3	*Tamarindus indica*	Tamarin	Dark green	*Trichoderma sp.*
4	*Cycas revolute*	Sago palm	Green	*Trichoderma sp.*
5	*Adonsonia digitata*	Boabab	Dark green	*Trichoderma sp.*

6	*Litchi chinensis*	Black coral	White	*Mucor sp.*
7	*Azadracta indica*	Neem	Black green	*Rhizopus sp.*
8	*Bougainvilliea spectabilis*	Boungainvillea	White	*Mucor sp.*
9	*Anageisus leocarpus*	African birch	Black and white Edge	*Aspergillus sp.*
10	*Vitellaria paradoxa*	Shea butter	Black green	*Rhizopus sp.*

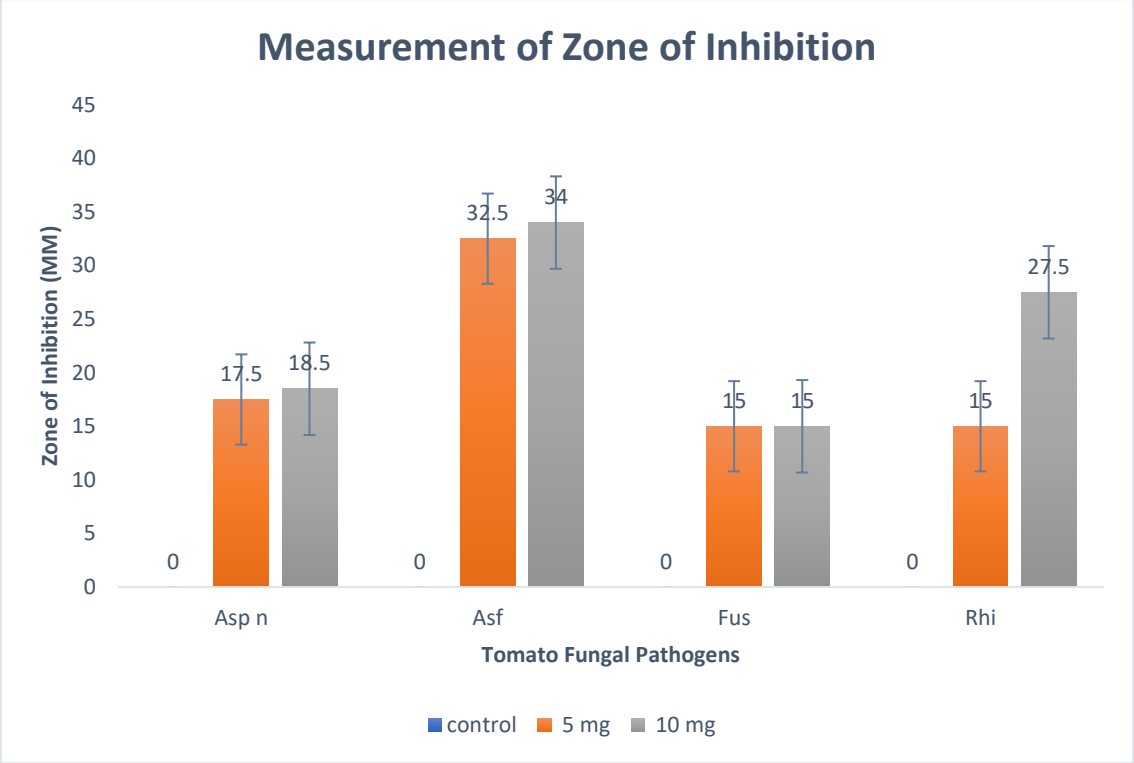

Figure 1: Different Zone of inhibition in millimeters at different concentrations of *Trichorderma sp*. on fungal pathogens of tomato.
KEY: Asp n = *Aspergillus niger*, Asf = *Aspergillus fumigatus*, Fus = *Fusarium oxysporum*, Rhi = *Rhizoctonia solani*

Discussion

Fungal species belonging to the genus *Trichoderma* are worldwide in occurrence and easily isolated from the soil. The potential of *Trichoderma* species as bioconrol agents against various plant diseases has been reported by several workers (Sundaramoorthy and Balabaskar, 2013; Sharon *et al.*, 2001). In the present investigation, fungal antagonist isolate caused highly significant reduction in fungal pathogens incidence under *in vitro* conditions. The inhibitory effect of these bioagents against tested pathogen was probably due to competition and antibiosis.

Demands for *in vitro* effectiveness of *Trichoderma* against species of *Fusarium* have been reported (Sundaramoorthy and Balabaskar, 2013). The antagonist *Trichoderma sp.* was reported to be equally antagonistic to *Fusarium sp.* under *in vitro* conditions (Sundaramoorthy and Balabaskar, 2013). Sundaramoorthy and Balabaskar, 2013 reported that *Trichoderma* sp. successfully controlled *Fusarium* sp. on cotton, wheat and muskmelon. Sesame seeds treated with three isolates of *T. viride* reduced

the pre and post emergence damping-off caused by *R. solani* and *F. oxysporum*.

In the present investigation, the fungal pathogens of tomato were cultured and later sub-cultured to attain a pure culture, on introduction of *Trichoderma* sp., the growth of tomato fungal pathogens dropped drastically in the cultured media at 10 mg and less at 5 mg concentration, these shows that at higher concentration of *Trichoderma*, inhibition of fungal pathogens was high.

However, this research is not in agreement with the report by Kucuk and Kivanc (2003) that none of the pathogens they tested were sufficiently inhibited by the filtrate of *T. harzianum* isolates, and that *R. solani* was the most sensitive plant pathogenic fungi against *Aspergillus fumigatus* found in this study.

Whereas, the result agreed with the report of Vargas (2006) that from all the media they evaluated, PDA was the most effective in the isolation of *Trichoderma* sp. and the use of biocides favoured its isolation from the soil. Chloramphenicol, Streptomycin and rose Bengal favoured the development of *Trichoderma* sp. and subsequently reduce that of the plant pathogenic fungi.

Conclusion

Based on the findings of this work we can conclude that *Trichoderma* species indicated strong inhibition to different fungal pathogens such as *Rhizoctonia solani, Fusarium oxysporum, Aspergillus niger,* and *Aspergillus fumigatus* and can equally be utilized as biocontrol agents for the manufacture of biopesticides to reduce the high rate of chemical toxicity to the soil environment as well as non-target organisms.

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