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Identification of Fungi Colonizing the Rhizopshere of Tomato (*Solanum lycopersicum*) Plants and their Pathogenicity on Healthy Tomato Fruits

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Abstract

Soil is a complex natural resource that represents a vast reservoir of biodiversity with several billion prokaryotic and eukaryotic microorganisms. A study was carried out to identify fungal organisms colonizing the rhizosphere of tomato plants in Makurdi and to test their pathogenicity on healthy tomato fruits. Soil samples were collected from the rhizosphere of tomato plantations at Akpehe, Wurukum area of Makurdi. The soil samples were placed in polyethylene envelopes and taken to the Botany Laboratory of the Benue State University Makurdi for isolation of fungi. Fungi isolation was done using the serial dilution method. Exactly 1 ml of 10^{-4} dilution level of the inoculums were placed in Petri dishes after which molten Sabouraud agar was aseptically introduced and incubated at room temperature for 5-7 days. Data collected were analyzed using Analysis of Variance and Chi-square. The results showed that six fungi were isolated from the soil samples namely; Aspergillus niger, Aspergillus fumigatus, Rhizopus stolonifer, Fusarium sp, Penicillium sp and Curvularia sp. The frequency of fungi occurrence in the soil samples was significant across species ($\div^2$ = 17.20, df = 5. P=0.01). Aspergillus niger had the highest occurrence 10 (26.32%), followed by Rhizopus stolonifer and Aspergillus fumigatus each with 7 (18.42%) respectively, Fusarium sp 6 (15.79), Penicillium sp. 5 (13.16%) and the least occurrence was Curvularia sp. 3 (7.89%). Pathogenicity test revealed that rot induced by Rhizopus stolonifer was significantly higher (10.52cm^2) on tomato fruits compared with that induced by Aspergillus fumigatus (7.30cm^2), Aspergillus niger (6.87cm^2), Penicillium sp. (5.20 cm^2), Curvularia sp. (5.00cm^2), Fusarium sp. (4.21cm^2) and the lowest was recorded in the uninoculated (control) (1.45cm^2) fruits. The isolated soil borne fungi were pathogenic when inoculated on healthy tomato fruits. Further research should be carriedout on the effect, identification and interaction of both pathogenic and beneficial rhizosperic fungi on growth and development of plants.

Keywords: Fungi, rhizosphere, tomato plants, Pathogenicity, tomato fruits.

Introduction

Tomato (*Solanum lycopersicum*) is a fruit which is basically consumed either as whole or freshly cut and used in salads, processed purees, pasta, powder, ketchup, soup or packaged in cans. It is widely grown around the world having a total annual production of approximately 159 million tons on a cultivated area of about 5 million hectares (Food and Agriculture Organization Statistics; FAOSTAT, 2013). Tomato has achieved tremendous popularity over the last century due to its variety of purpose. It is grown in practically every country of the world in outdoor fields, greenhouses and net houses. World production and consumption have grown quite rapidly over the past 25 years (Naika *et al*., 2005). According to Ebimieowei and Ebideseghabofa (2013), Nigeria was reported to be the second largest producer of tomato in Africa after Egypt and 13th in the world, with a production of 6 million tons annually prior to 1990. Tomato is grown in Nigeria in its diverse agro-ecological zones that range from humid in the south to sub-humid in the middle belt and semiarid/arid in the north. It is mostly cultivated in the northern regions of the country, between latitudes 7.5°N and 13°N, and within a temperature range of 25 – 34 °C.

Tomato is an important vegetable that plays a major role in the provision of vitamins and minerals for humans, hence, necessary in the preparation of many local dishes and very important in the diet of both rural and urban dwellers in Nigeria (Olayemi *et al*., 2010). The intake of tomatoes provide the body with nutrients like carotene, vitamin, lycopene which lower the risk of cancer and cardiovascular diseases; it also has antioxidant components that are medically useful in the area of cataracts, bone metabolism, asthma and helps to reduce the risk of prostate and breast cancer (Shankara *et al*., 2005). It serves as condiments for soups which is a regular feature of African meals and accounts for about 18% of the average daily consumption of vegetables in Nigeria (Ebimieowei and Ebideseghabofa, 2013). It has presently been considered as an important cash and industrial crop in numerous parts of the world (Saeed-Awan *et al*., 2012)

Statement of the Problem

Soil is a complex natural resource that represents a vast reservoir of biodiversity with several billion prokaryotic and eukaryotic microorganisms. These microbes significantly share biomass and ecosystem functions in both natural and managed agricultural soils (Kazerooni *et al*., 2017). Soil borne diseases are diseases that are caused by pathogens which persist (survive) in the soil matrix and in residues on the soil surface. Rhizosphere contain lots of organic substances which harbor a high content of microorganism especially fungi, Thus the soil is a reservoir of inoculum of plant pathogens, the majority of which are widely distributed in agricultural soil. However, some species show localised distribution patterns. Thus, these plant pathogens cause diseases which may not be noticed until the above-ground (foliar) parts of the plant are affected severely showing symptoms such as stunting, wilting, chlorosis and death. These diseases are often very difficult to diagnose accurately and the pathogens may be difficult to grow in culture and identify accurately. Tomato plant is attacked by various diseases that significantly affect its growth and yield. Soil borne diseases like *Fusarium* wilt, bacterial wilt, stem rot or white mould are the most serious diseases affecting its yield. These diseases are caused by *Fusarium oxysporum* f. sp. *lycopersic*i (Sacc.), *Ralstonia solanacearum* and *Sclerotium rolfsii*; resident in the soil matrix and the yield loss due to these fungal organisms are huge.

Justification of the Study

It has been estimated that one gram of surface soil contain 50,000 to a million fungi. The loss of organic material from root provides the energy for the development of these fungal population in the rhizosphere around the root (Shinkafi and Gobir, 2018). Fungi are the dominant eukaryotes among soil microbial communities where they play crucial and key roles in terrestrial ecosystems (Acosta-Martinez *et al*., 2014). Microbial abundance, diversity and activity largely have implications on sustainable productivity of agricultural land and production systems. Information on the microbial communities associated with

rhizospheres and their complex interrelationship is essential in the selection of sustainable crop rotations and management practices (Chen et al., 2017). This research will help to identify the types of fungi associated with rhizosphere of tomato plants in makurdi

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Materials and Methods

Experimental Location

The experiment was conducted at the Botany Laboratory of the Department of Biological Sciences, Benue State University, Makurdi. Makurdi is located in North central Nigeria along the Benue River, between latitude 07° 442 283 N and longitude 08° 322 443 E. It is situated within the Benue trough, at the lower Benue valley and found in the guinea savanna region. The rainy season lasts from April to October with five months for dry season (November-March). Annual rainfall in Makurdi town is consistently high, with an average annual total of approximately 1173mm. Temperature in Makurdi is however, generally high through the year, with February and March as the hottest months. Temperature in Makurdi varies daily from a minimum of 22°C to a maximum of 40°C. Makurdi is in the guinea savannah ecological zone made up of trees and grasses of various types.

Collection of Soil Samples

Soil samples used in this study were collected from a farmland at Akpehe, Wurukum Area of Makurdi, Benue State. The soil from the rhizosphere of the tomato plants were collected at a depth of 2-3cm and at three different points on the field into polyethylene bags and pooled together as described by Liamngee *et al*. (2015). These were taken to the Botany laboratory of the Benue State University for isolation of rhizospheric fungi.

Preparation of Culture Media

Sabouraud Dextrose Agar (SDA) was used for isolation of the fungal pathogens. This was prepared according to the manufacturer's recommended procedures where 62.0g of powdered SDA was dissolved in 1000 mLs of sterile distilled water and stirred vigorously to homogenize. The medium was heated over a heating mantle and autoclaved at 121°C for 15mins at 15psi. The sterile medium was allowed to cool to a temperature at which it could be held with hands and two to three drops of streptomycin sulphate were added to inhibit bacteria growth. The medium was introduced into sterile Petri dishes and allowed to solidify before inoculation

.

Isolation of Fungal Organisms from Soil Samples

The serial dilution technique as described by Liamngee *et al*. (2015) was in the study for the isolation of fungi from the soil samples and a 10^4 dilution of the soil sample was prepared. In this method, a stock suspension was prepared by adding one gram of the soil samples to 9mls of sterile distilled water in a sterile glass test tube. Exactly 1ml was further transferred from the first dilution and introduced in to another labeled test tube to obtain 10^{-1} dilution. This was repeatedly done to obtain 10^{-4} dilution. Exactly 1 ml of inoculum was placed in the Petri dish after which molten SDA was aseptically added and gently swirled to enhance an even mixture. The culture plates were incubated at room temperature for 5-7 days. The plates were examined daily and each colony that appeared was considered to be one colony forming unit (cfu). Plates were observed for growth and the occurrence of individual fungi was determined by counting the number of times each individual fungus occurred divided by the total number of fungi and expressed as a percentage using the formula adopted by Liamngee *et al*. (2016).

$$\frac{\text{Number of times each fungus occurred}}{\text{Total number of fungi per plate}} \times 100$$

Subculturing of Fungi Isolates

The individual fungal colony was picked with the aid of a sterile needle and inoculated on freshly prepared Sabouraud Dextrose Agar medium. The culture plates were incubated at ambient condition of light and temperature for 5-7 days and observed daily for fungal growth. After 5-7 days, sub-

culturing was done to obtain pure culture of the isolates. To subculture, a sterilized inoculation needle was used to pick a little quantity of the fungal growth on the old culture and transferred to the center of a freshly prepared SDA in another Petri dish. Sub-culturing was done repeatedly until pure cultures of each fungal organism was obtained as reported by Liamngee *et al*. (2015)

Identification of Fungi

The identification of fungi was done by observing the colony colour, nature of the colony and growth rate of the fungi macroscopically on the culture plates. Microscopic identification was done by staining a glass slide with a drop of Lactophenol in cotton blue. With the aid of a sterile needle, a small quantity of the fungal colony were placed on the stained glass slide, covered with a cover slip and viewed under the 40× objective lens of the light microscope. The observed characteristics of the fungi were compared with a standard chart for identification by Barnett and Hunter (1972) as reported by Liamngee *et al*. (2016).

Pathogenicity Test

The pathogenicity of the isolated fungi organisms was tested *in vivo* on healthy semi-ripe tomato fruits using agar plug method of inoculation described by (Liamngee *et al*., 2015). Fifteen healthy tomato fruits were surface sterilized with 5% Sodium hypochlorite for 30 seconds to one minute and washed in three changes of sterile distilled water. A 3mm cork borer was used to punch into the healthy tomato fruits and the bored tissues were removed. With the aid of a forcep, a small quantity of fungi from pure cultures were picked and introduced into the holes bored in the tomato fruits with cork borer (three tomato fruits were inoculated per replicate of each test fungus) and the tissue replaced. Lesion diameter was measured based on the symptoms induced at five days after inoculation at ambient conditions of light and temperature using a metre rule. Area of rot was calculated using the formula adopted by Ezeibekwe and Ibe (2010) as:

Area of rot = ðdl (Where ð = 22/7, d = diameter, l = depth)

Data Analysis

The data collected were analyzed using Analysis of Variance and Chisquare analysis.

Results

Morphological Description of Isolated fungi

The fungi organisms isolated from the rhizosphere of tomato plants were; *Aspergillus niger*, *Aspergillus fumigatus*, *Rhizopus stolonifer*, *Fusarium* sp., *Curvularia* sp. and *Penicillium* sp. The colony of *A. niger* on SDA was black in colour (Plate 1a) and the conidiophores were hyaline, inflated at the apex forming globose conidia when viewed under the microscope (Plate 1b). *Aspergillus fumigatus* on plate was powdery in nature with colony colour typically bluish green (Plate 2a). The conidia heads were columnar with blue colouration when viewed microscopically (Plate 2b).The colonies of *R. stolonifer* were fast growing with cotton like colouration on SDA (Plate 3a). The sporangiospores were hyaline, smooth walled and branched forming large terminal globose sporangia (Plate 3b). Colonies of *Curvularia* sp. were moderately fast growing and effuse grey to blackish brown in colour on SDA (Plate 4a). The Conidia were curved, obclavate, bipolar and slightly brown when viewed under the microscope (Plate 4b). The growth of *Penicillium* on SDA was light powderish green with traces of white edges (Plate 5a). When viewed under the microscope, the hyphae were hyaline and septate. The conidiophores produced brush like structures (Plate 5b). The colony colour of *Fusarium* sp. started as white cottony like structure and changed to pinkish white as days progressed (Plate 6a). The conidia had a septum, was cylindrical in shape, was hyaline with a smooth round end (Plate 6b).

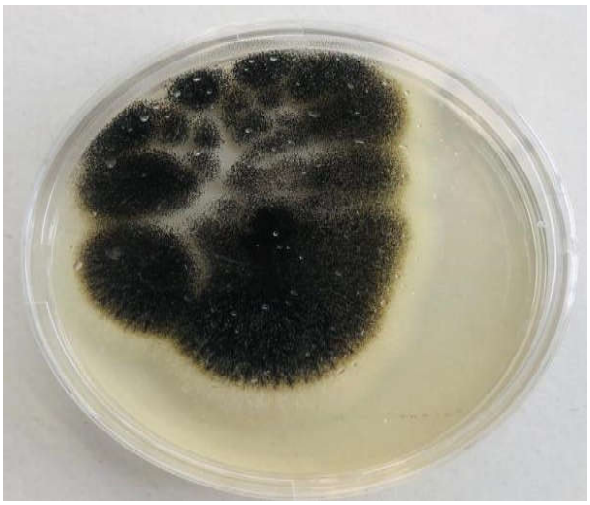

Plate 1a. Macroscopic view *A. niger* on SDA Colour colony was black.

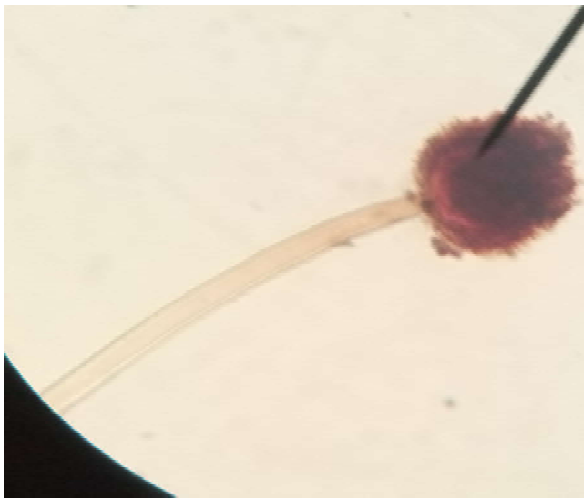

Plate 1b. Microscopic view of *A. niger* The conidia heads were black with transparent conidiophores.

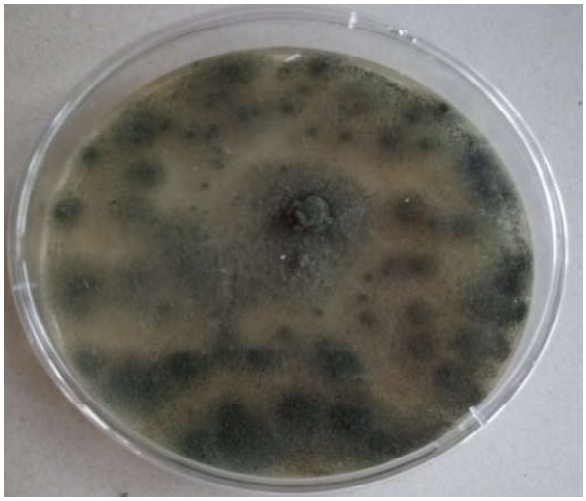

Plate 2a. Macroscopic view of *A. fumigatus* The colony colour was powderish green

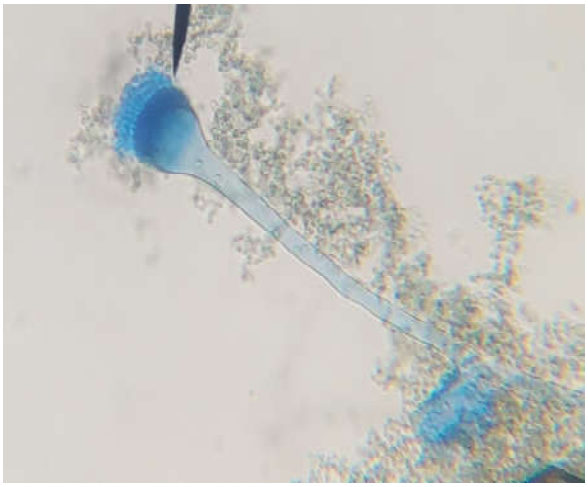

Plate 2b. Microscopic view of *A.fumigatus* The conidia heads were columnar with blue colouration.Conidiophores were transparent

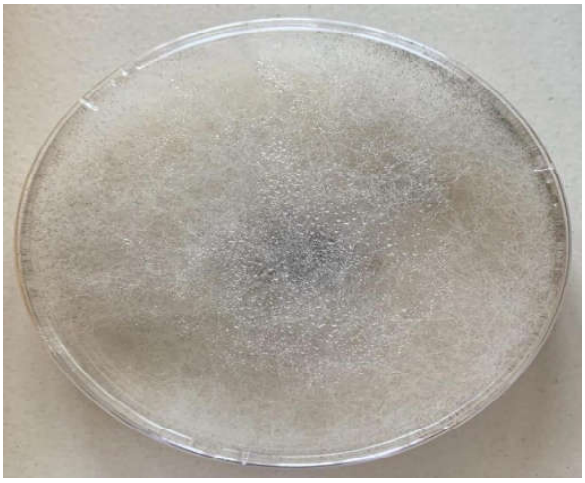

Plate 3a. Macroscopic view of *R. stolonifer* The colony had a cotton white fluffy colouration

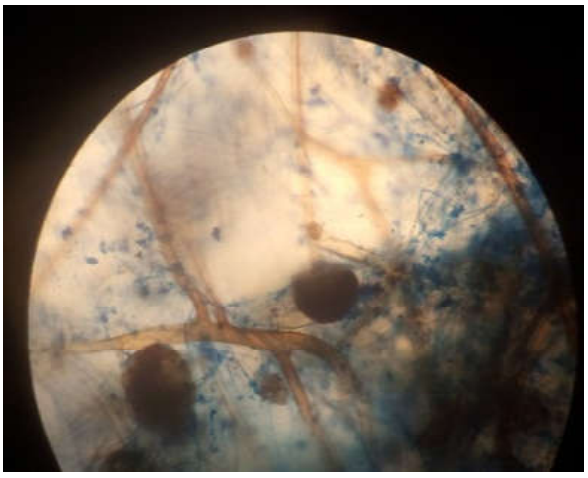

Plate 3b. Microscopic view of *R. stolonifer* The sporangiospores were branched with large globose sporangia

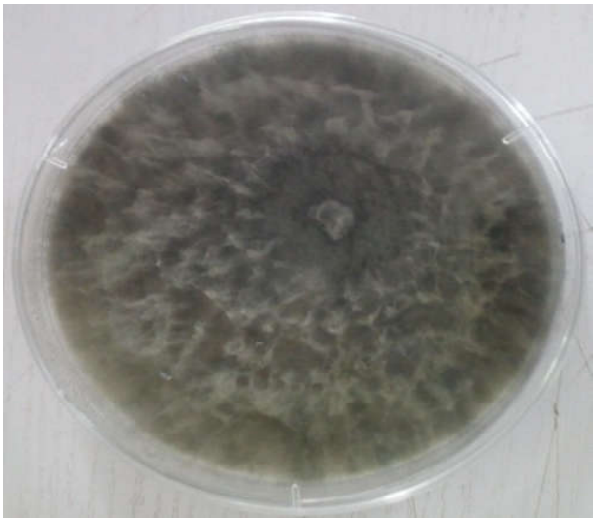

Plate 4a. Macroscopic view of *Curvularia* sp The colony colour was effuse grey to

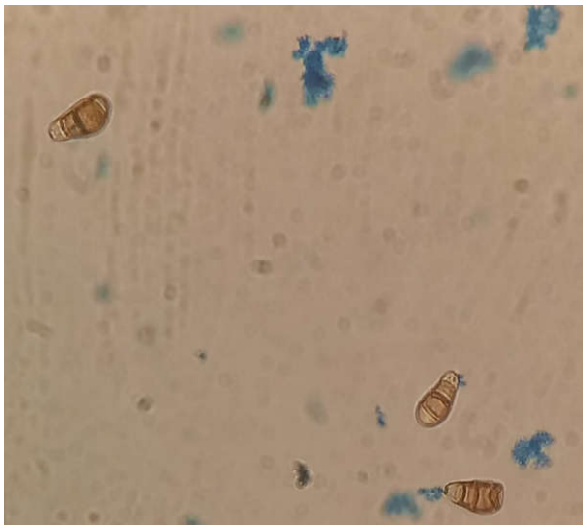

Plate 4b. Microscopic view of *Curvularia sp* The conidia were brown, curved and obclavate

Plate 5a. Macroscopic view of *Penicillium* sp

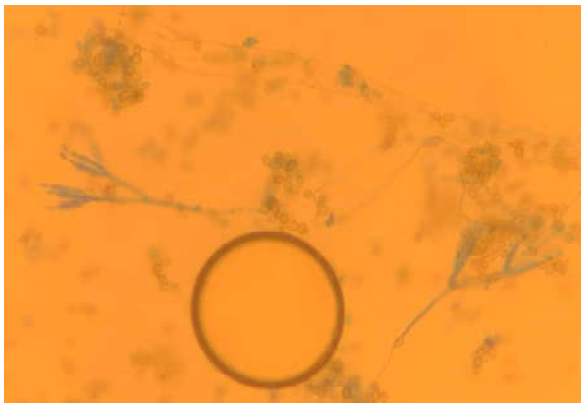

Plate 5b. Microscopic view of *Penicillium* sp The conidiophores produced brush like structures

Plate 6a. Macroscopic view of *Fusarium* sp. The colony colour was pinkish white

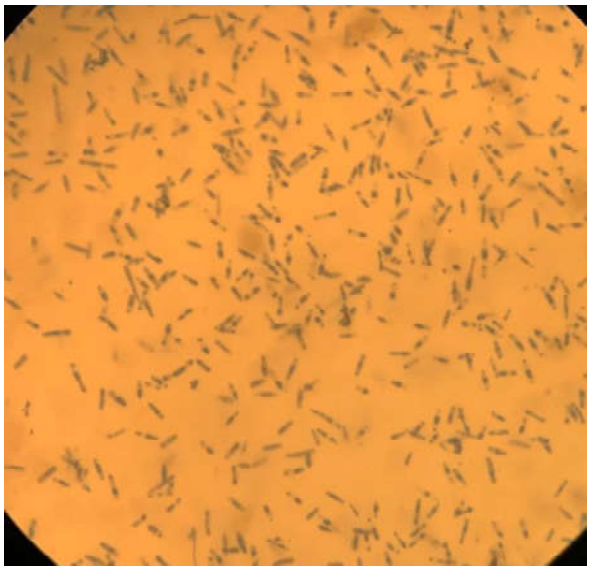

Plate 6b. Microscopic view of *Fusarium* sp.The conidia were cylindrical, single cell, hyaline with smooth round ends

The percentage fungi occurrence of isolates from soil samples is shown in Table 1. The result showed there was a significant frequency of fungi occurrence from the soil samples ($\div^2$ = 17.20, df = 5. P=0.01). *Aspergillus niger* had the highest occurrence 10 (26.32%), followed by *Rhizopus stolonifer* and *Aspergillus fumigatus* each with 7 (18.42%), *Fusarium* sp 6 (15.79), *Penicillium* sp. 5 (13.16%) and the least occurrence was *Curvularia* sp. 3 (7.89%).

Table 1. Percentage fungi occurrence of isolates from soil samples

Fungal Isolate	Number of isolates (%)
Aspergillus niger	10 (26.32)
Aspergillus fumigatus	7 (18.42)
Rhizopus stolonifer	7 (18.42)
Fusarium sp.	6 (15.79)
Penicillium sp.	5 (13.16)
Curvularia sp	4 (10.53)
Total	**38 (100)**

($\div^2$ = 17.20, df = 5. P=0.01)

The disease causing potential of the isolates is shown in Table 2. All the tested organisms caused significant rot on healthy tomato fruits. The rot induced by *Rhizopus stolonifer* was significantly higher (10.52cm^2) on tomato fruits, compared with rot induced by *Aspergillus fumigatus* (7.30cm^2), *Aspergillus niger* (6.87cm^2), *Penicillium* sp. (5.20 cm^2), *Curvularia* sp (5.00cm^2), *Fusarium* sp. (4.21cm^2) and the lowest was recorded in the uninoculated (control) (1.45cm^2).

Table 2. Pathogenicity of fungi isolates from soil samples on healthy Tomato fruits

Fungal Isolate	Area of Rot (cm^2)
Aspergillus niger	6.87
Aspergillus fumigatus	7.30
Rhizopus stolonifer	10.52
Fusarium sp.	4.21
Penicillium sp.	5.20
Curvularia sp	5.00
Control	1.45
FLSD (0.05)	**0.97**

Key; FLSD: Fisher's Least Significance Difference

Discussion

In this study, *Aspergillus niger*, *Aspergillus fumigatus*, *Rhizopus stolonifer*, *Fusarium* sp., *Penicillium* sp. and *Curvularia* sp. were isolated from the rhizosphere of tomato plants in Makurdi. A similar study was carried out by Shinkafi and Gobir (2018) in Sokoto where they isolated *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus oryzae*, *Rhizopus oryzae* and *Rhizopus stolonifer* from rhizosphere of tomato plants. Similar fungi have also been reported from other vegetable plants. Oyeyiola (2008) isolated *R.stolonifer*, *A. niger*, *A. fumigatus* and *Mucor racemosus* from rhizosphere of Okra plants. The differences in the fungi isolated in this study compared to other research findings could be attributed to geographical location, the method used in isolation and source of the soil sample used.

This study revealed that *A.niger* had the highest occurrence compared to other fungi isolated. This is similar to studies by Onaebi *et al*. (2020) and Dawar *et al*. (2014) who reported *A. niger* as the highest occurring fungi from rhizosphere of Okra plants. However, the finding from this study was in contrast to the findings by Shinkafi and Gobir (2018) who reported *A.fumigatus* as the highest occurring fungi from rhizosphere of tomato plants. The variations in occurrence of fungi reported in this study compared with other research findings could

be attributed to geographical location, type of plants and source of soil samples collected. Also the highest occurrence of *Aspergillus niger* compared with other isolated fungi in this study could be as a result of its survival in almost every environment and its ubiquitous nature.

The large number of fungal species isolated from the rhizosphere is not surprising. This may be due to production of substrates by growing roots in the form of root exudates containing amino acids, sugar, organic acid, nucleotide and other substrate necessary for their growth and survival. Hence, their proximity to the roots and subsequent proliferation and multiplication of the mycoflora in the region. Also high rate of microbial decomposition of both organic and sloughed off tissue (plant) is yet another factor which determine their abundance in the rhizosphere compared with the open field soil where such activities are minimal. This is because the fungal flora acts as universal agents of decay, from which new life continually arises and is nourished.

The disease causing potential of the isolated fungi in this study namely; *Aspergillus niger*, *Penicillium* sp., *Curvularia* sp., *Fusarium* sp. *Aspergillus fumigatus* and *Rhizopus stolonifer* showed that all the organisms were pathogenic on healthy tomato fruits causing different levels of decay. This was due to the ability of the fungal pathogens to utilize the nutrients of the tomatoes as a substrate for growth and development. The process of disease development during fungal invasion as facilitated by fungi penetrating tissue. The colonization process involves the ability of the microorganism to establish itself within the produce**.**

Studies by Sajad *et al*. (2017); Liamngee *et al*. (2016) reported that fungi species of *Aspergillus niger*, *Fusarium* spp., *Mucor* spp., *Botryodiplodia theobromae*, *Aspergillus flavus* ,*Penicillium* spp. all caused significant decay when they were inoculated into healthy tomato fruits. Susceptibility of tomato fruits to fungal infection could be largely due to differential chemical composition such as pH (near neutrality) and moisture content which predisposes them to fungal spoilage.

Conclusion

In this study, *Aspergillus niger, Aspergillus fumigatus*, *Rhizopus stolonifer*, *Fusarium* sp., *Penicillium* sp. and *Curvularia* sp. were isolated from the rhizosphere of tomato plants in Makurdi. *Aspergillus niger* had the highest occurrence from soil samples collected from the rhizosphere of tomato plants in Makurdi. The isolated soil borne fungi were pathogenic when inoculated on healthy tomato fruits. *Rhizopus stolonifer* induced the highest rot when inoculated on healthy tomato fruits compared with other fungi and the non-inoculated (control)

Recommendation

Based on the findings from this study, the following recommendation is made:

Further research should be carried out on the effect, identification and interaction of both pathogenic and beneficial rhizospheric fungi on growth and development of plants.

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