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ISOLATION AND IDENTIFICATION OF FUNGI ASSOCIATED WITH SOILS AND DISEASED TOMATO PLANTS ON FARMERS' FIELDS IN BENUE STATE NIGERIA

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ABSTRACT

A study was carried out to isolate and identify fungi associated with infected tomato plants and soils collected from tomato fields in major tomato growing Local Government Areas (LGAs) of Benue State, Nigeria. Fungi isolated from diseased tomato plant tissues and rhizospheric soils using the direct plate and soil dilution techniques respectively were identified through examination of cultural and microscopic features, aided by relevant identification keys and manuals. A total of 46 fungi belonging to 5 divisions and 14 genera were isolated from sampled plant and soil materials. Soil samples gave the highest number of fungal isolates, 31/46(67.39%) compared to plant materials, 15/46(32.61%). Plant and soil samples collected from Buruku LGA yielded the highest number of fungal isolates, 16 followed by Ushongo, 14, Gboko, 10, and lastly Tarka, 06. Fungi belonging to the Division Deuteromycota were the most frequently occurring, 76.09%, followed by Ascomycota, 13.04% and both Oomycota and Basidiomycota with 4.35% respectively. Members of the Zygomycota were the least occurring in the sampled materials with 2.17%. Fungi belonging to the genus *Aspergillus* were the most frequently occurring with 19.57%. The least frequently occurring fungi were those belonging to the genera *Colletotrichum, Scopulariopsis,* and *Mucor,* all having 2.17% frequencies of occurrence respectively. The study revealed the occurrence of fungi in tissues and rhizosphere of the tomato plant, which included several fungi genera of agricultural importance.

Keywords: Fungi, Identification, Infected Soils, Isolation, Tomato Plants

INTRODUCTION

The tomato plant (*Solanum lycopersicum* L.) native to tropical Central and South America (Cobley and Steele, 1976) is currently regarded one of the most important vegetables in the world, ranking second in importance to potatoes in most countries (Rick *et al*., 1990). The global importance of the tomato plant and its products are largely due to its rich nutritional content and medicinal properties (Cox, 2000; Feet, 2001; Bjarnadottir, 2019). Today, total annual world production of tomatoes is estimated at about 130 million tons (Eurofresh, 2016).

Soils contain large numbers of microorganisms – usually between one and ten million per gram of soil – with bacteria and fungi being the most prevalent (Bruehl, 1987; SARE, 2012). A good number of soil inhabiting microbes are able to complete their life cycles in the soil while most plant pathogenic microbes undertake certain aspects of their life cycles in the host plants (Bruehl, 1987). Aerial plant surfaces also provide suitable habitats for epiphytic micro-organisms which are influenced by the nutrients present on the leaf surfaces (Pandey *et al.*, 1993).

Fungi account for over 85% of all plant diseases (Raid, 2011) hence, their presence on plant parts and growth soils bear huge implications on total plant health and productivity. Accounts of effects of microbial populations on growth and productivity of the tomato crop have also been documented by several other authors (Rovira, 1963; Kim *et al.*, 1998; Koike *et al*., 2007; Ncube *et al.*, 2011; Terna *et al.*, 2015; Omolaran *et al.*, 2016).

Benue State, Nigeria is known as 'the food basket of the Nation' chiefly due to the vast scale of the production of important food crops including tomatoes by farmers in the State (Uja, 2013; Ososanya, 2018). Although tomatoes remain among the most cultivated crops in the State, there still exist paucity of knowledge on the microbial populations co-existing and interacting with the tomato crop. Not much has been reported on fungi infecting tomato plants and fields in Benue State since the studies on the fungal pathogens of field grown tomatoes in Benue State by Bem (2009). This study shall provide an update on the current status of the fungal diversity in infected tomato plant tissues and soils in tomato fields in selected farming locations in Benue State.

MATERIALS AND METHODS

Isolation and Identification of Fungi Associated with Diseased Plants

Naturally infected leaves, stems and roots of tomato plants showing various disease symptoms (Plate 1) as well as soil in the infected fields were collected and conveyed in sterile polyethylene bags to the Botany Laboratory of Benue State University, Makurdi, for further studies. Isolation and identification of fungi associated with diseased tomato plants was carried out in accordance with methods reported by Bem (2009) as follows:

Surface Sterilization of Diseased Plant Tissues

Sampled diseased plant tissues were thoroughly washed in flowing tap water and cut into smaller pieces of about $2mm^2$ – $4mm^2$ with the aid of a sterile scalpel to expose infected points. Cut pieces of plant tissues were carefully placed in appropriately labelled beakers into which 5% sodium hypochlorite was added and allowed to stand for a minute to ensure adequate surface sterilization without damaging plant tissues. Pieces of surface sterilized plant tissues were carefully removed from the sterilizing solution with a pair of sterile forceps and thoroughly rinsed in three changes of sterile distilled water.

Isolation of fungi from Stems, Roots and Leaves of Host Plants

Following surface sterilization, stem tissues were blotted dry using sterile Whatman no. 1 filter paper. Thin slices 2mm to 4mm thick of surface sterilized stem (xylem inclusive), root and leaf tissues were plated on solidified sterile Potato Dextrose Agar (PDA) with the aid of sterile forceps, and incubated at 27 $^{\circ}$C for 72 hours. Fungal isolates were sub-cultured unto freshly prepared PDA plates impregnated with 0.01mg of streptomycin sulphate to prevent bacterial growth. Repeated sub-culturing was carried out until pure cultures were obtained and preserved on PDA slants for further identification.

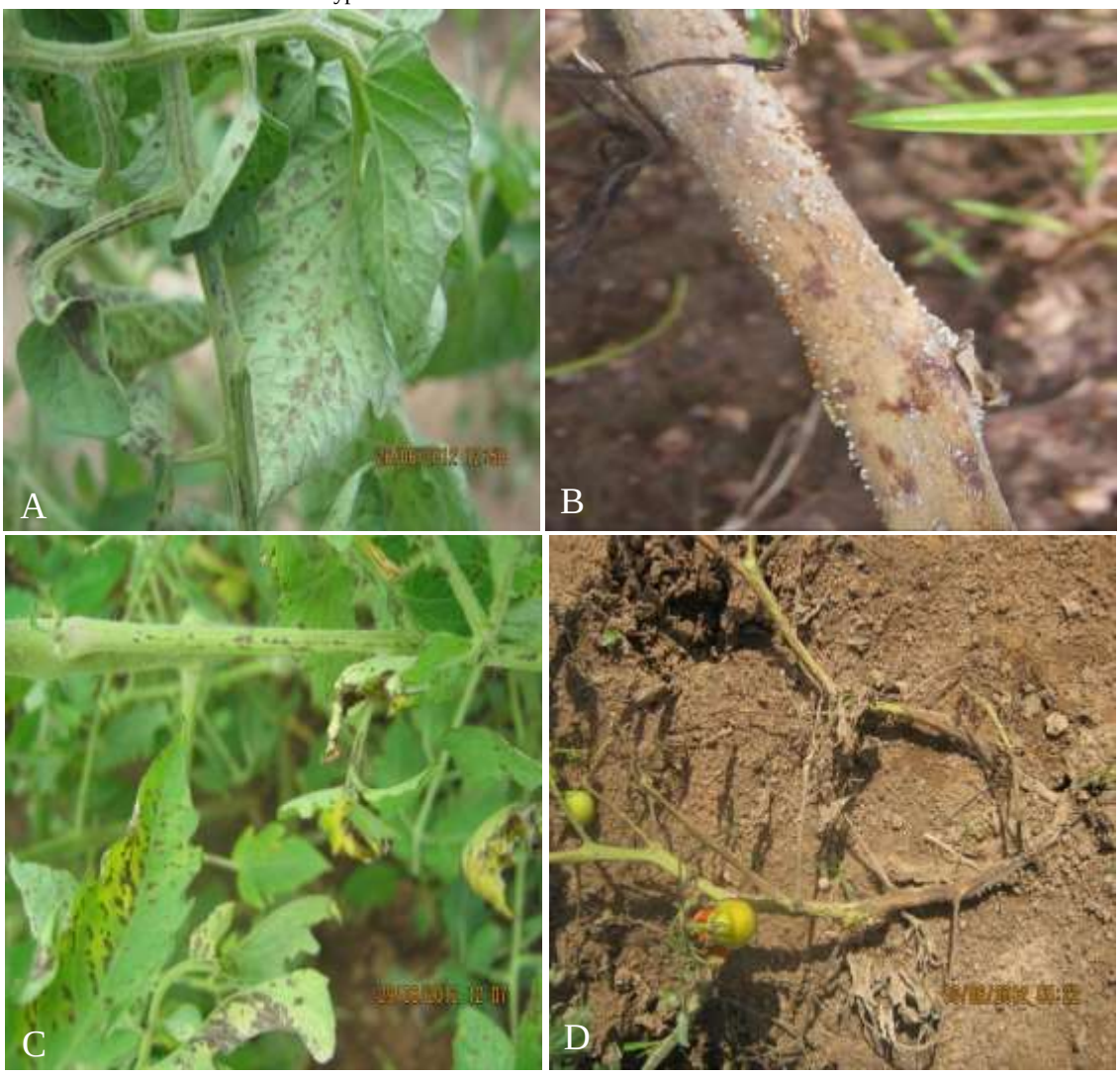

Plate 1. Tomato Plants Showing Disease Symptoms in: (A) Ushongo (Leaf necrosis), (B) Buruku (Presence of fungal mycelia and sclerotia on plant stem), (C) Tarka (Leaf yellowing, stem and leaf necrosis), (D) Gboko (Stem rot and whole plant wilt) (Terna, 2012).

Isolation of Fungi from the Soil.

Soil samples from the top 5cm layer of rhizosphere of infected plants were collected and diluted serially. Concentration 10^{-6} was collected with a sterile pipette and gently mixed by swirling with molten sterile PDA at 45°C (pour plate technique), allowed to set and incubated at 27°C for 72 hours.

Identification of Fungal Isolates

Identification of fungal isolates was based on cultural characteristics and microscopic observation of morphological features aided by taxonomic keys of Samson *et al.* (2010).

Data Analysis

Data obtained from the study were analyzed using simple percentages in the form of percentage frequencies and relative frequencies of abundance and presented in charts and tables.

RESULTS

Fungi belonging to different genera were isolated and identified from tomato tissues and infected field soils collected from the studied area. A total of 14 fungi genera were identified through observation of cultural and microscopic morphology of the isolated fungi. The identified fungi genera were; *Fusarium* spp., *Penicillium* spp., *Aspergillus* spp., *Aureobasidium* spp., *Chrysonilia* spp., *Colletotrichum* spp., *Pythium* spp., *Geotrichum* spp., *Saccharomyces* spp., *Trichoderma* spp., *Scopulariopsis* spp., *Alternaria* spp., *Mucor* spp., and *Sclerotium* spp (Table 1).

Soil samples yielded the highest number of fungal isolates, 31 making up 67.39% compared to tomato plant materials, 15 representing 32.61% of the entire fungal populations obtained in the study (Table 2). Among the isolates collected from plant materials, majority (08) were obtained from leaf samples followed by stem samples (04) and lastly root samples (03), representing 53.30%, 26.67% and 20.00% respectively. Plant and soil samples collected from Buruku LGA yielded the highest number of fungal isolates (16) followed by Ushongo (14), Gboko (10) and lastly Tarka (06).

Members belonging to the Division Deuteromycota were the most occurring with 76.09% frequency of occurence, followed by Ascomycota with 13.04%, and both Oomycota and Basidiomycota with 4.35% respectively. Members of the Zygomycota were the least occurring in the sampled materials having a percentage frequency of occurrence of 2.17 (Fig. 1).

The most represented genus was *Aspergillus* making up 19.57% of the species found followed by *Aureobasidium* constituting 13.04% of the species. The least occurring fungi were those belonging to the genera *Colletotrichum*, *Scopulariopsis*, and *Mucor* all having 2.17% frequencies of occurrence respectively (Fig. 2).

Table 1. Morphological Characteristics of Fungi Isolated from Infected Tomato Plants and Field Soils in Selected Locations in Benue State, Nigeria.

Cultural Characteristics	Conidia	Phialides	Chlamydospores	Identity
Cottony mycelia, slightly raised circular growth, ranging in colour from whitish, pink, yellow, red to shades of purple.	Macro and micro conidia present	mono and poly phialides present	Present	*Fusarium* spp.
Slow-growing mycelia in shades of green on PDA, irregular, and slightly raised towards centre.	Elipsoidal conidia in chains	flask-shaped phialides present, consisting of a cylindrical basal part and a distinct neck	Absent	*Penicillium* spp.
Fast growing circular colonies on PDA, ranging in colour from yellow, yellow-brown, brown to black and shades of green.	Rough walled conidia mostly in long chains and radiate configuration	Mostly flask-shaped phialides present	Absent	*Aspergillus* spp.
Colonies on PDA are flat, smooth, moist, yeast-like, mucoid to pasty, shiny and leathery in appearance. Surface yellow and turns brown to black with time.	Blastoconidia present	Distinct phialides absent	Absent	*Aureobasidium* spp.
Fast growing colonies on PDA, pale pink in colour, flat and circular.	Brightly coloured smooth-walled, ovoidal to ellipsoidal conidia arising in chains from inconspicuous conidiophores	Absent	Absent	*Chrysonilia* spp.

Table 1 (Continued)

Cultural Characteristics	Conidia	Phialides	Chlamydospores	Identity
Circular, woolly or cottony colonies with characteristic pale brown or greyish white colour.	Ovoid to oblong, slightly curved or dumbbell shaped conidia present	Cylindrical phialides present	Absent	*Colletotrichum* spp.
Whitish circular and flat colony growth on PDA.	Globose oospores present	Absent	Absent	*Pythium* spp.
Rapidly growing, white, powdery to cottony colonies on PDA which becomes yeast-like or slimy with age.	Conidia arise in chains from fragmentation of hyphae.	Absent	Absent	*Geotrichum* spp.
Colonies on PDA are flat, smooth, moist, glistening or dull, and cream in colour.	Blastoconidia globose, and ellipsoid to elongate in shape observed	Absent	Absent	*Saccharomyces* spp.
Colonies on PDA are circular, woolly, whitish in colour, forming scattered blue-green or yellow-green patches of conidia with age.	Smooth-walled conidia, ellipsoidal in shape present	Flask-shaped phialides inflated at the base present	Absent	*Trichoderma* spp.

Table 1 (Continued)

Cultural Characteristics	Conidia	Phialides	Chlamydospores	Identity
Colonies are irregular, granular to powdery in texture, whitish but become light brown or buff tan in time.	Rough-walled, globose conidia present	Absent	Absent	*Scopulariopsis* spp.
The colony is flat, circular, woolly and covered with greyish aerial hyphae.	Conidia have both transverse and longitudinal septations	Absent	Absent	*Alternaria* spp.
Colony is fluffy, circular, raised, white and becomes greyish brown in time.	Sporangiospores are hyaline, grey or brownish, globose to ellipsoidal, and smooth-walled	Absent	Present	*Mucor* spp.
Fan-shaped, rapidly growing, silky-white, slightly raised colonies on PDA, producing brownish sclerotia with age.	Absent	Absent	Absent	*Sclerotium* spp.

Table 2. Fungi Associated with Soils and Tomato Plants in Major Tomato Growing LGAs in Benue State Nigeria

S/No.	Isolate Code	Name of Organism	Division	Source	Location
1.	UshSL1	*Penicillium spp.*	Deuteromycota	Soil	Ushongo
2.	UshSL2	*Aspergillus spp.*	Deuteromycota	Soil	Ushongo
3.	UshSL3	*Aspergillus spp.*	Deuteromycota	Soil	Ushongo
4.	UshSL4	*Aureobasidium spp.*	Deuteromycota	Soil	Ushongo
5.	UshSL5	*Aspergillus spp.*	Deuteromycota	Soil	Ushongo
6.	UshSL6	*Aspergillus spp.*	Deuteromycota	Soil	Ushongo
7.	UshSL7	*Penicillium spp.*	Deuteromycota	Soil	Ushongo
8.	UshSL8	*Chrysonilia spp.*	Deuteromycota	Soil	Ushongo
9.	UshSL9	*Fusarium spp.*	Deuteromycota	Soil	Ushongo
10.	UshSL10	*Colletotrichum spp.*	Ascomycota	Soil	Ushongo
11.	UshSL11	*Pythium spp.*	Oomycota	Soil	Ushongo
12.	UshSL12	*Geotrichum spp.*	Deuteromycota	Soil	Ushongo
13.	UshSL13	*Aspergillus spp.*	Deuteromycota	Soil	Ushongo
14.	UshL1	*Saccharomyces spp.*	Ascomycota	Leaves	Ushongo
15.	TkR1	*Trichoderma spp.*	Deuteromycota	Roots	Tarka
16.	TkL1	*Aureobasidium spp.*	Deuteromycota	Leaves	Tarka
17.	TkSL1	*Scopulariopsis spp.*	Deuteromycota	Soil	Tarka
18.	TkL2	*Aspergillus spp.*	Deuteromycota	Leaves	Tarka
19.	TkSL3	*Saccharomyces spp.*	Ascomycota	Soil	Tarka
20.	TkL3	*Saccharomyces spp.*	Ascomycota	Leaves	Tarka
21.	GbkSL1	*Alternaria spp.*	Deuteromycota	Soil	Gboko
22.	GbkST1	*Alternaria spp.*	Deuteromycota	Stem	Gboko
23.	GbkSL2	*Penicillium spp.*	Deuteromycota	Soil	Gboko
24.	GbkSL3	*Alternaria spp.*	Deuteromycota	Soil	Gboko
25.	GbkST2	*Fusarium spp.*	Deuteromycota	Stem	Gboko
26.	GbkR1	*Pythium spp.*	Oomycota	Root	Gboko
27.	GbkSL4	*Aspergillus spp.*	Deuteromycota	Soil	Gboko
28.	GbkSL5	*Aureobasidium spp.*	Deuteromycota	Soil	Gboko
29.	GbkSL6	*Mucor spp.*	Zygomycota	Soil	Gboko
30.	GbkSL7	*Saccharomyces spp*.	Ascomycota	Soil	Gboko
31.	BkbR1	*Trichoderma spp.*	Deuteromycota	Root	Buruku
32.	BkbL1	*Chrysonillia spp.*	Deuteromycota	Leaf	Buruku
33.	BkbL2	*Alternaria spp.*	Deuteromycota	Leaf	Buruku
34.	BkbST1	*Sclerotium spp.*	Basidiomycota	Stem	Buruku
35.	BkbL3	*Aureobasidium spp.*	Deuteromycota	Leaf	Buruku
36.	BkbSL8	*Aureobasidium spp.*	Deuteromycota	Soil	Buruku
37.	BkbSL9	*Fusarium spp.*	Deuteromycota	Soil	Buruku

Table 2 (Continued)

38.	BkbST2	*Trichoderma spp.*	Deuteromycota	Stem	Buruku
39.	BkbSL10	*Geotrichum spp.*	Deuteromycota	Soil	Buruku
40.	BkbSL11	*Aspergillus spp.*	Deuteromycota	Soil	Buruku
41.	BkbL4	*Aspergillus spp.*	Deuteromycota	Leaf	Buruku
42.	BkbSL12	*Aureobasidium spp.*	Deuteromycota	Soil	Buruku
43.	BkbSL13	*Penicillium spp.*	Deuteromycota	Soil	Buruku
44.	BkbSL14	*Sclerotium spp.*	Basidiomycota	Soil	Buruku
45.	BkbSL15	*Saccharomyces spp.*	Ascomycota	Soil	Buruku
46.	BkbSL16	*Alternaria spp.*	Deuteromycota	Soil	Buruku

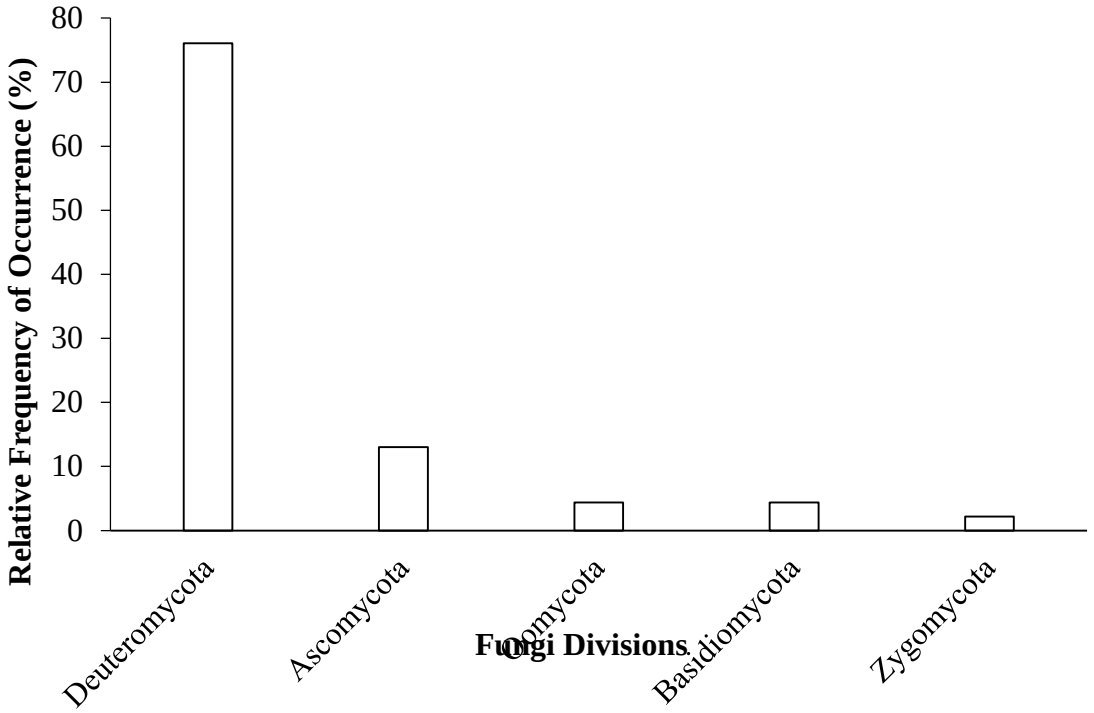

Fig. 1. Relative Frequency of Occurrence of Members of Different Fungi Divisions in Sampled Plant and Soil Materials

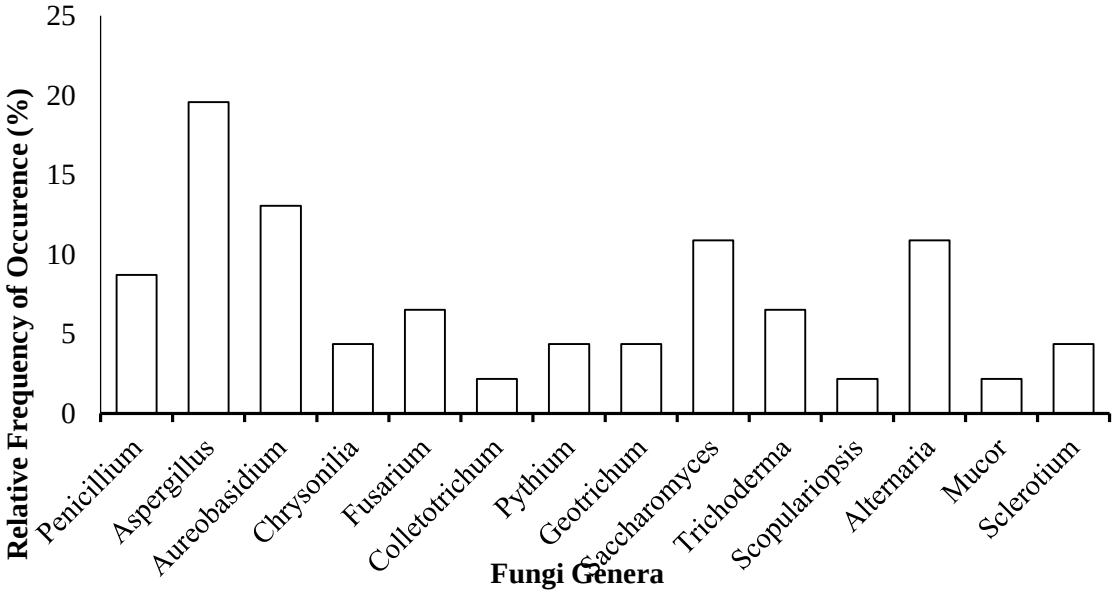

Fig. 2. Relative Frequency of Occurence of Different Fungi Genera on Sampled Soil and

DISCUSSION

The comparatively higher fungal populations and diversity observed in soil samples as opposed to plant materials reported in the study could be linked to the more heterogenous material composition of soil which supports a more diverse population of fungi as opposed to the relatively more homogenous plant tissues. This is supported by the report of Bollen (1959) who stated that soil is a colloidal complex of organic and inorganic materials more or less saturated with water and supported by mainly mineral grains which serves as a preferred culture medium for a number of microbes. Bruehl (1987) also reported that soils contain large numbers of microorganisms – usually between one and ten million per gram of soil – with bacteria and fungi being the most prevalent. Brady and Weil (2002) also mentioned that fungi comprise the greatest fraction of the soil biomass.

Fungi belonging to the Division Deuteromycota were the most frequently isolated from soil and plant materials in the reported study. Similarly, in a study by Swer *et al.* (2011) on fungal population and diversity in organically amended agricultural soils of Meghalaya, India, it was also reported that members of the Deuteromycotina were the most occurring of all fungi isolated. The success of members of the Division Deuteromycota in efficient colonization of soil and plant substrates is due in part to their evolved ability to undertake efficient asexual reproduction which ensures their continual multiplication in the absence of compatible mating structures as is the case with other sexually reproducing fungi. This is supported by the report of Muiz (2012), that a possible mechanism that provides an answer to the question of the success of the Deuteromycota in their environment is the parasexual cycle. This is a process in which plasmogamy, karyogamy and haploidization takes place, but not in any particular place in the thallus nor at any specific period during its lifecycle. Lifeofplant (2011) attributed the success of the Deuteromycota in soil colonization to their ability to produce antibiotic substances which inhibit microbial competition by soil bacteria and other fungi.

The comparatively higher occurrence of fungi belonging to the *Aspergillus* genus observed in the study can be attributed to their ease of adaptability and the presence of a wide range of substrates capable of supporting appreciable growth and reproduction of most member of the genus. DeVries *et al.* (2016) also reported that most *Aspergillus* species are adapted for the degradation of complex plant polymers as well as a diverse range of organic and synthetic substrates. According to Chehri (2013), in addition to growth on carbon sources, many species of *Aspergillus* demonstrate oligotrophy where they are capable of growing in nutrient-depleted environments, or environments with a complete lack of key nutrients.

Buruku had the highest fungal diversity compared to other sampled locations. This could be largely as a result of the high humidity characteristic of Buruku LGA as a result of its proximity to the River Buruku, a prominent river linking to the River Benue. High relative humidity is a requirement for optimum growth and reproduction by most fungi. This is in agreement with works of several researchers (Huber and Gillespie, 1993; Cooke and Whipps, 1993; Harrison *et al.*, 1994; Ibrahim *et al.*, 2011) in which it was stated that high relative humidity and several hours of free surface water are critical for both spore germination, growth and the successful completion of the life cycles of most fungi.

CONCLUSION

The study revealed a comparatively higher fungal population and diversity in soil samples as opposed to plant materials among the different sampled locations. Fungi belonging to the Division Deuteromycota were the most frequently isolated from soil and plant materials. Members of the genus *Aspergillus* had the highest frequency of occurrence in infected plant tissues and field soils compared to other fungi genera. Majority of fungi observed in this study belonged to economically important genera and could play important roles in determining tomato crop health and productivity in the study area. Species identification through molecular characterization and determination of the pathogenicity of the isolated fungi are required for in-depth understanding of the role played by members of the studied fungi genera in the pathogenesis of the tomato crop in Benue State and other tomato growing locations in Nigeria and beyond.

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