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RESEARCH ARTICLE

Effects of Different Carbon and Nitrogen Sources on the Biomass of Molecularly Identified Fungi Associated with Fruit Rot of Tomato

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Abstract: Tomato (*Solanum lycopersicum* L.) fruit is an important component of the diet but pathogens are a threat to its availability. The purpose of this research work was to identify fungi associated with tomato fruit rot and study their biomass in different carbon and nitrogen sources. Four varieties of tomato were employed in this study: two local varieties (Hausa and Yoruba land races) and two improved varieties (Tropimech and Roma VF). Freshly harvested tomato fruits were collected and stored at room temperature until rot sets in. Fungi were isolated from rotted fruits using Potato Dextrose Agar. The isolated fungi were identified using macromorphological and micromorphological features. Internal Transcribed Spacer (ITS) regions of the ribosomal DNA (rDNA) of fungi were amplified and sequenced. Pathogenicity tests and physiological studies were conducted using fructose, sucrose, and starch as carbon sources and calcium nitrate, sodium nitrate, ammonium chloride, and urea as nitrogen sources. The biomass of the isolates was assessed in response to carbon and nitrogen sources. *Aspergillus japonicus, Rhizopus oryzae, Curvularia geniculata, Fusarium proliferatum,* and *Fusarium oxysporum* were isolated from all the varieties. The isolates were differently pathogenic and local tomato varieties were more susceptible to the tested fungi than improved varieties. Comparatively, the fastest mycelial growth was observed in *R. oryzae* (8.30cm) in the PDA medium at Day 7, followed by *A. japonicus* (6.60cm). The two *Fusarium* species grew slower. Biomass of the fungi revealed that fungi showed differential abilities in utilizing different carbon and nitrogen sources.

Key words: *Fungi, Growth, ITS, Molecular, Mycelia, Solanum lycopersicum,*

Introduction

Tomato (*Solanum lycopersicum* L.) belongs to the Solanaceae (nightshade) family whose color can be red, yellow, or green depending on the variety and degree of ripeness (Helyes et al., 2006). Other members of Solanaceae include *Solanum melogena* (eggplant), *S. tuberosum* (Irish potato), *Capsicum annuum* (bell pepper), *C. frutescence* (chili pepper), and *Nicotiana tabacum* (tobacco). It is native to South America, especially Peru and Galapagos Island (Matthew, 2011).

Tomato fruits are highly perishable due to their high moisture content. Poor postharvest handling also accounts for fruit loss (Adeoye et al., 2009). In terms of pathological loss, *Aspergillus niger, Rhizopus nigricans, R. stolonifer,* and *Mucor* spp. have been recognized as the principal rot-causing agents of tomatoes (Matthew, 2011). A common bread-mold fungus (*Rhizopus stolonifer*), grows very aggressively even on refrigerated fruits. On tomatoes, *Rhizopus* rot is characterized by water-soaked and may exude a clear liquid. Also, the sour rot pathogen (*Geotrichum candidum*) turns the flavor of tomato to sour. Anthracnose, caused by *Colletotrichum coccodes, is a* common disease of tomato, occasionally attacking pepper and other solanaceous fruits (Tsitsigiannis et al., 2008).

One of the major challenges especially in developing countries is how to enhance food security and ensure its long-term sustainable development (Kiaya, 2014). Matthew (2011) reported that about 40% of tomato fruits stored in baskets are lost to fruit rot which is a severe financial loss to growers, middlemen, and retailers. Fungi are one of the groups of pathogens usually responsible for the decay of tomato (Etebu et

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al., 2013). Many of these achlorophyllous organisms are apparent and grow on the surface of the infected hosts with different macromorphological features. The extent and severity of damage caused by fungi depend on their nature and type. Fungal identification is difficult and can result in a diagnostic error (Sangoi et al., 2009). In addition, many fungi share similar macromorphological and micromorphological features making their identification a tedious task. The use of morphological approaches in fungal systematics may not be enough for lower-level (species) classification and become an enigma without molecular tools (Sangoi et al., 2009) . It becomes necessary to identify these organisms with a high degree of precision to reduce the risk of not only loss but also contamination of the fruits. The biology and physiology of fungi are dissimilar making their control cumbersome. However, detection, isolation, identification as well as physiological studies of fungal isolates become imperative to proffer appropriate management techniques and control strategies. The objectives of this research work were to use morphological and molecular tools to identify fungi associated with tomato fruit rot and study the mycelial dry weight of isolated fungi in carbon and nitrogen-rich media.

Materials and Methods

Collection of Tomato Fruits

Four (4) varieties of tomato *viz*: two local varieties (Hausa and Yoruba land races) and two improved varieties (Tropimech and Roma VF) were used in this study. Freshly harvested tomato fruits were collected and stored until rot sets in. The rotted fruits of each variety were aseptically collected from the storage structures into labeled sterilized plastic crates and taken to the Pathology Laboratory, Department of Plant Biology, University of Ilorin for pathological examinations.

Preparation of Culture Medium

Potato Dextrose Agar (PDA) was used to isolate fungi. This nutrient medium was commercially obtained in a dehydrated form. In preparing the medium, 39 g of powdery PDA was suspended in 1 L of distilled water. The powder was dissolved by heating with frequent agitation until complete dissolution was observed. This was sterilized in an autoclave at 121 ^{o}C for 15 minutes. After autoclaving, Chloramphenicol (30 mg/L) was added to inhibit bacterial growth (Amadi et al., 2014). The prepared medium was stored at 8-15 ^{o}C until used.

Isolation of Fungi from Rotten Tomato Fruits

The samples of spoilt tomato fruits were surface sterilized with 70% ethanol by swabbing method for two minutes, then rinsed with several changes of sterile distilled water and blotted dry with sterile filter papers. The sterilized fruits were put in a desiccator containing sterile cotton wool moistened with distilled water to create a micro-humidity chamber of 100% relative humidity which was measured by a direct air reading hygrometer. This was done to induce the growth of fungi. Cultures were established in Petri-dishes containing PDA supplemented with chloramphenicol and maintained at 25 ± 2 ^{o}C. After several sub-culturing, pure cultures of each isolate were obtained from emerging mycelial colonies and maintained on PDA slant in McCartney bottles (Al-hindi et al., 2011). The bottles were stored in a refrigerator at 4 ^{o}C.

Morphological Identification of Fungal Isolates from Rotted Tomato Fruits

The isolates were identified based on macroscopic and microscopic characteristics of the pure culture of each fungal isolate. The macroscopic characteristics observed were growth pattern (whether filamentous or colonial), the surface colour of the colony, and the colour of the reverse side of the plate. The nature of the hyphal wall (thick or thin and shape), presence or absence of spores, the colour of the hyphae, presence or absence of septa, type and nature of reproductive apparatus (sporangiophore or conidiophore), shape and colour of the spore were studied microscopically (Fawole and Oso, 2007). The monographs were used for identification (Barnett and Hunter, 2010; Cambell and Stewart, 1980).

Molecular Identification of Fungal Isolates from Rotted Tomato Fruits

The pure culture of each fungal isolate (7 days old) was used for the study. The genomic DNA of each isolate was extracted and purified using a Zymo Research DNA extractor kit (The Epigenetics Company, USA). Polymerase Chain Reaction (PCR) was done in the Central Research Laboratory, University of Ilorin, Ilorin, Nigeria. The Internal Transcribed Spacer (ITS) region of each isolate was amplified using universal primer pairs: ITS1 (5′-TCCGTAGGTGAACCTGCGG3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (Bechem and Afanga, 2018). The PCRs were done in a 25µl reaction volume containing DNA templates. Amplification was carried out on a thermal cycler PCR machine (Multigene) with initial denaturation (94 ^{o}C for 30s), 35 cycles of denaturation (94^{o}C for

30s), annealing (55 ^{o}C for 60s), and extension (72 ^{o}C for 60s), then the final extension was made to be 72 ^{o}C for 5 min.(Bechem & Afanga, 2018). The Sanger sequencing method was used to determine the chains of nucleotides in the DNA templates. The proliferated DNAs were sent for sequencing. This was done in Inqaba Biotec-Africa's Genomics Company, Pretoria, South Africa.

Pathogenicity Test of the Fungal Isolates

The pathogenicity test was carried out using the method of Tafinta et al. (2013). Healthy tomato fruits were surface sterilized with 75% ethanol. Cylindrical plug tissues were cut out from the fruits using a sterilized 2 mm sized cork borer. One-week-old agar disc of fungal culture was aseptically placed in these holes, covered, and sealed using petroleum jelly. The procedure was repeated separately across each of the fungal isolates. The control sample was inoculated with sterile distilled water. The inoculated samples and the control were placed in sterile plastic containers and incubated at 28 ± 3 ^{o}C for 7 days. The diameter of the lesion of the fruits was measured with a calibrated ruler and recorded accordingly. The fungi were later re-isolated from inoculated samples and compared with initial cultures.

Growth Rate and Performance of Isolated Fungi in Different Carbon and Nitrogen Sources

The growth rate of each fungal isolate was studied following the method of Ibrahim et al., (2011). From the 7-day-old pure culture of each fungal isolate, 4 mm agar discs were taken and aseptically placed in the center of sterile Petri dishes containing PDA medium. The inoculated plates were incubated at room temperature. Three replicates were done for each isolate. The linear growth of each isolate was measured using a calibrated ruler and the mean values were recorded.

The mycelial dry weight of each fungal isolate in carbon-rich and nitrogen-rich media was determined (Suleiman & Akaajime, 2010). The basal medium consisted of: 1.0g, KCl; 0.5g, Mg SO_4 $7H_2O$; 3.0 g, Ca $(NO_3)_2$; 1.0 g, K_2HPO_4; and 0.01 g, $FeSO_4$ $7H_2O$. The carbon sources were glucose, starch, and fructose while the nitrogen sources were calcium nitrate, sodium nitrate, ammonium chloride, and urea. Then, 10 g of each carbon source and nitrogen source was dissolved into 100 ml of sterile distilled water in sterile conical flasks. Ten milliliters of each solution were separately added to a 20 ml sterile basal medium in the flasks. Each flask was inoculated with 5 mm diameter disc of 7-day-old fungal culture growing on PDA such that the mycelial mats were uppermost and floated on the medium and incubated for 14 days. The mycelia were harvested by filtration method. The residues (mycelia) were oven dried and then weighed using a weighing balance (JA3003 Electronic Balance). Three replicate flasks were used for each carbon and nitrogen source.

Data Analysis

Data were analyzed using Statistical Package for Social Science (SPSS) software, version 16.00. One-way Analysis of Variance (ANOVA) was used to determine the differences within the variety. The level of significance used in the F ratio was $p < 0.05$. When the F ratio was significant, means were separated using Duncan's Multiple Range Test (DMRT). The consensus DNA sequence for individual isolates was generated using Seqtrace software (Seqtrace-win-0.9.0). The ITS nucleotide sequences for each fungal isolate were compared with those in the open-access database of the National Centre for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool for Nucleotides (BLASTN) Sequences.

Results and Discussion

Isolation and identification of Isolates

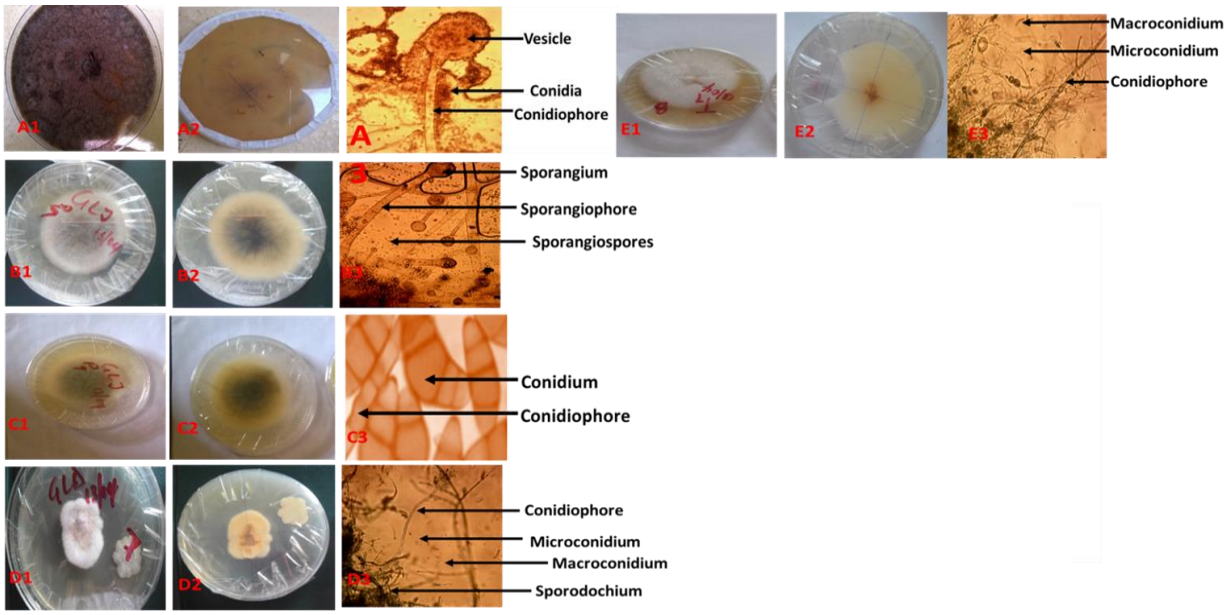
Five fungi were isolated in total and they were identified as *Aspergillus japonicus, Rhizopus oryzae, Curvularia geniculata, Fusarium proliferatum*, and *F. oxysporum.* All the varieties yielded these fungi. The morphological descriptions including morphocultural and microscopic features of each isolate are given in Table 1 and Fig. 1. The colonies of *A. japonicus* were first white and then changed to black with conspicuous conidiophores and conidia. *R. oryzae* appeared blackish-grey and sporangiophores, sporangia, and sporangiospores were visible under a light microscope. Black-brown colour characterized the colonies of *C. geniculata* and the conidia had septations. *F. oxysporum* and *F. proliferatum* were cotton-like in the PDA culture medium but later appeared with a tinge of pink.

The phylogenetic tree of isolate 1 showed a complete alignment with species of *A. japonicus* and was completely separated from *A. fumigatus, A. oryzae*, and *A. flavus* (Fig. 2). Isolate 2 clustered with other *R. oryzae* (synonym of *R. delemar*) species generated from NCBI (Fig. 3). *C. geniculata* (isolate 3) had 98% identity with reference species from NCBI with 0% gap and the phylogeny tree confirmed the identity of the fungus (Fig. 4). *Cochilobolus geniculatus* is the telemorph of *C. geniculata.* Molecular analysis corroborated the

morphological identity of isolate 4 to be *F. proliferatum* with 98% identity and a gap of 1%. The isolate showed a great affinity with *F. proliferatum* and was clearly segregated from *F. sporotrichioides, F. solani* and *F. oxysporum* (Fig. 5). Isolate 5 was clustered with *F. chlamydosporium, F. oxysporum, F. equiseti* and *F. lateritum*, and this suggested that the isolate might be one of those aforementioned *Fusarium* species (Fig. 6) but morphological identification indicated that the isolate was *F. oxysporum. Aspergillus niger, R. nigricans, R. stolonifer, Candida yeasts, Penicillium* spp., and *Mucor* spp. are responsible for the postharvest loss of most of the fruits during storage (Matthew, 2011). The isolates in this study agreed with the findings, which revealed that seven fungal species including *Aspergillus*, *Rhizopus*, and *Fusarium* were identified in tomato fruits (Kalyoncu et al., 2005). In general, the fungi that cause fruit rot are toxigenic and may cause infections (Monsa, 2004). *Aspergillus* spp. and *Fusarium* spp. are potential sources of harmful secondary metabolites called mycotoxins (Frisvad & Thrane, 2002; Akinmusire, 2011). *Aspergillus* spp. is known to produce these metabolites such as Ochratoxins which pose a health threat to both man and livestock. Ochratoxins affect the normal functions of the kidney in humans and animals (Pfohl-Leszkowicz & Manderville, 2012). *R. oryzae* is naturally ubiquitous and found in association with decaying organic substances (Meussen et al., 2012). It is pathogenic to plants and its pathogenicity is attributed to its ability in degrading enzymes such as pectinases, cellulases, and hemicellulases (Ghosh & Ray, 2011).

Table 1: Morphological description of fungal isolates

Isolate	Description	Identity
Isolate 1	The colonies grown on PDA were white first, then turned black with the formation of visible and distinct conidiophores and conidia The hyaline septate hyphae were conspicuous. The conidiophores were long and globose at a terminal end (Plate A).	*Aspergillus japonicus*
Isolate 2	The colonies of the isolates grown on PDA were cottony white at first, then turned brownish-grey to blackish-grey and grew aggressively. Both sporangia and sporangiophores were visible under the light microscope. The columella were globose. The sporangiospores were also visible but dispersed (Plate B).	*Rhizopus oryzae*
Isolate 3	The colony was black-brown velvety with black reverse. The conidia were bent at a sharp angle (geniculate) and assumed a boat shape. Conidia had three septations. Central cells were larger and darker than the end cells They were straw-coloured to dark brown. Condiogenous cells were polytretic and sympodial (Plate C).	*Curvularia geniculata,*
Isolate 4	The mycelium was extensive and cotton-like in culture with tinge of pink on PDA culture medium. The conidiophores were short, slender and branched irregularly. Conidia (phialospores) were hyaline. Macroconidia were several-celled, bent at the pointed ends and assume canoe-shaped. Microconidia were single-celled, oblong and borne singly (Plate D).	*Fusarium proliferatum*
Isolate 5	Sporodochium was present bearing conidiophores on its surface. The aerial mycelium appeared white and loosely floccose. The conidiophores are branched. The conidia are septate and gradually tapered toward both ends (Plate E).	*Fusarium oxysporum*



A1-A3 = The front view, reverse view, and microphotograph of *Aspergillus japonicus*
B1-B3 = The front view, reverse view, and microphotograph of *Rhizopus oryzae*
C1-C3 = The front view, reverse view, and microphotograph of *Curvularia geniculate*
D1-D3 = The front view, reverse view, and microphotograph of *Fusarium proliferatum*
E1-E3 = The front view, reverse view, and microphotograph of *Fusarium oxysporum*

Figure. 1 Morphocultural and microscopic identification of fungi isolated from rotted tomato fruits.

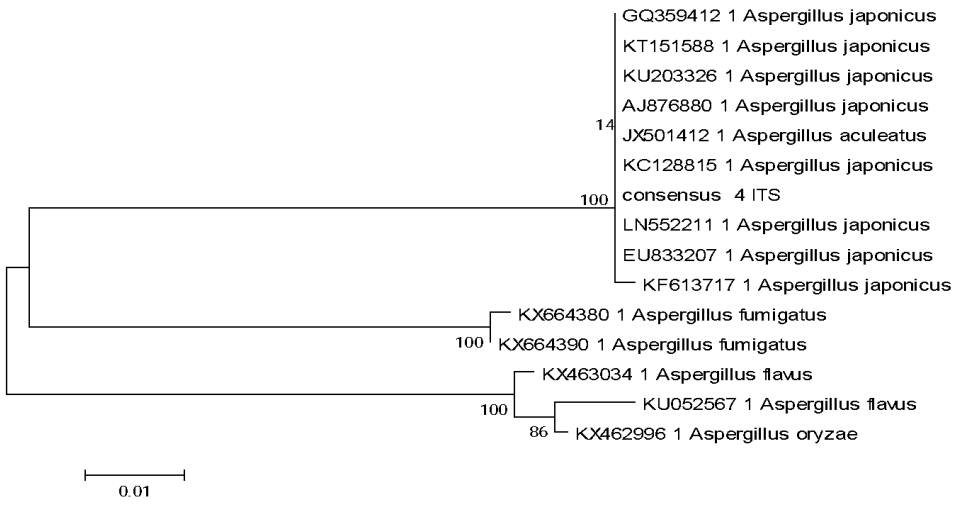


Figure. 2 Molecular Phylogenetic analysis of *Aspergillus japonicus* by Maximum Likelihood

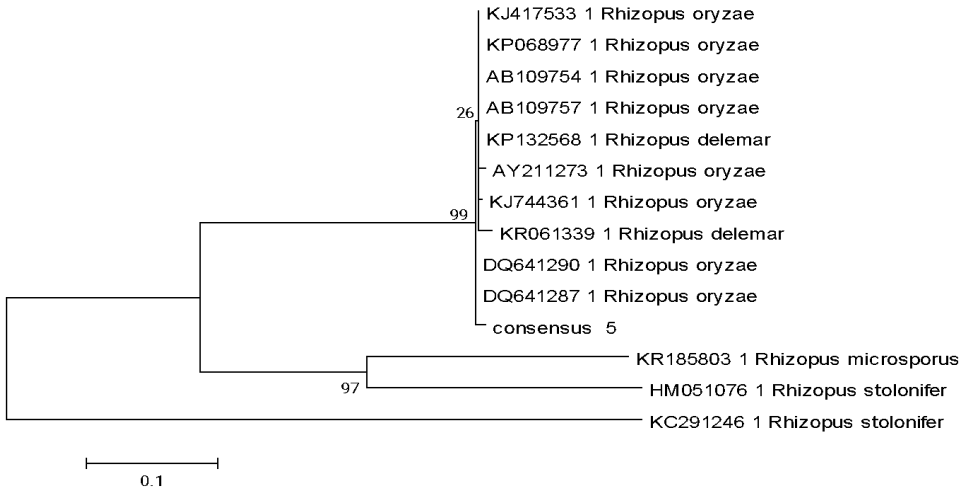


Figure. 3 Molecular Phylogenetic analysis of *Rhizopus oryzae* by Maximum Likelihood method

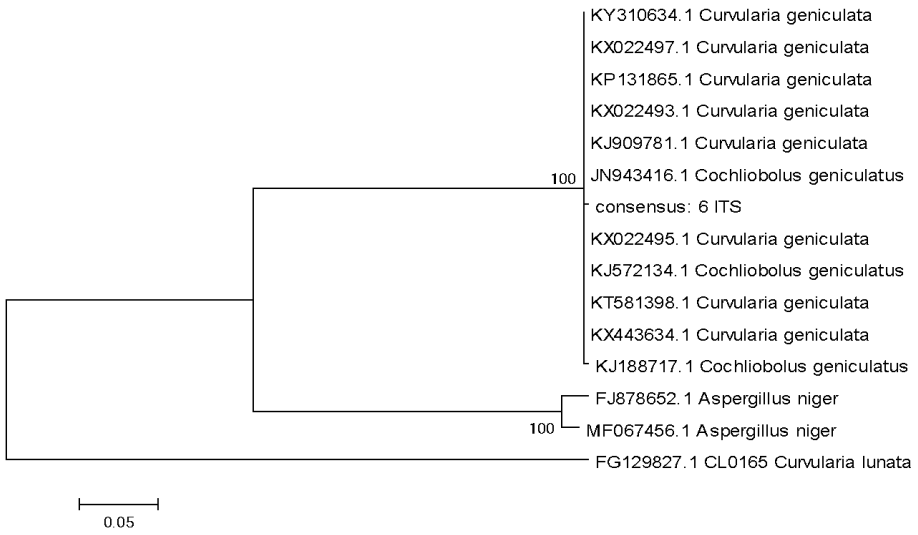


Figure. 4 Molecular Phylogenetic analysis *of Curvularia geniculata* by Maximum Likelihood

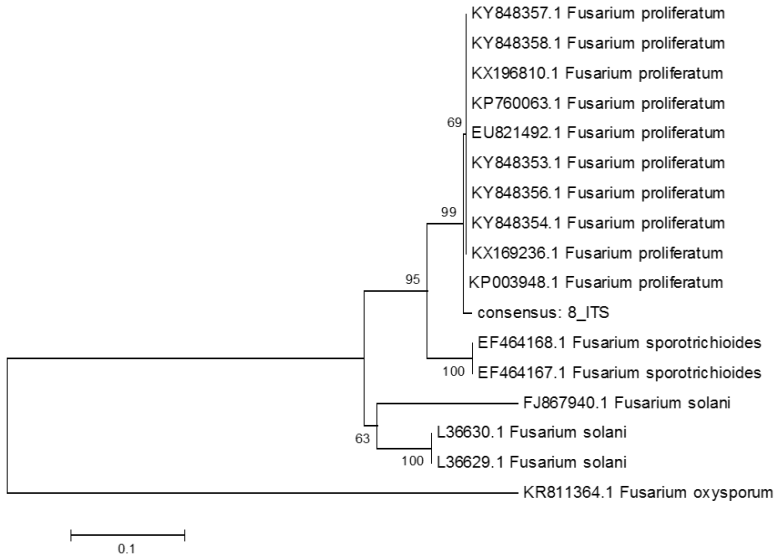


Figure. 5 Molecular Phylogenetic analysis of *Fusarium proliferatum* by Maximum Likelihood

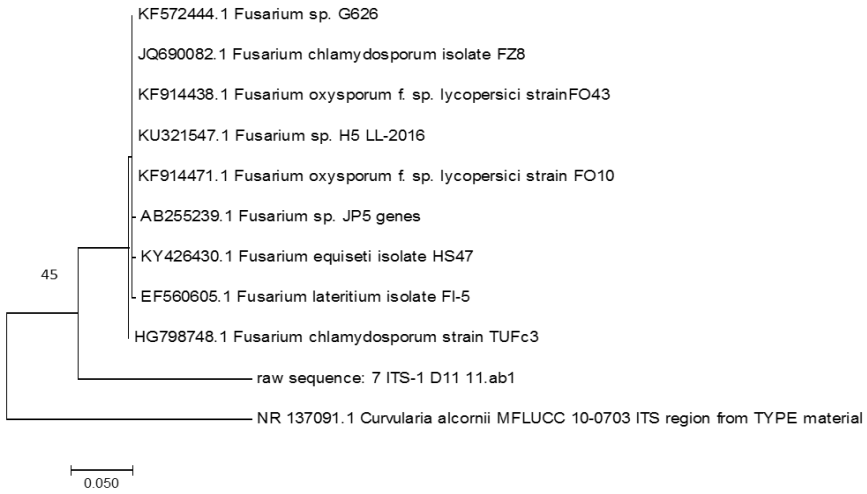


Figure. 6 Molecular Phylogenetic analysis of *Fusarium oxysporum* by Maximum Likelihood method

Pathogenicity Test of Fungal Isolates from Tomato Fruits

All the fungal isolates (*Aspergillus japonicus*, *Rhizopus oryzae*, *Curvularia geniculata*, *Fusarium proliferarum* and *Fusarium oxysporum*) were capable of causing lesions on healthy tomato fruits with varying degrees of severity. The two species of *Fusarium* displayed the highest pathogenicity (Table 2). *F. oxysporum* was identified as the most virulent species of *Fusarium* in the tomato field affecting both the vegetative phase and fruits (Steinkellner et al., 2005). Pathogenic species of *Fusarium* were also known to produce pectinase, cellulase, and α-amylase that disintegrate the cell wall component (Di Pietro et al., 2003). *R. oryzae* induced soft rot in apple and banana fruits (Kwon et al., 2011, 2012) and this supported the findings in this present study that the fungus is pathogenic. The essential pathogenicity factors of *A. japonicus* are attributed to xylases and polygalacturonases (Di Pietro et al., 2003). Although *A. japonicus* is pathogenic, the conidia of the fungus are useful in adsorption of a mycotoxin known as zearalenone as a way of decontaminating animal fodders (Jard et al., 2009). *C. geniculata* is pathogenic to not only plants but also mammals by invading mammalian tissues such as the liver and kidney (Vishnoi et al., 2005).

T**able 2: The rot diameter of fungal isolates in tomato fruits after 7 days of inoculation**

	Diameter of rot (cm)			
Fungi	**Hausa Variety**	**Yoruba Variety**	**Tropimech Variety**	**Roma VF Variety**
Aspergillus japonicus	3.23 ± 0.03^{b}	3.57 ± 0.03^{c}	2.27 ± 0.12^{c}	2.03 ± 0.03^{c}
Rhizopus oryzae	2.29 ± 0.04^{c}	2.32 ± 0.00^{d}	1.78 ± 0.04^{d}	2.01 ± 0.58^{c}
Curvularia geniculate	0.88 ± 0.02^{d}	1.16 ± 0.02^{e}	0.87 ± 0.00^{e}	0.84 ± 0.04^{d}
Fusarium proliferatum	3.22 ± 0.00^{b}	3.66 ± 0.01^{b}	3.15 ± 0.02^{b}	2.90 ± 0.04^{b}
Fusarium oxysporum	4.20 ± 0.05^{a}	3.88 ± 0.04^{a}	3.47 ± 0.04^{a}	3.10 ± 0.00^{a}

Means followed by the same letter(s) along the same column are not significantly different at $p < 0.05$

Table 3: Mycelial dry weight (g) of isolated fungi on different carbon sources

	Aspergillus japonicus	*Rhizopus oryzae*	*Curvularia geniculate*	*Fusarium proliferatum*	*Fusarium oxysporum*
Fructose	2.80 ± 0.06^{a}	0.85 ± 0.33^{c}	1.06 ± 0.13^{b}	0.71 ± 0.07^{b}	0.99 ± 0.07^{c}
Sucrose	0.88 ± 0.05^{c}	2.46 ± 0.02^{a}	1.71 ± 0.09^{a}	1.97 ± 0.35^{a}	1.38 ± 0.10^{b}
Starch	1.57 ± 0.12^{b}	1.97 ± 0.04^{b}	1.97 ± 0.19^{a}	2.24 ± 0.28^{a}	1.76 ± 0.10^{a}
Control	0.79 ± 0.08^{c}	0.61 ± 0.02^{d}	0.96 ± 0.15^{b}	0.90 ± 0.10^{b}	0.67 ± 0.06^{d}

Means followed by the same letter along the same column are not significantly different at $p < 0.05$

Table 4: Mycelial dry weight (g) of isolated fungi on different nitrogen sources

	Aspergillus japonicus	*Rhizopus oryzae*	*Curvularia geniculate*	*Fusarium proliferatum*	*Fusarium oxysporum*
Calcium Nitrate	0.76 ± 0.050^{cd}	1.62 ± 0.04^{c}	1.13 ± 0.13^{ab}	1.08 ± 0.01^{d}	0.97 ± 0.01^{b}
Sodium Nitrate	0.94 ± 0.05^{c}	2.94 ± 0.04^{a}	1.78 ± 0.22^{a}	1.51 ± 0.03^{c}	1.17 ± 0.14^{ab}
Ammonium Chloride	1.63 ± 0.05^{b}	1.88 ± 0.09^{b}	1.33 ± 0.10^{ab}	1.89 ± 0.11^{b}	1.82 ± 0.37^{a}
Urea	2.10 ± 0.08^{a}	0.86 ± 0.04^{d}	1.50 ± 0.32^{a}	2.40 ± 0.11^{a}	1.03 ± 0.20^{b}
Control	0.64 ± 0.03^{d}	0.42 ± 0.03^{e}	0.73 ± 0.18^{b}	0.46 ± 0.03^{e}	0.61 ± 0.15^{b}

Means followed by the same letter(s) along the same column are not significantly different at $p \geq 0.05$

Growth Rate and Responses of Isolated Fungi to Different Carbon and Nitrogen Sources

The mycelia of *R. oryzae* (8.30 cm) grew faster than those of other fungi in PDA medium on Day 7 and this was followed by the growth of *A. japonicus* (6.60 cm). The mycelial growth of the two *Fusarium* species was very slow compared to the other three isolates. On Day 7, the colony diameters of *F. proliferatum* and *F. oxysporum* were 3.75 cm and 4.78 cm respectively with a significant difference at $p \geq 0.05$ (Fig. 7).

Among all the carbon sources used in the present study, *A. japonicus* grew best in fructose with a mycelial dry weight of 2.80 g and this was significantly higher than those recorded in the other two carbon sources and the control (Table 3). Fructose enhanced the mycelial growth of *A. japonicus* and this result was corroborated by the previous finding (Suleiman, 2010). Bolla et al. (2010) stated that fructose is an effective carbon source for fungi to produce mycelial biomass and exopolysaccharides. The highest mycelial dry weight for *R. oryzae* (2.46 g) was observed in sucrose. Both sucrose and starch enhanced the weight of mycelia of *C. geniculata* as well as *F. proliferatum*, and no significant difference was observed between the two carbon sources. *F. oxysporum* had the highest mycelial dry weight of 1.76g in starch and the lowest (0.99 g) in fructose carbon media (Table 3) *F. proliferatum* grew best in starch medium and Zhou & Yang (2010) had earlier reported that the mycelial growth of a fungal colony of *F. acuminatum* was the best with soluble starch.

On the utilization of nitrogen, *A. japonicus* had 2.10 g of mycelial dry weight in urea which was significantly higher than the other three sources (Table 4). *R. oryzae* and *C. geniculata* utilized sodium nitrate better than other nitrogen sources and had mycelial dry weights of 2.94 g and 1.78 g respectively. Calcium nitrate appeared to be a poor source of nitrogen that the mycelia dry weight of *F. proliferatum* but strived best in urea. Also, 1.82 g of the mycelial dry weight of *F. oxysporum* was recorded in ammonium chloride. No significant difference in the mycelial dry weight of *F. oxysporum* in calcium nitrate, sodium nitrate, and urea media (Table 4). Nitrogen is useful in protein synthesis and other important functions. All fungal isolates were able to grow in the basal media supplemented with different nitrogen sources. Fungi are known to absorb inorganic forms of nitrogen and this may be due to anthropogenic sources (Itoo & Reshi, 2014). Urea and ammonium chloride favored the biomass production of *A. japonicus*. *Aspergillus* spp. are

capable of a wide range of nitrogen sources such as ammonium, nitrate, histidine, elastin, and collagen (Krappmann & Braus, 2005). *R. oryzae* had the lowest ability to utilize urea as the sole nitrogen source and this may be due to the absence of a specialized transport system or a urease enzyme (Ewase et al., 2007). Itoo and Reshi (2014) confirmed that ammonium is the best nitrogen source for the biomass production of fungi. Contrarily, the mycelial dry weight of *C. geniculata* in calcium nitrate and ammonium chloride was lower than that of other nitrogen sources and this agreed with the result of Pedria et al. (2015) who reported that ammonium sulfate and potassium nitrate did not support the growth of certain fungi. Nitrogen is one of the limiting factors that determine the growth of microbes (Farjalla et al., 2006). *F. proliferatum* and *F. oxysporum* utilized all nitrogen sources to a varied extent. According to Islam (2015), sodium nitrate and ammonium nitrate media supported the growth and sporulation of *Fusarium* spp. This result conformed to the observation that nitrates favored mycelial growth and varied among *Fusarium* species. (Khailare & Ahmed, 2012).

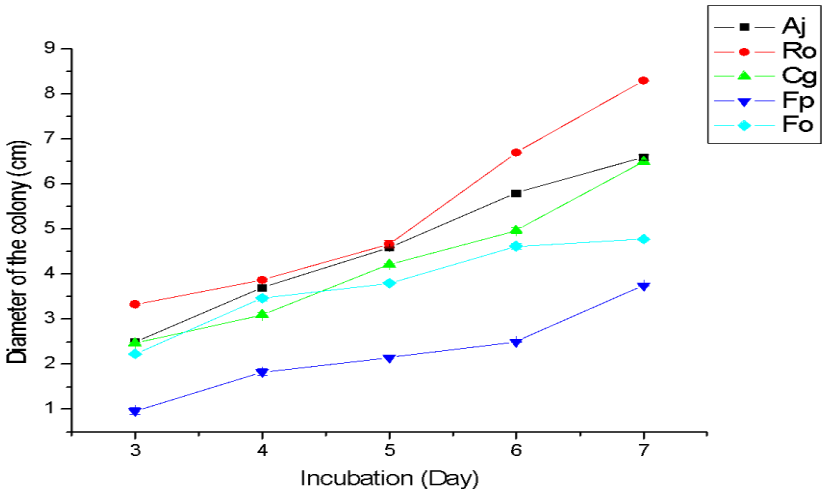


Figure. 7 Mycelial growth of fungal isolates on PDA medium

Aj = *Aspergillus japonicus* Ro = *Rhizopus oryzae* Cg = *Curvularia geniculata*
Fp = *Fusarium proliferatum* Fo = *Fusarium oxysporum*

Conclusion

Fungal spoilage of tomato fruits is common during postharvest activities. *Aspergillus japonicus, Rhizopus oryzae, Curvularia geniculata, Fusarium proliferarum,* and *Fusarium oxysporum* are associated with different forms of rot in the fruit during storage with varying degrees of pathogenicity. All the fungal isolates showed irregular patterns of responses to all carbon and nitrogen-based media. While the best carbon source was starch for the biomass production of *C. geniculata, F. oxysporum,* and *F. proliferatum,* fructose and sucrose were the best for *A. japonicus* and *R. oryzae* respectively. All nitrogen sources significantly supported the mycelial growth of all fungal isolates compared to the control. Further research on the physiological requirements of these fungi is encouraged to untangle intricacies in their studies.

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