Original Research Article

Isolation and Identification of Fungi Associated with Fruit Rot Disease of Tomato (*Solanum lycopersicum* L.*)* in the Southern Guinea Savannah, Nigeria

Abstract

Rot disease is a major threat to tomato fruit production and postharvest handling in major tomato growing areas in Nigeria. Rotted tomato fruits were randomly collected from farmers' farms for the purpose of isolation and identification of rot causing fungi in Tarka, Benue State, Nigeria between July and December, in 2015 and 2016. Results revealed that *Aspergillus flavus*, *A. niger*, *Alternaria solani*, *Phytophthora infestans*, *Oidium neolycopersici*, *Sclerotium rolfsii*, *Fusarium oxysporum*, *Verticillium* spp and *Colletotrichum* spp caused tomato fruit rot in the area. In both years, the lowest number of fungi was recorded in the months of July and December while the highest number was obtained in September. The mean number of fungi isolated showed that *S. rolfsii* was the least with 3.17 in 2015 and 1.66 in 2016 while *F. oxysporum* was the highest with 14.33 in 2015 and 9.33 in 2016. There were more fungi isolated in 2015 than in 2016. There were significant differences *($P \leq 0.05$)* in the mean number of *A. niger*, *P. infestans*, *O. neolycopersici* and *F. oxysporum* isolated in both years. In conclusion, farmers should handle tomato fruits with care, to reduce postharvest injury and damage in order to ensure the availability of disease free fruits and to increase the output for the global population.

Keywords: Disease; *Fusarium oxysporum*; isolation; rot; tomato

Introduction

Tomato (*Lycopersicon esculentum* L.) is an annual fruit vegetable crop in the family Solanaceae (Wani, 2011). The crop is cultivated as vegetable in the world; in Africa, Nigeria is the second

largest producer after Egypt (FAO, 2020). The fruit is eaten raw, cooked ripe or unripe, and put in several dishes, sauces and drinks; it is also dried and ground into different products for consumption (Onuorah, *et al.*, 2015).

Tomato fruit provides minerals, vitamins A and C, proteins, carbohydrates, fats, dietary fibre and potassium (Wogu and Ofuase, 2014); and it is widely consumed in Nigeria. It is the second most valuable vegetable fruit crop in the Nigerian Savanna, and its average consumption is about 18% daily (Yusuf *et al.*, 2020). It is a highly perishable crop and losses account for as high as about 50% between the points of production and consumption (Mbuk *et al*., 2011). The fruit is highly perishable due to high moisture content and susceptibility to plant pathogens both in the field and during postharvest handling. The post harvest losses in fresh tomato account for about 25.80% and these losses vary from time to time, season to season and even from one Region to another depending on the interaction of a susceptible host, a virulent pathogen and favourable environmental conditions (Mujib *et al.*, 2007; Sajad *et al.*, 2017). In all parts of the world, bacteria and fungi have been found to be the most frequently occurring rot causing pathogens of tomato fruits (Obetta *et al*., 2011). The most commonly isolated and identified fruit rot fungi pathogens are *Fusarium oxysporum*, *Alternaria solani* and *Aspergillus niger* (Nowicki *et al*., 2012; Sani and Gwa, 2018; Abdulkadir *et al.*, 2023). Other pathogens equally responsible for fruit rot disease in tomato include the following fungi, *A. flavus*, *Alternaria alternata*, *Botrytis cinerea*, *Curvularia spp*, *Fusarium moniliforme*, *Geotrichum* spp, *Mucor spp*, *Penicillium spp*, *Phytophthora* spp and *Rhizopus stolonifer*; and bacteria such as *Erwinia* spp (Onuorah and Orji, 2015; Yusuf *et al.*, 2020; Nizamani *et al*., 2021). The aim of this study was to isolate and identify the fungi which cause field rot disease of tomato fruits in Tarka Local Government Area, Benue State, Nigeria and also to determine the susceptibility of the fruits to the isolated pathogens.

Material and Methods

Study Area

The isolation and identification of pathogens which cause fruit rot disease of tomato were carried out at the Plant Pathology Laboratory, Department of Crop and Environmental Protection, Federal University of Agriculture, Makurdi, Benue State in 2015 and 2016.

Collection of Infected Tomato Samples

Infected fruits with different degrees of rot symptoms were randomly collected from tomato farms at Tarka Local Government Area and packaged as reported by Sani and Gwa (2018).

Sterilization of Samples

Glass wares were washed in running tap water and sterilized in an oven at 120^{o}C for 30 minutes. Infected samples were washed with tap water, cut with a sharp sterilized blade into small pieces approximately 2×2 mm^{2} in diameter at the interphase of healthy and infected tissues as reported by Lum and Takor (2021). Samples were further sterilized in 5% Sodium hypochlorite solution for about 20 seconds (Sani and Gwa, 2018). The small pieces were rinsed in four successive changes of sterile water in order to remove the chlorox chemical on them and were blotted on sterile paper for about 10 minutes before inoculation of the tissues.

Inoculation of Plant Tissues

Sterile Potato Dextrose Agar (PDA) (20 ml) was poured in 90 mm Petri dishes and left to solidify before inoculation. The medium was amended with 0.16 g of powdered streptomycin sulphate to suppress bacteria growth. Four pieces of the infected tissues were aseptically transferred to the Petri dishes containing the solidified PDA and incubated at 30±5^{o} C for five days. Plates were regularly monitored and growth colonies were examined to determine the frequency of occurrence of each of the pathogens identified.

Determination of Frequency

The frequency of occurrence of the isolated pathogens was calculated by counting the number of times each isolate occurred out of the total number of isolates. Colonies that grew on the plates were examined and aseptically sub-cultured in order to obtain pure cultures of the various isolates.

Identification of Pathogens

Identification of the pure cultures was done by preparing slides of fungal isolates from the different pure cultures and examining them with the aid of a compound microscope. Morphological and cultural characteristics of the isolated fungi were compared with identification guides to establish the identity of the isolated fungi (Agrios, 2005).

Pathogenicity Test of Isolated Fungi

Pathogenicity tests were carried out using the method of Sani and Gwa (2018) to ascertain the ability of the various isolates to cause rotting on apparently healthy looking tomato fruits free of disease symptoms and physical injuries. The fruits were washed in three successive changes of running tap water and 5% sodium hypochlorite solution was used to sterilize them for about 2 minutes to remove surface contaminants. Cylindrical discs about 5 mm in diameter were made on the ripe and healthy looking tomato fruits using a sterilized cork borer. Mycelial discs (4 mm in diameter) from five days old cultures of *Aspergillus flavus*, *A. niger*, *Alternaria solani*, *Phytophthora infestans*, *Oidium neolycopesici*, *Sclerotium rolfsii*, *Fusarium oxysporum*, *Verticillium* spp and *Colletotrichum* spp were aseptically obtained and inserted separately into the holes created. The holes were covered with Petroleum jelly to prevent contamination by other microbes. Tomato fruits treated with sterile PDA instead of the inocula of the various isolates served as the control. The tested isolates and the control were replicated three times. A total of 33

ripe healthy tomato fruits were used for the pathogenicity test. The treatments were completely randomized and the inoculated tomato fruits were incubated at ambient room temperature ($30\pm5^{o}C$) under sterile conditions for 5 days for growth of the fungi. Symptoms of rots obtained from fruits artificially inoculated with the fungal isolates were compared with those already observed when infected on the field. The artificially infected fruits were re-isolated aseptically, and cultured on PDA plates; proper morphological and cultural examination and comparisons were made with those infected on the field (Gwa and Richard, 2018).

Statistical Analysis

Data collected were analyzed by one-way analysis of variance (ANOVA) according to Gomez and Gomez (1984). The two-tailed paired Student's t-test was used for comparing the mean frequency of occurrence of fungal isolates for the two years at 5% level of probability.

Results

In this study, a total of nine different fungi namely *Aspergillus flavus*, *A. niger*, *Alternaria solani*, *Phytophthora infestans*, *Oidium neolycopersici*, *Sclerotium rolfsii*, *Fusarium oxysporum*, *Verticillium* spp and *Colletotrichum* spp were isolated as pathogens causing tomato fruit rot disease on the field between July and December, in 2015 and 2016 (Figure 1a–e).

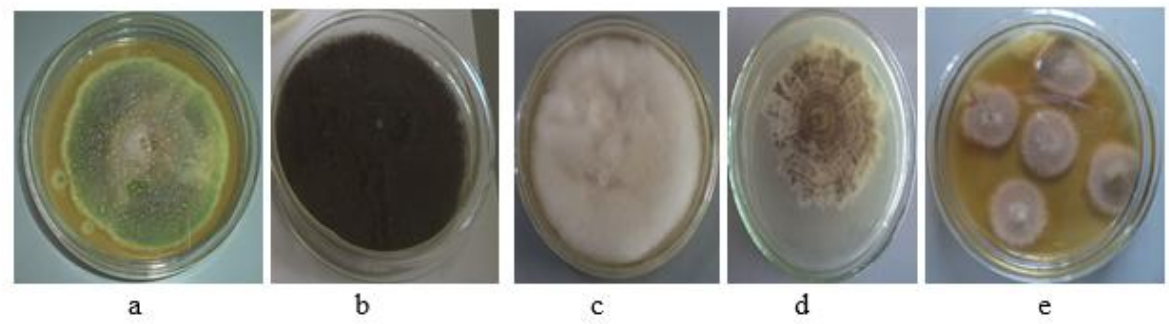

Figure 1. Colonies of some Fungi on Potato Dextrose Agar (a) *Aspergillus flavus*; (b) *A. niger*; (c) *Fusarium oxysporum*; (d) *Colletotrichum* spp; (e) *Alternaria solani*

The number of fungi isolated showed that *F. oxysporum* occurred most frequently while *S. rolfsii* was consistently the least from July to December, 2015 (Figure 2). The results further indicated that for each fungus, the least number was obtained in July and December while the peak number was recorded in September, 2015.

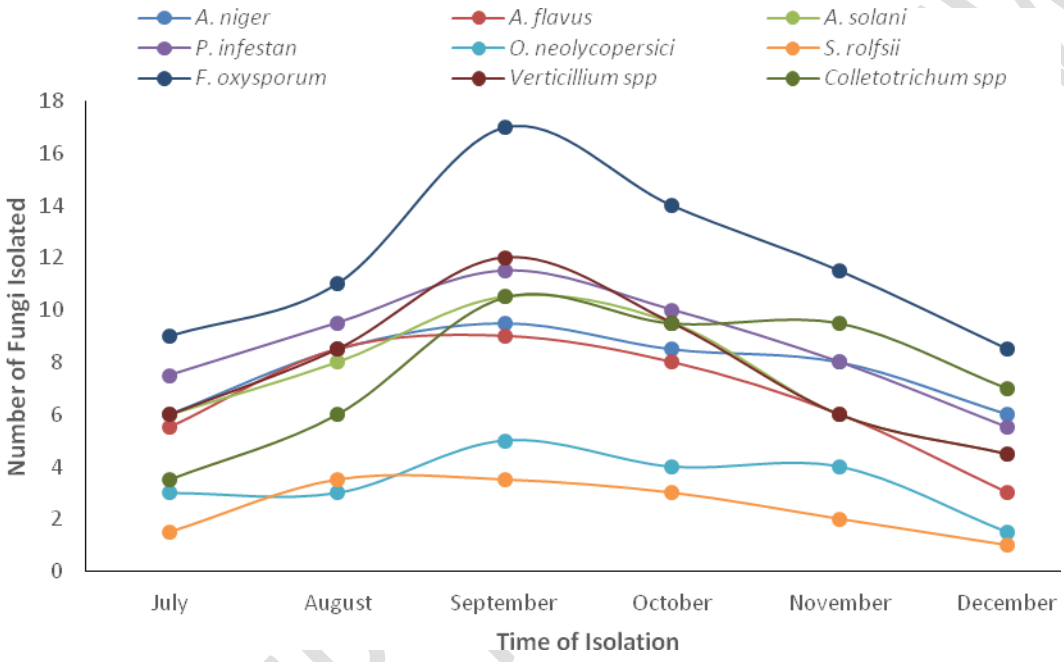

Figure 2: Number of fungi isolated on tomato fruits from July to December, 2015

The mean number of rot causing fungi isolated on tomato fruits from July to December in the 2015 and 2016 cropping seasons showed that *S. rolfsii* was the least while *F. oxysporum* was the highest (Table 1). The number of fungi isolated was more in the 2015 cropping season than in 2016. The mean number of *A. niger*, *P. infestans*, *O. neolycopersici* and *F. oxysporum* differed significantly ($P \leq 0.05$) in both years. The rest of the fungi did not vary significantly in both years.

Table 1: Mean variation of fungi isolated on tomato fruits in 2015 and 2016 cropping seasons

Fungi Isolated	Year		Df	T-Value	P-Value
	2015	2016			
Aspergillus niger	8.83±0.65	6.67±0.56	9	2.52	0.03*
Aspergillus flavus	7.17±0.79	6.17±1.10	9	0.75	0.47
Alternaria solani	8.83±0.95	6.00±0.97	9	2.10	0.06
Phytophthora infestans	10.00±0.86	7.33±0.88	9	2.17	0.05*
Oidium neolycopersici	4.33±0.56	2.50±0.43	9	2.61	0.02*
Sclerotium rolfsii	3.17±0.65	1.66±0.33	7	2.04	0.08
Fusarium oxysporum	14.33±1.70	9.33±0.95	7	2.60	0.03*
Verticillium spp	9.00±1.20	6.50±1.10	9	1.54	0.15
Colletotrichum spp	8.50±1.10	6.83±1.20	9	1.06	0.31

*indicates statistical significance at 95% CL

Results of the pathogenicity test indicated that all the nine isolates were able to elicit rotting in the tomato fruits (Table 2). The results however, revealed that *F. oxysporum* was more aggressive than the other pathogens and caused severe rotting of the fruits. The results further showed that *Oidium neolycopesici* and *S. rolfsii* caused slight rotting of the fruits while the rest of the isolates produced moderate rotting. There was no rotting observed in the control treatment (tomato fruits uninoculated with the pathogenic isolates).

Table2: Pathogenicity test of fungal isolates in artificially inoculated fruits of UC 82B variety of tomato

Isolates	Days to rotting	Rotting on tomato fruits	
		Inoculated fruits	Uninoculated fruits (Control)
Aspergillus niger	3	++	-
Alternaria solani	4	++	-
Aspergillus flavus	3	++	-
Verticillium spp	4	++	-
Oldium neolycopesici	4	+	-
Phytophthora infestans	4	++	-
Colletotrichum spp	4	++	-
Fusarium oxysporum	2	+++	-
Sclerotium rolfsii,	5	+	-

+ = slight rotting; ++ = moderate rotting; +++ = severe rotting; - = no rotting.

Discussion

The results of this study showed that *Aspergillus niger, A. flavus, Alternaria solani, Phytophthora infestans, Oldium neolycopesici, Sclerotium rolfsii, Fusarium oxysporum, Verticillium* spp and *Colletotrichum* spp are pathogens which cause tomato fruit rot in Tarka, Benue State. Similar results were obtained by Ibrahim *et al*. (2011); Matthew (2011); Laila *et al.* (2018); Yusuf *et al.* (2020); Nizamani *et al*. (2021) who indicated that fungi are major rot causing pathogens of tomato fruits. In another study, Sani and Gwa (2018) isolated *A. flavus, A. niger, F. oxysporum, F. moniliforme* and *Rhizoctonia solani* from rotted tomato fruits in Dutsin-Ma, Nigeria and found them pathogenic to healthy ones. Similarly, Onuorah and Orji (2015) isolated *A. niger, Rhizopus stolonifer, F. oxysporum, Saccharomyces cerevisiae, Alternaria alternata, Penicillium digitatum* and *Geotrichum candidum* from rotted tomato fruits in Awka,

Nigeria and found *A. niger* with the highest percentage of rots. Yusuf and Agieni (2020) isolated *Aspergillus* spp, *Fusarium* spp, *Penicillium* spp and *Rhizopus* spp from infected tomato in Anyigba, Kogi State and found *Aspergillus* spp with the highest frequency (38.89%) and *Fusarium spp* with the least (5.56%), contrary to these results which indicated that *F. oxysporum* was the most frequently isolated fungus in both cropping seasons (mean number=14.33 in 2015 and 9.33 in 2016) while *Sclerotium rolfsii* was the least (mean=3.17 in 2017; 1.60 in 2016).

Similarly, Abdulkadir *et al.* (2023) isolated *F. oxysporum* from tomato fruits in Makurdi, and found the fungus responsible for *Fusariu*m wilt disease of tomato. In another study carried out in Ethiopia, Lemma *et al*. (2014) isolated *Alternaria* spp, *Fusarium* spp, *Rhizopus* spp, *Penicillium* spp and *Erwinia carotovora* from infected tomato samples. In related studies, Mugao and Birgen (2021) isolated *Erwinia* spp, *Botrytis* spp, *Alternaria* spp, *Geotrichum* spp and *Rhizopus* spp from infected tomato fruits in Mwea, Kenya and found healthy tomato fruits susceptible to them. Nizamani *et al.* (2021) isolated *Erwinia* spp, *Botrytis* spp, *Alternaria* spp, *Geotrichum* spp and *Rhizopus* spp from rotted tomato fruits and observed that *A. solani* was the main cause of post-harvest tomato fruit rot in Tandojam, Pakistan, contrary to the results obtained in this study. Similar findings were reported by Sajad *et al*. (2017) and Cristina *et al*. (2018) that the major pathogens which caused tomato fruit rots were *Alternaria* sp., *Fusarium* sp., *G. candidum* and *R. stolonifer.*

This study revealed that fungal infection was more in September of both years than in any other month. This is probably due to favourable environmental conditions such as high rainfall, relative humidity and soil moisture which enhanced spore formation and dissemination of pathogens at the expense of the tomato plants which were susceptible.

Conclusion

This study showed that tomato fruits were susceptible to fungi pathogens such as *Aspergillus niger*, *A. flavus*, *Alternaria solani*, *Phytophthora infestans*, *Oidium neolycopersici*, *Sclerotium rolfsii*, *Fusarium oxysporum*, *Verticillium* spp and *Colletotrichum* spp. *Sclerotium rolfsii* had the least frequency while the most devastating pathogen was *F. oxysporum*. The results further revealed that in both years, the number of fungi isolated was least in July and December and highest in September. It is therefore, recommended that appropriate measures be taken during the growing, harvesting and postharvest handling of tomato to mitigate diseases and increase the output for sustainable crop production and to ensure food security for the global teeming populations.

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