Isolation and Identification of Fungi causing Spoilage of Tomato Fruits in Makurdi Benue State Nigeria

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ABSTRACT

Post-harvest losses from tomatoes is a huge challenge in Benue State. This study was designed to identify fungal pathogens responsible for post-harvest spoilage of tomato fruits in Makurdi, Benue State, Nigeria. Tomato fruits showing symptoms of decay were obtained from four (4) markets namely; Modern, Northbank, Wadata and Wurukum respectively. A total of forty (40) tomato fruits, ten (10) from each location were collected. Fungi were isolated on Potato Dextrose Agar using the direct plating technique. The disease-causing potential of the isolates were carried on healthy fruits and the incidence and severity of the decay caused by the fungi were recorded. Data on occurrence of fungi was analysed using Chi square ($\chi 2$) to show the relationship of fungal occurrence with respect to the location while the T-test was used to analyse the disease-causing potential (pathogenicity) of the isolated fungi. A total of five (5) fungi were isolated from the decaying tomato fruits which include *Aspergillus niger*, *Penicillium spp*., *Mucor spp*., *Bipolaris spp*. and *Cylindrocladium scoparium*.The results revealed Mucor spp. with the highest occurrence of 11(34.4%) and the least in a novel found fungi *Cylindrocladium scoparium* 1(3.10%), which was not statistically significant with respect to the different locations ($\chi 2 = 20.41$, $p > 0.05$) in this study. T-test revealed statistically significant differences in the pathogenicity of tomato fruits inoculated with the test fungi as compared to the control ($p<0.05$). The presence of these pathogenic fungi are a threat to human health on consumption.

Keywords: Tomato fruits, Fungi, Spoilage, Makurdi,

1.0 INTRODUCTION

In Nigeria, only 68.4% of tomatoes produced are consumed, about 45% are loss due to poor production processes by farmers, transportation and handling techniques, inadequate storage facilities and poor packaging and

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exposure to pests or diseases (Ugonna *et al.,* 2015). Several attempts such as proper sanitation and improvement in production through adoption of post-harvest techniques have been employed to increase its availability and increase farmers' income (Onowa, 2020 and Ozioko *et al.*, 2020).

Tomato produce can be contaminated with pathogenic or non-pathogenic microorganisms from the soil, animal sources or from humans and this can occur during growth, harvest, transportation, further handling and processing (Wani, 2011). Fungi such as *Altternaria species, Aspergillus spp., Fusarium spp., Mucor spp., Penicillium spp., Rhizopus spp. and Trichoderma spp.* have been incriminated as the main causes for tomato spoilage than bacteria (Ghosh, 2009). Fungal contamination of many agricultural products, including tomatoes starts in the field. The biological and physical damages occur mostly during harvest and transportation phases. The combination of large amount of water and a soft endocarp makes tomatoes more susceptible to spoilage by fungi (Onuorah and Orji, 2015). Fungi organisms are potential pathogens that adversely account for post-harvest losses as well as causing a devastating harm to human health. Exposure of humans to spores, mycelia and hyphael fragments of these pathogenic fungi could result to respiratory diseases and hypersensitivity such as increased asthma morbidity, rhinitis, allergic fungal sinusitis, bronchopulmonary mycoses and hypersensitivity pneumonitis whereas Its consumption could result to food poisoning as a result of the mycotoxins produced by these pathogenic fungi (Benedict *et al.,* 2017). This research was therefore designed to identify fungi associated with spoilage of tomato fruits in Makurdi as well as determine the level of pathogenicity from the spoilt tomatoes.

1.0 MATERIALS AND METHODS

2.1 Collection of Samples

Tomato fruits showing symptoms of decay were collected from four different markets in Makurdi namely; Modern, Northbank, Wadata and Wurukum markets. A total of forty (40) tomato fruits, ten (10) from each location were collected, packaged separately in polythene bags and labelled appropriately. They were taken to the Botany Laboratory of the Department of Biological Sciences, Benue State University for isolation of fungi pathogens.

2.2 Preparation of Potato Dextrose Agar (PDA) Medium

The medium used for the isolation of the fungal pathogens was potato dextrose agar (PDA). This was prepared by dissolving 39.6g of PDA powder in 1000ml of sterile distilled water. The mixture was stirred vigorously to homogenize and the flask content was heated on a heating mantle until the solution became clear. After heating, the flask was covered with foil paper and sterilized by autoclaving at 121^0C for 15mins at 15 pounds per square inch (psi). The sterile medium was allowed to cool to about 40^0C and two to three drops of streptomycin sulphate was added to inhibit bacterial growth. Approximately 20ml of the cooled medium was aseptically poured into the Petri dishes by flame sterilizing the mouth of the flask containing the medium. This was allowed to set before culturing the fungi.

2.3 Culture and isolation of fungal pathogens from the decaying tomato fruits

Small sections were cut from the decaying tomato fruits using a sharp knife and the excised portions were surface sterilized in 1% Sodium hypochlorite for 1 minute. The surface sterilized fruits were rinsed in two changes of sterile distilled water to remove residuals of the Sodium hypochlorite. After this, they were placed separately based on locations on top of filter paper to mop up the excess moisture (Ngongu *et al*., 2018). They were then placed on solidified Potato

Dextrose Agar medium. Three replications were made for each sample. The inoculated plates were incubated at room temperature and observations were made for microbial growth. After 6-7 days of growth, sub culturing was done to obtain pure cultures of the isolates as reported by Liamngee *et al*. (2015). 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 PDA in another Petri dish. Sub-culturing was done repeatedly until pure cultures of each fungal organism encountered were obtained.

2.4 Morphological characterization and identification of fungal isolates

The identification of the isolates was done macroscopically and microscopically. Macroscopic examination was done by observing colony characteristics for appearance, change in medium colour and growth on PDA. Microscopic identification was done by placing a drop of Lactophenol in cotton blue on a clean glass slide after which a small quantity of fungi isolates from the pure cultures were placed in the stain using a sterile inoculating needle, covered with a cover slip and viewed under 40x objective of a compound microscope. Identification of the organism was done by comparing the fungi using a standard identification manual by Barnett and Hunter (1972).

2.5 Pathogenicity Test

A pathogenicity test was conducted on healthy tomato fruits where they were washed with distilled water and thereafter sterilized in 1% Sodium hypochloride solution for thirty seconds. Mycelia discs of fungal isolates from five day old cultures were used to inoculate the tomato fruits. The cylindrical plugs (5 mm) were used to plug holes created in the tomato fruits by a cork borer. The discs of the tomato fruits in the cork borer were replaced and sealed with sterile PDA. On appearance of symptoms, the tissues at the margin of the healthy and diseased parts were excised, sterilized and placed on PDA and incubated at room temperature for 57 days. At the end of this period, morphological characteristics and growth patterns observed in each case were compared with the ones of the original isolates. Three tomato fruits were used for each fungal isolate, replicated three times and arranged in completely randomized design. Controls were tomato fruits inoculated with sterile PDA only. After 56 days of post inoculation, hand feel and visual examination of the exterior and interior of the fruits were used to ascertain the symptoms of fruit rots. Disease incidence and severity on the tomato fruits were determined. Disease severity was determined by applying the rating scale in which 0 = no disease symptom, 1 = 1 20% severity level on infected fruits, 2 = 21 40%, 3 = 41 - 60%, 4 = 61 80% and 5 = 81 100%.These were applied in the formula as reported by Liamngee *et al*. (2018).

Disease incidence (%) DI = X/N x 100

Where X = Number of infected fruits, N= Total number of fruits sampled.

Severity (%) DS = Σ (a+b) / NZ x 100

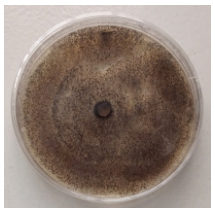
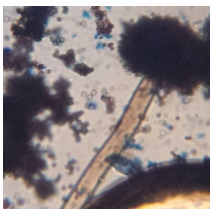
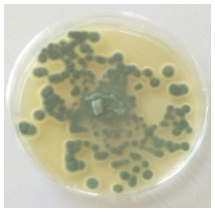
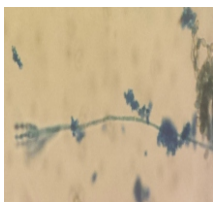
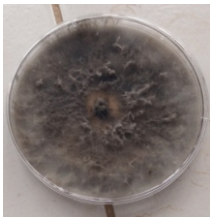
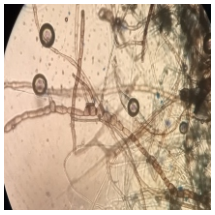
Where ∑(a+b) = sum of symptomatic fruits and their corresponding score scale, N= Total number of fruits sample and Z= Highest score scale

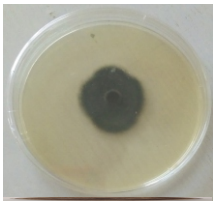
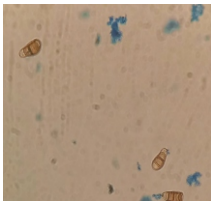
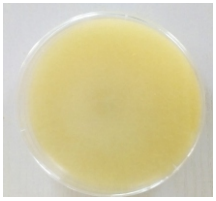
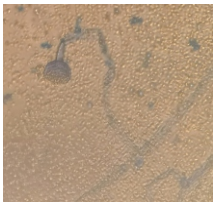
3. RESULTS

A total of five fungi were isolated from tomato fruits. They are; *Aspergillus niger*, *Penicillium* spp., *Cylindrocladium scoparium*, *Bipolaris* species and *Mucor* species**.** For *A.niger*, growth on PDA started with a round black colony with traces of white edges. As days progressed, the black colony increased in diameter with the edges turning to pale yellow. The growth was fast and the plate completely turned black as growth progressed. conidia head was globose with black colouration and the conidiophore was transparent. For *Penicillium* species, growth

started slowly and the colony colour was light powdery green with traces of white edges and had a scattered formation. As growth progressed, the colonies became compact with their edges first coming together before the colonies merged. Colony completely turned green as growth progressed. Hyphae were hyaline and septate. The conidiophore produced a brush- like structure which carried the spores. For *Cylindrocladium scoparium*, the colony grew fast on PDA forming cotton wool like effuse colonies. The colony was completely white at initial stage. As days progressed, there was a suppression of the cotton-wool like colour to ash. The bottom of the plate had a light brown colour. As the organism aged, the plate turned black retaining its cotton- like nature. The conidiophore was composed of two or more bifurnicate lateral branches to the main stipe. The primary branches give rise to secondary and sometimes tertiary branches with progressively smaller cells with each branch ending in two or more ovoid cells. For *Bipolaris* species, colony colour was dull green at the initial stage but as growth continued, the colony turned brown with a serrated edge and concentric rings. The hyphae were septate and conidia were curved having a nucleoloid like structure inside the conidia. For *Mucor* species, the colony colour was creamy on PDA with a gel- like covering at the initial stage. As growth progressed the covering became thicker but retained its creamy colour. The hyphae were broad, sporangiophores were long and terminated in a round spore filled sporangia as shown in Table 1.

Table 1: Characterization of fungal isolates from the decaying tomato fruits

Morphological Characteristics		Appearance on	Photomicrograph		Probable organism
Macroscopic	Microscopic	PDA			
Growth on PDA started with a round black colony with traces of white edges. As days progressed, the black colony increased in diameter with the edges turning to pale yellow. The growth was fast and the plate completely turned black as growth progressed.	Globbose-shaped conidia head with black colouration. The conidiophore were transparent.				*Aspergillus niger*
Growth started with a slow growth. colony had a scattered formation with light powdery green with traces of white edges. As growth progressed, the colonies became compact with their edges first coming together before the colonies merged. Colony completely turned green as growth progressed.	Hyphae were hyaline and septate. The conidiophore produced a brush- like structure.				*Penicillium* species
The colony grew fast on PDA forming a cotton wool like effuse colonies. The colony was completely white at initial stage. As days progressed, there was a suppression of the cotton- wool like colony.The bottom of the plate had a light brown colony and the cotton wool white colour turned ash. As this organism aged, the plate turned black retaining its cotton- like nature.	The conidiophore was composed of two or more bifurnicate lateral branches to the main stipe. The primary branches gave rise to secondary and sometimes tertiary branches with progressively smaller cells with each branch ending in two or more ovoid cells.				*Cylindrocladium Scoparium*

Morphological Characteristics		Appearance on PDA	Photomicrograph	Probable organism
Macroscopic	Microscopic			
Colony colour was dull green at the initial stage but as growth continued, the colony turned brown with a serrated edge and concentric rings.	The hyphae were septate and conidia were curved having a nucleoloid like structure inside the conidia.			*Bipolaris* species
The colony colour was creamy on PDA with a gel- like covering at the initial stage. As growth progressed the covering became thicker but retained its creamy colour.	The hyphae were broad, sporangiophores are long and terminated in a round spore filled sporangia.			*Mucor* species

The percentage occurance of fungal pathogens across the markets revealed that *Aspergillus niger* 4(50.00) was highest in Northbank, followed by Wadata 3(42.90), Modern market 2(20.00) and Wurukum with 1(14.00) respectively. When *Penicillum* spp was assessed, Wadata 3(42.90) had the highest fungal percentage occurrence followed by Modern market with 2(20.00) and Wurukum with 1(14.30) with the least occurrence. For *Cylindrocladium scoparium*, Wadata 1(14.30) had the highest percentage occurrence from the rest of the markets. For *Bipolaris* species., Wurukum 3(42.90) had the highest occurrence followed by Modern market 1(10.00).The percentage distribution of *Mucor* species. across the markets showed that Modern market 5(50.00) had the highest distribution followed by Northbank with 4(50.00) and Wurukum 2(28.60) respectively. There was no significant difference in the distribution of fungal isolates in all the five markets as shown in Table 2

Table 2: Occurrence of fungal pathogens in different locations

	Fungi (%)				
Markets	*A.niger*	*Penicillium* species	*Cylindrocladium scoparium*	*Bipolaris* species	*Mucor* species
Wadata	3(42.90)	3(42.90)	1(14.30)	0(0.00)	0(0.00)
Northbank	4(50.00)	0(0.00)	0(0.00)	0(0.00)	4(50.00)
Wurukum	1(14.30)	1(14.30)	0(0.00)	3(42.90)	2(28.60)
Modern	2(20.00)	2(20.00)	0(0.00)	1(10.00)	5(50.00)
Total	**10(31.20)**	**6(18.8)**	**1(3.10)**	**4(12.50)**	**11(34.4)**

χ^2= 20.41; df=12; P=0.06

The incidence of decay on tomato fruits inoculated with each test fungi was 100% respectively and this was significantly higher compared to the control which was 0% as shown in Table 3.

Table 3: Incidence of decay on healthy tomato fruits inoculated with test fungi

Treatment	*A.niger*	*Penicillium* species	*Cylindrocladium scoparium*	*Bipolaris* species	*Mucor* species
Inoculated	100.00	100.00	100.00	100.00	100.00
Control	0.00	0.00	0.00	0.00	0.00

T value= 9.00, P= 0.00

Severity of decay ranged from 15% - 35% and this was significantly higher than the control which showed 0 & severity as shown in Table 4

Table 4: Severity of decay on healthy tomato fruits inoculated with test fungi

Treatment	*A.niger*	*Penicillium* species	*Cylindrocladium scoparium*	*Bipolaris* species	*Mucor* species
Inoculated	15.00	25.00	30.00	25.00	35.00
Control	0.00	0.00	0.00	0.00	0.00

T value= 47.39, P= 0.00

4. DISCUSSION

The disease causing potential of the isolated fungi in this study showed that all the organisms were pathogenic on healthy tomato fruits causing different decay. This was due to the ability of the

fungal pathogens to produce resistant spores from the field which led to its subsequent growth and development during favourable conditions. Another factor could also be attributed to the ability of the fungi to utilize nutrients from the tomatoes which usually acts as substrates for growth (Ngongu *et al*., 2018). Sani *et al*. (2018) identified five fungal pathogens which were associated with the spoilage of onions, tomatoes and cabbage which include; *Aspergillus flavus*, *Mucor* species., *Rhizopus stolonifer*, *Aspergillus niger* and *Penicillium* species. Their findings on the fungal pathogens were similar except for *Bipolaris* spp. and *Cylindrocladium scoparium* which were identified in this study. A significant finding from this research was the isolation and identification of *Cylindrocladium scoparium*.

The occurrence of the fungi isolated from this study was low as compared to studies by Oyemaechi *et al*. (2014) and Sani *et al*. (2018). This could be attributed to environmental and seasonal variations of the different locations studied. The various fungi pathogens recorded in different studies compared to those across different locations is an evidence that tomato spoilage is affected by various fungi which depends on nutrient availability, host specificity and the environment they occupy. This is observed in the differences in the fungi organisms isolated from Sajad *et al*. (2017) in India and Abdullah *et al*. (2016) in Yemen compared to the isolated fungi in studies in Nigeria by Oyemaechi *et al*. (2014); Sani*et al*. (2014); Liamngee *et al*. (2016); Yaradua *et al*. (2018) and Mwekan *et al*. (2019).

The pathogenicity test result revealed that, all the fungal isolates had the ability to cause significant decay when they were inoculated into apparently healthy tomatoes at varying percentages of severity. The incidence of decay of healthy tomato fruits inoculated with fungi organisms as shown in Tables 2 and 3 showed a statistical significantly higher decay and severity between the inoculated tomato fruits and the control. Numerous microbial defects which include signs and symptoms of tomatoes are characterized by the type of micro-organism responsible for the deterioration, during the process of infection in the case of fungal invasion, the development of fungal penetrating structure follows. The colonization process involves the ability of the microorganism to establish itself within the produce**.** The contamination of tomato fruits by fungi could also be as a result of poor handling, storage conditions, distribution, marketing practices and transportation, distribution and changing physiological state of the fruits (Liamngee *et al*., 2016). The morphological characteristics both comprising macroscopic and microscopic were similar after re-isolation from the starter cultures. This could be due to the utilization of nutrients by the fungi which serve as substrates for their growth and development. A similar trend was reported by Liamngee *et al.* (2018). The presence of these fungal pathogens could pose a threat to life due to the production of mycotoxins which are capable of suppressing the immune system and exposing the consumer to ill health after consumption (Ahmed and Jutta, 2015).

This study revealed the presence of fungi associated with spoilage of tomato fruits after harvest or during storage. These pathogens pose a great threat to humans and animals after ingestion of the contaminated fruits as a result of the production of their potent mycotoxins which can suppress the immune system and also result in disease and death. The presence of *Cylindrocladium scoparium* remains a unique discovery in this work as there is no known published report of *Cylindrocladium scoparium* on tomato fruits in Benue State. Proper storage practices should be encouraged efficiently monitored. A further study on *C. scoparium* on tomatoes is recommended.

Competing interests

Authors have declared that there is no existing conflict of interest between them.

REFERENCES

Abdullah, Q., Mahmoud, A. and Al-harethi, A. (2016). Isolation and identification of fungal post harvest of some fruits in Yemen. *Pakistan Science Mission*, 1 (1): 36-44.

Abhinaba, G. (2009). Identification of Microorganisms Responsible For Spoilage of Tomato (*Lycopersiconesculentum*) Fruit**,** *Journal of Food Nutrition.* ***2****(10): 2830*

Ahmed, A.I. and Jutta, I. (2015) Papen brock Review Mycotoxins: Proucing Fungi and Mechanismsof Phytotoxicity.*www.mdpi.com*/j*ournal*/*agriculture*

Barnett, H.L. and Hunter, B.B. (1972). Illustrated Genera of Imperfect fungi 3rd edition Burgess, Minnesota, pp. 273

Benedict, K., Chiller, T. M. and Mody, R.K (2017). Invasive Fungal Infections Acquired from contaminated food or Nutritional Supplements: A review of the Literature. *Foodborne Pathogens and Dissease,* **13**(7): 343-349

FAO (Food and Agriculture Organization of the UnitedNations).Data, various years. 2013.Available from URL *http://faostat.fao.org/cgi-bin/nph-db.pl?subset=ag.*

Ghosh, A. (2009) Identification of Microorganisms Responsible for Spoilage of Tomato (*Lycopersiconesculentum*) Fruit.*Journal of Phytology*. 1 (6): 414-416

Liamngee, K., Akomaye, M.U. and Okoro, J.K. (2015). Efficacy of some botanicals in the control of fungal organisms causing post harvest rot of Yam in Katube Market, Obudu, Nigeria. *Journal of Pharmacy and Biological Sciences*, 10 (6): 33-41.

Liamngee, K., Ogo-Oluwa, A.T. and Akinyemi, B.K. (2016). Isolation and identification of seed borne fungi of common bean (*Phaseolus vulgaris*) from selected markets in Makurdi. *International Journal of Applied Agricultural Sciences*, 2 (5): 75-78.

Liamngee, K., Iheanacho, A.C. and Kortse, P.A. (2018). Isolation, identification and pathogenicity of fungal organisms causing post harvest spoilage of tomato fruits during storage. *Annual Research and Review in Biology*, 26 (6): 1-7.

Mwekaven, S.S., WanenIkyo, T., Alye, C.T. and Aorkwagh, M.T. (2019). Identification and enumeration of microorganisms associated with post harvest spoilage of tomatoes (*Lycopersiconesculentum*) sold in Gboko Metropolis. *Journal of Scientific and Engineering Research*, 6 (6): 153-158.

Ngongu, I., Ugese, D.F., Liamngee, K. and Agatsa, D.T. (2018).Isolation and identification of fungi causing postharvest decay of oranges (*Citrus sinensis*) during storage in Makurdi.*Mind Sourcing Bio*, 3 (12): 1-8.

Olayemi, F. F., Adegbola, J. A., Bamishaiye, E. I. and Daura, A. M. (2010).Assessment of Post-Harvest Challenges of Small Scale Farm Holders of Tomatoes, Bell and Hot Pepper in Some Local Government Areas of Kano State, Nigeria. *Bayero Journal of Pure and Applied Sciences,* 3 (2): 39 42.

Onowa, G. (2020). Mitigating Tomato post-harvest losses. Participation Responses to Recommended Technology. *Food Technology,* Pp 1-5.

Onuorah, S. and Orji, M.U. (2015). Fungi Associated with the Spoilage of Post-harvest Tomato Fruits Sold in Major Markets in Awka, Nigeria, *Universal Journal of Microbiology Research,* 3 (2): 11-16.

Oyemaechi, C.U.,Chukwuezi, F.O. and Ozougwu, V.E.O. (2014).Microbial agents of tomato spoilage in Onitsha metropolis. Advances in Biological Research, 8 (2): 87-93

Preedy, V. R. and Watson, R. R. (2008).*Tomatoes and Tomto Products:Nutritional,*

Medicinal and Therapeutic Properties, pp. 34-56. Boca Raton CRC Press

Ozioko, R. Enwelu, I., Ukwuani, R.A. and Chiemela, V.N. (2020). Post harvest losses among tomato farmers in Nsukka and Udi Local Government Area of Enugu State, Nigeria. *Business*

Sajad, A.M., Jamaluddin and Abid, H.Q. (2017). Fungi associated with the spoilage of post harvest tomato fruits and their frequency of occurrences in different markets of Jabalpur, Madhya-Pradesh India. *International Journal of Current Research Revolution*, 9 (5): 12-18.

Sani,N.M., Salihu, Y., Saidu, A.Y, Yousouf, S., Huhu, D.Z. and Rabiu, G.A. (2018). Isolation of fungi associated with spoilage of se;ected vegetables sold in Dutseultra modern market, Jigawa State, Nigeria. *Dutse Journal of Pure and Applied Sciences*, 4 (2): 349-358

Ugonna, C.U., Jolaoso, M.A. and Onwualu, A.P. (2015). Tomato value chain in Nigeria: Issues, Challenges and Strategies. *Journal of Scientific Research and Reports,* 7(7): 501-505.

Wani, A.H. (2011). An overview of thefungal rot of tomato. *Mycopathology*, 9 (1): 33-38

Yaradua, S.S., Kankara, S.L. and Yusuf, M. (2018). Isolation and identification of fungi from some selected vegetables in Kanakara Local Government Area, Katsina State. Journal UJMR .*Journal of MicrobiologyResearch,* 3 (1): 1-5)

Zdenka, P., Smerak, P., Demova, H. and Houska, M. (2010).Antimutagenic Effects of Lycopene and Tomato Puree, *Journal of Medicinal Food,* 13 (6): 1443-1450