

ISOLATION AND IDENTIFICATION OF FUNGAL PATHOGENS OF POST-HARVEST ROT OF TOMATO FRUIT IN BIU MARKETS, BIU LOCAL GOVERNMENT AREA OF BORNO STATE

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ABSTRACT

Tomato is considered as the most important vegetable crop in the world after potato. However, production losses in post-harvest tomato are caused by damages that occur during harvesting, handling which are caused by various micro-organisms, the most destructive of which are fungi and bacteria. Survey for incidence and severity of post-harvest rot of tomato (*Solanum lycopersicum L.*) was conducted in three different markets in Biu Local Government Area of Borno State. All the data obtained were analysed using analysis of variance (ANOVA) and the means were separated using the least significant difference (LSD). Biu main market showed rot incidence of 89 %; General hospital market had rot incidence of 87 % and Lashe money market had the highest percentage rot incidence of 90%. Eight fungal species were isolated and identified as *Aspergillus flavus*, *Basidiobolus ranarum*, *Bipolaris spicifera*, *Chaetomium globosum*, *Mallesezia furfur*, *Nigrospora sphaerica*, *Rhizopus microsporus* and *Rhizopus oryzae*. The most frequent fungus was *Rhizopus microsporus* (23.10 %). The pathogenicity test carried out showed *Chaetomium globosum*, *Mallesezia furfur* and *Rhizopus oryzae* were the most virulent of the other test organisms, with the highest mean rot percentage (73 %). Based on the findings in this study, it is recommended that further research be carried out on the effect of fungal infection on the nutritional value of tomato fruits and to reduce fungal pathogens infection by genetically engineering the tomato fruits to produce thick exocarp.

Key words: incidence; severity; post-harvest; rot; tomato; pathogenicity.

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INTRODUCTION

Tomato belongs to the genus *Solanum* and species *lycopersicum* (Wani, 2011). Tomato plants can grow up to 10 feet tall, but most species are less than three feet tall on the average (Dimphna, 2016). Tomato plants are perennial but are normally grown as annual, with a weak stem that often sprawls over the ground and vines over other plants (Sravanthi and Gangadhar, 2015). Fruits of tomato are diverse in size and shape, ranging from small and round to large and variable shapes such as pear-shaped, elongated, flattened and heart-shaped. The size of tomato varies depending on the plant species. Cherry tomato plants produce small, cherry-sized fruits (Dimphna, 2016).

Tomato fruits contain high amounts of carbohydrates, fats, organic acids, water, minerals, vitamins and pigments. Tomato is considered as the second most important vegetable crop in the world after potato. It is an important vegetable crop across the globe. It originated in west-south America. Now, it is widely grown throughout the world (Kimura and Sinha, 2008). The fruits of tomato are used in all kinds of soups, stew and are also eaten raw in salads, sandwiches, sauces, drinks. Burger tomato can also be dried and ground into pancakes (Onuorah, *et al.*, 2015). Tomato has been used globally not only as food, but also as research material. It is a major vegetable crop that has achieved tremendous popularity over the last century. It is grown in every country of the world indoor and outdoor, fields, greenhouses and net houses (Bihn and Gravani, 2006). Ripe tomato fruits have high nutritive values, being a good source of vitamin A, vitamin B, vitamin C and minerals. Due to the importance of tomato as food, it has been bred to improve productivity, fruit quality and resistance to biotic and abiotic stresses (Kimura and Sinha, 2008).

Tomato is a highly perishable crop. It has been reported that about 50% of the crop is usually lost between rural production and consumption channels in the tropical areas. One of the main causes of post-harvest losses is damage that occurs during harvesting and handling. The magnitude of post-harvest losses differs with region, season and time (Mujib *et al.*, 2007). Post-harvest losses of tomato in Nigeria are due to rot caused by various micro-organisms (Thirupathi *et al.*, 2006). Among the various micro-organisms responsible for the post-harvest decay of tomatoes, fungi and bacteria are the most destructive (Obetta *et al.*, 2011). Fungi are the most important and prevalent pathogens, infecting a wide range of fruits and causing destructive and economically important losses of the fruits during storage, transportation and marketing (Kator *et al.*, 2018). Pathogenic fungi such as *Alternaria*, *Aspergillus*, *Fusarium*, *Mucor* species, *Penicillium*, *Rhizopus* and *Trichoderma* have been implicated in some crop spoilage. Few studies on fungi associated with tomato spoilage have been reported in Nigeria. This study was carried out to isolate and identify fungal pathogens associated with post-harvest rot of tomato fruit in Biu markets, Biu Local Government Area of Borno State, Nigeria.

MATERIALS AND METHODS

Study Area

The survey was randomly carried out in Biu main market, Biu General Hospital market and Lashe Money market all in Biu Local Government Area. Isolation and identification were carried out in the Department of Biology laboratory, Nigerian Army University, Biu, Borno State in 2022. Biu is located in the north-eastern part of Nigeria and lies between latitude 10° and 11° north of the equator and between longitude 11° and 13° east of the greenwich meridian.

The detailed description of the climatic condition of the region in the southern part of Borno State varies yearly (Mudi *et al.*, 2019). Vegetation is Sudan savannah interspersed with tall trees and woodlands, arable lands and favourable weather for crop production. The people of the area are predominantly farmers and cultivate food crops like maize, millet, sorghum, rice, soya bean, cowpea and groundnut as major sources of livelihood (Mudi *et al.*, 2019). The area has tropical climate marked by dry and rainy seasons; the rainy season commences around May and ends in the middle or late October with an average rainfall of 800 mm-1200 mm. The dry season starts in October or November and lasts till April. Maximum temperature is about 40 °C around April while minimum temperature could be as low as 18.3 °C between December and early January. Relative humidity in the area is about 26 % in the month of January while February has the lowest value of 16 %. The months of July and August usually have the peak with relative humidity of about 80 %.

Sources of Samples

Samples of tomato fruits showing rot symptoms were collected from three locations: Biu main market, General Hospital market and Lashe Money market, all within Biu town, in sterile polyethylene bags, straight to the laboratory of the Department of Biology, Nigerian Army University, Biu, for isolation and identification. Biu is a Local Government Area in Borno State (Figure 1), with major and minor markets where different types of fruits (including tomato) are sold. One hundred (100) tomato fruits were collected from each of the three markets, making a total of 300 fruits from all the markets.

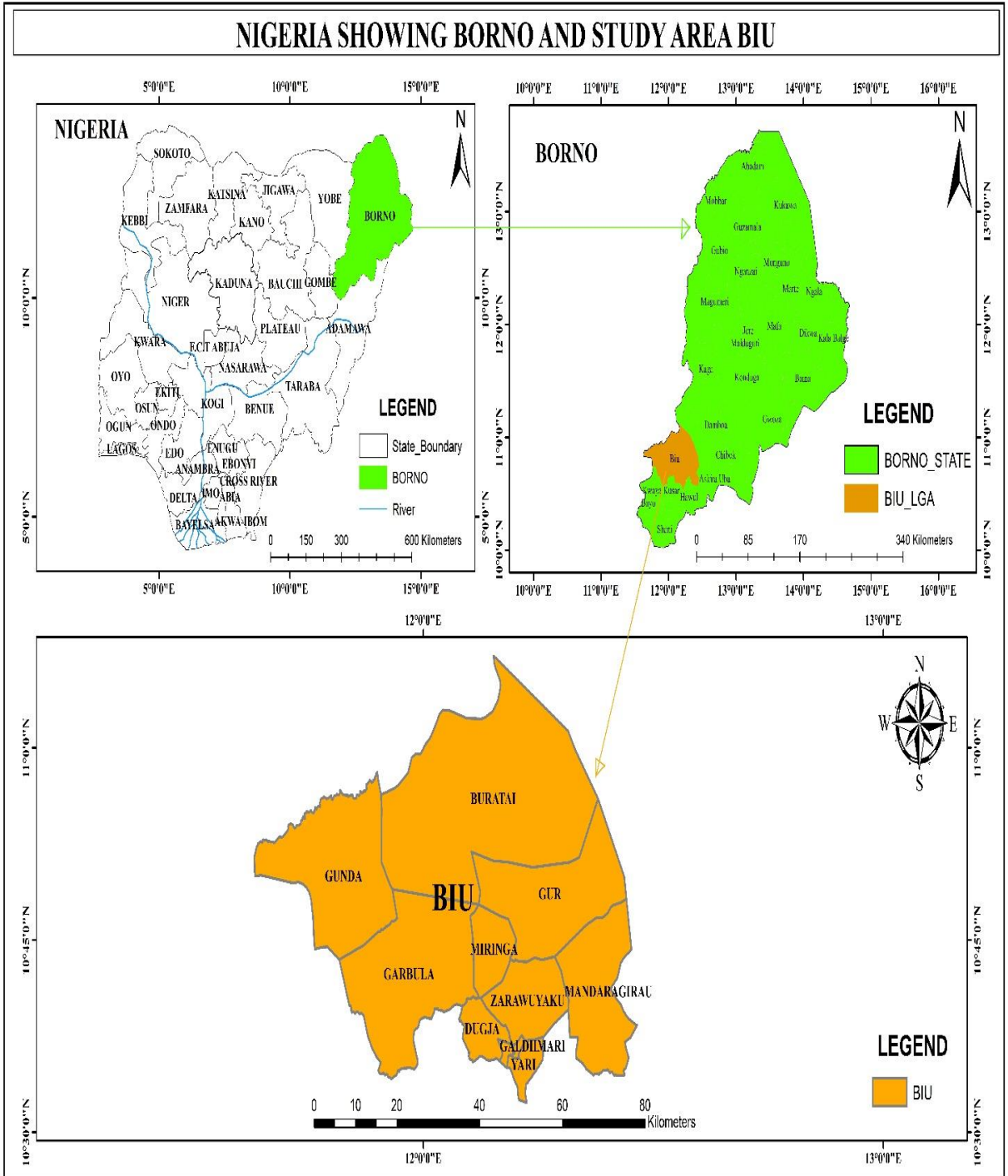


Figure 1: Map of Borno State, showing Biu
Source: Department of Urban and Regional Planning Modibbo Adama University, Yola

Determination of Tomato Rot Incidence and Severity in the Market

Samples of tomato fruits were collected at random from the three markets in Biu town. A total number of one hundred tomato fruits was collected from each market. The incidence of tomato fruit rot in each market was determined by counting the infected tomato fruits from the samples collected from each market, using the formula:

$$\% \text{Disease Incidence} = N / T \times 100$$

Where,

N = Number of infected tomato fruits

T = Total number of tomato fruits collected

The Severity of the tomato fruit rots was computed by measuring the lesion size of rot and in millimetres.

Sterilisation of Materials

The Petri dishes that were used for culturing were sterilised. The inoculating needle and the cork borer were also sterilised by flaming over a Bunsen burner and allowed to cool by dipping them in methanol. The prepared media was sterilised by autoclaving for 15 minutes at 10 lbs pressure at 121 °C and allowed to cool. The inoculation of the organisms was done in a sterilised environment in the inoculation chamber. The table in the inoculation chamber was wiped with 95 % alcohol and the ultraviolet light (UV light) was left on for 30 min. on the glass wares before inoculation (Kader, 2002).

Preparation of Culture Media

Potato Dextrose Agar (PDA) was used for culturing of the isolates. Thirty-nine grams (39 g) of PDA was dissolved in one litre of distilled water. The PDA was poured into a conical flask and 6 ml (0.1%) of streptomycin was added to the sterilised media, just before pouring into Petri dishes, to prevent bacterial growth. The Petri dishes were covered with cotton and wrapped with aluminum foil before autoclaving at 121° C for 15 minutes at 10 lbs. pressure. Pouring of media into the Petri dishes was done in the inoculation chamber. Twenty (20) ml of the media was poured into 9 cm diameter Petri dishes in three replicates, and allowed to cool and solidify under ultraviolet light (Suleiman and Michael, 2013).

Preparation and Plating of Inoculate

The diseased portion of the tomato was sliced into 5 mm square sections each and sterilised in 0.1 % mercuric chloride solution for 30 seconds in sterilised Petri dishes. These were then rinsed in three to five changes of sterile distilled water to wash off the sterilants. The sliced portions were then blotted dry using sterile filter papers and transferred into the solidified PDA plates using sterilised forceps. They were placed at the centre of the Petri dishes and incubated at room temperature (30 ±3 °C) in the laboratory for seven (7) days (Brownbridge *et al.*, 2012).

Isolation of Fungi

Under aseptic conditions, the diseased sample of tomato fruit was cut into approximately 5 mm with a neat sterile scalpel. Pieces were picked with flamed a sterilised pair of forceps. The pieces were immersed into 0.1 % sodium hypochlorite in a sterile 9 cm diameter Petri dish for surface-sterilisation for 30 seconds. The sterilised pieces were rinsed in three changes of sterile distilled water and then blot-dried between sterile filter papers. The sterilised piece of tomato fruit was then plated aseptically on 9 cm diameter Petri-dish containing sterile solidified Potato Dextrose Agar (PDA) and incubated at room temperature for 3-4 days (i.e. immediately when new colonies began to grow) before sub-culturing on fresh sterilised PDA (Liamngee *et al.*, 2015). Pure colonies were photographed and photomicrographs were prepared under the microscope (×40).

Preparations of Slides and Identification of Fungi

Microscopic examination was carried out to observe the structure and characteristics of the fungal isolates. A sterile needle was used to pick a little portion of the hyphae containing spores and placed on a sterile glass slide stained with Lactophenol in cotton blue and examined under the microscope (Liamngee *et al.*, 2015). Micrographs of the isolates showing conidia, sporangia, etc. were taken. The morphological and structural characteristics observed were compared with structures in the identification guides of Hunter and Barnett (Nizamani *et al.*, 2021).

Frequency of Occurrence

The prevalence of the isolated fungi of tomato fruit rot was calculated and expressed as percentage frequency as follows:

$$\% \text{ Frequency} = N \times 100 / T \times$$

Where,

N = Number of isolated organisms

T = Total number of isolated organisms (Kirk *et al.*, 2008)

Pathogenicity Test

Healthy tomato fruits were obtained and surface-sterilised with 1% sodium hypochlorite for 30 seconds and rinsed in three (3) changes of sterile distilled water (Chukwuka *et al.*, 2010). A sterile cork borer (5 mm in diameter) was used to puncture and inject healthy tomato fruits with spores of isolated organisms from the cultures prepared in three replicates. Vaseline jelly was smeared to completely seal each hole to avoid contamination. The cultures were kept at a room temperature. A similar set-up was placed as control using sterile distilled water in place of the fungal inocula (Nizamani *et al.*, 2021). The set up was arranged in a completely randomised design. It was kept for seven (7) days to allow for possible rot development and the isolates were re-isolated from the new host and shown to be the same as the originally inoculated pathogen. Data were taken after seven (7) days using visual scale and with the aid of hand lens. Severity scale of 1 to 5 was used, 0, 1, 2, 3, 4 and 5: No disease on leaf and pods, 1; Small brown spot covering <1 % lesion area (pin point spots on exocarp); 2: Brown sunken spots 1-10 % lesion area (< 1 % exocarp area); 3: Brown spots 11-25 % lesion area (1-10 % exocarp areas); 4: Circular brown sunken spots 26-50 % lesion area (11-25 % exocarp area); 5: Circular to irregular > 51 % lesion area (>26 % exocarp area).

Data Analysis

Data obtained were analysed using the analysis of variance (ANOVA) to test for significance using the Statistical Tool for Applied Sciences (SAS) version 8.3 and the means that were significant were separated using the least significant difference (LSD) at 5 % probability level (Schaffer *et al.*, 2010).

RESULTS

Incidence and Severity of Tomato Fruit Rot in Biu

Table 1 shows that the soft rot of tomato fruit was prevalent in all the three markets surveyed (Biu main market, Biu General Hospital market and Lashe Money market). The percentage incidence of rot varied significantly among the markets. The percentage incidence of rot in Lashe Money market was highest (90 %) followed by Biu main market (89 %) and the General Hospital market (87 %).

The Table 1 also shows the extent of damage (i.e. severity) on infected samples of tomato fruits in Biu. There was a significant difference in the severity of rot observed in the three markets, with the General Hospital market having the highest with a lesion size of 23.1 mm, followed by Lashe Money market with disease severity of 22.6 mm. Biu main market had the lowest disease severity of 18.8 mm. There was no significant variation between the General Hospital market and Lashe Money market.

Isolation and Identification of Fungal Species

The isolated fungi from tomato fruits were identified as *Aspergillus flavus*, *Basidiobolus ranarum*, *Bipolaris spicifera*, *Chaetomium globosum*, *Mellesszia furfur*, *Nigrospora sphaerica*, *Rhizopus microsporus* and *Rhizopus oryzae* (Plates 1-8). These were identified based on their colonial, shape, colour of appearance and morphological characteristics. The eight plates show the radial growth expansion rate of the eight isolates after seven days.

Plate 1 shows *Aspergillus flavus* colonies which were powdery masses of yellowish-green. The surface colour of the colony was greyish-green and the margins were entire. The reverse was hyaline. The colony growth was moderate to rapid. Micrographic structure revealed that conidiophores of *A. flavus* isolates were colourless, thick-walled, rough and bore vesicles hemispherical. Conidia covered the entire vesicle. Conidiophore was smooth, long and hyaline.

Plate 2 show broad, sparsely septate hyphae with an eosinophilic halo around the invading hyphae, referred to as the Splendore-Hoeppli phenomenon. Corneal confocal microscopy showed multiple round to oval-shaped lesions which looked like fungal zygospores in the corneal stroma.

Plate 3 shows colonies which were fast-growing and reached a diameter of 4 cm in 7 days. They were presented as velvety, brownish and flat. Microscopy revealed septate, pigmented hyphae and unbranched zigzagged conidiophores with thick-walled, darkly pigmented cylindrical conidia, which were predominantly three-septate. Plate 4 shows morphological characteristics of *Chaetomium globosum* which include brown-coloured septated hyphae, perithecia and ascospores. Plate 5 shows *M. furfur* colonies which were smooth, opaque and umbonate. Microscopically, all *M. furfur* isolates appeared as small, ovoid cells and buds were formed on a broad base. The cells had a bottle-like shape due to a small protrusion visible at the end of each cell. Plate 6 shows that the conidia were brownish-black, oblate spheroid and single-celled. On the average, they ranged from 16-18 µm in diameter. The initial white translucent-looking colony of *N. sphaerica* turned brown-black due to massive sporulation of conidia from the conidiophores. Plate 7 shows colonies which were dark greyish-brown, up to 10 mm high and with simple rhizoids. Sporangiohores were brownish, up to 400 µm high and 10 µm wide. They were produced in groups of one to four, usually in pairs. Sporangia were greyish-black, spherical, up to 100 µm in diameter. Plate 8 is characterised by the presence of stolons and pigmented rhizoids, the formation of sporangiophores, singly or in groups, from nodes directly above the rhizoids, and apophysate, columellate, multi-spored, generally *globose* sporangia.

Table 2 shows the occurrence of fungal pathogens of tomato fruit rot in Biu markets. *Aspergillus flavus* was present in Lashe Money market only. *Basidiobolus ranarum* was present in the General Hospital market only. *Bipolaris spicifera* was present in Lashe Money market. *Chaetomium globosum* was present in the General Hospital market. *Malassezia furfur*, *Nigrospora sphaerica* and *Rhizopus oryzae* were present in the General hospital market and Lashe Money market. *Rhizopus microsporus* was observed in all the three markets. Table 3 shows the distribution of fungal pathogens of tomato in Biu. There was no significant difference between *Aspergillus flavus*, *Basidiobolus ranarum*, *Bipolaris spicifera* and *Chaetomium globosum*, as well as between *Malassezia furfur*, *Nigrospora sphaerica* and *Rhizopus oryzae*, differed significantly from the other fungal species.

Table 1: Percentage Incidence and Severity of Tomato Rot in Biu, Borno State, Nigeria

Markets	Incidence of rot (%)	Rot diameter (mm)
Biu Main Market	89	18.8
General Hospital Market	87	23.1
Lashe Money Market	90	22.6
LSD (0.05)	1.0	1.4

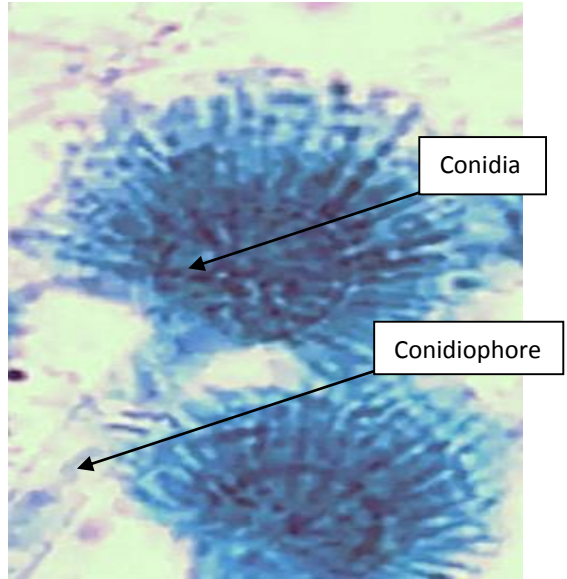
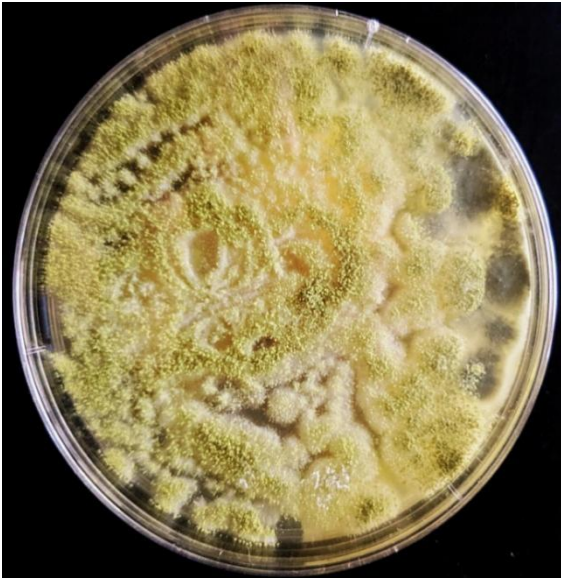
Table 2: Occurrence of fungal pathogens of tomato fruit rot in three markets in Biu, Borno State, Nigeria

Fungal isolates	Presence of isolates in each market		
	Biu Main Market	General Hospital Market	Lashe Money Market
<i>Aspergillus flavus</i>	-	-	+
<i>Basidiobolus ranarum</i>	-	+	-
<i>Bipolaris spicifera</i>	-	-	+
<i>Chaetomium globosum</i>	+	-	-
<i>Malassezia furfur</i>	-	+	+
<i>Nigrospora sphaerica</i>	-	+	+
<i>Rhizopus microsporus</i>	+	+	+
<i>Rhizopus oryzae</i>	-	+	+

Key:

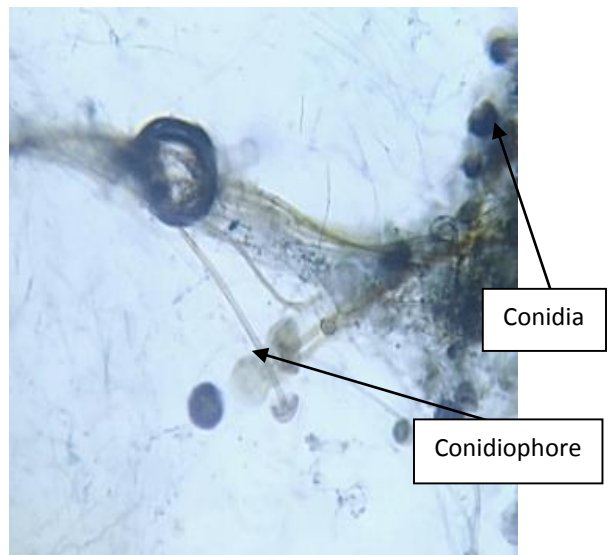
+ Presence

- Abscent



(b)

Plate 1 (a): Seven-day old culture of *Aspergillus flavus* (b): Micrograph of *A. flavus*



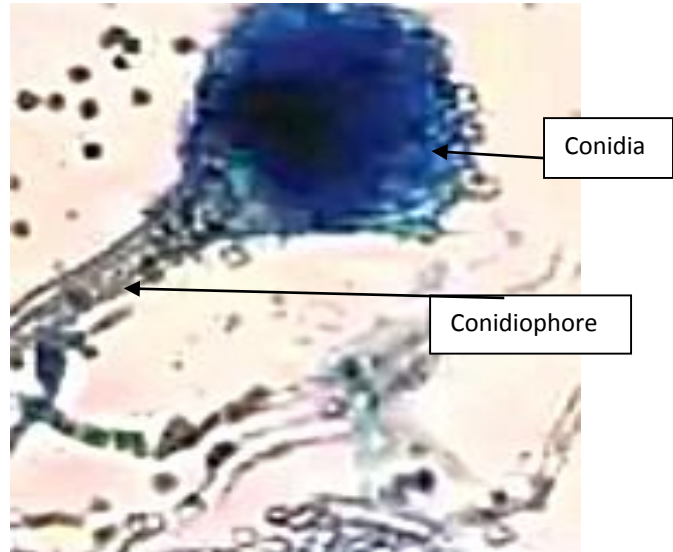
(a)

(b)

Plate 2 (a): Seven-day old culture of *Basidiobolus ranarum* (b): Micrograph of *B. ranarum*



(a)

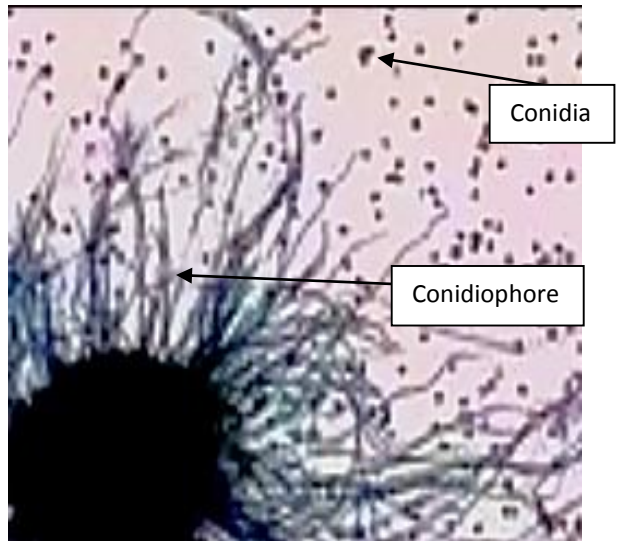


(b)

Plate 3 (a): Seven-day old culture of *Bipolaris spicifera* (b): Micrograph of *B. spicifera*

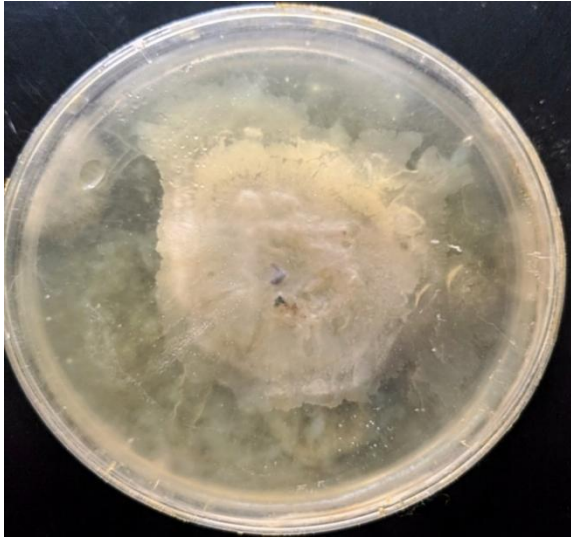


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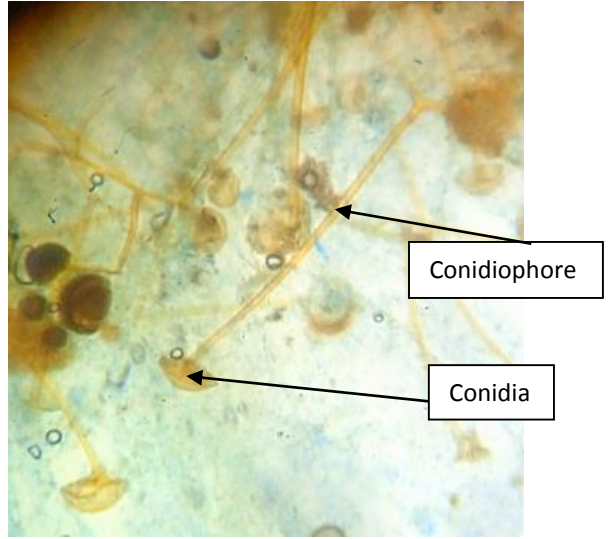


(b)

Plate 4 (a): Seven day old culture of *Chaetomium globosum* (b): Micrograph of *C. globosum*



(a)

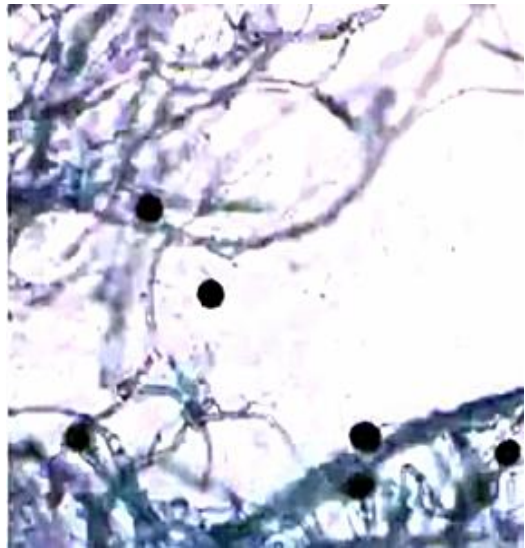


(b)

Plate 5 (a): Seven-day old culture of *Malassezia furfur* (b): Micrograph of *M. furfur*

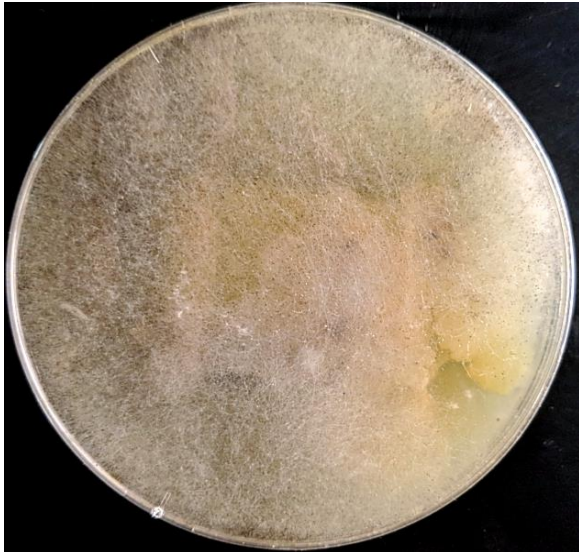


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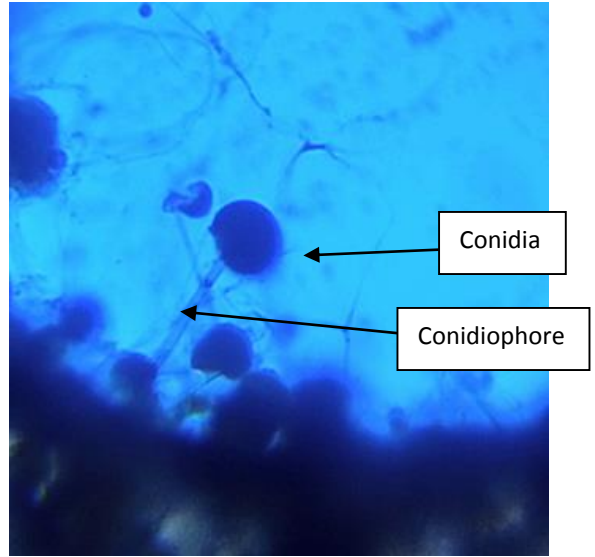


(b)

Plate 6 (a): Seven-day old culture of *Nigrospora sphaerica* (b): Micrograph of *N. sphaerica*



(a)

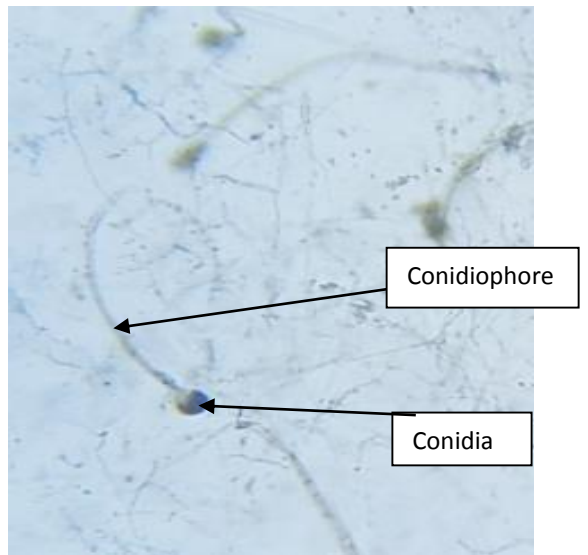


(b)

Plate 7 (a): Seven-day old culture of *Rhizopus oryzae* (b): Micrograph of *R. oryzae*



(a)



(b)

Plate 8 (a): Seven-day old culture of *Rhizopus microsporus*
(b): Micrograph of *R. microsporus* with spore from conidiophore

Pathogenicity of Fungal Isolates on Tomato Fruits

All the isolates were pathogenic, since there was a highly significant difference between the means of the infected fruits and the non-infected ones (control) (Table 4). *Chaetomium globosum*, *M. furfur* and *R. oryzae* had the highest virulence with a lesion size of 73 %, followed by *B. ranarum* and *R. microsporus* with a lesion size of 67 %. *Aspergillus flavus*, *B. spicifera* and *N. sphaerica* differed significantly with a lesion size of *Aspergillus* 60, 46 and 40 % , respectively.

Table 3: Frequency of distribution of fungal pathogens of tomato fruits in Biu

Fungal Isolate	Frequency of isolates (%)
<i>Aspergillus flavus</i>	7.7
<i>Basidiobolus ranarum</i>	7.7
<i>Bipolaris spicifera</i>	7.7
<i>Chaetomium globosum</i>	7.7
<i>Malassezia furfur</i>	15.4
<i>Nigrospora sphaerica</i>	15.4
<i>Rhizopus microsporus</i>	23.1
<i>Rhizopus oryzae</i>	15.4
Control	100.0
LSD (0.05)	1.9

Table 4: Virulence of the isolates on fresh tomato fruit (mm)

Fungal Isolate	Lesion size (%)
<i>Aspergillus flavus</i>	60
<i>Basidiobolus ranarum</i>	67
<i>Bipolaris spicifera</i>	46
<i>Chaetomium globosum</i>	73
<i>Malassezia furfur</i>	73
<i>Nigrospora sphaerica</i>	40
<i>Rhizopus microspores</i>	67
<i>Rhizopus oryzae</i>	73
Control	0.0
LSD (0.05)	4.1

DISCUSSION

This study was carried out in three different markets in Biu Local Government Area of Borno State. The incidence of fungal rot disease of tomato showed significant variation in the markets. Lashe Money market had the highest rot incidence and severity followed by Biu main market and the General Hospital market. Koka *et al.* (2022) conducted a similar study on the incidence and severity of fungi responsible for spoilage of products between harvest and consumption in Kashmir. Mailafia *et al.* (2017) carried out a study on isolation and identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria in which *Aspergillus niger*, *Fusarium avenaceum*, *Penicillium digitatum* and *Rhizopus stolonifer* were identified. The higher incidence may be due to the use of susceptible tomato varieties coupled with favourable environmental conditions for the growth and reproduction of the pathogenic fungal species in the area. The findings are in agreement with Palencia *et al.* (2010) who reported that *Aspergillus niger* has been found to be associated with and pathogenic to grapes and onions.

This study identified eight fungal species in Biu markets, namely *Aspergillus flavus*, *Basidiobolus ranarum*, *Bipolaris spicifera*, *Chaetomium globosum*, *Malassezia furfur*, *Nigrospora sphaerica*, *Rhizopus microsporus* and *Rhizopus oryzae*. Kator *et al.* (2018) identified *Aspergillus flavus*, *Penicillium waksmanii*, *Botryodiplodia theobromae*, *Fusarium oxysporum* and *Colletotrichum asianum*. Liamngee *et al.* (2015) isolated *Aspergillus flavus*, *Botryodiplodia theobromae*, *Aspergillus niger* and *Aspergillus fumigatus* from decaying yam tubers in storage. Dimphna (2016) identified *Penicillium*, *Aspergillus*, *Fusarium*, *Cladosporium* and *Rhizopus*.

The eight fungi identified in this study were not all present in a single market. In Biu General Hospital market, *Malassezia furfur*, *Nigrospora sphaerica* and *Rhizopus oryzae* were identified. In Biu main market, *Basidiobolus ranarum*, *Rhizopus microsporus* and *Chaetomium globosum* were identified and in Lashe Money market, *Bipolaris spicifera*, *Rhizopus microsporus*, *Aspergillus flavus* and *Nigrospora sphaerica* were observed, eight (8) fungal pathogens, namely *Aspergillus niger*, *Aspergillus flavus*, *Penicillium expansum*, *Fusarium oxysporum*, *Aspergillus fumigatus*, *Alternaria porri*, *Rhizopus stolonifer* and *Mucor species*. Nizami *et al.* (2021) isolated six different fungi namely *Alternaria solani*, *Alternaria alternata*, *Aspergillus niger*, *Geotrichum candidum*, *Fusarium oxysporum* and *Rhizopus stolonifer* from harvest.

In this study, *Rhizopus microsporus* had the highest frequency, followed by *Malassezia furfur*, *Nigrospora sphaerica* and *Rhizopus oryzae*. *Aspergillus flavus*, *Basidiobolus ranarum*, *Bipolaris spicifera*, and *Chaetomium globosum* had the same frequency. Mailafia *et al.* (2017) reported that *Aspergillus niger* had the highest frequency of occurrence in pineapple, watermelon, orange, pawpaw and tomato. Nizami *et al.* (2021) reported the highest frequency of occurrence in *A. solani* followed by *A. niger* and *G. candidum*. The lowest percentage infection was observed in *F.oxysporum*, followed by *A. alternata* and *R. stolonifer*.

The result of the pathogenicity test showed that there was a significant difference between the fungal isolates. All the isolates were very aggressive. *Chaetomium globosum*, *Malassezia furfur* and *Rhizopus oryzae* had the highest virulence, followed by *Bipolaris ranarum* and *Rhizopus microsporus*. *Aspergillus flavus*, *Basidiobolus spicifera* and *Nigrospora sphaerica* differed significantly. Result of the pathogenicity test showed that *Aspergillus ochraceous*, *A. flavus*, *Sclerotium rolfsii* and *P. citrinum* were highly pathogenic with the first three causing the rapid disintegration of treated fruits in 3-5 days. *A. niger* was moderately pathogenic, while *H. fulvum* was the least pathogenic on tomato fruits. These findings are in line with those of Chuku *et al.* (2008) who reported that *Fusarium spp.*, *R. stolonifer* and *Aspergillus spp.* were responsible for soft rot of tomato.

CONCLUSION

There is high incidence and severity of tomato fruit rot in Biu markets caused by pathogenic fungi. Toxigenic fungi namely *Aspergillus flavus*, *Basidiobolus ranarum*, *Bipolaris spicifera*, *Chaetomium globosum*, *Malassezia furfur*, *Nigrospora sphaerica*, *Rhizopus microsporus* and *Rhizopus oryzae* were found associated with tomato fruit rot. *Rhizopus microsporus* is a threat to tomato fruit storage in Biu because of its high frequency of occurrence and virulence level.

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