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**Fungal Foliar Pathogens of Tomato (*Lycopersicon esculentum* M.) in  
 Birnin Kebbi Local Government Area, Kebbi State, Nigeria**

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**ABSTRACT**

A study was conducted to identify fungi pathogens associated with diseased leaves of tomato cultivated in Birnin Kebbi Local Government Area Kebbi State, Nigeria from March to August, 2019. A total of One hundred (100) diseased leaves samples were collected from five locations namely; Harasawa, Kardi, Makera, Ambursa and Gulumbe of the study area. A total of eight (8) species of fungi namely; *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Alternaria porri*, *Rhizopus stolonifer*, *Fusarium oxysporum*, *Penicillium citrinum* and *Mucor* specie were identified from the samples collected using standard methods. From these results obtained *Aspergillus niger* had the highest frequency of occurrence (30%) followed by *A. flavus* (20%) while *Mucor* species had the lowest (2.5%). Based on locations, Ambursa had the highest fungal load 87.5% followed by Gulumbe 62.3% while Harasawa had the lowest 37.5%. The leaves of tomatoes could get infected easily due to the activity of fungal pathogens, there is need to sensitize the farmers regarding the effects of fungal infections on tomato plants and means of obtaining the resistant tomato cultivars so as to minimize the incidence of the disease in the study area.

**Keywords:** Fungi, Foliar, Pathogens, Tomatoes, Birnin-Kebbi

## 1.0 INTRODUCTION

Tomato (*Lycopersicon esculentum*), is an annual plant, having a weak woody stem covered with glistering reddish yellow glandular hairs that can grow up to 10 feet tall, but most species are less than three feet tall on average which known as “*Tumatori*” in Hausa. It is an important vegetable crop across the world that originated from West South America but distributed all over the world (Kimura and Sinha, 2008). These plant is widely cultivated in many parts of Birnin Kebbi and world that range from outdoor fields, greenhouses and net houses. They have a weak stem that often sprawls over the ground and vines over other plants (Sravanthi and Gangadhar, 2015). Fruit have smooth skin with diverse in size and shape, ranging from

small and round to large and variable shapes (Baiyewu *et al.*, 2007). However, it is green when

immature but becomes bright red or yellow as it ripens.

The fruits of tomato are popular throughout the world and are used in all kind of stews, soups and also eaten raw in salads. Ripe tomato fruits have high nutritive values, being a good source of vitamin A, B, C and minerals (Sabongari and Aliero; Anonymous, 2002). However, it contains high amount of carbohydrates, fats, organic acids, water, minerals, vitamins and pigments. Tomato fruits are used in garnishing various cooked food in Nigerian dishes as well as dishes in many other parts of the world. It is estimated that ripe tomato fruits contain

approximately 94 % of water, 4.3 % carbohydrates, 1 % protein, 0.1 % fat, 0.6 % fiber and vitamins. Antioxidant phytochemicals such as the carotene and lycopene are contained in tomatoes (Wogu and Ofuase, 2014; Sravanthi and Gangadhar, 2015). They are good sources of natural antioxidants which include carotenoids, vitamins, phenolic compounds, flavonoids, dietary glutathione, and endogenous metabolites and have been shown to eliminate free radicals (Sravanthi and Gangadhar, 2015; Pitchaon *et al.*, 2007). Because of 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 has been widely used not only as food, but also as research material. Tomato is a major vegetable crop in Birnin Kebbi, Nigeria and all over the world that had achieved tremendous popularity over the last century.

Root and crown rot as well as wilt diseases of tomato caused by *Fusarium oxysporum* f. sp. *Lycopersici* and *Fusarium oxysporum* f. *spradicislycopersici* pathogens which survive in soil are responsible for serious losses in vegetable crop yield (Lobo, 2000; Radwan and Al-masri, 2012; El-mohamedy *et al.*, 2013a; Amira *et al.*, 2013). Moreover, Early and late blights caused by *Alternaria solani* and *Phytophthora infestans* are the most important diseases attacking tomato plants under open field or plastic houses conditions (Khurana, 1998; Abd-el-kareem *et al.*, 2001; Abd-el-kareem *et al.*, 2002). Under plastic houses conditions tomato is reported to be infected with different root and foliar diseases, *i.e.* damping-off, root rot, white rot, wilt, early and late blights as well as leaf spots (Abd-el-kareem *et al.*, 2002; Simone and Momol, 2001; Hochmuth, 2003; El-mough *et al.*, 2012).

Pathogenic fungi, such as *Alternaria*, *Aspergillus*, *Fusarium*, *Mucor* sp., *Penicillium*, *Rhizopus* and *Trichoderma* have been implicated in some crop spoilage (Beuchat, 1995). Fungi contamination of many agricultural products, including tomatoes starts in the fields (Aran *et al.*, 1987). Few studies on fungi associated with tomato spoilage are available in Nigeria (Wokoma, 2008; Eni *et al.*, 2010; Wogu and Ofuase, 2014; Bashir *et al.*, 2016). Tomato plant shows susceptibility to the pathogen *Alternaria alternate* sp. *lycopersici*. This pathogen causes leaf

spot disease of tomato plants (*Lycopersicum esculentum*) which leads to reduction in photosynthetic yields. *Alternaria* weak pathogen, attacking already stressed plants, but may also be pathogenic on healthy plants, grows in long chains, with dark brown conidiophores and conidia (Bashir *et al.*, 2016).

*Lycopersicum esculentum* is a useful plant with nutritional, medicinal and health benefits. In spite of all these benefits, the plant is besieged by lots of diseases both in the field and post-harvest diseases which result in yield losses thus making its valuable components unavailable. Information on the occurrence of fungal foliar pathogens of tomato is limited in Kebbi State, which could be the basis for its management. To this point and to the best of our knowledge, there is no any documented record available on fungal diseases affecting the production of *Lycopersicum esculentum* in Birnin Kebbi. Cultivation and management of *Lycopersicum esculentum* are concept with great relevance to current economic issues at States, national, and global scale, not only in Kebbi State. Cultivation of tomato would benefit agricultural economy and boost financial income as crop vegetable productions generate higher profit, provides employment and income to the farmers. This study would provide a base line data regarding the fungal foliar disease of tomato which will aimed in developing management strategies thereby increasing the production and boost the financial income to the farmers in the study area.

## 2.0 MATERIALS AND METHODS

### 2.1 Study Area

The study was conducted in Birnin Kebbi Local Government Area, Kebbi State, Nigeria. Birnin Kebbi is a city situated in northwestern Nigeria with coordinated 12°27'13"N, 4°12'01"E. It is the capital city of Kebbi State and headquarters of the Gwandu Emirates. As of 2006 the city has an estimated population of 125,594. Birnin Kebbi is situated on the Sokoto River and connected by road to Argungu (45 km northeast), Jega (35 km southeast) and Bunza (45 km southwest) with the relatively abundant surface water resources in form of rivers. These rivers provide good sources of water for irrigation, domestic use, fishing and transportation. Birnin Kebbi has a tropical continental-type climate with a

wet season that lasts from April to October in the South and from May to September in the north, the dry season lasts for the remaining period of the year. Agriculture is the main occupation of the people especially in rural areas, including farming, fishing and livestock rearing. The annual temperature varies from 21°C to 38°C. The soil type found in the area ranges from heavy clay in the fadama areas to loamy, sandy loam and sandy soils. Mean annual rainfall is about 800mm. The fadama crops include tomato, onion and watermelon (Keta *et al.*, 2016).

### **2.2 Diseased Tomatoes Field Survey**

A survey was carried out in major tomato producing areas of Birnin Kebbi Local Government Area in five locations viz: Harasawa, kardi, Makera, Ambursa and Gulumbe. One farm per village was evaluated and noted in this study. Symptoms were closely observed and recorded as described by Kehinde (2011).

### **2.3 Sample Collection**

Fresh leaf samples of tomatoes showing disease symptoms were collected randomly from the five farms. Twenty (20) leaves samples especially from fresh leaves were obtained from each farm, which make a total of One hundred samples. Only the leaves that show any of the symptoms such as spots, blight, wilting and water soaked lesions were collected from each farm using sterilized razor blade. The leaves were placed in sterile plastic bags, labelled and kept cool to prevent them from desiccation and subsequent drying out. The collected samples were taken to Department of Plant Science and Biotechnology Laboratory, Kebbi State University of Science and Technology, Aliero for analysis. However, dead plants or organs were not included during the time of collection, because the tissue may have been invaded by decomposing fungi and bacteria, and the original pathogen would no longer be detectable.

### **2.4 Sterilization of Glasswares**

All glasswares such as Petri-dishes, test tubes, conical flasks, were washed thoroughly with detergent and sterilized by dry heat in hot air oven at a temperature of 160°C held for 45-60 min. Cooling time was allowed to enable the items in the oven to cool slowly. The oven door was not opened until the

temperature inside the oven has fallen below 50°C, to avoid cracking of glassware and air containing contaminated organism being drawn into the oven (Cheesbrough, 2002).

### **2.5 Preparation of Potato Dextrose Agar Medium**

Potato Dextrose Agar (PDA) was used. Its preparation was according to manufacturer's specification and instruction. 39.0 g PDA powder was weighed with weighing balance and homogenized in 1000ml distilled water inside one litre conical flask by gentle heating and stirring on laboratory hot plate. The homogenate was distributed equally in three (3) 500ml conical flasks, the mouth plugged with cotton wool and covered with aluminium foil. The homogenate was then sterilized by autoclaving at 121°C for 15 minutes. The medium was allowed to cool to about 45°C. Just before pouring it in aliquot of 20ml in Petri dishes, 0.1% streptomycin was incorporated to prevent growth of bacteria. Poured Petri dishes were left overnight to solidify and be certain of those free of contamination (Cheesbrough, 2002).

### **2.6 Inoculation and Incubation of the Symptomatic Leaves**

Symptomatic leaves that were collected from the field were rinsed in running tap water and small pieces, up to 2mm was cut out from the affected portions of the leaves. They were surface sterilized in 0.5% of sodium hypochlorite (NaClO) for 5 minutes, rinsed twice in sterile distilled water and the small pieces were inoculated aseptically on 90 mm diameter Petri dishes containing PDA (Potato Dextrose Agar) medium using flame-sterilized inoculation loops. Cultures were incubated at room temperature (28±2°C) for up to 7 days (Kehinde, 2011).

### **2.7 Isolation and Identification of the Fungal Organisms**

Sub culturing from the distinct colonies of mixed growth on freshly prepared PDA plates was carried out to obtain pure cultures. Slides of the mycelia observed from different fungal isolates were prepared as follows: A drop of lactophenol cotton blue solution was placed in the centre of a grease free clear glass slides. A small portion of unidentified fungal culture was cut out with inoculation needle.

The portion was placed in the lactophenol cotton blue droplet on the slide and teased out with another needle. A cover slip was lowered over teased portion and was mounted on the stage of a binocular microscope for viewing and examination. The characteristics such as structure of mycelia, fruiting bodies, color and shape of the upper thallus as well as the production of the pigment on the underside spore structure was noticed and identified with the help of standard mycology atlas (Ricciardi *et al.*, 2012).

**2.8 Determination of Frequency of Occurrence**

The frequencies of occurrence of fungi associated with disease leaves of tomato was determined using the formula described by Keta *et al.* (2016).

Percentage of frequency =

$$\frac{\text{Number of the identified isolate}}{\text{Total number of fungi isolated}} \times 100$$

**2.9 Data Analysis**

Statistical Package for Social Science was used for the data analysis. The fungi isolated were recorded

as frequency using tables as a descriptive statistical tool.

**3.0 RESULTS**

The fungal isolates associated with diseased leaves of tomato were identified based on spore appearance, morphology and cellular characteristics as shown in (Table 1). The isolates include *Aspergillus niger*, *Aspergillus flavus*, *Penicillium expansum*, *Fusarium oxysporum*, *Aspergillus fumigatus*, *Alternaria porri*, *Rhizopus stolonifer* and *Mucor* species. However, the results of frequency of occurrence of the fungal isolates associated with diseased tomato leaves were presented in (Table 2), where *Aspergillus niger* had the highest percentage (30%) followed by *A. flavus* (20%) while *Mucor* species were obtained with the lowest (2.5%) frequency of occurrence respectively. The distribution of the fungi isolates based on the locations were seen in (Table 3). Ambursa has the highest fungal load of 87.5% followed by Gulumbe with 62.3% while Harasawa had the lowest 37.5

**Table 1: Macroscopic and Microscopic Characteristics of the Fungi Species in the Study Area**

Fungal isolate	Macroscopic Examination	Microscopic Examination
<i>Aspergillus niger</i>	Black color with white edges	Large conidial heads, dark brown becoming radiate and split to columns
<i>Aspergillus flavus</i>	Dark colony The conidia were black in color. The hyphae were well developed, profusely branched, septate and hyaline. Flat and smooth conidia are borne in chains at the tips of sterigmata	It has ball head, two hyphae with ball heads. Presence of conidiophores with a lot of spores
<i>A. fumigates</i>	Yellowish green spore. The conidia were green in color. The hyphae were well developed, profusely branched, septate and hyaline flat and smooth conidia are borne in chains at the tips of sterigmata.	Spores are scattered, cytoplasm is coiled and has strands. Cell wall is thin
<i>Rhizopusstolonifer</i>	Whitish becoming brown-black	Non-septate mycelia with branching sporangiosphores
<i>Penicillium expansum</i>	Dark green spore. The culture was green diamond in color and sparkling as it matured	The conidia are globe-like and resemble glass beads under the microscope. The hyphae appeared branching and septate conidiophores were erect, septate and faint
<i>Alternaria porri</i>	Spore on MEA at 25c attaining a diameter of 6cm in 7 days, black or olivaceous-black or grayish	Conidiospores 1-3 septate, simple or sometimes branched, straight or flexuous or sometimes geniculate, with one or several apical spores, medium brown, smooth walled
<i>Mucor</i> species	Grey spore becoming brownish	Non-septate mycelia with branches sporangiosphores

**Table 2: Frequency of Occurrence of Identified Fungal Isolates from diseased leaves of Tomato in the Study Area**

Isolates	Frequency	Percentage (%)
<i>Aspergillus niger</i>	12	30
<i>Aspergillus flavus</i>	8	20
<i>Penicillium expansum</i>	2	5
<i>Alternaria porri</i>	6	15
<i>Aspergillus fumigates</i>	4	10
<i>Rhizopus stolonifer</i>	5	12.5
<i>Fusarium oxysporum</i>	2	5
<i>Mucor</i> species	1	2.5
<b>Total</b>	<b>40</b>	<b>100</b>

**Table 3: Distribution of Fungi Isolates with Respect to Locations**

Locations	Fungi isolate	Distribution (%)
Harasawa	<i>Aspergillus niger</i> , <i>Rhizopus stolonifer</i> , <i>Aspergillus flavus</i>	37.5
Kardi	<i>Aspergillus niger</i> , <i>Fusarium oxysporum</i> , <i>Aspergillus flavus</i> and <i>Mucor</i> species	50
Makera	<i>Penicillium expansum</i> , <i>Aspergillus fumigates</i> , <i>Aspergillus niger</i> , <i>Fusarium oxysporum</i> , <i>Aspergillus flavus</i> and <i>Mucor</i> species	75
Ambursa	<i>Alternaria porri</i> , <i>Aspergillus fumigates</i> , <i>Aspergillus niger</i> , <i>Fusarium oxysporum</i> , <i>Aspergillus flavus</i> , <i>Mucor</i> species and <i>Penicillium expansum</i>	87.5
Gulumbe	<i>Aspergillus niger</i> , <i>Fusarium oxysporum</i> , <i>Aspergillus flavus</i> , <i>Mucor</i> species and <i>Alternaria porri</i>	62.5

#### 4.0. DISCUSSION

This study was carried out in five (5) localities, namely, Harasawa, Kardi, Makera, Ambursa and Gulumbe in Birnin Kebbi LGA to determine the fungi species associated with the diseased leaves of tomatoes. Eight (8) fungal pathogens were isolated from the samples collected and they include: *Aspergillus niger*, *Aspergillus flavus*, *Penicillium expansum*, *Fusarium oxysporum*, *Aspergillus fumigatus*, *Alternaria porri*, *Rhizopus stolonifer* and *Mucor* species. These findings were in line with the study conducted by Keta *et al.* (2016) who reported that, *Aspergillus niger*, *Phaeoacremonium parasiticum*, *Rhizopus oryzae*, *Scopulariopsis fusca*, *Aspergillus flavus*, *Trichophyton rubrum*, *Syncephalastrum racemosum* and *Rhizopus stolonifer* are foliar fungi associated with Watermelon in Kebbi State. Thus, some fungi species were not detected in this present study, these might be due to difference in the study area and samples used. The higher number of these fungi in this present studied could probably due to environmental conditions such as temperature and relative humidity that favor the growth and activity of these fungi species in the area. These were conformity with the research of (Baiyewu *et al.*, 2007). Similar study was conducted by Ibrahim (2005) and Joon *et al.* (2001), indicated that *Aspergillus* species

were most frequently encountered during isolations in many parts of the tropical and humid regions of the world. Studies have shown that *Aspergillus* produce aflatoxins. Aflatoxins are associated with some diseases in livestock's and humans throughout the world. *Aspergillus flavus* is the main producer of the well-known carcinogenic aflatoxins and its presence in food is of huge concern in terms of food safety, they are toxic at low concentrations (Ghosh, 2009). The dominance of *Aspergillus* species in leaves of tomatoes could pose a serious health risk. *Fusarium* wilt of tomato caused by *Fusarium oxysporum* and *Rhizoctonia solani* causing damping off, cankers, root rots, fruit decay, foliage disease causes serious economic loss. *Fusarium oxysporum* penetrates the roots mainly through wounds and proceeds into and throughout the vascular system, leading to functional collapse, systemic wilting and often the death of the infected plant in the greenhouse (Latha *et al.*, 2009). Moreover, fungal phytopathogens are known to be the cause of many plant diseases and much loss of crop yields, especially in tropical and subtropical regions (Latha *et al.*, 2009)

It is clear from the result of this study that Ambursa has the highest fungal load (87.5%)

followed by Makera (75%) while Harasawa had the lowest (37.5%). The higher incidence may be due the use of susceptible tomato varieties coupled with favorable environmental conditions supporting the growth and reproduction of the pathogenic fungi species in the area. The findings are in accordance with Palencia *et al.* (2010) in his report that *Aspergillus niger* has been found to be associated with and pathogenic to grapes and onions. Based on *in vitro* culture and toxin production, it was cited as the pathogen causing such diseases as blight.

### 5.0 CONCLUSION

Several genera of fungi have been identified in this study as being associated with the leaves of tomato fruits. The understanding of the unique symptoms and the causal pathogen of tomatoes

leaves diseases in Kebbi State might or could facilitate easy management by farmers. The present study provides essential awareness on the symptoms and distribution of fungal pathogens in the selected fields of study.

### 6.0 RECOMMENDATIONS

Since these leaves of tomatoes can get infected easily by the activity of fungal pathogens (spore), there is need to sensitize the farmers regarding the effects of fungal infections on tomato plants and means of obtaining the resistant tomato cultivars. Similarly, Continuous monitoring of these diseases by plant pathologist is required to provide appropriate management strategies of the disease.

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