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INCIDENCE AND PATHOGENICITY OF FUNGAL PATHOGENS AFFECTING OKRA (*ABELMOSCHUS ESCULENTUS*) FRUIT WITHIN SOKOTO NORTH, SOKOTO

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ABSTRACT

Okra (*Abelmoschus esculentus*) is a widely cultivated vegetable that is highly susceptible to fungal infections, leading to significant post-harvest losses. This study investigated the incidence and pathogenicity of fungal pathogens affecting okra fruits in Sokoto North. Fresh samples of okra were obtained directly from three (3) local farms and stored for one week to observe signs of spoilage. Potato Dextrose Agar (PDA) was prepared at a concentration of 39 g per liter of distilled water according to the manufacturer's instructions and incubated. Identification was done based on morphological and microscopic characteristics using a compound microscope. Pathogenicity tests were conducted by inoculating healthy okra fruits with fungal isolates. Data were analyzed using descriptive statistics with percentage of occurrence to determine fungal prevalence, while mean and standard deviation for mycelial growth measurements. The study identified four fungal species: *Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus fumigatus*, and *Candida tropicalis*. Among these, *Rhizopus stolonifer* had the highest occurrence rate (40.00%), while the other species had (20.00%) each. Pathogenicity tests showed that *Aspergillus niger* had the highest incidence with (64.35%), followed by *Aspergillus fumigatus* with (60.26%), *Candida tropicalis* with (52.56%), and *Rhizopus stolonifer* with (42.56%). The study emphasizes the substantial risk posed by these fungal pathogens to okra crops, leading to post-harvest losses. It is

recommended that proper post-harvest handling and antifungal treatments should be explored to mitigate these pathogens.

KEYWORDS: (Okra, Fungal pathogens, Pathogenicity, *Aspergillus niger*, *Aspergillus fumigatus*).

INTRODUCTION

Okra (*Abelmoschus esculentus* L. [Moench]) is a popular herbaceous hirsute plant in *Malvaceae* family (also known as he mallow family) grown for its edible and fibrous seed pods. It has the typical floral characteristics of that family originating from Africa and widely distributed in the tropics including Nigeria. Okra has two colours: red and green, and is at times referred to as lady‘s finger’ (Onaebi *et al.*, 2020). The edible part is the juvenile fruit and when fully matured; can become fibrous and unsuitable for consumption. Indian, Nigeria, Pakistan, Ghana and Egypt are the most important okra producing countries of the world (Ibrahim *et al.*, 2021).

Okra as an economically important fruit vegetable plays a vital role by contributing carbohydrates, proteins, fats, minerals and vitamins in the precise proportion to maintain growth and development in human diet (El- Sayed and El-Sayed, 2012). Millions of Nigerians consume Okra as soup because of its low calories and high dietary fiber content. Okra mucilage is essential for certain medical and industrial applications. It helps to reduce blood cholesterol level and it can help maintain intestinal health (Helen-Sheeba and Sundar, 2018; Qusai *et al.*, 2020; Onaebi *et al.*, 2021).

Okra is susceptible to several diseases due to poor storage and postharvest handling from the farm to the market. Some varieties are highly susceptible to root decaying/root rot organisms while some are associated with deterioration of the fruits. Reported causal agents of okra fruit rot include the Genera *Fusarium, Altenaria, Curvularia, Helmithosporium, Chaetomium, Cladosporium, Penicillium, Aspergillus, Rhizopus, Rhizoctonia* (Helen-Sheeba and Sundar, 2018; Abubakar *et al.*, 2019; Qusai *et al.*, 2020; Ibrahim *et al.*, 2021). The inability to prolong the shelf life as a result of lack of information on organisms associated with the rot of okra, ultimately reduce the yield and income or net return of the farmer. However, losses are generally small during processing, storage and handling using equipment that are more efficient, good quality storage facilities, and close control of critical variables by a highly knowledgeable cadre of managers. Identification of organism (fungi) associated with Okra or

diseases of okra will be a pointer to proffering solution to postharvest rot or diseases of okra and improve its shelf life (Abubakar *et al.*, 2019).

The most serious fungal diseases of okra in Africa are damping-off (*Macrophomina phaseolina*, *phythium aphanidermatum*, and *rhizoctonia solani*), vascular wilt (*Fusarium oxysporum*), Cercospora blight (*Cercospora Abelmoschus, Cercospora malayensis*) and powdery milded (*Erysiphe cichoracearum, Oidium abelmoschi*) (Ricciardi *et al.*, 2012).Okra mosaic virus (OKMV), transmitted by flea beetles (*Podagrica*), is widespread in Africa but damage is much less important than that caused by okra leaf curl disease (OLCV), transmitted by whitefly (*Bemisia tabaci*).

Okra is a perishable commodity which deteriorates and this can cause reduction in its quality and quantity during handling and storage processes. Traditionally, the fresh okra chips are indiscriminately sun-dried on roof tops, concrete constructions, and along roadsides for some weeks depending on the intensity of sunlight.

Different control measures including chemicals, plant extracts and biocontrol have been used to reduce diseases in different crops and increase yield. Generally, the use of plant extracts for control of diseases is favoured (Akueshi *et al.*, 2002; Olusanmi and Amadi, 2009; Amadi *et al.*, 2010) because they are non toxic and are easily biodegradable. Some then pour into sterile Petri dishes. This medium is suitable for the cultivation and differentiation of fungi and is often used with antibiotics for the isolation of pathogenic fungi from material containing large numbers of other fungi or bacteria.

The aim of this study is to isolate and characterize fungal pathogens associated with Okra (*Abelmoschus esculentus*) diseases in Sokoto metropolis. The specific objectives are; to identify and characterize fungal pathogens associated with Okra fruit (*Abelmoschus esculentus*) in the Sokoto North and to determine the frequency of occurrence of fungal pathogens.

MATERIALS AND METHODS

Study Area

This research work was carried out in Biology Laboratory of Sokoto State University, Sokoto located within the latitude 13° 12’ 98’ N, longitude 5° 20’ 36’ E and altitude 302 m above the

sea level. Sokoto is located in the North West of Nigeria, between latitudes 13° 4' 07' north and longitudes 05° 14' 49' east and above 265 m the sea level.

Sample Collection

Spoilt Okra fruits were collected from three different places: Wamakko, Sokoto north and Sokoto south in Sokoto. The symptoms were carefully noted; completely rotten fruits were avoided for isolation as they contained mostly secondary pathogens. The collected fruits were transferred to microbiology laboratory, Sokoto State University for analysis (Ricciardi *et al.*, 2012). Twenty-five (25) grams portion of the sample was sterilized and wrapped in nylon and allow to undergo deterioration over a period of 5-7 days in the laboratory.

Media Preparation

The culture medium used in this research work was prepared in accordance to the manufacturer's instructions using standard aseptic technique (Cheesbrough, 2000).

Inoculation and Incubation

The direct plating technique described by Pitt and Hocking (1985) was employed. The affected tissues were surfaced-sterilized with 10% ethanol using a cotton wool. Four small pieces from each sample were directly inoculated aseptically on prepared sterile plates of Potato dextrose agar (PDA) and incubated at 28^{o}C for 7 days.

Sub-culturing

When fungal growth from the tissue was visible, fungi were sub cultured onto freshly prepared sterile PDA plates to obtain a pure cultures for identification. Where there is a mixed culture, fungi were continuously sub cultured until pure isolates were obtained. Stock cultures of the pure isolates were prepared and preserved at 4^{o}C in the refrigerator (Ricciardi *et al.*, 2012).

Sterilization of Glass Wares

The glass wares were washed thoroughly with detergent and sterilized using a hot air oven at 160°C for one hour. Then, the oven was allowed enough time to cool, to prevent, the glass from cracking and for ease of handling.

Sample Preparation

About one gram of the deteriorating okra sample was aseptically transfer into sterile 20 cm^2 beaker and thoroughly mixed with glass rod to form an homogeneous suspension and 1 ml of

the aliquot of the sample was pipetted into sterile test tubes containing 9 ml of the sterile distilled water, each suspension was serially diluted in another 8 sets of test tube to dilution ration of 10-8 about 0.1 ml portion of the sample suspended from the 6th and 7th dilution factors was aseptically pipetted separately into different sterile petri dishes and thoroughly mixed with 20 ml of the cool molten Agar medium. The plate was gently swirled for even distribution of the sample aliquot with the media and allowed to set and incubated at optimum temperature. PDA plates for fungi were incubated at 27±2 °C for 48 to 72 hours or more. At the end of the incubation period, the colonies forming from the viable fungi spores were counted and recorded appropriately (Ricciardi *et al.,* 2012). When fungal growth from the tissue was visible, fungi were sub-cultured onto freshly prepared sterile PDA plates to obtain a pure culture for identification. Where there is a mixed culture, fungi were continuously sub cultured until pure isolates were obtained. Stock cultures of the pure isolates were prepared and preserved at 4 °C in the refrigerator (Ricciardi *et al.,* 2012).

Isolation and Identification of Fungi

Successive hyphae tip was transferred to fresh media until pure cultures of each of the fungi were obtained. Pure isolate of the fungal culture was obtained by aseptically transferring fungal hyphae to freshly prepared SDA plates and incubated for seven days. This was done until pure cultures were obtained using single spore isolation technique. The pure isolates obtained were transferred to freshly prepared SDA slants for further studies. Each fungal colony was viewed under the microscope using x 40 magnification and then compared to Color Atlas of Mycology (Mutegi *et al.,* 2009; Negedu *et al.,* 2010; Adetunji *et al.,* 2012).

Identification of Fungi Isolates by Cultural and Biochemical Test

Preliminary identification: colony characteristics of the pure fungi isolate were examined on the solid agar surface after 48-72 hours of the incubation. These characteristics include the colour, shape, opacity, translucency, elevation, edges and surface texture.

Identification of Fungal Isolates

The fungal isolates were subjected to certain comparative morphological studies by an image and analysis system using published descriptions in a mycological atlas contained in the Microbiology Laboratory of Department of Biological Sciences Sokoto State University. This was followed by a slide mount of each isolate. The characteristics observed were matched with those available in the aforementioned mycological atlas and were identified accordingly (Adebayo and Okonko *et al.,* 2012).

Microscopic Observation

Pure isolates of fungi obtained were identified by staining with lacto phenol cotton blue stain and observed microscopically. A wet mount of the fungi isolate was prepared by placing a drop of lacto phenol blue in the centre of a clean slide. Mounting needle was sterilized in the Bunsen flame, allowed to cool and used to transfer little of the fungal mycelia into the drop of the stain solution on the slide. The hyphae were carefully teased with the needle. Then the preparations were covered with cover strips avoiding air bubbles and thereafter examined under the low power objectives of the microscope (X10-X40 objective). Cultural characteristics of the fungal isolated, the shape and colour of the hyphae, microscopic and macroscopic observation of the individual spores condiophores, phialides were employed in the identification and characterization of different species of fungal isolate.

Determination of Percentage Occurrence of the Fungal Isolates

The frequency of occurrence of the different fungal isolated from the three major market in Sokoto metropolis was determined for each isolate at each location. Isolation was made from different okra fruits showing rot symptoms. The frequency of occurrence of each isolate in each location was calculated as described by Carlson (2014). % Frequency = Number of identified fungi/Total number of fungi x 100.

Data Analysis

Data collected from the study were subjected to descriptive analysis by interpreting the data into table, frequency and chart.

RESULTS

Morphological and Microscopic Characteristics of Fungal Pathogens Isolated from from Okra Fruit (*Abelmoschus esculentus*)

Four species from three genera of fungal pathogens, including *Candida*, *Aspergillus*, and *Rhizopus*, were identified. The *Aspergillus* species include *Aspergillus niger* and *Aspergillus fumigatus*, while the genus *Rhizopus* is represented by *Rhizopus stolonifer*. Additionally, the genus *Candida* is represented by *Candida tropicalis* (Table 1).

Table 1: Morphological and Microscopic Characteristics of Fungal Pathogens Isolated from from Okra Fruit. (*Abelmoschus esculentus*)

Specimens	Front View Appearance on Petri Plate	Rear View Appearance on Petri Plate	Microscopy Features	Description (Common Features)	Organism Identified
A	Creamy, smooth, and moist colonies	Pale yellow or off-white	(A) Opaque cells appear clonal and polymorphic (B) White cells appear circular and elongated.	Forms yeast-like colonies, characterized by polymorphic cells ranging in size from approximately 2–10 micrometers.	*Candida tropicalis*
B	Black, powdery, and circular colonies	Yellowish to pale brown pigmentation	Conidiophores are short (2-3 µm), smooth, colored, or rough; vesicle is round with a convex head.	A filamentous fungus forming dark spores with filamentous hyphae, giving colonies a powdery texture.	*Aspergillus niger*
C	Greenish-gray, velvety colonies	Pale yellow to light green	Conidiophores are smooth-walled, unseptated, up to 300 µm long, and terminate in dome-shaped vesicles 20-30 µm wide.	Produces smooth, velvety colonies with unseptated hyphae and characteristic green pigmentation.	*Aspergillus fumigatus*
D	White, fluffy colonies with black spots	Brownish-black pigmentation	Composed of branched, non-septate hyphae (length 497–1400 µm, diameter 27–32 µm).	A filamentous mold producing stolons and black sporangia, giving colonies a fluffy white appearance with dark dots.	*Rhizopus stolonifer*

Frequency of Fungal Pathogens Isolated from Okra Fruit (*Abelmoschus esculentus*) in Sokoto North Local Government Area, Sokoto

The results showed that *Rhizopus stolonifer* had the highest percentage of occurrence with (40.00%) while *Candida tropicalis, Aspergillus Aspergillus niger and Aspergillus fumigatus* had the least percentage of occurrence with (20.00%) each (Table 2).

Table 2: Frequency of Fungal Pathogens Isolated from Okra Fruit (*Abelmoschus esculentus*) in Sokoto North Local Government Area Sokoto

Fungal Isolates	**SOKOTO NORTH**			
	S/N A	**S/N B**	**S/N C**	**Total (%)**
Candida tropicalis	0(0.00)	1(100.00)	(0.00)	1(20.00)
Aspergillus niger	0(0.00)	0(0.00)	1(100.00)	1(20.00)
Aspergillus fumigatus	1(100.00)	0(0.00)	0(0.00)	1(20.00)
Rhizopus stolonifer	1(50.00)	0(0.00)	1(50.00)	2(40.00)
Total (%)	**2(40.00)**	**1(20.00)**	**2(40.00)**	**5(100.00)**

KEYS: S/N = SOKOTO NORTH

Length and Percentage of the Mycelia Growth of Fungal Pathogens in Treatment Replica (Pathenogenicity)

Among the four fungal isolates studied, *Aspergillus niger* exhibited the highest level of pathogenic impact on okra fruits with (64.35**%**) damage rate, followed by *Aspergillus fumigatus***,** with (60.26**%**) damage, and *Candida tropicalis* with (52.56**%**) damage rate**.** *Rhizopus stolonifer* showed the lowest percentage of mycelial growth with (42.56**%**) damage on okra fruits (Table 3).

Table 3: Length and Percentage of the Mycelia Growth of Fungal Pathogens in Treatment Replica. (Pathenogenicity)

Test fungus	**Original length of the apple fruit(mm) (A)**	**Length of mycelia growth in Okra fruit (mm)**			**Average (B)**	**% damage (B)/(A) × 100**
		A	**B**	**C**		
Candida tropicalis	65	21.00	30.5	51.00	34.17±15.33	52.56%
Aspergillus	65	34.50	40.50	50.50	41.83±8.08	64.35%

niger						
Aspergillus fumigatus	65	27.00	21.00	69.50	39.17±26.44	60.26%
Rhizopus stolonifer	65	18.02	20.00	45.00	27.67±15.04	42.56%

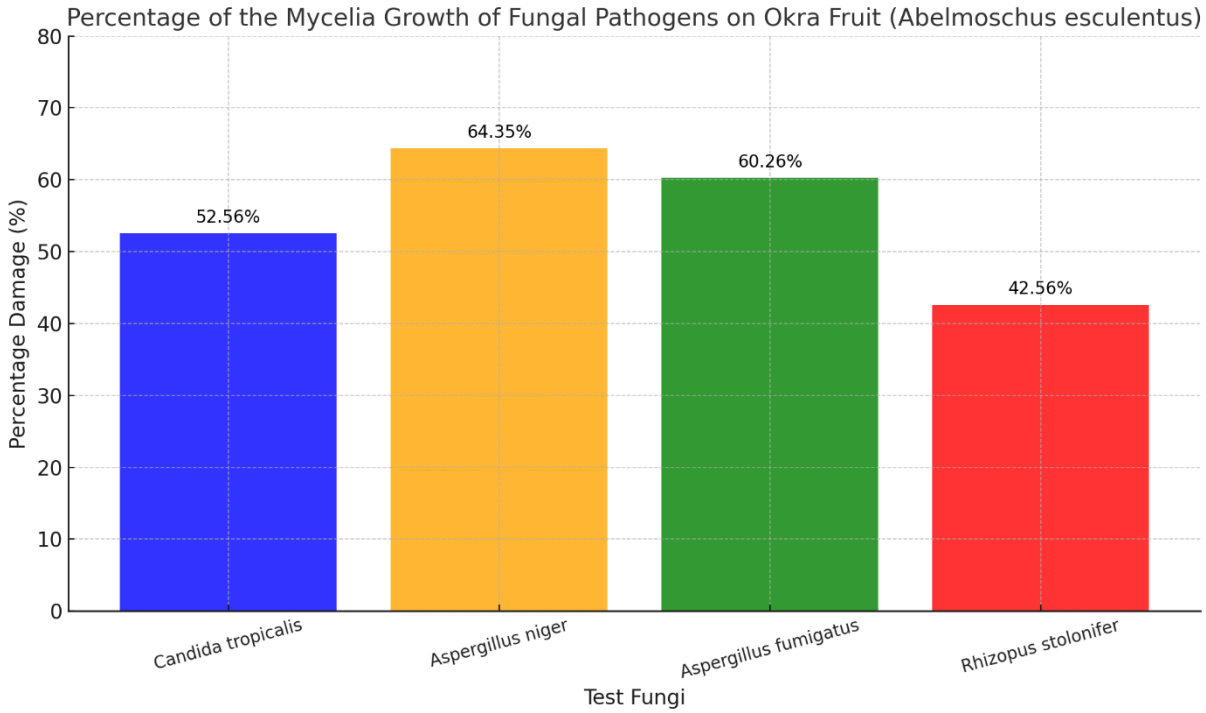

Figure 1: Percentage of the Mycelia Growth of Fungal Pathogens in Treatment Replica. (Pathenogenicity)

DISCUSSION

The fungal pathogens associated with Okra (*Abelmoschus esculentus*) diseases in Sokoto north four different fungi was identified; *Aspergillus niger*, *Aspergillus fumigatus*, *Rhizopus stolonifer* and *Candida tropicalis*. These organisms are commonly implicated in the postharvest deterioration of many crop produce and have been reported severally (Oyetunji *et al.*, 2012). Henz *et al.* (2007) have reported *R. solani* in okra fruits in Brazil. These organisms generally gain access into the crops by several means. While some of them utilize wounds created in different ways on the surface of the plant materials others may access the crops through natural openings on the surface of the produce (Akinyele and Ikotun, 1989).

Rhizopus stolonifer had the highest percentage of occurrence, while others had the least occurrence. These organisms are similar to those isolated by Nartey (2011); Kone *et al.*

(2012); Lamichhane *et al.* (2015) and Catarino *et al.* (2016) who reported the isolation of fungal species from blighted vegetables. Similar fungal species have been isolated from blighted tomato plants by Muhammed and Muhammad (2013). *A. niger, Fusarium oxysporum* and *Rhizopus nigricans* were isolated from blighted tomato, okra and cabbage seedlings as reported by Muhammed *et al.* (2016).

Among the four fungal isolates studied, *Aspergillus niger* exhibited the highest level of pathogenic impact on okra fruits with (64.35**%**) damage rate, followed by *Aspergillus fumigatus***,** with (60.26**%**) damage, and *Candida tropicalis* with (52.56**%**) damage rate**.** *Rhizopus stolonifer* showed the lowest percentage of mycelial growth with (42.56**%**) damage on okra fruits. These fungal species lead to loss of yield and quality of the crop (Muhammed and Muhammad, 2013). Proper sterilization of the soil to deactivate the effects of soil and surface borne fungi species associated with the rhizosphere and phyllosphere causing blight disease.

A considerable number of fungal pathogens belonging to the genera of *Fusarium, Aspergillus, Colletotrichum, Rhizopus* and Penicillium have been detected in okra by many researchers (Alam, 2011; Jamadar *et al.,* 2013). The presence of these fungal pathogens on okra fruits suggests that they used compromised surfaces of the fruits such as wounds to cause rots.

CONCLUSION

- Four species of fungal pathogens belonging to three genera *Candida, Aspergillus,* and *Rhizopus* were isolated from okra (*Abelmoschus esculentus*) fruits.
- Pathogenicity tests showed that *Aspergillus niger* had the highest damage on okra fruits and *Rhizopus stolonifer* had the least damage.

Recommendations

1. Farmers and traders should adopt proper postharvest handling methods to reduce mechanical injuries on okra fruits, as wounds serve as entry points for fungal pathogens such as *Aspergillus niger* and *Candida tropicalis*.
2. Eco-friendly bio-fungicides derived from medicinal plants or other natural sources should be explored and applied to control fungal pathogens without harmful chemical residues on produce.
3. Careful postharvest management should be adopted by farmers and marketers to reduce the fungal load and possible spoilage of okra fruit in the study area.

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