



Isolation and Identification of Fungi Species Responsible for Leaf Spot Disease of *Abelmoschus Esculentus* L. (Okra) in Donga lga Taraba State.



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ABSTRACT

Cercospora leaf spot (CLS) is among the most economically important foliar diseases affecting okra, *Abelmoschus esculentus* L., reducing photosynthetic capability, losses of yield and premature defoliation. This work was designed to isolate and identify fungi associated with leaf spot of okra in farms in Donga, Taraba State. Samples of disease leaves were collected from five farms randomly in the study location. The infected leaves were collected using a sterile paper bag and conveyed to the Microbiology laboratory of Federal University Wukari, under aseptic condition for the isolation of the causal organisms. Small pieces of lesions were surfaced sterilized with alcohol and inoculated on prepare plates of potato dextrose agar (PDA). After 48 to 62 hours of inoculation, when fungal growth was visible, fungi were sub cultured on to a fresh PDA media to obtain pure cultures. Pure isolated fungi were identified according to recommended references and fungi atlas. Two fungal species associated with leaf spot of okra were isolated. The isolated fungi were *Cercospora abelmoschi* and *Cercospora malayensis*. The findings showed the role of *Cercospora* spp. as the major causal organism of leaf spot disease in okra within Donga. Okra production is in great danger if the fungi are not properly managed as they destroy the leaves affecting photosynthesis leading to low yield in okra fruit production in Northern Nigeria. Fungal diseases like damping-off, powdery mildew, Cercospora leaf spot and fusarium wilt significantly reduce okra production in northern Nigeria by causing wilting, leaf damage, stunted growth and fruit rot. These diseases lead to yield losses, decrease quality and can even cause seedlings death, impacting both the quantity and market value of okra. Integrated pest management strategies such as crop rotation and resistant varieties are recommended to reduce the spread and incidence of CLS in okra production.

Keywords:

Isolation, Identification, Cercospora, Leaf Spot, Disease.

INTRODUCTION

Okra was earlier included in genus *Hibiscus*, section *Abelmoschus* in the family Malvaceae (Wang *et al.*, 2023). The section *Abelmoschus* was subsequently proposed to be raised to the rank of distinct genus by. The wider use of *Abelmoschus* was subsequently accepted in the taxonomic and contemporary literature. This genus is distinguished from the genus *Hibiscus* by the characteristics of the calyx, spatulate, with five short teeth, connate to the corolla and caduceus after flowering (Akinyele *et al.*, 2021; Wang *et al.*, 2023). The taxonomic status of okra has been widely accepted, with *Abelmoschus esculentus* being the most economically important species in the genus (Oladipo *et al.*, 2023). According to Malvaceae Info (Hinsley, 2024),

there are about eight-fifteen species of *Abelmoschus* namely: *A. moschatus*, *A. manihot* (L.) *A. esculentus* (L.) *A. tuberculatus*, *A. ficulneus* (L.) *A. crinitus*, *A. angulosus*, *A. caillei*, and *A. esculentus* is the most important of all these species. In Nigeria, there are two distinct seasons for okra, the peak and the lean seasons. During the lean season (November to June) okra fruit are produced in low quantities, scarce and expensive to get. In the peak season (July to October), it is produced in large quantities much more than what the local populace can consume (Adedeji *et al.*, 2020). Okra (*Abelmoschus esculentus* L.), commonly known as lady's finger is a warm-season vegetable crop belonging to the Malvaceae family, widely cultivated in tropical and subtropical regions (Akinyele *et al.*, 2021).

In Nigeria, okra is a significant horticultural crop, valued for its role in income generation, cultural diets (Usman *et al.*, 2023) and food for humans and organisms (Abdul *et al.*, 2025).

Cercospora leaf spot (CLS), caused by the fungal pathogen *Cercospora* species, is a significant disease affecting various crops, including soybeans, peanuts, and sugar beets, leading to substantial yield losses globally (Shrestha *et al.*, 2020). The disease manifests as small, grayish-white lesions with dark borders on leaves, often progressing to defoliation under favorable warm, humid conditions. It is a highly virulent fungal disease affecting multiple crops (Jardine, 2020), with severity influenced by environmental condition, pathogen virulence factors, and host susceptibility (Shrestha *et al.*, 2020). Its destructiveness stems from necrotic lesions reducing photosynthetic capacity, leading to defoliation, lower yields, and reduced quality (Jardine, 2020). Key virulence factors include Cercosporin, a light-activated toxin, and effector proteins like CbNip1, which enhance necrosis, particularly in sugar beets. *Cercospora* leaf spot is a globally prevalent fungal disease caused by species of the genus *Cercospora*, primarily affecting a wide range of crops including sugar beet, soybean, and leafy vegetables (Abbas *et al.*, 2020). The disease manifests as circular to irregular brown or gray lesions on leaves, often leading to premature defoliation and significant yield losses (Kumar *et al.*, 2022). *Cercospora beticola*, the most studied species, is notorious for affecting sugar beet and is transmitted through airborne conidia, crop residue, and infected seeds (Li *et al.*, 2023). Environmental conditions such as high humidity, leaf wetness moderate temperatures and salinity favor the development and spread of disease and pathogen (Nouri *et al.*, 2019; Mustapha *et al.*, 2023). Effective disease management relies heavily on integrated approaches, including the use of resistant cultivars, fungicide application, crop rotation, and sanitation practices (Zhang *et al.*, 2021). However, resistance to commonly used fungicides such as QoI and DMI groups has been increasingly reported, complicating control measures (Fernandez *et al.*, 2021). The aim of the study is to isolate and identify the fungi species associated with leaf spot disease of okra in Donga, Taraba State.

MATERIALS AND METHODS

Study Area

The study was conducted at the Microbiology laboratory, Department of Microbiology, Federal University Wukari, Taraba State.

Sample collection

Diseased leaves samples showing symptoms of *Cercospora* leaves spot were collected randomly from five selected farms in Donga local Government area of Taraba State, Nigeria located on Latitude 7°15'N - 7° 85'N and longitude 9°47'E - 9°78'E in the morning

between 8 to 10 am, diseased samples labelled were conveyed using sterile paper bags under aseptic condition to the Microbiology Laboratory of Federal University Wukari for further investigation. Leaves were identified as okra leaves with the help of a Botanist in the Department of Biological Sciences, Federal University Wukari, Taraba State.

Sterilization of Glass Wares

All the glass wares first washed with tap water and detergent solution. They were then rinsed with distilled water and air dried. The glass petri-dishes were wrapped with aluminium foil and autoclave at 160°C for one hour (1 hr). They were allowed to drop for 30 minutes before usage to avoid cracking.

Preparation of media

Potato Dextrose Agar (PDA) was used for fungal cultures, and it was prepared according to manufacturer's instructions, 3.9 g in 100 ml of distilled water and autoclaved at 121°C for 15mins. It was allowed to cool to 37 °C before pouring in to 90 mm petri dishes, and then kept to solidify. Streptomycin was added as a bacteriostatic agent to inhibit the growth of bacteria in the medium.

Isolation of fungi associated with leaf spot disease of okra in the study area

Infected okra leaves showing typical *Cercospora* leaf spot symptoms (brown spots with yellow halos) were collected and placed in paper bags and labeled appropriately. The Chiejina (2008) isolation method was used. Surface-sterilization (70% ethanol) s performed to remove external contaminants (dust, other fungi). Small sections approximately 2 mm in diameter from the margin of the lesions (area between healthy and diseased tissue) were cut and dipped into 70% ethanol for 30 seconds and transferred to 1% sodium hypochlorite (NaOCl) solution for 2 minutes. It was rinsed three times in sterile distilled water to remove any chemical residues. Through the use of sterile filter paper, the sample (diseased leaf sample) was blot-dried and inoculated onto Potato Dextrose Agar (PDA) medium for sporulation for 7 days. Daily observation of the plates for fungal growth was noted.

Sub-Culturing

When fungal growth from the lesions was visible, fungi were sub-cultured on to freshly prepared PDA to obtain pure cultures for identification. Fungi were continuously sub-cultured until pure isolates were obtained. The pure fungal cultures were stored safely in the refrigerator at 4°C to prevent any further fungal growth in the plate.

Once fungal colonies emerged, small portions of the growing fungus were transferred onto fresh PDA plates using a sterile needle to obtain pure isolates without contaminants.

Identification of the Isolates

The fungal isolates were subjected to certain comparative morphological studies by an image and analysis system using published descriptions in a mycological atlas. This was followed by a slide mount of each isolate. The cultural and morphological identification was carried out based on macroscopic and microscopic characteristics using standard mycological keys (Watanabe, 2002; Alexopoulos *et al.*, 2021).

Pathogenicity Test

Koch's postulates were evaluated to establish the pathogenicity of the isolated fungi. The isolates were used in the pathogenicity test. A hyphae suspension of 7-day old culture grown on potato dextrose agar plates were homogenized in distilled water. Healthy leaves were inoculated with the hyphal suspension and some healthy leaves were sprayed with sterile distilled water. The leaves were individually covered with plastic bags to maintain a relative humidity of 100% for 24 hrs and then maintained in a screen house. Typical symptoms of *Cercospora* leaf spots appeared on the inoculated leaves 10 days after inoculation and were identical to those observed in the field. Fungi re-isolated from the symptomatic leaf tissues were morphologically identical to the original isolates. The pathogenicity test was conducted twice and showed similar results, fulfilling Koch's postulates. No symptoms were observed on control plants which were not inoculated with disease.

RESULTS AND DISCUSSION

Isolated fungi

The following fungi were isolated from the disease okra leaves collected from the field:

Cercospora abelmoschi and *Cercospora malayensis*. The colonial morphology on PDA and photomicrographs of isolated fungi as shown in plate (1 to 4).



Plate 1 Seven-day old culture of *Cercospora abelmoschi* (Back of petridish showing white mycelia of *Cercospora abelmoschi* growing on PDA)



Plate 2. Micrograph of *Cercospora abelmoschi*



Plate 3. Seven-day old culture of *Cercospora malayensis*

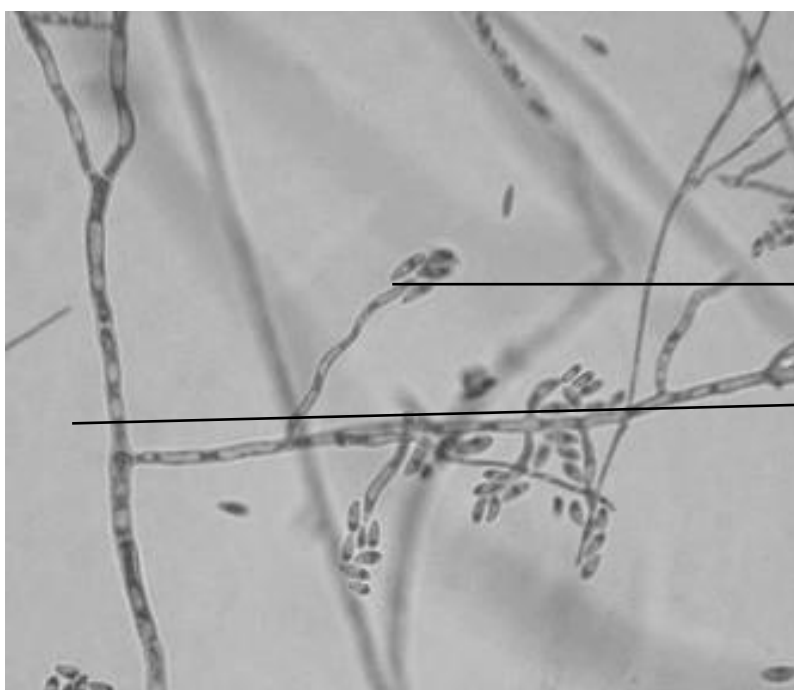


Plate 4. Micrograph of *Cercospora malayensi* X 40 showing conidia and conidiophores.

From infected okra leaves exhibiting characteristic *Cercospora* leaf spot symptoms, a number of fungal isolates were isolated, they include *Cercospora abelmoschi* and *Cercospora abelmoschi*. According to Nwankwo *et al.*, (2021), *Cercospora* spp., *Fusarium* spp.,

and *Aspergillus* spp. were the commonest fungi found based on microscopic and cultural traits with infected leaves of okra with leaf spot. Giri *et al.* (2022) reported that integrated use of botanicals and fungicides effectively managed *Cercospora* leaf spot disease in okra.

One of the most damaging foliar diseases of okra, *Cercospora* leaf spot (CLS) is caused by *Cercospora* species and results in early leaf defoliation and decrease in yield (Olawale *et al.*, 2024). The pathogen favours spore germination and infection in humid, moderately heated settings (Shrestha *et al.*, 2020; Li *et al.*, 2023).

The *Cercospora* species also produces a toxin called Cercosporin which led to further development of disease which is in agreement with the report of Urezula *et al.* (2020).

Leaf spot disease of okra is a major economic important disease especially in Donga local government where there is a reduction in the production of okra fruits up to 60 % due to this foliar disease. It became necessary to know the causative agents of this foliar disease and proffer solutions to reduce the loss been experienced by farmers in the area.

The results of this investigation align with worldwide findings that *Cercospora* species can endure in agricultural leftovers, underscoring the importance of cultural practices such residue management (Kumar *et al.*, 2022). Additionally, Koch's postulates were fulfilled by the pathogenicity test, which verified the isolated fungi's causative involvement in producing symptoms identical to field infections. This is consistent with comparable findings in related crops including sugar beetroot and soybean (Abbas *et al.*, 2020). Leaf spot disease of okra is a major economic important disease especially in Donga local government where there is a reduction in the production of okra fruits up to 60 % due to this foliar disease. It became necessary to know the causative agents of this foliar disease and proffer solutions to reduce the loss been experienced by farmers in the area. Symptoms of leaf spot disease cause by *C. abelmoschi* and *C. malayensis*, which were encountered in the study area during the research included, leaf spot, irregular brown or angular black spots.

CONCLUSION

Isolation of fungi were made from infected okra leaves with symptoms of leaf spot in Donga by tissue isolation and associated fungi were identified as *C. abelmoschi* and *C. malayensis* which were confirmed by comparative macroscopic and microscopic analysis, cultural and morphological analysis. Symptoms of leaf spot disease cause by *C. abelmoschi* and *C. malayensis*, which were encountered in the study area during the research included, leaf spot, irregular brown or angular black spots. Adoption of resistant cultivars, fungicide rotation, and proper field sanitation are recommended to mitigate disease impact.

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