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Research Article

Fungicidal Effects of Neem Plant Parts Extracts Against Two Phytopathogenic Fungi Responsible for Leaf Spot of Okra (*Abelmoschus esculentus* L) in Donga, Taraba State, Nigeria

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ABSTRACT

Fungicidal effects of aqueous extract of *Azadirachta indica* plant parts (leaves, bark and roots) were determined on fungi that cause leaf spot of okra in Donga. In the study, the phytopathogenic fungi were isolated from infected okra leaves and identified based on morphological and cultural characteristics, namely: *Cercospora abelmoschi* and *Cercospora malayensis,* as confirmed by pathogenicity test using Koch's postulate. Different concentrations (25, 50, 75 and 100 % w/v) of aqueous extracts of the leaves, bark and roots were separately added to growth media prior to inoculation (poisoned food method). All aqueous extracts of *Azadirachta indica* plant parts significantly ($P < 0.05$) reduced mycelia growth of the fungal pathogens and these effects gradually increased with increasing concentration. The highest inhibition was at 100% concentration, while the lowest inhibition percentage was observed at 25% concentration for each of the extracts. Statistically, there was a significant difference in the inhibition of radial growth in all the fungal isolates at all concentrations compared to the control. However, aqueous extracts of neem leaves were more effective on *Cercospora abelmoschi* with a higher inhibition percentage of 83.00% at 100% concentration, while aqueous neem root extracts had the lowest inhibition percentage of 25.13% at a concentration of 25% against *Cercospora malayensis*. It could be emphatically concluded that aqueous extracts of neem plant parts can effectively control fungal disease of okra leaves. This makes them potential biocides in disease control in that they are available, cheap, and safe.

Keywords: Aqueous; Extracts; Fungicidal effect; Inhibition; Pathogenic

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INTRODUCTION

Okra was earlier included in genus *Hibiscus,* section *Abelmoschus* in the family Malvaceae. The section *Abelmoschus* was subsequently proposed to be raised to the rank of distinct genus by the wider use of *Abelmoschus* which was subsequently accepted in the taxonomic and contemporary literature. This genus is distinguished from the genus *Hibiscus* by the characteristics of the calyx, spathulate, with five short teeth, connate to the corolla and caduceus after flowering (Tripathi *et al.,* 2011). Taking classification of Van Borssum Waalkes as the starting point, an up-to-date classification was adopted at the International Okra Workshop held at National Bureau of Plant Genetic Resources (NBPGR) in 1990 (IBPGR, 1991).

According to Oyenuga and Fetuga (1975), there are about eight (8) 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.* However, *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 (Bamire and Oke, 2003). During the lean season (November to June) okra fruits are produced in low quantities, scarce and expensive to get (Bamire and

Oke, 2003). In the peak season (July to October), it is produced in large quantities, much more than what the local populace can consume. It has been reported by Gemede *et al.* (2015) that proper processing, preservation, marketing and utilization of okra is necessary to arrest the wastage being experienced during the peak season due to problem of storage facilities and diseases. 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 (Gemede *et al.,* 2015). Native to Africa, okra is a versatile crop grown for its tender pods, which are consumed fresh, cooked, or processed. Gemede *et al*. (2015) also reported that there are 2,283 accessions out of which 2,029 were collected from the African continent of which 1,769 were from West Africa. The crop is therefore far more heavily represented in West Africa than in any other part of the world (Gemede *et al*., 2015). It is an annual crop with a life-span ranging from 1 to 5 months depending on the species. The seeds are round, greyish and relatively large (about 1.5 to 3 mm). Its adaptability to diverse agroecological conditions, including high temperatures and moderate drought, makes it a staple vegetable in many farming systems (Schippers, 2000). In Nigeria, okra is a significant horticultural crop, valued for its role in food security, income generation, and cultural diets.

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 favourable warm, humid conditions (Shrestha *et al.,* 2020). It is a highly virulent fungal disease affecting multiple crops (Jardine, 2020), with severity influenced by environmental conditions (Shrestha *et al.,* 2020), pathogen virulence factors and host susceptibility (Nouri *et al.,* 2019). Its destructiveness stems from necrotic lesions reducing photosynthetic capacity, leading to defoliation, lower yields, and reduced quality (Shrestha *et al*., 2020). Key virulence factors include Cercosporin, a light-activated toxin, and effector proteins like CbNip1, which enhance necrosis, particularly in sugar beets (Jardine, 2020). *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, and moderate temperatures favour the development and spread of the pathogen (Nouri *et al.,* 2019). 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 study is aimed at investigating the antifungal effects of the different neem plant parts in the control of mycelia of the fungi responsible for the leaf spot of okra from selected farms in Donga, Taraba State, Nigeria.

MATERIAL AND METHODS

Study Area

Experiments were carried out in the Department of Microbiology, Federal University Wukari, Taraba Sate. The okra leaves with symptoms of leaf spot disease were randomly collected from different farms in Donga Local Government area Taraba state, Nigeria. Five farms l**oc**ated in different locations within the study area were randomly selected.

Preparation of Culture Media

Potato Dextrose Agar (PDA) was prepared by dissolving 39 grams in 1 liter capacity flask and then made up to 1 liter using sterile distilled water. The medium was autoclave at 121^0 C for 15 minutes at 15lb. The sterilized medium was allowed to cool to about 45^0 C, before being supplemented with streptomycin sulphate (3 grams) and aseptically dispensed in to sterilized 9 cm diameter glass petri dishes.

Isolation and Identification of Fungal Pathogens

Infected leaves were randomly collected from five different farms. The Chiejina (2008) isolation method was adopted. Sections of 2 mm diameter were cut from the lesions of the infected leaves and surface sterilized in 0.1% mercuric chloride for 2-3 minutes, after which they were rinsed in three changes of sterile distilled water. The sections were plated in water agar and mycelium was transferred unto PDA plates. The plates were incubated at room temperature (25± 2°C) for 7 days, subcultures were made aseptically from the plates unto fresh PDA plates and incubated under at room temperature to obtain pure cultures. The identification of the isolated fungi was done macroscopically and microscopically. Macroscopic identification was based on observed culture growth patterns and mycelia

colour. Small portions of the fungal culture were teased and mounted in lactophenol cotton blue stain on a grease-free slide, covered with clean cover slip and then viewed microscopically. Fungal identification was confirmed with the aid of mycological atlasses by Barnett and Hunter (1999), Alexopoulou *et al.* (2002), Agrios (2005), Ellis *et al*. (2007) and identification atlas by Watanabe (2002).

Pathogenicity Test

Each of the fungal isolates obtained from infected leaves were tested for their ability to cause the same condition previously observed on healthy plant leaves. Spore suspension was prepared from seven-day old culture of the isolates and introduced unto healthy leaves of same okra cultivars in the screen house. Disease symptoms produced were similar to those symptoms observed on infected leaves collected from the field. On establishment of the conditions, inocula were taken from the infected leaves in the screen house and cultured. The organisms were re-isolated and identified as previously isolated organisms. This was taken as evidence that they incited the disease. This proved Koch's postulate.

Sources of Plant Materials and Preparation of Extracts

Parts of neem (*Azadirachta indica*), the leaves, bark and roots were used in the experiment. The plant parts were washed in tap water then surface–sterilized with (1 % NaClO for 5 minutes and rinsed in three changes of distilled water) and air dried at (27± 2°C). They were ground using mortar and pestles. Then 25 grams, 50 grams, 75 grams and 100 grams were dissolved separately in 100 ml distilled water, and then filtered through a Whatman No.1 filter paper separately into separate Erlenmeyer flasks of 250ml capacity to produce 25, 50, 75 and 100 % extract concentrations.

Effects of Plant Extracts on Fungi Mycelial Growth

The method of Amadioha and Obi (1999) and Ijato (2011) were used to evaluate the effect of the extracts on the fungal growth. This was done by creating four equal sections on each plate by drawing two perpendicular lines at the bottom of the plate; the center of the plates was indicated by point of intersection of the lines. This was done before dispensing the PDA unto the plates. 2ml of the extract were separately introduced into the Petri dish containing equal amount (20 ml) of the PDA media (poisoned food method) (Nene and Thalpiyal, 2000). Each plate was inoculated with 2mm plug of pure isolate taken from margins of actively growing culture of pathogens using 2mm cork borer. The plates were incubated at (25± 2°C). The control plates were only added with distilled water. Streptomycin was added in the medium to inhibit growth of fungi. Mycelia growth diameter of each isolate was measured and recorded for 120 hours (5 days). Lesion size was determined by rule measurement (mm). Design was completely randomized design in repetitions. Each treatment was repeated three times. Mean radial mycelia growth of each isolate was recorded and data transformed into inhibition percentage by using the following formula adopted by Naz *et al.* (2006):

Inhibition percentage % = $\frac{\mathrm{DC}-\mathrm{DT}}{\mathrm{Dc}}\mathrm{x}100$

Where DC = Average diameter of fungal spore in control,
DT = Average diameter of fungal spore with treatment

Data Analysis

Data were subjected to analysis of variance (ANOVA) and significant means were separated using the least significance difference (LSD) at 5% level of probability using SPSS (Version 27).

RESULTS AND DISCUSSIONS

Inhibition of Mycelia Growth of the two Fungi Using Neem Leaf, Bark and Roots Aqueous Extracts

All concentrations of aqueous neem plant parts extracts suppressed the mycelial growth of the two tested pathogens; the effect was proportional to the concentration of the extract (Tables 1 - 3). The highest inhibition was at 100% concentration, while the lowest inhibition percentage was observed at 25% concentration. Statistically, there was significant difference in inhibition of radial growth in all the fungal isolates at all concentrations compared to the control. Aqueous extracts of neem leaves were more effective against *Cercospora abelmoschi* with higher inhibition percentage of (83.00%) at 100% concentration (Table 1) while aqueous neem roots extracts had the lowest inhibition percentage of (25.13%) a 25 % concentration against *Cercospora malayensis* (Table 3). All the three aqueous extracts of the neem plant parts were able to inhibit the mycelial growth of the fungi *in vitro* at different concentrations, though the higher the concentration the higher the inhibition percentage. There was significant difference between the different concentrations in all the different extracts. Neem leaves show the highest inhibition which may be due to difference in the intensity of the different bioactive components.

Table 1. Effect of Aqueous Extracts of Neem leaves on two Fungi i*n vitro*

Concentration (g/v)	Inhibition (%) mm of Fungal mycelia growth *Cercospora abelmoschi*	*Cercospora malayensis*
25%	28.50	28.37
50%	48.83	48.23
75%	58.15	58.53
100%	83.00	82.20
Control	0.00	0.00
Mean	43.70	43.47
LSD	0.33	0.33
P-value	0.05	0.05

Key: LSD = Least Significant Difference

Table 2. Effect of Aqueous Extracts of Neem Bark on phytopathogenic fungi

Concentration (g/v)	Inhibition (%) mm of Fungal mycelia growth *Cercospora abelmoschi*	*Cercospora malayensis*
25%	26.37	26.23
50%	46.31	46.23
75%	56.32	56.21
100%	80.23	80.12
Control	0.00	0.00
Mean	41.85	41.76
LSD	0.05	0.05
P-value	0.05	0.05

Key: LSD = Least Significant Difference

Table 3. Effect of Aqueous Extracts of Neem Roots on phytopathogenic fungi

Concentration (g/v)	Inhibition (%) mm of Fungal mycelia growth *Cercospora abelmoschi*	*Cercospora malayensis*
25%	25.27	25.13
50%	43.38	42.43
75%	54.31	54.56
100%	78.34	77.81
Control	0.00	0.00
Mean	40.26	39.99
LSD	1.20	1.20
P-value	0.05	0.05

Key: LSD = Least Significant Difference

The mycelial growth inhibition of the pathogens by the different extracts of neem plant parts investigated in this study indicated that the plant parts extract had inhibitory effect on the growth of the two fungi, *Cercospora abelmoschi* and *Cercospora malayensis.* These results further revealed that antifungal activities of the extract were enhanced by increasing the concentration from 25 to 100 % (w/v); hence the inhibition activities of the extracts were concentration dependent. This is in agreement with reports of Ilondu (2012) and Chiejina and Ukeh (2013) who indicated that increase in antifungal activities had corresponding increase in concentration of plant extracts. *Azadirachta indica* leaves aqueous extract exhibited high effect inhibiting mycelia growth reduction against the two pathogenic fungi.

The antifungal activity of *A. indica* parts conforms to the results of Ogbebor and Adekunle (2005); Coventry and Allan (2001) that these extracts are very effective in inhibiting the growth of fungi. Antifungal properties of *A. indica* parts could be attributed to the presence of saponin and alkaloids, chemical components which has been identified as antifungal agents in the plants (Kumar *et al*., 2008). The fungicidal effects of plant extracts on different pathogens of crop plant have been widely reported (Amadioha and Obi, 1999; Okigbo and Ogbonnaya, 2006; Olufolaji, 1999; Onifade, 2002). However, the difference in the efficacy of the extracts

could be attributed to the differences in their active ingredients (Onifade, 2002; Okigbo *et al*., 2005). Major compounds of plants extracts are phenol, flavonoids, alkaloids, quinones, saponins, tannins and sterols (Halama and Van Haluwin, 2004) and their fungicidal or fungistatic properties against various plant pathogens have been established (Scheurella and Mahaffee, 2002). These products might either have direct inhibitory effects on pathogens, exhibiting fungicidal or fungistatic properties. They could help in establishment of favourable condition for antagonistic microbes (Scheuerella and Mahaffee, 2002).

CONCLUSION

The study demonstrated that *A. indica* plant parts have antifugal effects against *Cercospora abelmoschi* and *Cercospora malayensi.* This shows that extracts of neem plant can be harnessed as potential biocide againt plant fungal pathogens. It is anticipated that further research in to these extracts would identify the bioactive components responsible for their fungicidal activity.

Conflict of interest

There was not any conflict of interest between the authors from beginning of the study to the end. Everything was well designed and agreed upon the proposal.

Authors contributions:

Conceptualization, K.J.T and H.S.P.; methodology, K.J.T.; validation, K.J.T and Z.G.B.; formal analysis, K.J.T and C.I.B.; investigation, K.J.T resources, K.J.T and Z.G.B.; data curation, K.J.T and C.I.B.; writing original draft preparation, K.J.T.; writing review and editing, K.J.T and Z.G.B.

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