



DIVERSITY OF FUNGI ASSOCIATED WITH LEAF SPOT ON SOME WILD PLANTS IN ZARIA,  
KADUNA STATE

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ABSTRACT

Leaf spot is a common descriptive term applied to a number of diseases affecting the foliage of plants. Foliar diseases are one of the most important limiting factors for cultivation of crops in tropical and subtropical areas. Fungi associated with leaf spot symptoms on some wild plants (*Chlorophytum blepherophyllum*, *Ipomoea nil*, *Trianthema portulacastrum*, *Senna singuena*, *Combretum molle*, and *Clematis hirsuta*) were studied. Potato Dextrose Agar (PDA) was prepared according to the manufacturer's instructions and used throughout the study. Fungi were isolated by tissue plating method on Potato Dextrose Agar (PDA) containing streptomycin sulphate. Percentage occurrence of each fungal isolate was calculated. Pathogenicity test was carried out on the pathogenic fungi isolated *in vitro* using leaf disk method. Fungi isolated include: *Nigrospora aerophila*, *Choanephora* sp, *Monocheatia* sp, *Rhizoctonia solani* and *Fusarium equiseti*. Percentage occurrence of fungi isolated from wild plants were determined with *Nigrospora aerophila* having the highest (23.08%) followed by *Rhizoctonia solani* (15.39%). This is the first report of *Nigrospora aerophila* in the Northern Guinea Savannah on wild plants. Six different types of leaf spot symptoms were observed on leaves of six wild plants which lead to identification of five fungal species which indicate that these wild plants serve as reservoir hosts of these fungi.

**Keywords:** Fungi, Leaf spot, Potato Dextrose Agar (PDA), Wild plants

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INTRODUCTION

Leaf spot is a common descriptive term applied to a number of diseases affecting the foliage of plants. Foliar diseases are one of the most important limiting factors for cultivation of crops in tropical and subtropical areas [1]. Leaf spot diseases are induced by various pathogenic groups; fungi and bacteria [2]. Fungi causing leaf spot disease include; *Septoria* sp, *Alternaria* sp, *Cladosporium* sp, *Rhizoctonia* sp, *Nigrospora* sp, *Cercospora* sp, *Phyllosticta* sp, and *Ascochyta* sp [3, 4, 5]. Leaf spot diseases may cause yield loss of up to 70%, *Septoria* sp and *Alternaria* sp cause yield losses of 70% and 100% respectively [6].

Weeds and native plants should be considered when endeavoring to manage and control plant pathogens of cultivated plants. Weeds can significantly influence disease incidence. Weeds can interact with pathogen management in several ways, including provision of weed biological control, parasitic weeds can directly serve as vectors of plant pathogens, weeds can serve as reservoir alternative hosts for pathogens and vectors, weeds may be obligate or alternate hosts for some pathogens, and herbicides used for weed control can interact with plant pathogens [7, 8, 9].

Fungi affecting weeds and wild plants have been studied in different part of the world [11]. The interaction between wild plants and cultivated crops has a relevance to the management of plant pathogenic fungi. *Alternaria* sp and *Septoria* sp causes major losses due to leaf spot diseases on a wide range of crops [12]. The losses from the disease may approach 100% in warm, wet seasons where control measures are not practiced. Harvestable fruits number, size and quality are all reduced by these diseases [6]. Even though wild plants and weeds have constrained in cultivation of crops they also serve for medicinal purposes [13, 14, 15].

The aim of this to isolate and identify fungi associated with leaf spot symptoms on leaves of some weeds and wild plants. The information obtained will enhance the understanding of diversity and epidemiology of fungi inducing leaf spot on both weeds and wild plants in Zaria area. The information can be used by Quarantine stations and as biological control for weeds.

MATERIALS AND METHODS

Sample collection

Leaves of wild plants with leaf spot (*Chlorophytum blepherophyllum*, *Ipomoea nil*, *Trianthema portulacastrum*, *Senna singuena*, *Combretum molle*,

and *Clematis hirsuta*) were collected from Zaria Area (Katanga and Shika Dam villages). Katanga is located at longitude 11°10'13" and latitude 7°46'39". Shika dam is located at 11.4°N and longitude 7.75°E. The collected plants were taken to herbarium Department of Botany, A.B.U. Zaria, for identification.

#### Preparation of media

Potato Dextrose Agar was prepared as described by the manufacturer's instructions (Oxoid, England) and used throughout the study.

#### Isolation of fungi

Lesions were isolated by cutting infected leaves with sterile surgical blades. All lesions were surface sterilized in 0.1% mercury chloride (HgCl) for three minutes and rinsed three times with sterile distilled water. Lesions were plated on Petri dishes (four lesions per plate) containing Potato Dextrose Agar (PDA) amended with streptomycin sulphate (1.5g/l) to inhibit bacterial growth. Each sample was in duplicate. The Petri dishes were incubated at room temperature. Observation was made daily for seven days, for the presence of colonies of fungi. The fungi colonies were sub cultured on fresh PDA for subsequent identification [16].

#### Identification of isolated fungi

Identification was carried out by both macroscopic and microscopic observations of pure cultures. Fungi were identified by macroscopic characters such as colony texture, color, and its cultural characteristics on PDA. Fungi were also identified by microscopic characters such as conidiophores morphology and shape. Confirmation of the fungal identification was done using identification key [17], they were also compared with already described isolates in the Department of crop protection diagnostic unit Institute of Agricultural Research, ABU Zaria. Some of the isolates were send to Commonwealth Agricultural Bureaux international (CABI), UK.

#### Pathogenicity tests

Leaf disc inoculation method was carried out on leaves of weeds and wild plants found around zaria area. 8mm diameter disc was cut using a sterile cork borer from healthy leaves of weeds and wild plants. Inoculation with fungal spores was carried out using a micropipette by depositing 25µl droplet of conidial suspension on the leaf disc centre which had been superficially wounded using sterile needle. To prepare conidial suspension, isolates were sub cultured on fresh PDA, once sporulating abundantly, it was flooded with sterilize distilled water, macerated in a blender and the suspension pass through four- folded layers of cheese cloth inside a funnel, to obtain a conidial suspension, each fungus was standardized to the appropriate concentration using a haemocytometer. The leaf disc were placed in petri- dishes under humid condition (moistened filter paper) and covered with foil paper to prevent light. They were incubated at 22°C temperature in an incubator for seven days before evaluation [18].

#### RESULTS

Six different types of symptoms that differ in size, color and shape were observed on six different species of uncultivated (wild) plants. *Trianthema portulacastrum* have circular white spot with brown margin leads to isolation of *Monocheatia* sp. *Chlorophytum blepherophyllum* have large circular spots with grey spot with brown margin, *Fusarium equiseti* and *Choanephora* sp. were isolated. *Ipomoea nil* have small circular spots with white center brown margin *Rhizoctonia solani* was isolated. *Clematis hirsute* shows circular spots with grey center brown margin that leads to isolation of *Nigrospora aerophila*. *Senna singuena* have dark brown scattered spots with irregular shape, *Nigrospora aerophila* was isolated. *Combretum molle* with grey spots that make hole when older leads to *Rhizoctonia solani*. These symptoms were described in Table 1, Plates 1 and 2.

**Table 1:** Fungi isolated from leaves spot symptoms on wild (uncultivated) plants in Zaria area

Name of plant	Description of symptoms	Fungi Isolated
<i>Trianthema portulacastrum</i>	Circular white spot with brown margin.	<i>Monocheatia</i> sp.
<i>Chlorophytum blepherophyllum</i>	Large circular spots With grey spot with brown margin.	<i>Fusarium equisetum</i> <i>Choanephora</i> sp.
<i>Ipomoea nil</i>	Small circular spots with white center brown margin	<i>Rhizoctonia solani</i>
<i>Clematis hirsute</i>	Circular spots with grey center brown margin	<i>Nigrospora aerophila</i>
<i>Senna singuena</i>	Dark brown scattered spots with irregular shape	<i>Nigrospora aerophila</i>
<i>Combretum molle</i>	Grey spots that make hole when older	<i>Rhizoctonia solani</i>



**Plate 1:** Uncultivated plants showing spot symptoms. **Plate 1A:** *Trianthema portulacastrum*. Circular white spot with brown margin. *Monocheatia* sp. was isolated. **Plate 1B:** *Chlorophytum blepherophyllum* large circular spots with grey spot with brown margin. *Fusarium equisetum*, and *Choanephora* sp. were isolated. **Plate 1C:** *Ipomoea nil*. small circular spots with white center brown margin. *Rhizoctonia solani* was isolated.



**Plate 2:** Wild plants showing spot symptoms. **Plate 2A:** *Clematis hirsuta*. Circular spots with grey center brown margin, *Nigrospora aerophila* was isolated. **Plate 2B:** *Senna singuena*. Dark brown scattered spots with irregular shape, *Nigrospora aerophila* was isolated. **Plate 2C:** *Combretum molle*. Grey spots that make a hole when older, *Rhizoctonia solani* was isolated.

#### Identification of fungi isolated from spot symptoms on wild (uncultivated) plants

Five different types of fungi were isolated from spot symptoms on wild plants. Each fungus was found to produce a definite symptom except *Nigrospora aerophila* and *Rhizoctonia solani* which were associated with two types of symptoms. *Nigrospora aerophila* macroscopic character shows upper surface light brown, reverse light brown, microscopic

character shows globose black conidia with vesicle and hyphae. *Monochaetia* sp. macroscopic character upper surface brown, reverse brown, microscopic character shows conidia of different shapes with slender conidiophore. *Choanephora* sp. macroscopic character shows upper surface black with white center, reverse black, microscopic character shows one celled ellipsoid brown conidia. *Fusarium equiseti* macroscopic character shows upper surface white, reverse pink, microscopic character shows macroconidia with septation. *Rhizoctonia solani*

macroscopic character shows upper surface grey with pink wave edge, reverse dark brown, microscopic character shows branched hyphae with point of septation. These characters are described in Table 2, Plates 3 to 7. These were compared with already described species using keys and already described isolates at IAR, ABU, Zaria. To ascertain the identity of *N. aerophila* and *R. solani* the isolates were sent to Common Wealth Agricultural Bureaux International (CABI), UK.

**Table 2:** Cultural and microscopic characters identification of fungi isolated from uncultivated plants in Zaria Kaduna state

<b>Fungi</b>	<b>Macroscopic character</b>	<b>Microscopic character</b>
<i>Nigrospora aerophila</i>	Upper surface light brown, reverse light brown.	Short conidiophores, black conidia, 1-celled globose, vesicle present.
<i>Monochaetia</i> sp.	Upper surface brown, reverse brown.	Conidia dark, several celled with pointed end cell.
<i>Choanephora</i> sp.	Upper surface black with white center, reverse black.	Conidia one celled ellipsoid brown.
<i>Fusarium equiseti</i>	Upper surface white cottony, Reverse pink.	Macroconidia with septation and Microconidia.
<i>Rhizoctonia solani</i>	Upper surface grey with pink wave edge, Reverse dark brown.	Branched hyphae with point of septation.



**Plate 3:** *Nigrospora aerophila*. A= Upper surface light brown, B=Reverse light brown, Ca=Vesicle, Cb= Globose black conidia, Cc= Hyphae. Mg= X 40 Stain; Lactophenol cotton blue



**Plate 4:** *Monochaetia* sp. A= Upper surface brown, B= Reverse brown, Ca = Conidia dark, Cb= several celled with pointed end cell. Mg = X 40 Stain; Lactophenol cotton blue



**Plate 5:** *Choanephora* sp. A= Upper surface black with white center. B=Reverse black, C= Conidia one celled ellipsoid brown. Mg x40 Stain; Lactophenol cotton blue



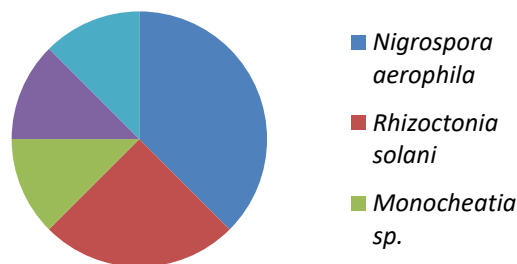
**Plate 6:** *Fusarium equisetum* A= Upper surface white B= Reverse pink, Ca= Macroconidia with septation. Mg X 40 Stain; Lactophenol cotton blue



**Plate 7:** *Rhizoctonia solani*. A= Upper surface grey with pink wave edge. B= Reverse dark brown. Ca= Branched hyphae, Cb= point of septation Mg X 40 Stain; Lactophenol cotton blue

**Percentage occurrence of fungi from wild plants**

*Nigrospora aerophila* has the highest percentage occurrence of 23.08%, followed by *R. solani* with 15.39%. *Fusarium equiseti*, *Choanephora*, and *Monocheatia* spp. have the lowest percentage occurrence of 7.69%. These are shown in Figure 1.



**Figure 1:** Percentage occurrence of fungi isolated from leaf spot of wild plants in Zaria area

**In vitro pathogenicity using leaf disk**

*Fusarium equiseti*, *Rhizoctonia solani*, *Nigrospora aerophila* and *Choanephora* spp. were tested on leaves of wild plants found to be producing the same symptoms when inoculated as shown in Table 3.

**Table 3:** *In vitro* pathogenicity test using leaf disk on wild plants in Zaria area

Fungi	<i>Clematis hirsute</i>	<i>Senna singuena</i>	<i>Combretum molle</i>
<i>Nigrospora aerophila</i>	+	+	-
<i>Rhizoctonia solani</i>	-	-	+
<i>oanephora</i> sp	-	-	+

**Key:** + = Pathogenic. - = Not pathogenic

**DISCUSSION**

The investigation on fungi causing wild plants diseases has been reported by many researchers in different part of the world [19, 20, 21, 9]. Six different wild plants (*Chlorophytum blepherophyllum*, *Trianthema portulacastrum*, *Clematis hirsuta*, *Combretum molle*, *Senna singuena* and *Ipomoea nil*) collected from the vicinity of cowpea farms around Zaria, all showed spots symptoms which were similar in shape but with minor variations in margin and centre coloration. From these symptoms however one or more taxonomically different fungi were isolated. So variation in color might be attributed to difference in types of fungi associated with the symptoms. These fungi (*Nigrospora aerophila*, *Monochaetia* sp., *Choanephora* sp., *Rhizoctonia solani*, and *Fusarium equiseti*) have been reported on other plant species [22 – 27]. *Fusarium equiseti* and *R. solani* were also isolated from cowpea pods and seeds by Ibrahim et al. [5, 28]. This shows that *Chlorophytum blepherophyllum*, *Combretum molle* and *Ipomoea nil* may serve as reservoir hosts of these fungi which are a threat to cowpea.

*Nigrospora aerophila* was isolated from *Clematis hirsuta* and *Senna singuena* although the

spot types were different. The difference may be attributed plant and/or variation in fungal biological race. Other species of *Nigrospora* had been reported to cause leaf spot on many other cultivated and wild plants [4, 23, 29]. This association of *N. aerophila* is being reported with leaf spot for the first time on *Clematis hirsuta* and *Senna singuena*.

Percentage occurrence of fungi isolated from weeds and wild plant shows *Nigrospora aerophila* has the highest followed by *Rhizoctonia solani*. This may be due to the fungi having wide host range. *In vitro* pathogenicity test carried out on some leaves wild plants showed that *Clematis hirsuta*, *Senna singuana* and *Combretum molle* were susceptible hosts of *Fusarium equisetum* and *Choanephora* sp. because inoculation with these pathogens produced symptoms.

## CONCLUSIONS

Six different types of leaf spot symptoms were observed on leaves of six wild plants. Five fungi were found to be associated with leaf spot symptoms on wild plants (*R. solani*, *N. aerophila*, *Choanephora*, *F. equisetum* and *Monocheatia* sp.).

## RECOMMENDATIONS

Wild plants growing around cultivated crops should be managed properly to avoid transmission. The different fungi isolated can be used for bio control of weeds plants reported among the wild plants.

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