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Diversity of Fungi Inducing Leaf Spot on *Spigelia anthelmia* and *Porphyrostemma chevalieri*

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Abstract
Leaf spot is a common descriptive term applied to a number of diseases affecting plants. The majority of spots are caused by fungi. Different leaf spots were observed on *Spigelia anthelmia* and *Porphyrostemma chevalieri* in Zaria area, Nigeria. The fungi were isolated by direct plating method on Potato Dextrose Agar (PDA) amended with streptomycin sulphate (1.5g/l). *In vitro* inoculation of healthy leaves of these plants produced spots symptoms. *Macrophoma* sp., *Nigrospora aerophila* and *Trichoderma* sp were isolated from *Spigelia anthelmia* while *Macrophomina* sp., *Alternaria* sp., and *Helicosporium* sp were isolated from *Porphyrostemma chevalieri*. These uncultivated plants serve as reservoir hosts of these fungi as the fungi isolated have been reported on cultivated plants. It can be concluded from this research that *Spigelia anthelmia* and *Porphyrostemma chevalieri* can serve as reservoir hosts of the isolated fungi which may infect the cultivated plants by producing leaf spots. It is therefore recommended that proper weeding is required to prevent the growth and establishment of the uncultivated plants (*Spigelia anthelmia* and *Porphyrostemma chevalieri*).
Keywords: Fungi, Leaf spot, *Spigelia anthelmia, Porphyrostemma chevalieri*

INTRODUCTION

Leaf spot is a common descriptive term applied to a number of diseases affecting plants. The majority of spots are caused by fungi, but some are caused by bacteria. Some insects also cause damage that appears like a spot disease. Leaf spot may result in defoliation in some plants (Nix, 2014). A spot disease causes yield loss of up to 70-100 % (RPD, 1999).

Spigelia anthelmia also called pinkroot is a tropical annual weed belonging to the family Loganiaceae and used in many herbal medicines. Some fungi species such as *Cercospora apii* and *Curvularia lunata* were reported to cause leaf spot in this plant from India and Nigeria (Kamble *et al.,* 2012; Amadi 2001).

Porphyrostemma chevalieri belongs to the family Compositea. It is erect hispid herb and is widely distributed in tropical Africa (Wild, 1980). These 2 species are found growing abundantly among cultivated plants such as cowpea and sorghum and in gardens in Zaria area. Uncultivated plants should be considered when endeavoring to manage and control plant pathogens of cultivated plants which can significantly influence disease incidence. There is no available documented report of fungi isolated from leaf spots of *Spigelia anthelmia* and *Porphyrostemma chevalieri* from Zaria. The aim of this research was to isolate and identify fungi from leaf portions with spot symptoms in *Spigelia anthelmia* and *Porphyrostemma chevalieri* growing among cultivated plants in Zaria.

MATERIALS AND METHODS

Study Site and Collection of Samples

Leaves of *Spigelia anthelmia* and *Porphyrostemma chevalieri* containing leaf spot were collected from Zaria Area. The collected plants were identified at the Herbarium of Department of Biological sciences, A. B. U. Zaria.

Inoculation and Identification of Fungal Species

Potato Dextrose Agar (PDA) was used throughout the study. Lesions were isolated by cutting leaf spots with sterile surgical blades and were then 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 duplicated .The Petri dishes were incubated at room temperature (22-25°C) and were daily observed for seven days for the presence of fungal colonies. The fungi colonies were sub cultured on fresh PDA for subsequent identification (Mungo, 1996).

Identification was carried out by both macroscopic and microscopic observations of pure cultures using identification key described by Barnett and Hunter (1999). Macroscopic characters such as colony texture, color, and its cultural characteristics on PDA were considered while microscopic characters such as conidiophores morphology and shape were observed. Identified fungal isolates were compared with already described isolates available at the Department of Crop Protection Diagnostic Unit, Institute of Agricultural Research, ABU Zaria for confirmation.

Leaf disc inoculation method was carried out on healthy leaves of *Spigelia anthelmia* and *Porphyrostemma chevalieri*. Eight milimeter diameter disc was cut using a sterile cork borer from healthy leaves. 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. The leaf disc was placed in petri- dishes under humid condition (moistened filter paper) and covered with foil paper to prevent light. They were incubated at 22^{o}C for seven days before evaluation (Batta, 2003).

RESULTS

Spigelia anthelmia had circular spots with white center and brown margin and fungi isolated were *Macrophoma* sp., *Nigrospora aerophila* and *Trichoderma* sp. *Porphyrostemma chevalieri* had brown spot dispersed with irregular shapes and fungi isolated were *Macrophomina* sp., *Alternaria* sp. and *Helicosporium* sp. These symptoms were described in Table 1 and shown in Plate 1.

Macroscopic characters observed in *Macrophoma* sp. Showed that the colony upper surface was grey and the reverse was black while microscopic character showed dark pycnidia. In *Nigrospora aerophila,* macroscopic character showed that both the upper surface and reverse of the colony were light brown while microscopic character showed globose black conidia with vesicle and hyphae. In *Trichoderma* sp. both the upper surface and reverse of the colony were green while microscopic character showed branched conidiophore and ovoid conidia. *Macrophomina* sp. macroscopic character showed colony with upper surface grey colour and black reverse colour while the microscopic character showed pycnidia with hyphae. In *Alternaria* sp., the colony morphology showed upper surface black with grey margin and reverse brown while conidiophores with both cross and longitudinal septa were observed. In *Helicosporium* sp. macroscopic character showed that both the upper surface and reverse were grey while the microscopic character showed tall and slender conidiophores. These are shown in Table 2 and Plates 2 to 7.

In vitro inoculation of *Spigelia anthelmia* and *Porphyrostemma chevalieri* with *Macrophoma* sp., *Nigrospora aerophila* and *Macrophomina* and *Alternaria* spp., respectively produced black spots *on* both plants (Plate 8).

Table 1. Fungi isolated from leaves spot symptoms

Name of Plant	Description of Symptoms	Fungi Isolated
Spigelia anthelmia	Circular spots with white center and brown margin	*Macrophoma* sp. *Nigrospora aerophila* *Trichoderma* sp.
Porphyrostemma chevalieri	Brown spot dispersed with irregular shapes	*Macrophomina* sp. *Alternaria* sp. *Helicosporium* sp.

Table 2. Cultural and microscopic characters used for identification of fungi isolated

Name of Fungi	Macroscopic characters	Microscopic Charaters
Macrophoma sp.	Upper surface grey, Reverse black.	Dark pycnidia.
Nigrospora aerophila	Upper surface light brown, reverse light brown.	Short conidiophores, black conidia, 1-celled globose, vesicle present.
Trichoderma sp.	Upper surface green, reverse green.	Conidiophores branched, conidia 1-celled ovoid
Macrophomina sp.	Upper surface grey cottony, reverse black.	Presence of hypha and pycnidia
Alternaria sp.	Upper surface black with grey margin, reverse brown.	Conidiophores with both cross and longitudinal septa.
Helicosporium sp.	Upper surface grey, reverse grey.	Conidiophores tall slender.

DISCUSSION

Spigelia anthelmia and *Porphyrostemma chevalieri* showed spots symptoms. From these symptoms however, one or more taxonomically different fungi were isolated. So variation in colour might be attributed to difference in types of fungi associated with the symptoms. Three fungi were isolated from leaf spot symptoms on *Spigelia anthelmia* (*Macrophoma* sp., *Nigrospora aerophila* and *Trichoderma* sp). Other species of *Nigrospora* have been reported to cause leaf spot on many other cultivated and wild plants (Emechebe *et al.,* 1980; Nutsugah *et al.,* 2004; Verma and Gupta, 2008; Zheng *et al.,* 2012). To the best of our knowledge, this is the first report of association of these fungi with *Spigelia anthelmia.*

Three different fungi were isolated from leaves of *Porphyrostemma chevalieri; Macrophomina* sp.*, Alternaria* sp., and *Helicosporium* sp. To the best of our knowledge, this is the first report of association of these fungi with *Porphyrostemma chevalieri.*

Macrophoma sp., *Alternaria* sp. and *Macrophomina* sp. have been reported to cause leaf spot on a number of cultivated and wild plants (Ibrahim *et al*, 1975; Emechebe *et al.,* 1980; Abdon *et al.,* 1980; Singh *et al.,* 1997; Kucharek, 2000; Reis and Boiteux, 2010; Kehinde, 2011; Motlagh, 2011; Fuhlbohm *et al*, 2012). This suggests that these plants may serve as reservoir hosts for these fungi.

The production of symptoms on inoculation with *Macrophoma* sp. and *Nigrospora aerophila* on *Spigelia anthelmia* showed that these fungi were responsible for the spots observed. Also *Alternaria* and *Macrophomina* spp were found to be responsible for the spots on *Porhyrostemm achevalieri.*

Conclusion

Different leaf spots were observed on uncultivated plants of *Spigelia anthelmia* and *Porphyrostemma chevalieri* in Zaria area, Nigeria, which are growing among the cultivated plants and therefore act as reservoir hosts of the pathogens. Six fungi species were isolated from the two plants. *Macrophoma* sp., *Nigrospora aerophila*and *Trichoderma* sp were isolated from *Spigelia anthelmia* while *Macrophomina* sp., *Alternaria* sp., and *Helicosporium* sp were isolated from *Porphyrostemma chevalieri. In vitro* inoculation of *Spigelia anthelmia* and *Porphyrostemma chevalieri* with *Macrophoma* sp., *Nigrospora aerophila* and *Macrophomina* and *Alternaria* spp. respectively produced black spots on both plants.

Recommendation

Proper management of weed should be adopted in order to pevent possible transmission of pathogens from the uncultivated plants which serve as the possible reservoir host to the cultivated plants.

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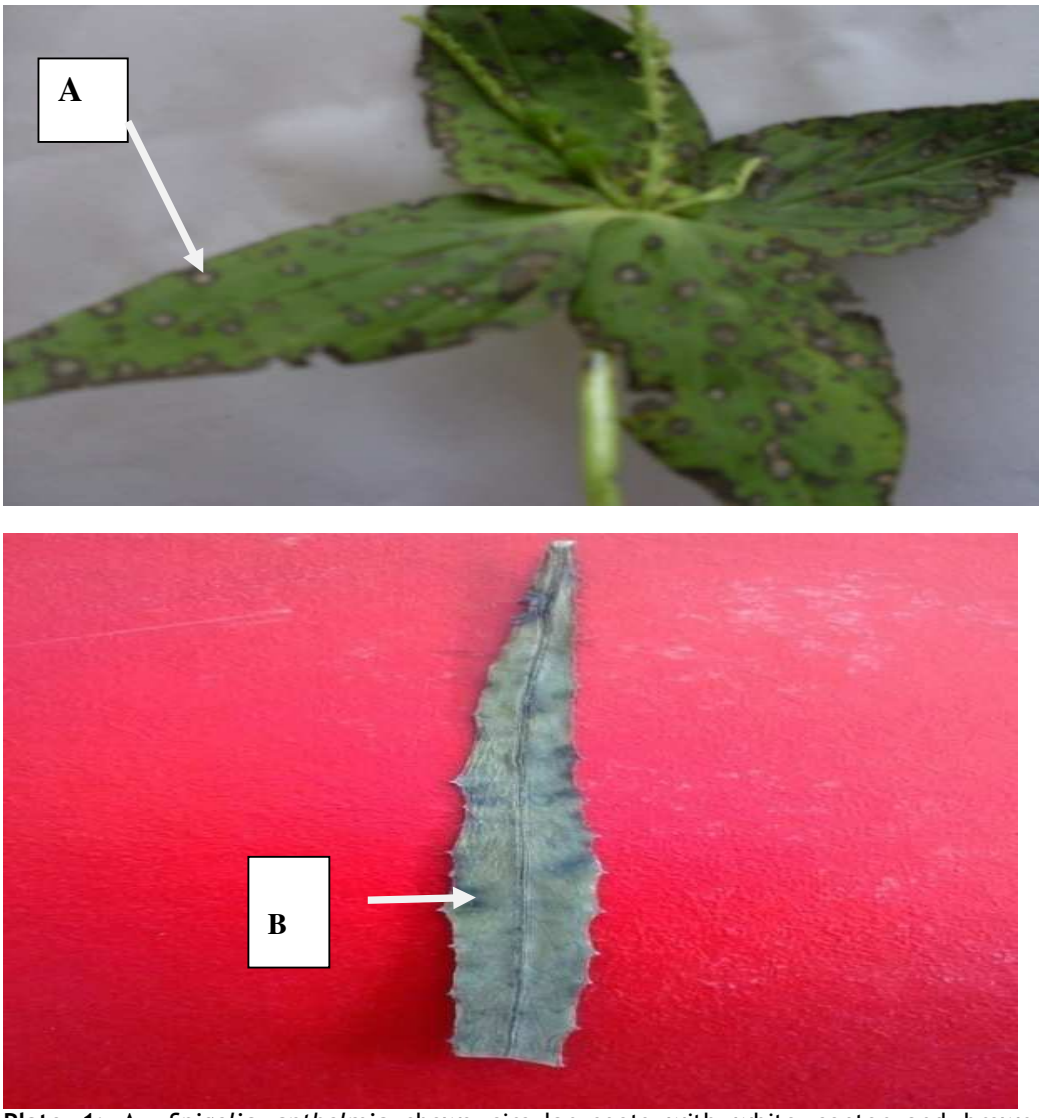


Plate 1: A= *Spigelia anthelmia* shows circular spots with white center and brown margin. *Macrophoma* sp., *Nigrospora aerophila* and*Trichoderma* sp were isolated from it
B= *Porphyrostemma chevalieri shows* brown spots dispersed with irregular shapes. *Macrophomina* sp., *Alternaria* sp. and *Helicosporium* sp. were isolated.

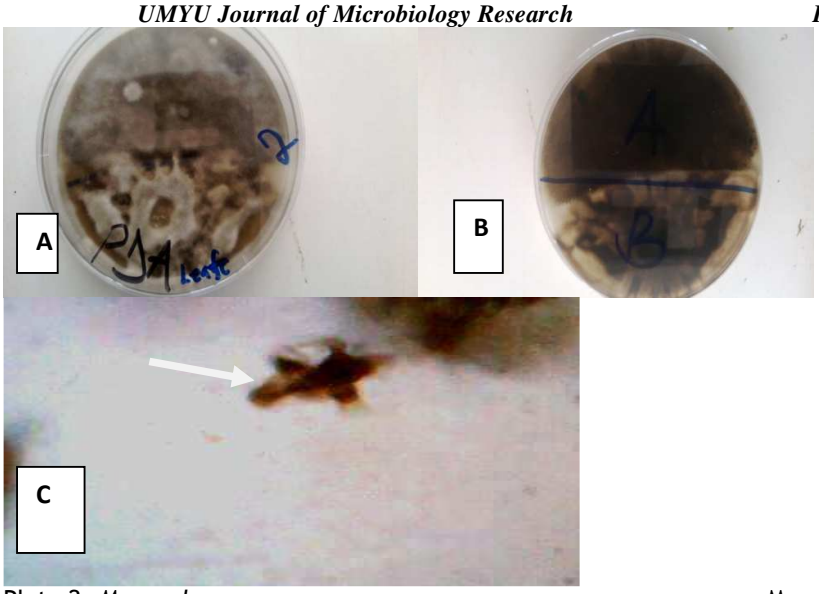


Plate 2: *Macrophoma* sp. Mg x40
A= upper surface grey, B=Reverse black Stain; Lactophenol cotton blue.
C=Dark pycnidia.

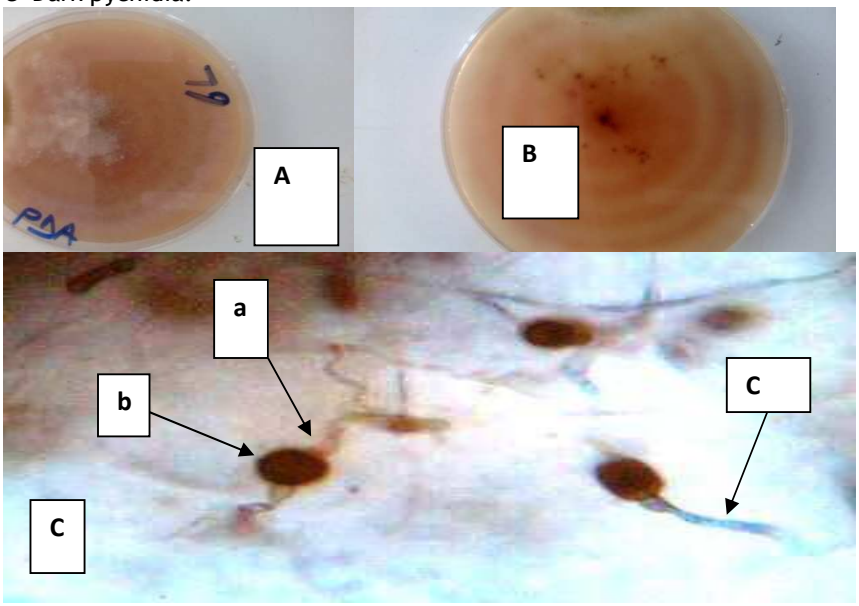


Plate 3: *Nigrospora aerophila*. Mg x40
A= Upper surface light brown, B=Reverse light brown. Stain; Lactophenol cotton blue
Ca=Vesicle, Cb= Globose black conidia, Cc= Hyphae.

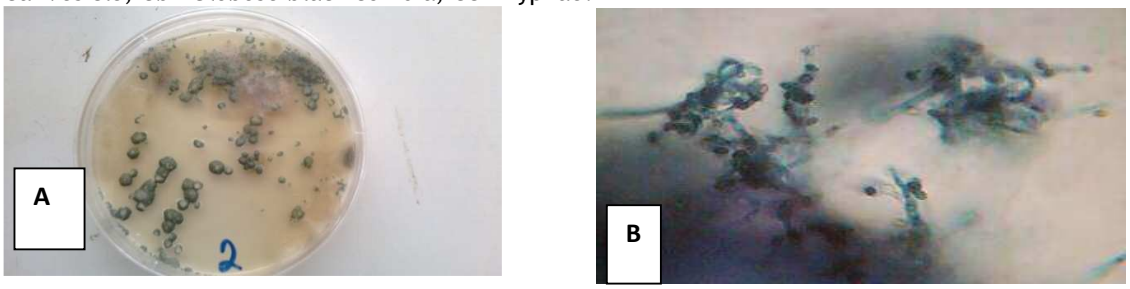


Plate 4: *Trichoderma* sp. Mg x40
A= Upper surface green, Stain; Lactophenol cotton blue
B= Conidia

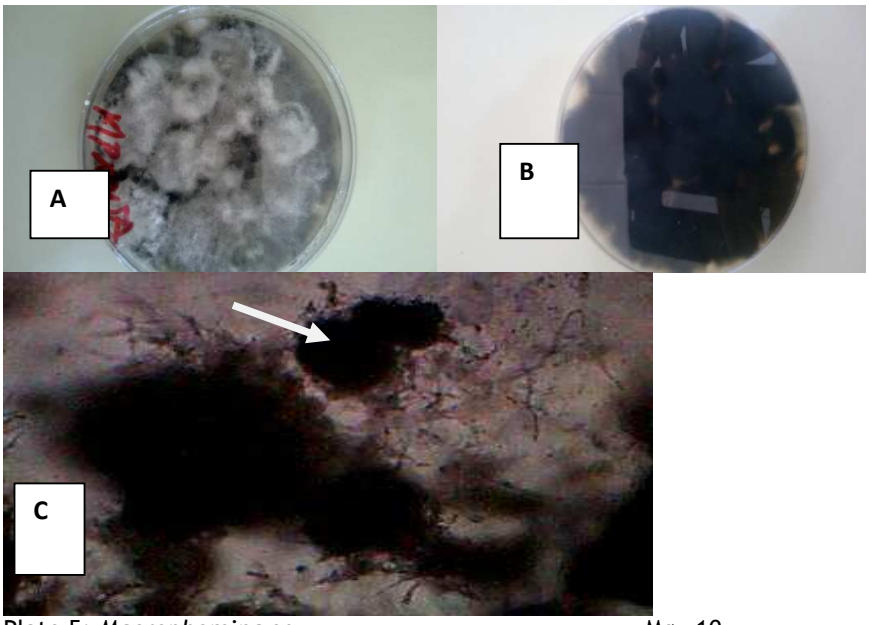


Plate 5: *Macrophomina* sp. Mg x10
A= Upper surface grey. B= Reverse black. Stain; Lactophenol cotton blue
C =Pycnidia,

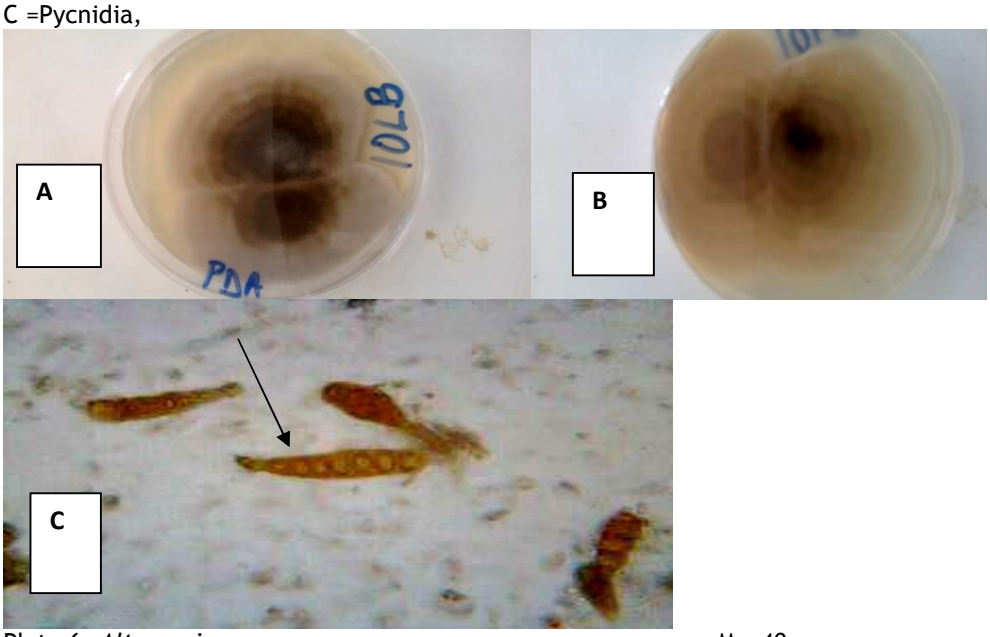
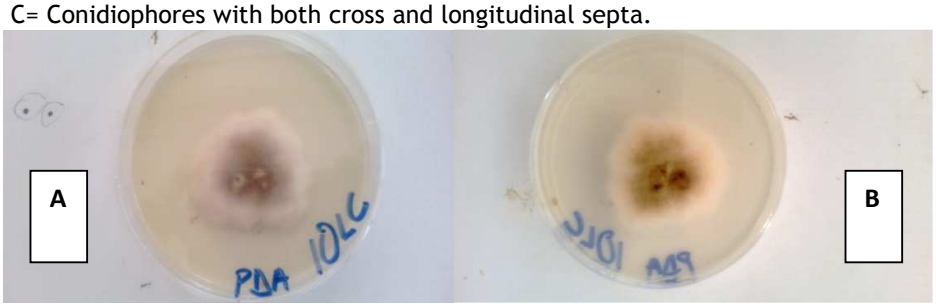


Plate 6: *Alternaria* sp. Mgx40
A= Upper surface black with grey margin. B= Reverse brown. Stain; Lactophenol cotton blue
C= Conidiophores with both cross and longitudinal septa.



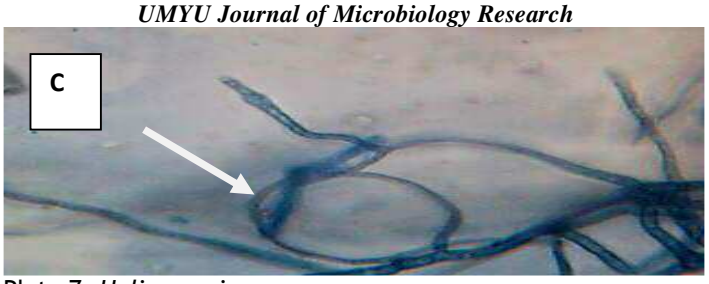


Plate 7: *Helicosporium* sp. Mg x40
A= Upper surface grey. B= Reverse grey. Stain; Lactophenol cotton blue
C= Conidiophores tall slender

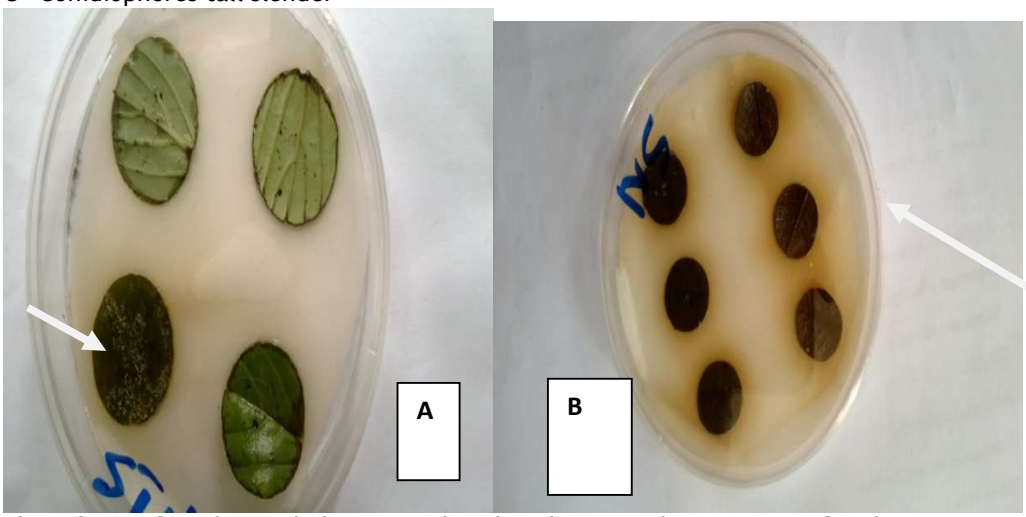


Plate 8: A= *Spigelia anthelmia* inoculated with *Macrophoma* sp. B= *Porphurostemma chevalieri* inoculated with *Alternaria* sp.