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FUNGI ASSOCIATED WITH DISEASES OF *DICHROSTACHYS CINEREA* AND *PARKIA BIGLOBOSA* TREES IN AMURUM FOREST RESERVE

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ABSTRACT

This study was carried out to determine the identity of fungal pathogens associated with diseases of *Dichrostachys cinerea* (L.) Wight & Arn and *Parkia biglobosa* (Jacq.) R.Br. ex G. Don in the Dry Savannah habitat of Amurum forest reserve. Random sampling was used to lay down the temporary sample plot and the survey involved sampling leaves and stems from tender and old trees that show symptoms such as abnormal growth, branch splits, chlorosis, stem canker, dieback, spots on leaves, galls and stunted growth. The diseased samples were taken to the laboratory for culture, isolation and identification of pathogens. Eight fungal species were isolated and identified: *Aspergillus niger*, *Choanephora cucurbitarum*, *Fusarium oxysporum*, *Fusarium solani*, *Lasiodiplodia theobromae*, *Pestalotiopsis* spp, *Thermomyces lanuginosus* and *Trichoderma harzianum* from the diseased trees. This study provides a foundation for future work to develop management strategies aimed at reducing the impact of these pathogens in the Reserve because, without knowledge of the identity of these disease-causing agents, it is not possible to accurately design control methods aimed at reducing the inoculum level of the pathogenic organisms.

Keywords: Fungi, diseases, *Dichrostachys cinerea*, *Parkia biglobosa*, forest

INTRODUCTION

Dichrostachys cinerea (L.) Wight & Arn and *Parkia biglobosa* (Jacq.) R.Br. ex G. Don both belong to the family *Fabaceae* and are the most abundant tree species in the Dry Savannah habitat of Amurum forest reserve (Dawang *et al.,* 2010). *D. cinerea* is a thorny, semi-deciduous to deciduous leguminous shrub that can reach a height of 3-7 m with an open crown, 3 m wide (Orwa *et al*., 2009), a deep taproot and many lateral horizontal roots that make eradication difficult (US Forest Service, 2011) while *P. biglobosa* is a perennial deciduous tree with a height ranging from 7 to 20 m, although it can reach 30 m under exceptional conditions (Orwa *et al*., 2009; PROTA, 2016). These trees are an important component of the forest reserve because they help reduce the effect of erosion, provide oxygen, improve air quality, ameliorate climate, conserve water, preserve soil and provide habitat and food for animals and plants (Ollinger, 2002; Shvidenko *et al*., 2005).

The importance of these trees cannot be overemphasized thus, their protection is of paramount importance in the reserve due to continual threat such as emerging tree diseases (pests and pathogens), shifts in climate conditions and other global change stressors (Wingfield *et al.,* 2015). Although these trees have evolved structural and chemical defences such as thick bark, waxy leaf coatings, root secretions and anti-microbial toxins to provide general protection from all microorganisms (Agrios, 2005; Casadevall, 2007), certain fungal pathogens have developed virulence factors that enable them to overcome general plant defences (Money, 2007; Loo, 2009).

Protection against diseases requires a much more integrated approach incorporating specialized knowledge on types and nature of the diseases. Full utilization of such an approach has not yet been achieved, due to insufficient knowledge about diseases and organisms associated with diseases of many tree species growing

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in the reserve. This implies also that if such knowledge were available it would be incorporated in formulating management programmes involving protection of forest against potential disease outbreaks.

Fungal pathogens, if not controlled in one way or the other, can have devastating effects on biodiversity and forest structure, which is why there is the apparent need to conserve and protect these trees. The aim of this study was to investigate fungal pathogens associated with diseases of these trees in the Dry Savannah habitat of Amurum forest reserve.

MATERIALS AND METHODS

Study site

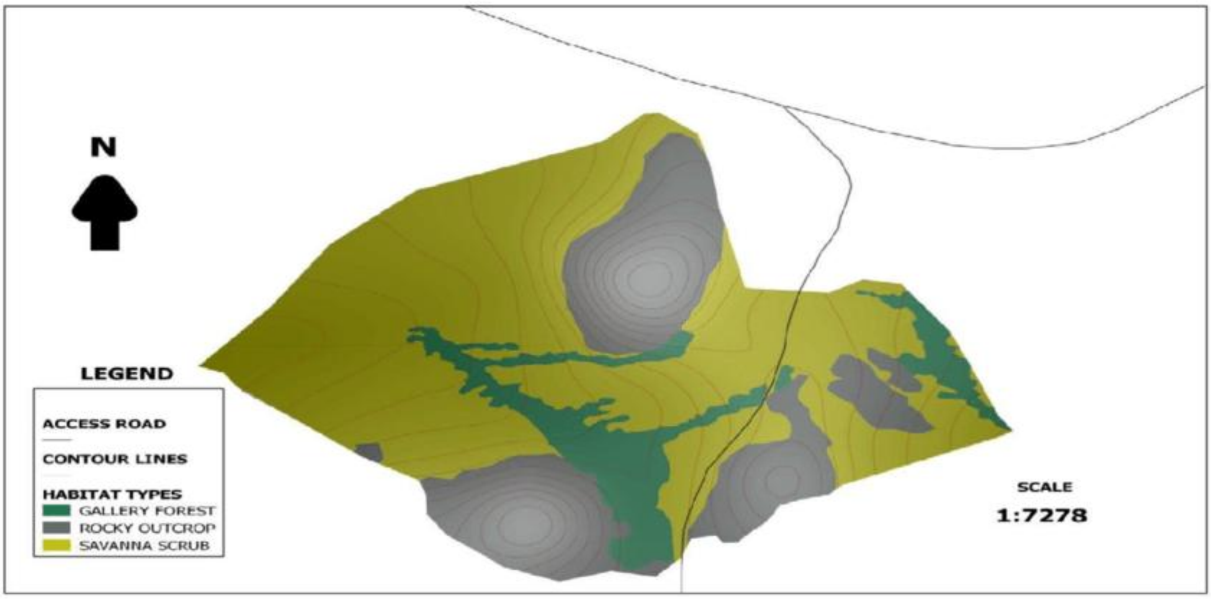

Figure 1. Map of Amurum reserve showing topography and distribution of major habitat types

The study was carried out in the Dry Savannah habitat of Amurum forest reserve recognized internationally as an important bird area (IBA) because of the rich avifauna such as *Lagonosticta sanguinodorsalis* (Rock Firefinch) and *Vidua maryae* (Jos Plateau Indigo Bird) (Dawang *et al*., 2010), located North East of Jos at latitude 09° 53' N and longitude 08° 59' E with an altitude of 1,280 m above sea level and being a small protected forest fragment with an area of about 300 hectares which holds one of the few remnants of natural vegetation typical of the Jos plateau (Vickery and Jones, 2002). The Reserve consists of three broadly identified habitat types (Figure 1): Rocky outcrops, Dry Savannah and Gallery forests. The Dry Savannah habitat which is a mixed woodland and grassland ecosystem is characterised by trees being sufficiently widely spaced so that the canopy does not close (Yessoufou *et al*., 2012).

Field survey and sample collection

Field surveys were carried out on 174 trees to observe symptoms on *Dichrostachys cinerea* and *Parkia biglobosa* trees which occur in the Dry Savannah habitat of Amurum forest reserve. Random sampling was used in the laying of the temporary quadrat points. Standardized quadrat plots of 20 m x 20 m in size were established at 30 m interval, giving a total of 10 sample quadrat points in the study area. The location of each quadrat point was recorded and established in the Dry Savannah using a Garmin Etrex® Global Positioning System (GPS) device. The mean annual rainfall in the areas surveyed was 1,411 mm and mean annual temperatures were between 8^{o} C and 38^{o} C (Table 1).

Table 1. Description of location surveyed to collect samples used for the identification of fungi on the Dry Savannah habitat

Sampling site	Number of focal trees encountered	Average rainfall (mm/year)	Altitude (m)	GPS Location	
				Latitude	Longitude
A001	16	1411	1311	**N**09.87558	**E**008.97797
A002	6	1411	1317	**N**09.87496	**E**008.97807
A003	3	1411	1320	**N**09.87498	**E**008.97858
A004	3	1411	1321	**N**09.87733	**E**008.98047
A005	9	1411	1328	**N**09.87686	**E**008.98072
A006	46	1411	1330	**N**09.87698	**E**008.98134
A007	30	1411	1324	**N**09.87744	**E**008.98194
A008	26	1411	1319	**N**09.87679	**E**008.98251
A009	25	1411	1327	**N**09.52510	**E**008.58963
A010	10	1411	1334	**N**09.52471	**E**008.58895

GPS = global positioning system.

Diseased samples including leaves and barks were collected in sterilized polyethylene bags during the survey and were brought to the laboratory at the University of Jos for isolation and identification of the pathogens.

Isolation and Identification

Isolation from leaves: A section of 10 mm square containing unhealthy and healthy tissues was cut and surface-sterilized by soaking in ethanol (70%, v/v) for 40 seconds, followed by rinsing in sterile distilled water three times and blot-dried on clean, sterile paper to remove sterilant. The pieces were transferred with a sterile tweezer, shaking off excess water onto a plate of Potato Dextrose Agar supplemented with streptomycin sulphate at 50 mg/l to prevent bacterial growth. Six pieces of leaf tissues were placed on two Petri plates and incubated in an inverted position at 25°C for 5 days as described by Aneja (2007).

Isolation from stems: The infected barks and stems were used for isolation. Samples were surface-sterilized with a cotton swab dipped in alcohol (70%, v/v) followed by lightly flaming of the tissue. A flamed scalpel was used to peel back the outside layer of the tissue exposing tissues not previously exposed to contaminants. Three small pieces of the bark sample were dug out from the freshly exposed area and transferred onto a Petri dish containing Potato Dextrose Agar supplemented with streptomycin sulphate at 50 mg/l to prevent bacterial growth under aseptic conditions and plates were incubated in an inverted position at 25^{o}C for 5 days (Aneja, 2007).

Identification: Fungi were identified based on macro and micro-morphological characteristics using keys of Domsch *et al*. (1980), Barnett and Hunter (1998) and Sajeewa *et al*. (2012). Further, the fungal hyphae growing from the diseased leaf tissues and barks were subcultured to obtain a pure culture. The percentage frequency of fungi was estimated using the following formula:

$$\textbf{PFC (\%)} = \frac{\text{Number of leaves /bark pieces colonized by a pathogen}}{\text{Total number of leaves /bark pieces}} \times 100\%$$

RESULTS

From the field survey carried out, observed data from 174 trees were recorded in the 10-quadrat sampled plot in the Dry savannah habitat of Amurum forest reserve. Among 174 recorded trees, only 54.35% *Dichrostachys cinerea* and 33.33% *Parkia biglobosa* trees did not show external macroscopic disease symptoms, while 43.26% of *Dichrostachys cinerea* trees and 66.67% *Parkia biglobosa* trees were diseased and 6.38% dead trees were only recorded for *Dichrostachys cinerea* (Figure 2).

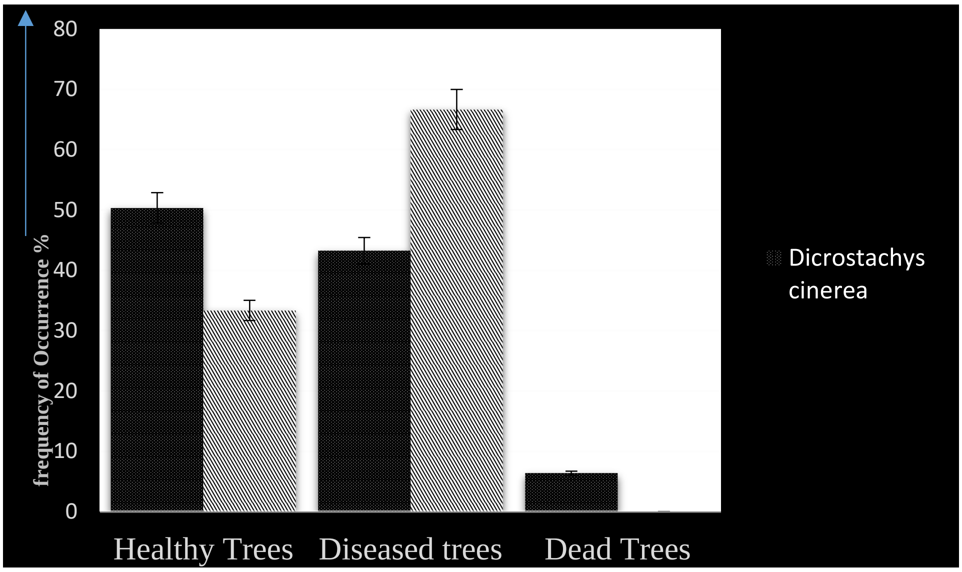

Figure 2. Percentage frequency of healthy, diseased and dead trees recorded in Dry Savannah habitat of Amurum Forest Reserve with 95% level of confidence intervals

In this study, several disease symptoms viz.: abnormal growth, branch splits, chlorosis, stem canker, dieback, spots on leaves, galls and stunted growth were observed on both *Dichrostachys cinerea* (L.) Wight & Arn and *Parkia biglobosa* (Jacq.) R.Br. ex G. Don in Amurum forest reserve (Figure 3).

Leaf spot symptoms which were observed on the focal tree species consist of different shapes which include angular, rounded, raised, sunken, smooth or fringed edges, had the highest percentage frequency of occurrence with 40.85%, followed by stunted growth with 14.02%, branch split and canker with 10.98%, chlorosis with 9.15%, abnormal growth such as presence of witches broom or parasitic plant with 5.49% frequency of occurrence, galls on leaves with 3.66%, branch decay with 3.05% while dieback had the least frequency of diseased symptom occurrence with 1.83% (Figure 3).

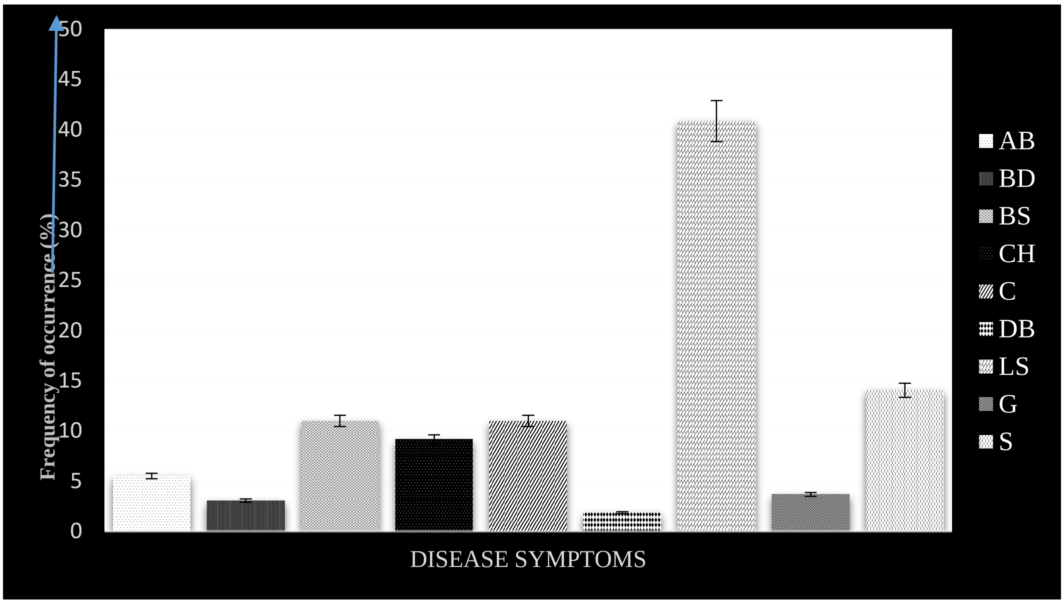

Figure 3. Percentage Frequency of occurrence of disease symptoms found on both *D. cinerea* and *P. biglobosa* with 95% level of confidence intervals

Key

AB- Abnormal growth **BD**=Branch decay **BS**=Branch split **CH**=Chlorosis
C=Canker **DB**=Dieback **LS**=Leaf spot **G**=Galls
S=Stunted growth

Isolation of fungi

Table 2 shows a total of eight fungi isolated from the bark and foliar part of the focal tree species. The fungal isolates included *Aspergillus niger*, *Choanephora cucurbitarum*, *Fusarium oxysporum*, *Fusarium solani*, *Lasiodiplodia theobromae*, *Pestalotiopsis* spp, *Thermomyces lanuginosus* and *Trichoderma harzianum* (Plate 2).

On the leaf isolation, *Pestalotiopsis* spp (50% on both *Dichrostachys cinerea* and *Parkia biglobosa*) had the highest frequency of colonization, followed by *Thermomyces lanuginosus* (33.33% on *Parkia biglobosa*), *Choanephora cucurbitarum* and *Trichoderma harzianum* (both had 25% on *Dichrostachys cinerea* and *Parkia biglobosa*, respectively), *Fusarium oxysporum* (12.50% on *Dichrostachys cinerea*), *Fusarium oxysporum* and *Trichoderma harzianum* (both had 8.33% on *Parkia biglobosa* and *Dichrostachys cinerea*, respectively), while *Fusarium solani* had the lowest percentage frequency of colonization (4.17% on *Dichrostachys cinerea*).

On bark isolation, *Lasiodiplodia theobromae* had the highest frequency of colonization with 75% on *Parkia biglobosa* and 58.33% on *Dichrostachys cinerea*, followed by both *Aspergillus niger* (16.67% on *Dichrostachys cinerea*) and *Trichoderma harzianum* (16.67% on *Parkia biglobosa*), while the lowest frequency of colonization was recorded for both *Aspergillus niger* (8.33% on *Parkia biglobosa*) and *Trichoderma harzianum* (8.33% on *Dichrostachys cinerea*).

Table 2. Fungi isolated from leaves and bark of *Dichrostachys cinerea* and *Parkia biglobosa* trees found in the Dry Savannah habitat of Amurum forest reserve.

Fungi isolated	COLONIZATION (%)			
	Dichrostachys cinerea		*Parkia biglobosa*	
	L	B	L	B
Aspergillus niger	0	16.67	0	8.33
Choanephora cucurbitarum	25.00	0	0	0
Fusarium oxysporum	12.50	0	8.33	0
Fusarium solani	4.17	0	0	0
Lasiodiplodia theobromae	0	58.33	0	75.00
Pestalotiopsis spp	50.00	0	50.00	0
Thermomyces lanuginosus	0	0	33.33	0
Trichoderma harzianum	8.33	8.33	25.00	16.67

L= Leaves, B= Bark

DISCUSSION

This study shed more light on the identity of fungal pathogens associated with disease of *Dichrostachys cinerea* (L.) Wight & Arn and *Parkia biglobosa* (Jacq.) R.Br. ex G. Don in the Dry Savannah habitat of Amurum forest reserve. Understanding the biology of these pathogenic agents will help in proposing possible control measure that will ensure that healthy vegetation is maintained in this habitat.

The present investigation revealed that out of 174 recorded trees, 83 (47.70%) trees were diseased and 9 (5.17%) were dead trees, making a total of 92 (52.87%) trees that were affected in the Dry Savannah habitat of the reserve. As a result of high prevalence in the reserve, forest managers need to develop control measures in order to help reduce if not possibly eliminate the causes of diseases on these trees in the habitat. During the course of this work, it was not known when and how the pathogenic organisms penetrated their hosts. However, it is well known that many pathogenic organisms enter their hosts through wounds, lenticels, or stomata (Agrios, 2005).

The majority of pathogens identified in this study represent first records for Amurum forest reserve. Hence, they contribute to a better understanding of the impact of these pathogens on the Dry Savannah habitat of the reserve.

During the investigation, leaf spot disease was the most severe disease occurring on both trees in the reserve and has been recorded as the most common disease of shade and ornamental trees as stated by Allen *et al*. (1996). These spots developed as small, scattered, circular to oval dead areas in the leaves and identifications revealed the pathogens associated were fungi of different species. The fungi were *Choanephora cucurbitarum, Fusarium oxysporum, Fusarium solani* and *Pestalotiopsis* spp. These pathogens have been recorded to be associated with foliar disease of other tree species (Rajput *et al.*, 2010; Sajeewa *et al*., 2012). This may be as a result of leaf wetness and under suitable temperature for the propagule germination of phytopathogenic fungi which weaken trees by interrupting photosynthesis, reduce the tree growth and increases its susceptibility to other diseases (Allen *et al.*, 1996; Agrios, 2005).

During our investigation, *Aspergillus niger* and *Trichoderma harzianum* were found to be associated with branch decay on both trees in the reserve. Ajay *et al*. (2011) reported *Aspergillus niger* as a strict saprophyte and according to Gary *et al*. (2004), the genera *Trichoderma* is also a saprophytic fungus.

Stem canker disease observed on both tree species revealed the associated pathogen to be *Lasiodiplodia theobromae*. It is a widespread unspecialized canker pathogen with a wide host range worldwide including Africa (Slippers and Wingfield, 2007) and has been recorded to be responsible for canker disease of tree species such as Dogwood (Mullen *et al*., 1991) and other woody plants (Slippers and Wingfield, 2007). *Lasiodiplodia theobromae* belongs to the family *Botryosphaeriaceae*, known as opportunistic, often stress-associated pathogens, causing disease and death of trees suffering from drought or other stress (Mullen *et al.*, 1991; Slippers and Wingfield, 2007).

CONCLUSION AND RECOMMENDATION

In conclusion, this study has provided useful information about the identity of important fungi associated with diseases of trees, particularly *Dichrostachys cinerea* and *Parkia biglobosa* in Amurum forest reserve. Many of these fungi represent first reports for the reserve and are well-known fungi elsewhere in the world. These trees serve important ecological functions; therefore, it is important to ensure that the forest is healthy because if left unchecked, the impact of these diseases will likely increase.

It is, therefore, recommended to put in place effective control measures that can help minimize disease development on these trees found in the Dry Savannah habitat of Amurum forest reserve.

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Plate 1: Disease symptoms observed on *D. cinerea* and *P. biglobosa* in the Dry Savannah habitat of Amurum forest reserve. On **panel A**: Dead tree of *D. cinerea*; **panel B**: Branch split on *D. cinerea*; panel **C**: *Leaf galls & chlorosis on D. cinerea*; **panel D**: Leaf galls on *P. biglobosa*; **panel E**: Leaf spot on *P. biglobosa*; **panel F**: Dieback on *P. biglobosa*

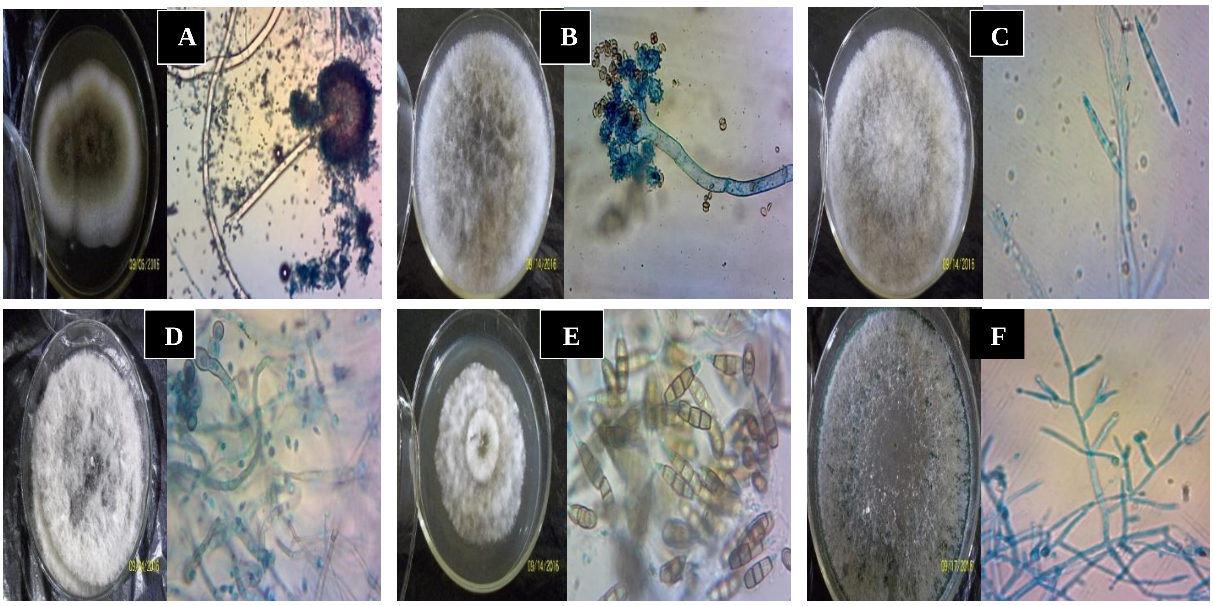

Plate 2. Some of the characteristics of fungal isolates. On **panel A**: Macroscopic & Microscopic view of *A. niger*; **panel B**: Macroscopic & Microscopic view of *C. cucurbitarum*; **panel C**: Macroscopic & Microscopic view of *F. oxysporum*; **panel D**: Macroscopic & Microscopic view of *L. theobromae*; **panel E**: Macroscopic & Microscopic view of *Pestalotiopsis* spp; **panel F**: Macroscopic & Microscopic view of *T. harzianum.*