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Fungal Microflora Biodiversity of Healthy and Diseased *Adansonia digitata* and *Sclerocarya birrea* Trees in Kenya

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Abstract: A study was conducted in Eastern Kenya to assess incidence and severity of *Adansonia digitata* and *Sclerocarya birrea* diseases under seasonal variations, and to assess associated fungal genera and their distribution. Asymptomatic and symptomatic tissues were sampled from 175 randomly selected trees. Isolations were done from leaves, twigs and bark following laboratory standard procedures. Samples were plated on Malt Extract Agar (MEA) and incubated at 25°C for 7 days. Fungal colonies were evaluated, and pure cultures were obtained using a single hypha. Fungal pathogens were identified based on morphological characteristics of cultures and spores. Statistical analysis were done using GENSTAT version 18. Fungal morphotypes isolated included: *Pestalotia* (39.0%), *Botryosphaeria* (41.0%), *Fusarium* (12.0%), *Alternaria* (7.9%) and *Cladosporium* (0.1%). There were no stastically significant differences ($p<0.01$) in number of isolated fungi among different plant samples and sampling locations. This is the first detailed study on fungal diversity associated with diseased and healthy A. *digitata* and *S. birrea* trees in Kenya and it clearly indicates the need for detailed studies of fungal species isolated to develop mitigation strategies.

Keywords: *Adansonia digitata*, *Botryosphaeria*, *Fusarium*, fungal microflora, *Pestalotia, Sclerocarya birrea*.

1. Introduction

Indigenous fruit trees in Africa contribute to nutritional security and income for the dryland communities (Gebauer et al. 2016). Across Sub-Saharan Africa, wild fruits are used for wide range of purposes (Jamnadass et al. 2011), they improve nutrition, boost food security, foster rural development and support sustainable landscape management (Chivandi et al. 2015). *Sclerocarya birrea* (A. Rich.) Hochst. (marula), is a multipurpose tree which is a source of nutrients, livelihood and food security (Mkwezalamba et al. 2015) for developing countries. The plant parts can be used for food, medicine and beverages (Gebauer et al. 2014), with key nutrient of Vitamin

A. *Adansonia digitata* L. (baobab) occurs in dry areas of Sub-Saharan Africa, the plant parts are used for food and medicine. The fruit pulp is of high nutritional value, especially regarding calcium and vitamin C (Stadlmayr et al. 2013). Therefore, baobab and marula are highly prioritized tree species for domestication and utilization in Africa (Kehlenbeck et al. 2013).

Diseased *A. digitata* and *S. birrea* trees, with various symptoms: canker, dieback and leafspots/blight were observed on farms. Branch and stem cankers which varied in size were characterized by visible lesion with exudation of gum which was initially creamish turning yellow and dark brown with time and vascular discoloration; dieback with dying of peripheral branches and leaf spots were characterized by development of necrotic lesions. Many woody plants host a high richness of fungi with most being latent, but they shift to pathogenic phase due to unfavourable conditions for the host (Pavlic-Zupanc et al. 2017). Previous studies have linked *Botryosphaeria* spp. to be the predominant causative agent of canker and dieback of trees worldwide (Burgess et al. 2019). Several Botryosphaeria spp. were isolated from *S. birrea* in South Africa (Mehl et al. 2017), and few also from baobab in South Africa, Namibia, Botswana, Mozambique and Madagascar (Cruywagen et al. 2017). *Pestalotia* spp. have also been associated with dieback of various tree species various dieback of trees; tree decline of *Mangifera indica* in India (Kumad et al. 2017), twig blight of blueberry (*Vaccinium corymbosum*) in China (Zhao et al. 2014), twig dieback and canker on blueberry (*Vaccinium corymbosum*) in Spain (Borrero et al. 2018) but no isolates have been obtained from baobab and marula. *Fusarium* species have also been linked to canker and dieback of Tea (*Camellia sinensis*) in Sri Lanka (Sinnah et al. 2017), and canker disease of *Dalbergia tonkinensis* in Vietnam (Nhung et al. 2018*).* However, knowledge of fungal communities associated with diseased baobab and marula trees in Kenya is unknown.

The objectives of this study were to: (1) assess incidence and severity of major diseases of *A. digitata* and *S. birrea* in Eastern Kenya, (2) to determine distribution and diversity of associated fungal microflora with focus on potential pathogens.

2. Material and methods

2.1. Study sites

Study sites were in Tiva, Ikanga (Kitui County) and Mukange (Makueni County) in Eastern Kenya which are the dry areas where baobab and marula are native trees. Kitui County is hot and dry, located between latitudes 0°10' S - 03°0' S and 37°50' E - 39°0' E and is located between 400 m and 1800 m above sea level, with annual temperatures ranging from 14°C to 34°C (Jaetzold et al. 2012), experiencing hot months between September – October and January – March. Rainfall on the other side ranges between 250 mm and 1050 mm per annum. Makueni Country stretches from latitude 01°35' S - 03°01' S from north to south and longitudes 37°10' E - 38°30' E from East to West. Majority of Makueni county lies within agroecological zone 5 (AEZ 5) in the semi-arid region of Eastern Kenya which is hot and dry receiving mean annual rainfall of 231 mm and 361 mm during long and short rains respectively. The mean maximum temperature of the area is 25°C and the mean minimum temperature is 13°C (Jaetzold et al. 2010).

2.2. Disease incidence and severity assessment

The survey was conducted in two seasons: 1. January to February, and 2. April to May 2018. Disease incidence was estimated by the total number of trees with canker and dieback symptoms expressed as the percentage of the total number of trees counted per farm. Disease severity was recorded as the percentage of the tree showing canker and dieback disease symptoms rated on six grade severity scale as described by Njuguna et al. (2011).

$$\text{Percentage of disease incidence (PDI)} = \frac{\text{Total number of infected trees}}{\text{Total number of trees assessed}} \times 100$$

$$\text{Percentage of disease severity (PDS)} = \frac{(1 \times a) + (2 \times b) + (3 \times c) \ldots + (6 \times f)}{N\ (\text{number of trees assessed} \times \text{maximum scale})} \times 100$$

where: 1, 2, 3, 4, 5 and 6 are severity categories, and a, b, c, d, e and f are the numbers of trees examined in each severity category.

2.3. Sampling and fungal isolation

A total of 150 symptomatic and 25 asymptomatic trees were sampled. From each selected tree, three samples were collected showing any of the disease symptoms; leaf spots and blight, dieback and stem canker with resin flow. In addition, three samples were also taken from leaves, branches and stems of healthy trees. Samples were cut from the trees and placed in bags, labelled and stored in a cooled box. Fungal isolations were performed within 24 hours from sample collection. Pieces were cut from edges of a diseased tissue and from healthy samples; surface sterilized by immersing them in 70% Ethanol for 1 min, then immersed in 33% hydrogen peroxide for one minute and rinsed three times in sterile distilled water and blotted dry with sterile filter papers. Pieces were then plated on petri dishes containing 2% malt extract agar (MEA) amended with streptomycin sulfate (100 mg/l) (Merck, Germany) and incubated at 25°C for a week under alternating light and dark cycles of 12 hours. Emerging hyphae were aseptically isolated under a dissecting microscope, transferred onto 2% MEA, and cultures were assigned a unique number.

2.4. Isolate grouping and identification using colony morphology

Two weeks after sub-culturing, fungal colonies were grouped using colony morphological characteristics like colony color and mycelia texture using a dissecting microscope (NIKON SM-2B, Japan). Spores were mounted on microscope slides in 85% lactic acid and examined microscopically (× 1000 magnification) to group and further identify the obtained fungi. The number of times each morphological group (frequency) occurred from a sample was recorded. Pure single hyphae cultures were transferred to potato dextrose agar (PDA) and stored at 4°C for further studies. Colony colors (upper surface and reverse) were determined with the charts of Rayner (1970).

2.5. Data analysis

Statistical analysis of data was performed using GenStat version 18. One-way ANOVA was used to test significance in occurrence of pathogens on different parts of the two tree species and on different sites and means separated using Tukey Test.

3. **Results**

3.1. Survey of disease incidence and severity on A. digitata and S. birrea trees under seasonal variation in Eastern Kenya

Canker and dieback disease were widespread in the two Agro-ecological zones (Makueni and Kitui), and in total 30% of the 150 trees assessed showed symptoms of canker and dieback. Disease incidence varied between 20 and 47% during wet season and between 30 and 69% during

dry season. Disease severity varied between 6 and 12.5% during wet season and between 7 and 25.2% during dry season as shown in Table 1. *Sclerocarya birrea* trees showed the highest incidence and severity of the disease compared to *A. digitata* trees across the sites.

Table 1. Incidence and severity of canker and dieback symptoms on *A. digitata* and *S. birrea* trees in Eastern Kenya.

Location	Tree species	Incidence [1] (%)		Severity [2] (%)	
		wet season	dry season	wet season	dry season
Tiva	*Adansonia digitata*	20	30	6.0	7.0
	Sclerocarya birrea	26	70	6.7	21.3
Ikanga	*Adansonia digitata*	42	54	12.3	21.8
Kibwezi	*Adansonia digitata*	40	57	10.8	23.0
	Sclerocarya birrea	47	69	12.5	25.2

[1] The incidence was determined per trees inspected per farm
[2] The severity was estimated according to damage index

3.2. Fungal morphotypes

The morphotypes identified corresponded to five genera, including; *Botryosphaeria*, *Pestalotia*, *Fusarium*, *Alternaria* and *Cladosporium* mainly. Isolates of *Botryosphaeria* produced aerial mycelium that was initially whitish turning greyish white, dark greenish grey or blackish grey within two weeks and it also produced light brown ovoid conidia (Figure 1c). *Cladosporium* species produced olive-green to brown or black colonies (Figure 1d). *Pestalotia* sp. were characterized by white, cottony mycelia with black fusiform acervuli having two apical and one basal appendages (Figure 1a and Figure 1b).

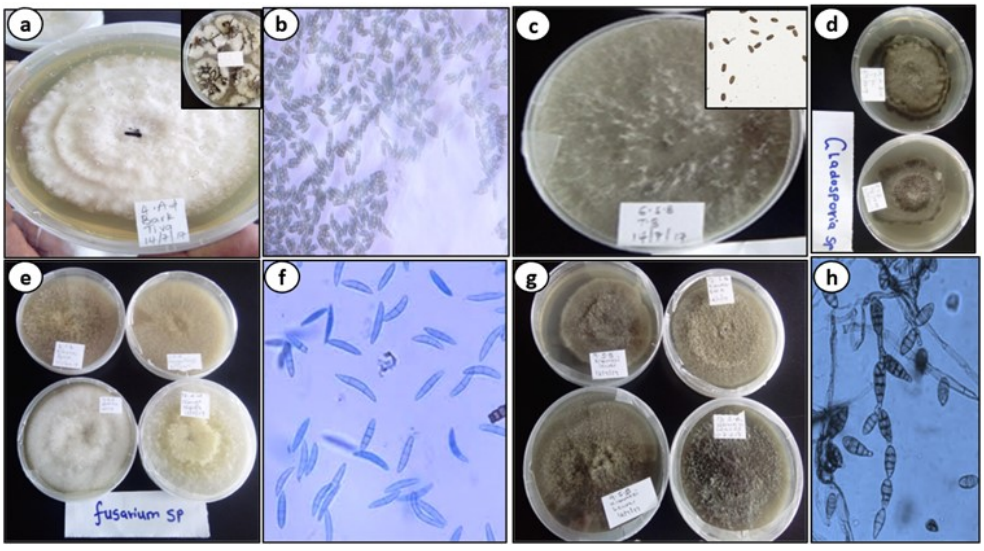

Figure 1. (a and b) cultures and spores of *Pestalotia* sp., (c) 5-day old culture of *Botryosphaeria* sp., and spores, (d) cultures of *Cladosporium* sp., (e and f) cultures and spores of *Fusarium* sp., (g and h) cultures and spores of *Alternaria* sp.

Fusarium sp. was characterized by white to creamish and pink colonies with typically curved and 3-7 septate macroconidia (Figure 1e and Figure 1f). *Alternaria* spp. isolates produced dark grey to brown mycelia with long chained conidia (Figure 1g and Figure 1h).

3.3. Fungal distribution in A. digitata and S. birrea trees in Eastern Kenya

There was no significant difference ($p<0.01$) on fungal infestation between plant parts and across the sites, ($p=0.259$ and $p=0.756$, respectively). Analysis of fungi occurring in different plant parts showed that most of the fungi were isolated from diseased stem bark (50%) followed by twigs and branches (35.2%), leaves (9.2%) and healthy plant parts (5.6%) (Figure 3).

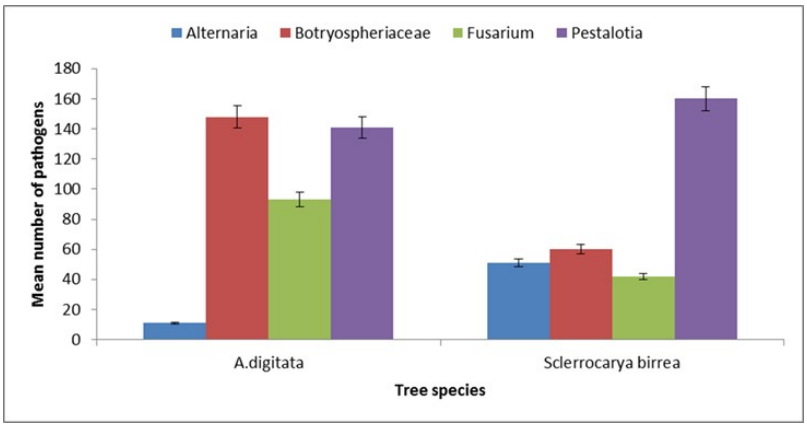

Figure 2. Frequency of fungi isolated from *A. digitata* and *S. birrea* trees in (Tiva, Ikanga and Mukange) Eastern Kenya and their distribution.

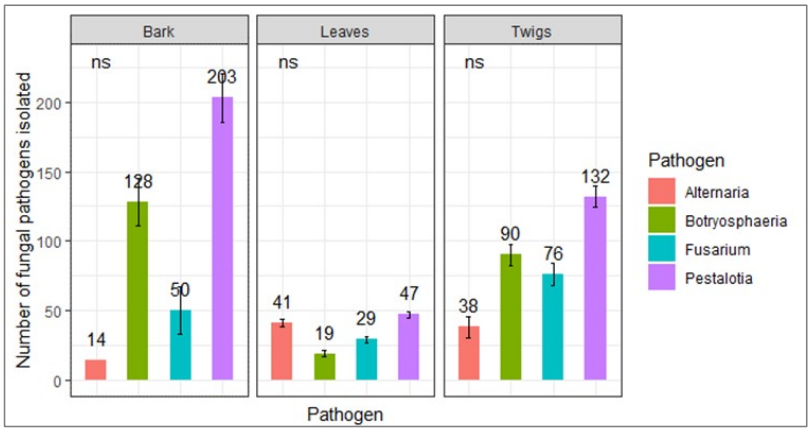

Figure 3. Fungi isolated from different plant parts of *A. digitata* and *S. birrea* trees.

Botryosphaeria and *Pestalotia* spp. were isolated more frequently on diseased tissues than healthy tissues. *Botryosphaeria* spp. occurred more often on *A. digitata* (71.4%) than on *S. birrea* (28.6%) while *Pestalotia* spp. occurred predominantly on *S. birrea* (53.3%) than on *A. digitata* (46.7%). *Fusarium* spp. occurred more on *A. digitata* (69.2%) than on *S. birrea* (30.8%), while *Alternaria* spp. Occurred more frequently on *A. digitata* (8.3%) than *S. birrea* (16.7%) (Figure 2). *Pestalotia* spp. occurred predominantly in Tiva than in Kibwezi and Ikanga, while *Botryosphaeria* spp. occurred more frequently in Kibwezi than any other regions (Figure 4).

Table 2. Significance of occurrence of fungal pathogens on healthy and diseased parts of *A. digitata* and *S. birrea* in Eastern Kenya.

Tree species	Fungal species	Healthy			Diseased			Occurrence* (%)
		Leaves	Branch	Bark	Leaf spots and blight	Dieback	Stem canker	
A. digitata	*Botryosphaeria*	–	++	+++	++	+++	++++	17.3
	Pestalotia	–	++	+	–	++	+++	16.1
	Fusarium	–	+	+	–	++	+++	10.3
	Alternaria	++	++	++	++	++	+	1.2
	Cladosporium	–	–	+	+	–	–	0.3
S. birrea	*Botryosphaeria*	++	++	++	+	+++	++++	6.9
	Pestalotia	++	++	++++	++	+++	++++	18.4
	Fusarium	–	+	+	+	+	++	5.8
	Alternaria	+++	++	++	+	++	++	5.8
	Cladosporium	–	–	–	–	–	–	0.0

* Percentage occurrence was calculated from total isolations of each fungal genera against isolations realized

\- Pathogen not detected in the tissue

\+ occurrence not significant at <1%

++ 1-5% occurrence in the disease symptoms

+++ 5-10% occurrence

++++ >10% occurrence

Species with an occurrence of > ++ were considered potentially important pathogen in the disease type

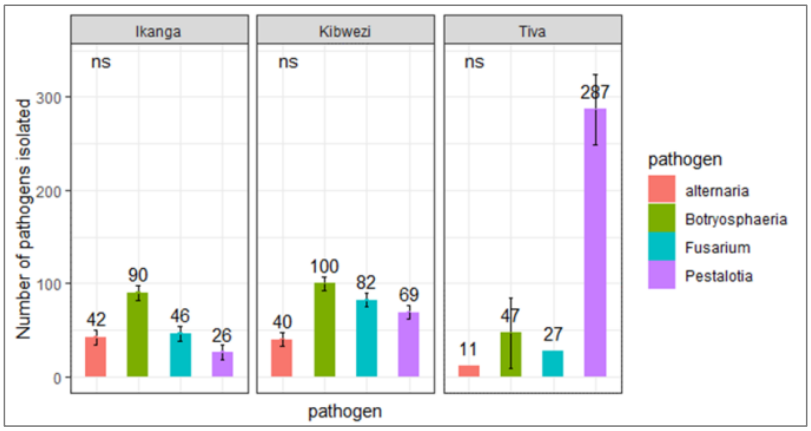

Figure 4. Fungal pathogens isolated from different sites.

4. Discussion

In this study disease incidence and severity was high during dry season than wet season, since high temperatures influence disease magnitude favouring disease development. The warm environmental conditions may have favoured proliferation of the disease impacting positively on the growth and survival of various pathogens resulting in increased incidence and severity. The observed high disease incidence and severity in *S. birrea* than *A. digitata* could be explain by marula planted closely compared to baobab possibly providing easier disease spread. since most *S. birrea* were planted on farms, pruning of trees seemed to aid disease spread within farms from infected plants to healthy plants through pruning tools. It also potrays marula to be highly susceptible to infections, therefore *A. digitata* could be better adaptive species for agroforestry systems in semi-arid areas than marula.

The fungal pathogens isolated co-occurred in the same sample which may increase the ability of the pathogens to overcome host's resistance especially under stressful conditions. The fungal isolates were also associated with both diseased and asymptomatic samples supporting previous observations that most fungi occur as latent pathogens but shifts to pathogenic phase due to unfavorable environmental conditions. In this study, prolonged drought periods could have predisposed marula and baobab trees in semi-arid areas to infection by fungal pathogens. *Botryosphaeria* and *Pestalotia* spp. were isolated most predominantly on diseased tissues than healthy samples and possibly play a role in the disease. Members of *Botryosphaeria*, *Pestalotia*, *Fusarium* and *Alternaria* are known to cause diseases on trees in Africa. *Botryosphaeria* causing canker and dieback disease was reported in Grevillea *robusta* in Kenya and on *Melia volkensii* as main hosts (Njuguna et al. 2011; Muthama et al. 2017).

The isolation frequency of *Pestalotia* spp. from marula could play a significant role in the canker and dieback disease although no record of isolations has been done on baobab. Members of *Botryosphaeria* species have been associated with canker and dieback diseases on many woody trees (Masberg et al. 2017). Recently, *Botryosphaeria* species were reported to cause canker disease on baobab and marula trees (Cherotich et al. 2019). Therefore, since *Botryosphaeria* species were isolated with the highest frequency from diseased tissues, it points to the possible that these fungi could be the cause of canker and dieback of baobab and marula trees in Kenya.

The emergence of such disease with a wide host range is a threat to agroforestry trees and will reduce the productivity of agroforestry systems. Further studies on ecology and implication of these pathogens is needed.

5. Conclusions and recommendations

Adansonia digitata and *Sclerocarya birrea* trees were hosts of numerous fungal pathogens, including *Botryosphaeria*, *Pestalotia*, *Fusarium*, *Cladosporium* and *Alternaria*, some of which cause serious diseases of woody trees. These trees species could be a serious threat to agroforestry systems because they could act as a source or reservoir of fungal inoculums. The wide spread of canker and dieback disease observed on farms is a threat to domestication of these indigenous fruit trees and therefore there is a need for improvement of our understanding of host-pathogen dynamics and ecology of these pathogens in order to develop mitigation strategies.

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