Indian Forester, 143 (9) : 890-893, 2017
http://www.indianforester.co.in

ISSN No. 0019-4816 (Print)
ISSN No. 2321-094X (Online)

BOTRYOSPHAERIACEAE FUNGAL SPECIES AS POTENTIAL PATHOGENS OF MELIACEAE IN THE ARID AND SEMI-ARID LANDS OF KENYA

ANGELA M. MUTHAMA, JANE W. NJUGUNA AND FRANCIS K. SANG[1]

Kenya Forestry Research Institute, 20412-00200 Nairobi, Kenya.
E-mail: Angelamuthama2@gmail.com

ABSTRACT

Botryosphaeriaceae are significant and cosmopolitan pathogens worldwide. In Kenya, the fungal family has been reported on *Grevillea robusta* on-farm. Two *Meliaceae* tree species, exotic *Azadirachta indica (*A. Juss.*)* and indigenous *Melia volkensii (*Geurke) were sampled across Kenya. Symptoms found in the field included shoot die-back (45%), canker 14%, both canker and dieback (25%) while 16% of the trees sampled were healthy. *Botryosphaeriaceae* was most prevalent in *Melia volkensii* mortality rate of 28%. DNA results of the ITS and Tef1-alpha gene region for 86 isolates classified into 6 species of the *Botryosphaeriaceae.* 3 of these species belonged to the genus *Lasiodiplodia* namely *L. pseudotheobromae, L. theobromae* and *L. parva*. This is the first report of species *Spencermartinsia viticola* and *Macrophoma theicola* in Kenya. Pathogenicity tests done under glass house conditions showed that the *L. pseudotheobromae* species was most virulent to both *M. volkensii* and *A. indica* while *L. theobromae* was least virulent to both tree species. Wilting and necrosis was recorded within 7 days of inoculation but wound healing occurred on both species after 12 weeks. This study gave insight into disease resistance and tolerance of these dryland species for plantation establishment. It also revealed wider host diversity for *Botryosphaeriaceae* in the drylands.

Key words: Botryosphaeriaceae, Meliaceae, Disease tolerance.

Introduction

Over 60% of Kenya's land areas has been classified as arid or semi-arid under agro-ecological zoning. These areas receive low rainfall 250-500 mm annually, have high minimum and maximum temperatures 23-24^{0}C and low to poor fertility of soils. The vastness of these areas in Kenya has led to afforestation efforts in a bid to create a green wall and prevent further desertification and as adaptation strategies to the effects of climate change. *Meliaceae* is a family of leguminous trees that consists of trees, shrubs and sometimes shrublets. It has about 50 genera and 650 species in tropical, subtropical and sometimes warm temperate regions of both hemispheres. The family has unique characteristics in the chemicals present in its plants *i.e.* limonoids, alkaloids and phenols that have been seen to contribute to the trees resistance to insect damage (Agrios, 2005). Most commonly planted species of *Meliaceae* in Kenya include native *Melia volkensii* and exotic *Azadirachta indic*a (unpublished data) which have been widely planted in the ASALs as drought resistant and termite resistant tree species. They also have multiple uses such as timber production, fodder for livestock, medicinal use in treating several ailments. *Botryosphaeria* species have been a common isolate as a causative agent of tree diseases in Kenya for the past 7 years. *Botryosphaeria* is a genera of fungi that has many members and has been found to be endophytic to many tree species (Slippers *et al.*, 2004a). Its first report was on *Grevillea robusta* (Njuguna *et al.*, 2011) common used as a shade tree for tea plantations and alley cropping. It has also been isolated from Eucalyptus tree species. The family contains 17 genera and over 150 species of fungus with a wide host range across the globe (Phillips *et al*., 2013). As environmental conditions worsen due to changing weather patterns this family of Fungi is crucial as it becomes a virulent pathogen at the onset of physiological stress. Presence of the pathogen on some existing plantations in the country also poses the risk of cross infection of native tree species from the exotic tree plantations. The present study was a survey on the presence of *Botryosphaeriaceae* on the existing plantations of representative species of *Meliaceae* and recommend management and control measures to be taken in controlling the disease and preventing an epidemic.

Material and Methods

248 trees in total of both natural stands and

Representatives of *Meliaceae* in Kenya showed susceptibility to species of *Botryosphaeriaceae* with symptoms of canker and die-back but showed resistance/tolerance during the pathogenicity test and have been recommended for climate change mitigation.

[1] Department of Forestry and Wood Science, University of Eldoret 1125-30100, Eldoret Kenya.

woodlots were sampled. Disease incidence and severity was scored by two people standing 5 m away from the tree and assessing the percentage of the tree's above ground biomass covered by symptoms such as stem and branch cankers, shoot and tip dieback, bark cracking as well as gum exudation. Disease incidence was scored as a percentage derived from the portion of trees that exhibited disease symptoms out of the total number of trees in the plantation or cluster used. Samples of twigs and bark were then collected using sterilized secateurs and blades and taken to the lab for analysis. Tools used to collect samples were sterilized with 70% ethanol to avoid infecting other trees. Trees whose trunks or stems were scraped were sprayed with fungicide to prevent re-infection by other pathogens.

Laboratory analysis

Both moist chambering and surface sterilization were used to obtain cultures from the samples collected. For moist chamberingthe cut sections were placed in surface sterilized glass petri dishes. The petri dishes were then placed in sterile tins containing wet blotting papers and the tin covered to form a damp chamber to encourage microbe growth. The tins were left at room temperature (21^{0}C) for at least 5 days until the fungi grows on the sampled material. In surface sterilization Sections of both symptomatic and asymptomatic tissue were cut into 6 small pieces washed with 33% Hydrogen peroxide for 2 minutes before rinsing them three times with distilled water. The pieces were then dried by blotting on sterile filter papers under a sterile laminar flow bench before being placed onto 2% Malt Extract Agar (Sigma-Aldrich) which was amended with 0.05% Streptomycin (Sigma-Aldrich) to inhibit bacterial growth. The petridishes were then sealed with Parafilm and incubated at 25^{0}C in 12 hour alternating light and dark cycles. Mycelial growth was monitored and colonies developed counted after 3 days. Conidial Morphology was studied under a microscope and used to classify the isolates into their genus level. These characteristics were studied under a compound microscope to define the shape, form, size, pigmentation (colour), elevation, opacity and the colony morphology to identify the pathogen.

DNA based identification

Three day old mycelia was grinded using a mechanical grinder and steel balls to make a fine powder. Tris was then added to the mixture and incubated for 50 minutes in a warm bath. CTAB was then added to the mixture and incubated at 40^{0}C for 30 minutes before adding 70% Sodium Chloride. PCR amplification using specific primers for ITS and Tef1-alpha were used. The sequences were then analyzed using Gene Sequencer 5000 and the sequences edited to match them to the nearest possible species. Instead that sequences were edited using MEGA 4.0 software and a cladogram produced to show the same. A series of reactions with specific chemicals and reagents is used to extract rDNA from cells of the fungi which is then analyzed using a sequencer machine into bands made of base pairs of DNA. The downloaded sequences are then compared with existing sequences from Genbank and other culture collections to find the closest relative at 100%.

Results and Discussion

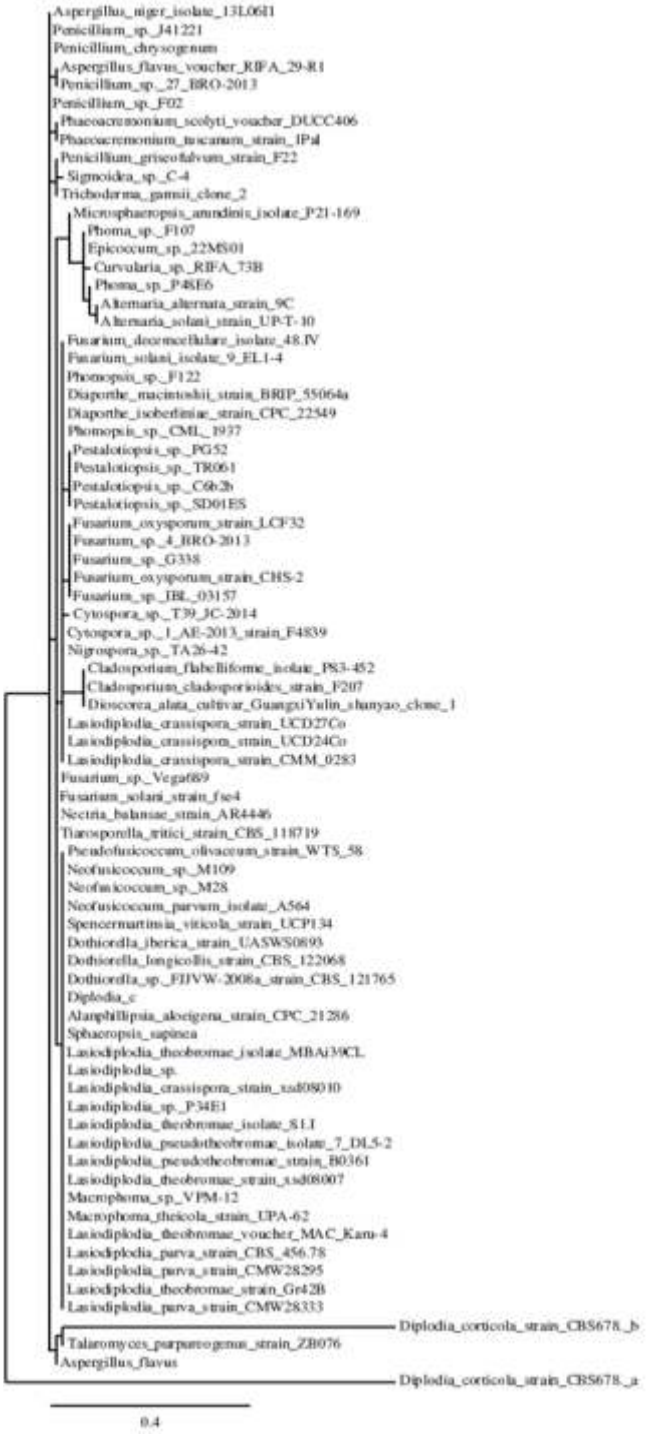
50.3% of the isolated fungal species were made up of *Botryosphaeriaceae* followed by *Nectriaceae* (32.3%) and then *Pestalotiopsis* (3.6%) *Botryosphaeria* species was also isolated from healthy trees characterizing it as an endophyte that would become pathogenic in the event of physiological stress on the tree. Well silviculturally managed trees had less disease symptoms than those in the wild.

Grand Total	247
Botryosphaeria sp.	125
Fusarium sp.	63
Pestalotia sp.	22
Nectria sp.	18
Phomopsis sp.	4
Alternaria sp.	3
Phoma sp.	3

Disease severity was higher in *Melia volkensii* than in *Azadirachta indica* however, there was no mortality recorded for *A. indica* and a few recorded for *M. volkensii.*

DNA results

Melia Volkensii isolates	*Azadirachta indica isolates*
Alternaria alternata	*Cladosporium flabelliforme*
Aspergillus flavus	*Cytospora* sp.
Diaporthe isoberliniae	*Fusarium oxysporum*
Diaporthe macintoshii	*Lasiodiplodia crassispora*
Lasiodiplodia crassispora	*Lasiodiplodia parva*
Lasiodiplodia parva	*Lasiodiplodia pseudotheobromae*
Lasiodiplodia parva / Botryosphaeria sp	*Lasiodiplodia pseudotheobromae*
Lasiodiplodia pseudotheobromae	*Lasiodiplodia* sp. KR1
Lasiodiplodia sp. KR1	*Lasiodiplodia theobromae*
Lasiodiplodia theobromae	*Lasiodiplodia theobromae*
Lasiodiplodia theobromae	*Macrophoma theicola*
Neofusicoccum parvum	*Pestalotiopsis* sp.
Nigrospora sp.	
Penicillium chrysogenum	*Phoma* sp.
Pestalotiopsis sp.	
Spencermartinsia viticola	
Talaromyces purpureogenus	



Botryosphaeriaceae Theiss & P. Syd. (Fungi, Acsomycota, Pezizomycotina, Dothideomycetes, Botryosphaeriales) is a family of fungi with a cosmopolitan distribution across the globe except in the Polar Regions known to cause severe die-back and canker on many woody plants. The characteristic of some of its species as endophytes makes a crucial species of interest especially as more and more wood lots are being established in lands that already experience moisture stress.

Lasiodiplodia, Dothiorella, Macrophoma, Sphaeropsis, Fusicoccum, Diplodia and *Botryodiplodia* include some of the most common genera linked to *Botryosphaeria* (Denman *et al*., 2000).

The family has 17 identifiable genera and over 103 species known from culture (Phillips *et al.,* 2013). Species from this family have been isolated from native, exotic and naturalized tree species in most tropical countries from both plantation and agro-forestry systems. Many genera of plants including angiosperms and conifers are susceptible to *Botryosphaeria* attack. In Kenya *Botryosphaeriaceae* has been isolated from many exotic tree species commonly established in plantations across the country. A major predisposing factor has been poor species site matching by planting tree species outside their optimal ecological zone limit. These species include *Eucalyptus camaldulensis*, Eucalyptus clones, *Grevillea robusta, Azadirachta indica, Senna siamea*. An attack and epidemic of *Diplodia pini* (renamed to *Sphaeropsis sapinea)* on Pine plantations in the country led to the ban of planting of *Pinus radiata* in eastern Africa. Known to have some chemical properties that make them disease resistant *Meliaceae* continued to fight the symptoms of the disease. The high disease severity but no mortality in some of the trees sampled was seen as perceived disease resistance of the target tree species.

Conclusion

Control measures against *Botryosphaeriaceae* species has been proper species-site matching during tree establishment and management of trees planted to ensure optimum growth conditions with little or no physiological stress on the plants. This reduces the susceptibility of the plants to infection by *Botryosphaeriaceae*. Treatment of pruning wounds with fungicides has also proved to be helpful in control of new infections in plantations. Once affected the mechanical removal and burning of infected stumps is advised to remove the source of inoculums which can lead to an epidemic through wide spread of the fungal infection. Further research should be done on these tree species with field trials to ascertain the perceived versus the real threats for seedlings once planted in the field.

Acknowledgement

Authors wish thank to the Swedish Research Council for their support in this research. We also wish to thank Prof. Pia Barklund and Prof. Jan Stenlid both from SLU (Swedish University of Agricultural Sciences) for their guidance and collaboration.

केन्या के शुष्क एवं अर्ध-शुष्क भूमियों में *मीलिएसीया* के एक सक्षम रोग जनक के रूप में *बॉट्रीओस्फेरिएसीया* कवकी प्रजाति

एंजीला एम. मुथेमा, जेनी डब्ल्यू जूगूना एवं फ्रान्सिस के. सांग

सारांश

बॉट्रीओस्फेरिएसीया विश्वभर में महत्वपूर्ण एवं सर्वदेशीय रोगजनक हैं। केन्या में कवक कुल फार्म में *ग्रीविलीया रॉबुस्टा* पर सूचित किया गया है। दो *मीलिएसीया* वृक्ष प्रजातियों, विदेशज *ऐजैडिरैक्टा इंडिका* (ए. जस) और देशज *मीलिया वोल्कीन्सी* (गूर्की) का सम्पूर्ण केन्या में सैम्पल किया गया। क्षेत्र में पाए गए रोग लक्षण में प्ररोह पश्च क्षय (45%), कैंकर (14%), कैंकर और पश्च क्षय दोनों (25%), शामिल थे जबकि सैम्पल किए गए 16 प्रतिशत वृक्ष स्वस्थ थे। बॉट्रीओस्फेरिएसीया 28 प्रतिशत की मर्त्यता दर के साथ *मीलिया वोल्कीन्सी* में सबसे ज्यादा प्रचलित था। 86 आइसोलेटों के लिए इसके जीन क्षेत्र के डी.एन.ए. परिणामों को *बॉट्रीओस्फेरिएसीया* की 6 प्रजातियों में वर्गीकृत किया गया। इन प्रजातियों में से 3 जीनस *लेसिओडिप्लोडिया*, यथा- एल. *स्यूडोथीओब्रोमेई, एल. थीओब्रोमेई* और *एल. पर्वा* से संबंधित हैं। केन्या में प्रजाति *स्पेन्सरमार्टिन्सिए विटिकोला* और *मैक्रोफोमे थीकोला* की यह पहली रिपोर्ट है। ग्लास हाउस अवस्थाओं के तहत रोग जनकता जाँच ने दर्शाया कि स्यूडोथीओब्रोमेई प्रजाति *मीलिया वोल्कीन्सी* एवं *ऐजैडिरैक्टा इंडिका* दोनों के लिए सबसे विषाक्त थी जबकि एल. *थीओब्रोमेई* दोनों वृक्ष प्रजातियों के लिए न्यूनतम विषाक्त थी। संरोपण के 7 दिन की भीतर म्लानी और ऊतक क्षय अभिलिखित किया गया किन्तु दोनों प्रजातियों में 12 हफ्ते के उपरान्त घाव भरने शुरू हुए। इस अध्ययन में रोपण स्थापना के लिए इन शुष्क भूमि प्रजातियों के रोग प्रतिरोध एवं सहनशीलता में अन्तर्दृष्टि दिया। इसने शुष्क भूमियों में *बॉट्रीओस्फेरिएसीया* के लिए व्यापक परपोषी विविधता को भी उद्घाटित किया।

References

Agrios G.N. (2005). *Plant Pathology*, 5th Edition. Elsevier, Academic Press.

Denman P.W., Crous J.E., Taylor J.C., Kang I., Pascoe and Wingfield M.J. (2000) An overview and taxonomic history of *Botryosphaeria* and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. *Studies in Mycology*, 45(2000): 129-140.

Njuguna J., Barklund P., Ihrmark K. and Stenlid J. (2011). A canker and dieback disease threatening the cultivation of Grevillea robusta on small scale farms in Kenya. *African J. Agricultural Research,* 6(3): 748-756.

Phillips A.J.L., Alves A., Abdollahzadeh A., Slippers B., Wingfield M.J., J.Z. Groenewald J.Z., and Crous P.W. (2013). "The *Botryosphaeriaceae*: genera and species known from culture" *Studies in Mycology*, 76(1): 51–167.

Slippers B., Crous P.W., Denman S., Courtinho T.A., Wingfield B.D. and Wingfield M.J. (2004a). Combined multiple gene genealogies and Phenotypic characters differentiate several species previously identified as *Botryosphaeriadothidea*. *Mycologia*, 96:83-101.