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Original scientific paper

Fungal Diseases Attacking Floral Phenology of *Terminalia brownii* in Drylands, Kenya

Michael M. Okeyo [1,2,*], Gilbert O. Obwoyere [2], Dickson L. Makanji [2], Jane W. Njuguna [3], Jackline A. Omondi [2]

[1] Kenya Forestry Research Institute, P.O. Box 892 - 90200 Kitui, Kenya
[2] Egerton University, P.O. Box 536 - 20107 Egerton, Kenya
[3] Kenya Forestry Research Institute; P.O. Box 20412- 00200 Nairobi, Kenya

* Corresponding author: Michael M. Okeyo; E-mail: mokeyo@kefri.org

Abstract: *Terminalia brownii* is among the dominant multipurpose tree species in the Kenyan drylands whose regeneration is hampered by poor seed germination. It is used for building, fencing, herbal medicine, wood carving, and woodfuel among others. A study to assess fungal pathogens that infest *T. brownii* floral phenophases was conducted in Baringo, Kendu Bay and Kitui Bay. Flower-buds, flowers and immature were sampled for culturing from 30 trees selected randomly within 5.0 ha area in each site and GPS coordinates recorded. Samples of 100 flower-buds, flowers, immature and mature fruits were surface sterilized using 10% sodium hypochlorite for 2 minutes and rinsed in distilled water. These were plated on Malt Extract Agar (MEA) media and incubated at 28 ± 2°C for seven days; fungal colonies were evaluated and sub-cultured to obtain pure cultures and pathogens were identified using morphological characteristics. Statistical analyses were carried out using GENSTAT version 18 and means separated using Turkeys test. Common fungal pathogens isolated were *Pestalotia* (53-57%), *Fusarium* spp (12-19%), *Rhizopus* (16-21%) and *Cladosporium* species (1-5%). While that of *Bostrosphaeria*, *Trichoderma* and *Alternaria* species were less than 2.5% in all sampled floral phenophases. There were significant differences ($p<0.05$) of fungal infestation between flower-buds, flowers, immature and mature fruits, but not across sites. Flower-buds had the least of isolated fungal pathogens, thus indicating that infestation took place during and after flowering. These fungi may affect seeds and germination by either causing seed deterioration or affecting the germinants hence lowering seed quality.

Keywords: *Terminalia brownii*, agroforestry, domestication, fungal-pathogens.

1. Introduction

Terminalia brownii is among the major indigenous tree species which are widely distributed in the arid and semi-arid lands of East, Central and West Africa (Mosango, 2013; Orwa et al. 2009; Beentje, 1994), on which most of the communities there, depend on for their livelihood. *Terminalia* species are among the important timber and charcoal producing tree species in the drylands mainly for cooking fuel in majority of Kenyan households. Fuelwood is an important energy

source in the cottage industries such as tobacco curing, brick making, fish smoking and bakeries (Orwa et al. 2009; ICRAF, 1992). Most dryland forests, including *T.brownii* species, and woodlands are under intense pressure from agriculture, human settlement, overgrazing, wild fires, illegal logging and overexploitation (Mutinda, 2014; FAO, 2014), thus the population of this species has been reported to be reducing at an alarming rate.

Tree growing has a potential of improving livelihoods of communities in arid areas because large tracts of land are available. Indigenous tree species are well adapted to the ecological setup in the drylands; have become increasingly important in not just mitigating deforestation but also in increasing land productivity and providing a base for commercial/industrial growth of these ecosystems (Awodoyin et al. 2015; Jama and Zeila, 2005). Despite *T. brownii* being one the important species in indigenous forests being encroached, its propagation is also being hindered by low germination percentages.

Seeds may fail to germinate due to poor pollination and synchronization of flowering in dioecious plant species, when seeds are harvested before maturity and infestation by insects and diseases (Yongeesha et al. 2005; Bewley, 1997). This has been reported to be observed in *T. brownii* seed germination. It is therefore necessary to evaluate the effect of incidence of diseases on floral phenology of *T. brownii* trees and their impacts.

1.1. Objective

To identify fungal pathogens that affect floral phenophases of *Terminalia brownii* growing in Kitui, Baringo and Homa Bay Counties, Kenya.

1.2. Study justification

Previous studies focused on germination and overcoming physiological dormancy of *T. brownii* seed (Omondi, unpublished report, 2011), but little is known on how seeding patterns, floral phenology and infestation by insect pests and fungal diseases affect seed viability. Information on infestation by fungal diseases of *T. brownii* will directly and indirectly promote conservation activities (Omondi et al. 2016). This paper examines some of the common fungal pathogens which may cause plant diseases thus affecting *T. brownii* fruits, seeds and seed germination processes.

2. Materials and Methods

2.1. Study area

Study sites were Nduumoni in Kitui, Kendu Bay in Homa Bay and Kimose in Baringo, representing the drier parts of Kenya where *T. brownii* is native. Kitui County is located between latitudes 0°10′ and 3°0′ South and longitudes 37°50′ and 39°0′ East of Greenwich and is located between 400 m and 1800 m above sea level, with annual temperatures ranging from 14°C to 34°C, experiencing hot months between September - October and January-March. Rainfall on the other side ranges between 250 mm and 1050 mm per annum. Kendu Bay is within Homa Bay County is between Latitude: 0° 40' 60.00" N and longitude: 34° 27' 0.00" E, and 1166 meters elevation above the sea level. Whereas the daily temperatures ranges between 26°C during the coldest months (April and November) and 34°C during the hottest months (January to March). The county receives between 250 mm and 1200 mm of rainfall annually, with the average annual rainfall estimated at 1,100 mm. It has two rainy seasons; March-April-May (long rains) and October to December (short rains) (http://www.kenya-information-guide.com/homa-bay-county.html).

Baringo County falls between longitudes 35°30' and 36°30' East and between latitudes 0°10' and 1°40' South in the former Rift Valley County (County Annual Development Plan, 2015/2016). It has two distinct weather patterns with temperatures in the southern part ranging between 25°C during the cold months (June and July) and 30°C during the hot months (January and February) while in the northern parts, temperatures range between 30°C to 35°C. The county receives between 1000mm and 1500mm of rainfall annually in the highlands and 600mm in the lowlands. Baringo experiences two rainy seasons; March to June (long rains) and November (short rains) (http://www.kenya-information-guide.com/baringo-county.html).

2.2. Collection of samples

A total of 30 trees were randomly selected from which samples of five trees were selected and inflorescences on top, middle and lower crowns tagged for sample collection throughout the floral phenophases. Flower-buds, full bloom flowers and immature fruits samples were then collected from the tagged inflorescences one's a week, labeled and transported in ice cold boxes to KEFRI pathology laboratories for culturing and identification.

2.3. Isolation of fungal pathogens

Fungal pathogens were isolated using Potato Dextrose Agar (composition; peeled potato 100.0g, glucose 20.0 g, agar 15.0 g, water 1000.0 ml) obtained from Oxoid (Basingstoke, UK). In surface sterilization, to sterilize the surface, the samples were placed in 10% sodium hypochlorite for 2 minutes followed by rinsing in sterile distilled water. The samples were blot dried using previously sterilized Whatman filter papers and directly plated on Potato Dextrose Agar (PDA) media. The plates were incubated at a 28°C for up to 7 days (Henry et al. 2015). Colonies showing typical fungal characteristics were sub-cultured on PDA until pure cultures were obtained. The pure cultures were preserved in PDA slants at 4°C and glycerol until further processing. The fungal pathogens and colonies were identified using cultural, morphological and biochemical characteristics (Binyam and Girma, 2016). Data on types of fungal pathogens, number of colonies and frequency of the pathogens fungal diseases infesting flower buds, flowers and fruits samples were collected and recorded.

3. Results

Common fungal pathogens isolated from floral phenophases of *T. brownii* flower-buds, full bloom flowers and immature fruits across the three study sites included: *Pestalotia, Fusarium*, *Rhizopus*, *Cladosporium, Bostrosphaeria* and *Alternaria* species among others (Figure 1).

There were significant differences ($p<0.05$) of fungal infestations between flower-buds, flowers, immature and mature fruits phenophases, but not across sites. Common fungal organisms isolated were *Pestalotia*, *Fusarium*, *Rhizopus* and *Cladosporium* species (Figure 2). Other organisms were *Bostrosphaeria* and *Alternaria* species were in very small amounts across all the study sites.

Similar fungal pathogens were isolated from flower buds, open flowers and immature fruits of *T. brownii*; these were: *Pestalotia* spp (53 – 57%), *Fusarium* spp (12 – 19%), *Rhizopus* (16 – 21%) and *Cladosporium* (1 – 5%). While that of *Bostrosphaeria*, *Trichoderma* and *Alternaria* species were less than 2.5% in all sampled floral phenophases (Figure 3). There were no significant differences ($p<0.05$) of isolated fungal infestations from flower-buds, flowers and immature fruits across study sites.

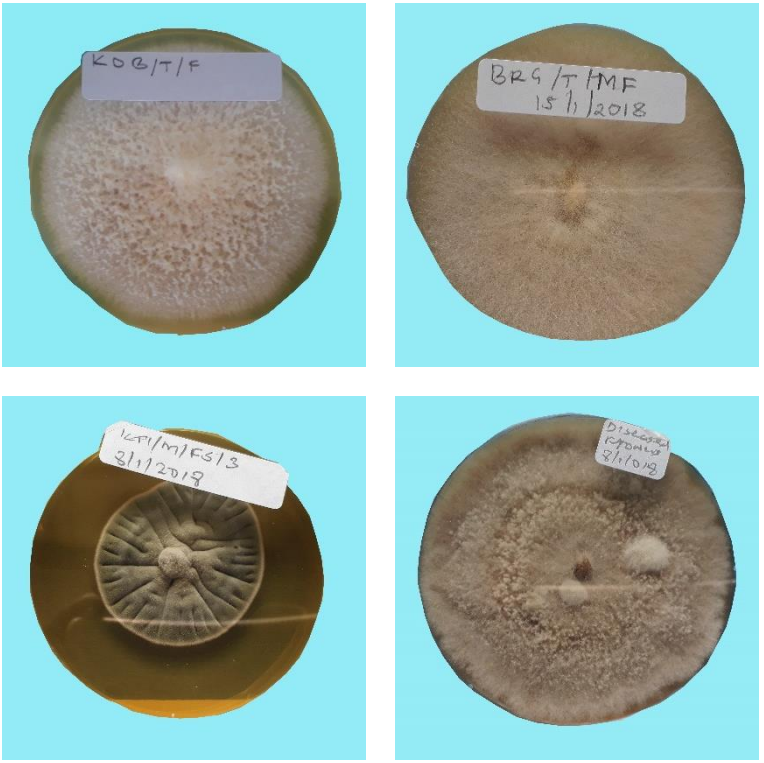

Figure 1. Some of the common fungal pathogens isolated from *T. brownii* floral phenophases: *Pestalotia* (A), *Fusarium* (B), *Cladosporium* (C) and *Alternaria* (D) species

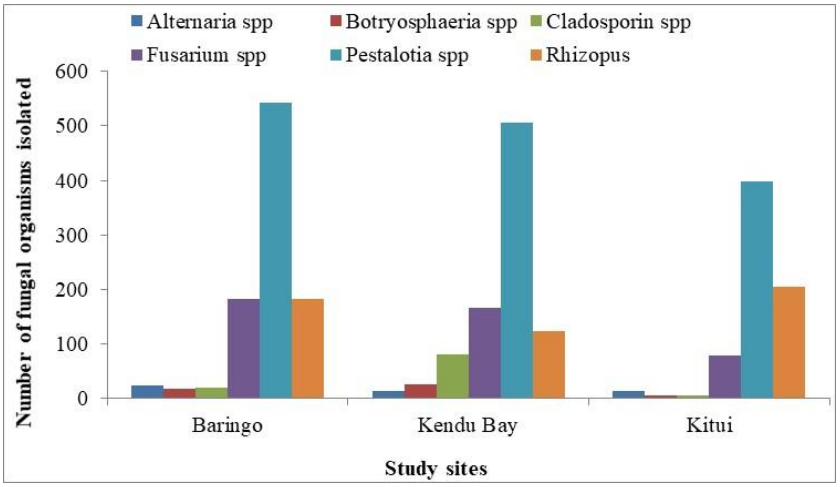

Figure 2. Number of fungal organisms isolated from *Terminalia brownii* floral phenophases within the study sites of Baringo, Kendu Bay and Kitui

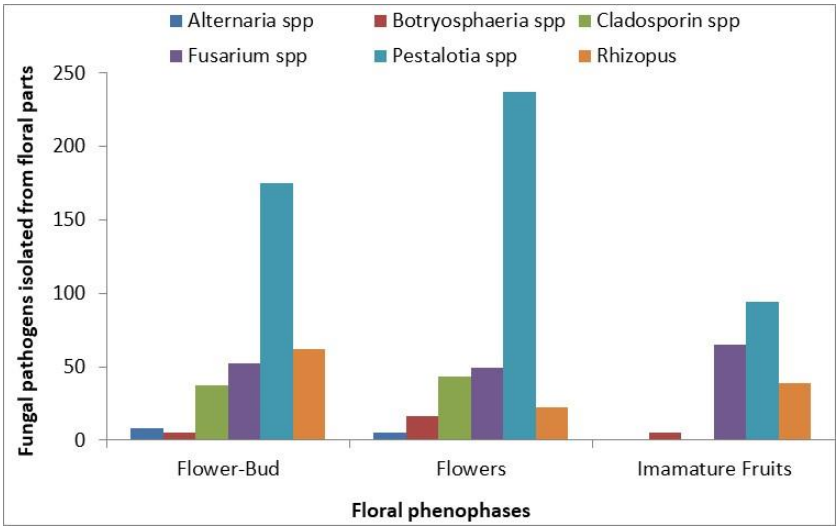

Figure 3. Percentage of major fungal organisms isolated from *Terminalia brownii* floral phenophases: flower buds, open flowers and immature fruits across study the three sites

4. Discussion

Most fungal pathogens occur in nature without causing any diseases unless the plant is susceptible and conditions are conducive for disease to occur. Many genus including members of *Cladosporium*, *Bostrosphaeria*, *Alternaria* and *Fusarium* are known to cause plant diseases in many regions of the world including the African continent (Binyam and Girma, 2016; Ivanová, 2016; Franke et al. 2014; Grasso and Granata, 2010). Members of *Botryosphaeria* species cause dieback and are associated with cankers on the branches and main tree stems (Grasso and Granata, 2010). It is therefore possible that many of the cankers found on *T. brownii* may be caused by members of the genus *Botryosphaeria*. However, it's not clear how these species may affect *T. brownii* fruits and seeds.

Fungi of the genus *Cladosporium* are cosmopolitan organisms whose spores are commonly found in water, air and soil. They have been reported to affect both plants and humans alike (Ogórek et al. 2012). In many plants they affect leaves, leaf petioles, branches, stems and fruits. It's therefore possible that the disease may affect T. brownii seedlings in the nursery and plants in the field leading to reduced or stunted growth. However, more studies on the same are needed.

Alternaria and *Fusarium* species are rot fungi and may cause rot in seeds during storage, germination and after germination (Ivanová, 2016; Franke et al. 2014). Strains of *Alternaria* fungi were found to significantly reduce the germination capacity of wheat seeds (Perelló and Larrán, 2013). The same was found to affect wheat-seedlings growth suggesting that *Alternaria* fungus could be seed borne. Similarly, members of *Fusarium* genus have been reported to cause seed rot diseases, often as part of a complex of diseases that affects different stages of the host, such as seeds, seedlings, and the crowns of developing plants (Franke et al. 2014). Therefore, *Alternaria* and *Fusarium* species are likely to affect and reduce germination capacity of *T. brownii* seeds and also affect seedling growth in the nursery by reducing the photosynthetic area through leaf spotting.

Members of *Pestalotia* genus have been reported to cause a variety of diseases in plants, including canker lesions, shoot dieback, leaf spots, leaf and stem blight, needle blight, tip blight, grey blight, scabby canker, severe chlorosis, fruit rots and leaf spots (Espinoza et al. 2008; Zhang et al. 2013). This also cause fruit and seed rots in *T. brownii* whenever conditions are conducive and in susceptible plants.

5. Conclusions and Recommendations

Terminalia brownii floral phenophases are affected by various fungal pathogens of the genuses; *Pestalotia*, *Fusarium*, *Rhizopus*, *Cladosporium, Bostrosphaeria* and *Alternaria* which were isolated from flower-buds, full bloom flowers and immature fruits. These are likely to affect seed production, reduce germination and affect growth of seedlings in the nursery. Their effect on seed formation, storage longevity and germination may be clear after identifying the individual species of each pathogen and subjecting them to more tests. These information will contribute seed biology and eventually promote domestication of *T. brownii* in drylands of Kenya and East Africa.

In order to understand the negative effects associated with various fungal pathogens isolated from *T. brownii* floral phenophases, the authors recommend the following detailed studies to be undertaken.

1. Carry out more research on specific fungal pathogens found infesting *T. brownii* floral phenophases,
2. More studies needed to determine the negative effects of each fungal pathogens isolates from *T. brownii* on seed production, storability and germination of fruits and seeds,
3. Research on the effect of fungal pathogens isolates on *T. brownii* seedling growth in the nursery and field conditions,
4. Carry out more research to identify the possible control measure for each fungal pathogen isolated.

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6. References

1. Awodoyin, R.O., Olubode, O.S., Ogbu, J.U., Balogun, R.B., Nwawuisi, J.U., Orji, K.O. (2015): Indigenous Fruit Trees of Tropical Africa: Status, Opportunity for Development and Biodiversity Management. Agricultural Sciences 6: 31-41.
2. Beentje, H.J. (1994): Kenya Trees, Shrubs and Lianas. National Museums of Kenya, Nairobi.
3. Bewley, J.D. (1997): Seed germination and dormancy. The plant Cell 9: 055-1066.
4. Binyam, T., Girma A. (2016): Detection and identification of major storage fungal pathogens of maize (Zea mays) in Jimma, Southwestern Ethiopia. European Journal of Agriculture and Forestry Research 4(2): 38-49.

5. Boyogueno, B.A.D. (2009): Characterization of Botryosphaeriaceae and Cryphonectriaceae associated with *Terminalia SPP*. in Africa. Diss. University of Pretoria.
6. Espinoza J.G., Briceno E.X., Keith L.M., Latorre B.A. (2008): Canker and twig dieback of blueberry caused by *Pestalotiopsis* spp. and a *Truncatella* sp. in Chile. Plant Disease 92: 1407–1414.
7. FAO (2014): The State of The World's Forest Genetic Resources. Commission on Genetic Resources for Food and Agriculture, FAO Rome.
8. Franke, J., Geary, B., Meyer, S. E. (2014): Identification of the Infection Route of a *Fusarium* seed pathogen into non-dormant *Bromus tectorum* seeds. Phytopathology 104(12): 1306-1313.
9. Grasso, F.M., Granata, G. (2010): First report of *Botryosphaeria dothidea* associated with dieback of aspen (*Populus tremula*) in Italy. Plant Pathology 59: 807.
10. http://www.kenya-information-guide.com/Baringo-county.html
11. http://www.kenya-information-guide.com/homa-bay-county.html
12. http://www.kenya-information-guide.com/kitui-county.html
13. ICRAF (1992): A Selection of Useful Trees and Shrubs for Kenya: Notes on Their Identification, Propagation and Management for Use by FarmingaAnd Pastoral Communities. ICRAF, Nairobi.
14. Ivanová, H. (2016): Comparison of the fungi *Pestalotiopsis funerea* (Desm.) Steyaert and *Truncatella hartigii* (Tubeuf) Steyaert isolated from some species of the genus *Pinus* L. in morphological characteristics of conidia and appendages. Journal of Forest Science 62(6): 279–284.
15. Jama, B., Zeila A. (2005): Agroforestry in the Drylands of Eastern Africa: A Call to Action. ICRAF Working Paper – no. 1. Nairobi: World Agroforestry Centre.
16. Mosango, D.M. (2013): *Terminalia brownii* Fresen. In: Schmelzer, G.H. and Gurib-Fakim, A. (Editors). Prota 11(2): Medicinal plants/Plantes médicinales 2. PROTA, Wageningen, Netherlands.
17. Mutinda, J. (2014): Assessment of the Impact of the Woodcarving Industry on the Environment: A Study of Wamunyu Location, Mwala District. Kenya. Journal of Environment and Earth Science 4(6): 17.
18. Ogórek, R., Lejman, A., Pusz, W., Anna Miłuch, A., Miodyńska, P. (2012): Characteristics and taxonomy of *Cladosporium* fungi. Mikologia Lekarska 19(2): 80-85.
19. Omondi, S. F., Odee, D.W., Ongamo, G.O., Kanya, J.I., Khasa, D.P. (2016): Synchrony in Leafing, Flowering, and Fruiting Phenology of *Senegalia senegal* within Lake Baringo Woodland, Kenya: Implication for Conservation and Tree Improvement. International Journal of Forestry Research (Hindawi Publishing Corporation) 2016: pp 11.
20. Omondi, W. (2011): Comparison of *Terminalia brownii* germination across provenances in Kenya, Unpublished report.
21. Orwa, C, Mutua, A., Kindt, R., Jamnadass, R., Anthony, S. (2009): Agroforestree Database: a tree reference and selection guide.
22. Perelló, A. E., Larrán, S. (2013): Nature and effect of A*lternaria* spp. Complex from wheat grain on germination and disease transmission. Pak. J. Bot. 45(5): 1817-1824.
23. Plant Resources of Tropical Africa. https://uses.plantnet-project.org/en/Terminalia_brownii (PROTA) Accessed 19.9.2018.
24. Yogeesha, H.S., Shivananda, T.N., Bhanuprakash, K. (2005): Effect of seed maturity, seed moisture and various pre-treatments on seed germination of annatto (*Bixa orellana* L.). Seed Science and Technology 33**:** 97–104.
25. Zhang, Y.M., Maharachchikumbura S.S.N., Tian Q. (2013): *Pestalotiopsis* species on ornamental plants in Yunnan Province, China. Sydowia 65: 59–74.