IJABR Vol. 15(1): 156 - 163 (2024)

Original article

Isolation and identification of mycoflora associated with *Gmelina arborea* wood in some selected timber sheds within Biu, Biu Local Government Area, Borno State NIGERIA

Adamu, M. and Bello, A.A.

Department of Biology, Nigerian Army University Biu, Borno State, Nigeria

Submitted: February, 2024; Accepted: March, 2024; Published: June, 2024

ABSTRACT

Gmelina arborea plantation is one of the largest plantations in Nigeria from which raw materials for paper industries, buildings, constructions and carpentry works is obtained. This research was aimed at investigating the mycoflora associated with the wood of Gmelina arborea, many of which led to wood decay in Biu Local Government Area of Borno State. Samples were collected from the affected part of the wood in three strategic study areas (Timber sheds) namely: Kasuwar Katako, Main market, and Muhammad Bwala timber shed. Isolation was carried out by inoculating the different samples on Potato Dextrose Agar (PDA) plates in the laboratory of the Department of Biology, Nigerian Army University Biu; and incubated at a temperature of 28^0C for a week from which pure cultures were obtained after subculturing the different fungal colonies. Identification of the fungal species was carried out using macroscopic and microscopic examinations. These are *Absidia corynbifera*, *Microsporum jypseum*, *Microsporum fulvum*, *Rhizopus microsporus* and *Rhizopus orizae*. The percentage frequency of each of these fungi was also determined using the means. The result obtained from this research shows that Rhizopus microsporus is the most prevalent found in all the three study areas while the other four species were encountered only once in one study area or the other. Therefore, further research should be carried out on the treatment and proper storage of wood decay to minimise spoilage and boost the economy.

Key words: Mycoflora, wood, fungal colony, Inoculation, Gmelina arborea

Corresponding author's email: adamumusamdg@gmail.com

INTRODUCTION

Gmelina arborea Roxb, a plant belonging to family Verbanaceae, is reported to be a widely grown deciduous tree of moderate to large size with an arborescent habit hence the specific name "arborea" [5]. It is a fast growing tree, which grows faster than some exotic species under the same conditions. It is medium sized, reaching a height of about 30 – 40 meters, with a bole averaging 40 centimetres in diameter but sometimes attaining 50 centimetres. The leaves are more or less heart-shaped, 10 – 25 centimetre by 5 – 18 centimetres and globrous or velvety beneath, the corolla is bright yellow and the ovary glabrous. Mycoflora could be described as the fungi of a particular area or habitat. Fungi are the most diverse group of microorganisms that inhabit different environmental sources such as soil, plant parts (leaves, root and fruits), water and food sources [12]. The growth and distribution of fungi are affected by different environmental factors that include temperature, pH, moisture, degree of aeration, amount and type of nutrients [8]. The morphology of a fungal colony in filamentous fungi results from growing as fibers (hypha), that are cylindrical, threadlike (2-10 um in diameter), long up to several centimeters, with different observations of colony features such as colour, size, shape visible by the naked eye which was used classically to identify fungi [10].

The morphology of fungi was observed under a compound microscope to examine the shapes forming from the arrangement spores [8]. The morphological and biochemical identifications of fungi sometimes face many problems such as: the need for a great time, requiring high skill, and generating various morpho/biotypes within one species. The use of molecular identification is fast, sufficient, reproducible, and can provide high specificity to distinguish between the species and subspecies of fungi unlike the morphological and biochemical tests used in the laboratory diagnosis of fungi [15].

Wherever bacteria and fungi co- occur, they must interact with and influence each other yet, although wood-decay fungi are well known for being highly competitive [2], relatively little attention has been paid to the fungus-bacteria relationship [3]. Fungal bacterial interactions have already been studied in other contexts for environment.

Fungi are the principal agents of wood decay in terrestrial habitats and hence they open up the wood resource for most other organisms living in dead wood [2, 3]. Wood inhabiting fungi constitute a highly diverse group of organisms. A large group of species are obligatorily associated with dead wood, as active wood decayers or mycoparasites, but also litter decomposing, mycorrhizal and bryophile species are associated with decaying wood. Fungal diversity is far lower in wood than in soil, and is highly influenced by the underlying soil type: nonetheless, there is a high level of intrasite heterogeneity. The fungi community varies depending on the wood's state of decay, with fungi richness increasing as the wood decomposes [9]. Heartwood and sapwood contain markedly different bacterial communities, but communities in heartwood are apparently more diverse, nonetheless, fungi may be more abundant in sapwood.

There are indications that bacterial communities differ between tree species. The water content, pH and CN ratio of the wood affect the bacterial community, as does the forest management regime [9]. Fungal abundance and richness is highest

Adamu and Bello International Journal of Applied Biological Research 2024

at advanced stages of wood decay, but does not show a clear pattern for phylum-level community composition [14]. These studies offer a tantalizing insight into saproxylic bacterial communities, but the field is still young and the conclusions are tentative.

Gmelina arborea is an economic tree with vast uses as timber and is a major source of raw material for construction, instrument and paper industries [7]. [16] reported that *G. arborea* timber is reasonably strong for its weight. It is used in constructions and making of furniture, carriages, sports and musical instruments and artificial limbs. It is also resistant to attack by termites. Its timber is also highly esteemed for door and window panels, joinery and furniture especially for drawers, wardrobes, cupboards, kitchen and camp furniture and musical instruments because of its light weight, stability and durability. It is similarly used for bentwood articles. In boat building it is used for decking and for oars. The timber from *G. arborea* is likewise popular in the making of picture and slate frames and various types of brush backs, brush handles, toys and handles of chisels, files, saws, screw drivers and sickles. The wood is also used for manufacturing tea chests and general-purpose plywood, blackboards, frame core and cross bands of flush door shutters. In the instrument industry, the timber from the plant is widely employed for the manufacture of drawing boards, plane tables, instrument boxes, thermometer scales and cheaper grade metric scales. It is also used in the production of artificial limbs, carriages and bobbins [17]. Therefore, the aim of this study was to isolate and identify the mycoflora associated with G.arborea timber within some selected timber shades in Biu Local Government Area of Borno State, Nigeria.

MATERIALS AND METHODS

Study Area

This study was conducted in three timber sheds within Biu metropolis, Biu Local Government Area of Borno State. The Biu plateau region of Borno State is located approximately between Latitude 10° 36' 40"N of the equator and Longitude 12° 11' 32"E. It has a tropical climate with distinct wet and dry seasons from May to October and November to April respectively [4].

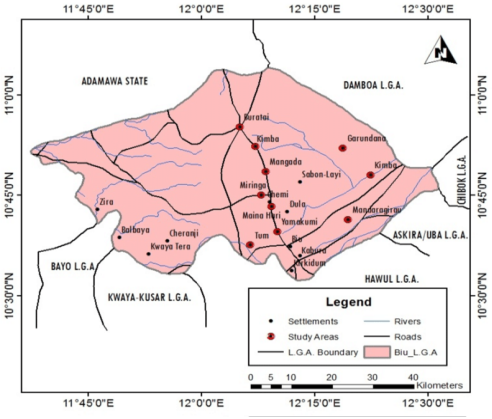

FIGURE 1: Map of The Study Area

Collection of Wood Samples

The decayed wood sample of *G. arborea* plant was obtained from three different locations within Biu metropolis. The locations are: Kasuwan katato, Main market and Muhammad Bwala timber sheds. A sterilized knife was used to collect the decayed part and was placed in a sterilized polythene bag. This was taken to the laboratory unit of the Department of Biology, Nigerian Army University Biu, for further analysis.

Preparation of Culture Media

About 20mL of streptomycin was measured using syringe and was introduced into the prepared media so as to inhibit the growth of bacteria and promote fungal growth. The media was then poured and distributed into six (6) plates, each carrying 20mL which was allowed to solidify before inoculation.

Isolation of Fungi

Isolation was made from the affected wood parts. This was done according to the method described by [13]. Pieces/parts of the affected wood was cut with a sterile scalpel and placed separately. These was later washed several times with distilled water and sterilized with 95 % ethanol. Sterile inoculating needle was used to pick the parts and placed on Potato Dextrose Agar (PDA).These were incubated for seven days at 28 $\pm$ 1˚C and then sub cultured until pure cultures were obtained for identification.

Sub-Culturing

After seven (7) days, fungal growth was seen in the petri-dishes. Another potato Dextrose Agar (PDA) was prepared and 6mL of streptomycin was mixed with ethanol which was added using a syringe to the prepared agar solution and stirred using stirring rod. The prepared agar solution was distributed inside the petri dishes 20mL each, beside a flammable spirit lamp for 21 proper and faster solidification (gel-like substance). Inoculation wire loop was surfaced sterilized over a flame and was used in picking the different types of cultured fungi from the plates and transferred into the freshly prepared plates which was placed in an incubator at room temperature (28°C) for four (4) days to sub-culture

Identification of Fungi

The fungal identification was carried out using the microscopic and macroscopic features of the fungal isolates as described by [1] in the book Illustrated Genera of Imperfect Fungi, Fourth Edition.

Wet mounts (tease mount) method for fungal hyphae identification:

Procedure of wet mount preparation:

i. Take a grease free slide and plate with fungus culture.
ii. ii. With the help of sterile scalpel bent wire, remove fungal colonies from plate (which might contain a small amount of supporting agar.
iii. iii. Place the portion of culture into a slide to which has been added to a drop of lacto phenol cotton blue or aniline blue.
iv. iv. Place the coverslip into position and apply gentle pressure to dispose the general growth and agar.
v. v. Examine microscopically

RESULTS

Fungi isolated and identified from the different timber sheds

Five fungal species were successfully isolated and identified from the three different timber sheds under study. This is illustrated in the following table.

Table 1: Fungal isolates associated with different G.arborea wood in the three timber sheds

S/N	Fungi	Source of innoculum

	Main Market	Kasuwan Katako	M. Bwala
1. *Absidia corynbifera*			√
2. *Microsporum fulvum*			√
3. *Mycrosporum jyps*eum			√
4.*Rhizopus microspores*	√	√	√
5. *Rhizopus oryzae*		√	

From the above table, *Rhizopus microsporus* was the only fungi isolated from the affected part of *G.arborea* wood in Main market. *Absidia corynbifera*, *Microsporum fulvum, Microsporum jypseum* and *Rhizopus orizae* were not found in this sample. *Microsporum jypseum , Microsporum fulvum* and *Rhizopus microsporus* were isolated from *G.arborea* wood sample from M. Bwala timber shed. *Absidia corynbifera* and *Rhizopus orizae* were not found in this sample. *Absidia corynbifera*, *Rhizopus microsporus*, and *Rhizopus orizae* were isolated from sample collected from Kasuwar Katako. *Microsporum jypseum* and *Microsporum fulvum* were not found to be isolated in this sample.

Percentage frequency (occurrence) of the fungal isolates

After isolating and identifying the different types of fungi in all the three study areas, the total number of each species found were counted and the percentage frequency of each species were calculated using the Total count method and the formula used by Ebele, 2011

$$\frac{\text{Number of times a fungus is encountered}}{\text{Total fungal isolation}} \times 100$$

Table 2: Percentage frequency of fungi isolated from the three samples.

S/N	Specie	Total Number	% frequency
1.	*Absidia corynbifera*	1	14.29%
2.	*Microsporum fulvum*	1	14.29%
3.	*Mycrosporum jyps*eum	1	14.29%
4.	*Rhizopus microspores*	*6*	14.86%
5.	*Rhizopus oryzae*	*1*	14.29%

The above table shows the result of the different five fungal species isolated and identified alongside their percentage frequencies of occurrence from all the three timber sheds. All the different five fungal species identified were not present in all the samples with the exception of *Rhizopus microsporus* that happens to be much more common than the other species, it was isolated from all the samples from all the different timber sheds with percentage frequency of 60%. *Absidia corynbifera, Microsporum jypseum, Microsporum fulvum* and *Rhizopus orizae* have similar percentage frequency of 10% each by which they were all encountered once from all the samples. There are a few potential explanations for why Rhizopus microsporus might be more common than the other four fungi.

One possibility is that Rhizopus microsporus might be better adapted to the conditions of the wood, such as the temperature, humidity, or nutrients available. Another possibility is that Rhizopus microsporus might have a faster growth rate or be more resistant to environmental stressors than the other fungi. It is also possible that the other fungi might be present in the wood, but in such small quantities that they were not frequently detected.

Adamu and Bello International Journal of Applied Biological Research 2024

Morphology and Pattern of growth in the different fungal isolates

In order to have an accurate Identification of the fungi isolated from the different timber sheds, an account was taken into both the Microscopic and Macroscopic morphology of these fungi as well as their pattern of growth in the culture media. Under the microscope, Absidia corynbifera has a distinctive appearance. It is a Zygomycete fungus, so it has a coenocytic hyphal network with aseptate hyphae (hyphae without cross walls). The hyphae are ribbon-like, with a width of 10-30 micrometers and in a length. In addition to the hyphae and vesicles, Absidia corynbifera also has rhizoids, which are root-like structures that help the fungus to attach to its substrate. The hyphae may also have swellings called sporangia, which are structures that produce spores. The spores are asexual, and they are released when the sporangia burst open [11]. They are typically light brown or yellowish-brown in color of up to several millimeters. The hyphae have terminal vesicles that are filled with spores.

DISCUSSION

The wood of *Gmelina arborea* is suitable for general utility purposes especially in construction and structural work such as carpentry, packaging utility, furniture, light flooring, musical instruments, electric poles, valuable source of timber, pulp and fodder. This species has been extensively used in afforestation programmes and has wider involvement in folk medicine.

Wood of *G.arborea* is very important and useful for the community of Biu as it is used in the making of roofs of many buildings; and in carpentry work which is often imported by heavy trucks in large quantities into Biu. The wood is usually deposited at the various timber sheds within Biu where it is processed into different sizes and sold. An observation was made in regard to the dacay and deterioration on this valuable raw material cause by some microorganisms most of which are fungi. The results of this

study showed that the mycoflora of Gmelina *arborea* wood in Biu consists of the following five species which was isolated and identified: *Rhizopus microsporus, Rhizopus orizae, Absidia corynbifera, Microsporum jypseum, and Microsporum fulvum.* Of these five species, *Rhizopus microsporus* was the most frequently found species in all the three sheds, while the other four species were found in only one or two sheds. This suggests that *Rhizopus microsporus* is better adapted to the environmental conditions of Biu more than the other four species.

This study further revealed that, all the fungi species isolated were said to cause wood decay in *Gmelina arborea,* although, *Microsporum fulvum and Microsporum jypseum* are typically associated with infections on skin of humans and animals which is possible as fungi are cosmopolitan with diverse habitat. The results of this research have provided valuable information about the fungi associated with *Gmelina arborea* wood decay in Biu. This information could be used to develop new methods for identifying and treating wood decay fungi, as well as to inform best practices for storing and using wood products.

CONCLUSION

Fungal species associated with *Gmelina arborea* wood in different locations within Biu metropolis, Borno State, Nigeria were investigated. Five fungal species (*A. corymbifera, R. oryzae, R. microspores, M. fulvum* and *M. jypseum)* associated with *G. arborea* wood were isolated. Of the five isolates, *Rhizopus. microsporus* is the most prevalent fungal species found in all the three study area. This is because of its best adaptability to the environment than the other fungal species. Due to the major role played by this plant in the economy of the study area, and the damage caused by these fungi, further research will be carried out on the proper storage and treatment of wood decay fungi

REFERENCES

1. Barnett, H. L. and Hunter, B. B. (1998). Illustrated genera of imperfect fungi 4th edition, APS press, St. Paul Minnesota, p. 32
2. Boddy, L., Preston, C. M., & Jones, T. H. (2017). Ecology of Wood: Characterization and Decay Processes. In The Fungi (pp. 239-272). Academic Press.
3. Boer, W. D., Folman, L. B., Summerbell, R. C., & Boddy, L. (2005). Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS microbiology reviews, 29(4), 795-811.
4. Bwala, M. J., Patrick, S. A., Yohanna, L., & Ambrose, Z. A. (2019). Analysis of Rainfall Trends and Water Balance Characteristics of Southern Borno Northeast Nigeria. Jalingo Journal of social and Management Sciences, 1(3), 37-49.
5. Cromer, R. N., Kriedemann, P. E., Sands, P. I. and Stewart, L. G. (1993). Leaf growth and photosynthetic response to nitrogen and phosphorus in seedling trees of Gmelina arborea. Plant Physiology, 20: 83 – 98.
6. Dawson, S. K., Boddy, L., Halbwachs, H., Bässler, C., Andrew, C., Crowther, T. W., ... &

Ovaskainen, O. Appendix 1: Protocols for the measurement of wood basidiomycete traits.

7. Duke, J. A. (2002). Gmelina arborea Roxb. Purdue University, Centre for New Crops and Plant Product's. http:/www.hot.purdue.edu/newcrop/ Duke energy/Gmelina arbrea.html. Retrival Date: 3/10/2002.
8. Gaddeyya G, Niharika PS, Bharathi P and Kumar PKR. (2012). Isolation and identification of soil mycoflora in different crop fields at Salur Mandal. AdvAppl Sci Res., 3:20202026. Henselt M and Holden DW. 1996. Molecular genetic approaches for the study of virulence in both pathogenic bacteria and fungi. Microbiol., 142:1049-1058.
9. Hoppe, B., Krüger, D., Kahl, T., Arnstadt, T., Buscot, F., Bauhus, J., & Wubet, T. (2015). A pyrosequencing insight into sprawling bacterial diversity and community dynamics in decaying deadwood logs of Fagus sylvatica and Picea abies. Scientific Reports, 5(1), 9456.
10. Lima RF and Borba (2001). Viability, morphological characteristics and dimorphic ability of fungi preserved by different methods. Rev Iberoam Mycol.,18:191- 196.
11. Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., & Anthony, S. (2009). Agroforestree Database: a tree reference and selection guide version 4.0. World Agroforestry Centre, Kenya.
12. Rebecca LJ, Dhanalakshmi V, Sharmila S, Susithra G, Kumar S and Bala S. (2012). Isolation, identification and characterization of fungi from rhizosphere soil of *Barleria cristata*. Inter J Hort Crop Sci Res., 2: 1-6.
13. Richter, H. G. and Dallwitz, M. J. (2000). Commercial Timbers: Descriptions, illustrations, identification and information. Retrieval: English Version 4th May, 2002. http:/biodiversity.uno.edu/delta
14. Rinta-Kanto, J. M., Sinkko, H., Rajala, T., Al-Soud, W. A., Sørensen, S. J., Tamminen, M. V., & Timonen, S. (2016). Natural decay process affects the abundance and community structure of Bacteria and Archaea in Picea abies logs. FEMS microbiology ecology, 92(7), fiw087.
15. Sugita, T., & Nishikawa, A. (2003). Fungal identification method based on DNA sequence analysis: reassessment of the methods of the pharmaceutical society of Japan and the Japanese pharmacopoeia. Journal of Health Science, 49(6), 531-533.
16. Umana, E. J., Akwaji, P. I., Markson, A. A., & Udo, S. E. (2015). Gmelina arborea Roxb: associated mycoflora and diseases in Cross River State, Nigeria. Global J Sci Front Res, 15, 1-11.
17. Webster, J., & Weber, R. (2007). Introduction to Fungi. New York, NY: Cambridge University Press.