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## Fungal Pathogens Affecting Seedlings of *Gmelina arborea* Roxb and *Tectona grandis* L.F. and Effect of Three Plant Extracts

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Fungal pathogens affecting seedlings of *Gmelina arborea* Roxb and *Tectona grandis* L.f. and effect of three plant extracts was investigated. Twenty plant stands were surveyed in five compartments in two locations. Four fungal diseases identified in the field during the survey were stem canker, leaf spot, butt and root rot and damping-off. A total of ten fungi were isolated from different parts (root, stem and leaf) of *G. arborea* and *T. grandis* as well as the rhizosphere (soil). The fungal isolates were; *Aspergillus flavus* Link ex Grey, *Aspergillus niger* van Tieghem, *Botryodiplodia theobromae* Sacc., *Fusarium oxysporum* Schlecht, *Geotrichium* sp Link, *Penicillium* sp Link: Fries, *Rhizoctonia solani* D.C., *Rhizopus stolonifer* Ehrenberg ex corda, *Sclerotium rolfsii* Tode, *Trichoderma viride* Persoon ex Grey. The most frequently occurred fungi were *Rhizopus stolonifer* (23.73%), *Botryodiplodia theobromae* (22.03%), *Aspergillus niger* (15.25%) and *Trichoderma viride* (10.17%). Pathogenicity test revealed that *A. niger*, *B. theobromae* and *R. stolonifer* were pathogenic on on healthy seedlings of *G. arborea* and *T. grandis*. Extracts of *Allium sativum* L., *Carica papaya* L. and *Zingiber officinale* Rosc. were employed to control the growth of fungal pathogens *in vitro*. Ethanol and cold water were used as solvent for extraction. The inhibitory effects of the ethanol and aqueous extracts of *A. sativum*, *C. papaya* and *Z. officinale* on the fungal isolates were conducted using food poisoning technique of antimicrobial assay. Standard antibiotics (Grisovid) was used as positive control comparative to plant extracts, while sterile distilled water was used as a negative control. All the plant extracts inhibited the growth of the test organisms at varying degrees. The effect of concentration of extracts on the test organism was significant ( $P < 0.05$ ). Colony diameter of the inhibition zone increased as the concentration of the extract increased (2.5% > 5.0% > 7.5% > 10.0%). Ethanol and aqueous extracts of *Allium sativum* had the highest inhibitory effects on the mycelia growth of all test fungi, while the lowest inhibitory effects were recorded by ethanol and aqueous extracts of *Carica papaya*. Ethanol plant extract proved to be more potent than aqueous plant extracts. In all, 10.0% extract concentration gave the maximum inhibition on the three test fungi. The result of this research study showed that *A. sativum*, *C. papaya* and *Z. officinale* have effective inhibitory potentials against the fungi identified. Hence, their biological active ingredients can be exploited for the control of fungal pathogens affecting forestry nursery seedlings. The significance of these findings is discussed in relation to phytochemical as a means of disease control and the substitutions of plant extracts as

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potential antifungal agents and thus recommend their use to foresters as an alternative to synthetic fungicides.

**Keywords:** Fungal pathogens, Plant extracts, Isolates, Phytochemical, Inhibition, fungicide

## **Introduction**

*Gmelina arborea* Roxb. is a fast-growing deciduous tree, occurring naturally throughout greater parts of India at altitudes up to 1,500 meters (Anon, 2002). Cromer *et al.*, (1993), noted that *Gmelina arborea* Roxb. (gmelina) belonging to family Verbanaceae, is reported to be widely grown deciduous tree of moderate to large size with an arborescent habit hence the specific name “arborea”. Anon (2002) opined that it is a fast-growing tree which grows faster than some exotic species under the same conditions. The leaves are more or less heart shaped, 10 to 25 centimeter by 5 to 18 centimeter and globous or velvety beneath, the corolla is bright yellow and the ovary glabrous. Anon (2002) reported that flowering takes place between February to April when the tree is more or less leafless, whereas fruiting starts from May onward up to June. The fruit is up to 2.5 centimeter long, smooth, dark green, turning yellow when ripe and has a fruity smell.

*G. arborea* is native to tropical moist forest from India, Burma, and Sri Lanka to southern China. It is widely introduced in Brazil, Gambia, Honduras, Ivory Coast, Malaysia, Malawi and Sierra Leona (Anon, 2002; Duke, 2002). Ademiluyi and Okeke (1973) cited in Umana *et al.*, (2015), described *G. arborea* as one of the widely grown plantation species in Nigeria. Best development of gmelina in Nigeria occurs where air temperature ranges from 18°C to 35°C, with distinct dry season and relative humidity above 40 %. The occurrence of these climatic features in West coast of Africa accounts for the success of *G. arborea* in Nigeria, Cote d’Ivoire, Sierra Leone and Ghana (Chijioko, 1986; FAO, 1989 cited in Umana *et al.*, 2015).

*G. arborea* Roxb. is an economic tree with vast uses as timber and is a major source of raw materials for the construction, instrumentation and paper industry (Duke, 2002; Anon, 2002). Anon (2002); Duke (2002) reported that *G. arborea* is reasonably strong for its weight. It is used in constructions, furniture, carriages, sports, musical instruments and artificial limbs. *G. arborea* is used in paper making and matchwood industry. *G. arborea* leaves are considered good for cattle (crude protein-11.9%). Besides these great economic potentials, *G. arborea* Roxb. is susceptible to a number of diseases. Umana *et al.*, (2015), reported that most fungal attack occurs at the seedling stage, which is very delicate stage of growth of this plant. Umana *et al* (2015) cited that Julian

(1982) reported that a whole bed of seedlings could be affected by fungal diseases. Damping off, moulds and stem rot are types of fungal attack.

*Tectona grandis* L.f. (teak) is one of the most valuable timbers in the world on account of its outstanding properties (Robertson, 2002). Teak is one of the most valuable timber trees in Southeast Asia due to its excellent wood properties. The wood is durable, has sound dimensional stability, an attractive natural colour and is valuable for high quality furniture and interior finishing (Mohd Farid *et al.*, 2005). Hashim and Mohd Noor (2002) reported that this relatively fast-growing species of the family Verbabaceae, can achieve a maximum height of approximately eighty five (85) metres.

*T. grandis* is found naturally in parts of India, Myanmar, LaO PDR and Thailand and it is naturalized in Java, where it was probably introduced some 400 to 600 years ago (Kadambi, 1972; White, 1992). It has been widely established in plantations as an exotic species for producing high quality poles and timber outside the countries of its natural distribution. Teak was first introduced outside Asia into Nigeria in 1902 (Horne, 1966) with seed first from India and subsequently from Myanmar (Kadambi, 1972).

Robertson (2002) reported that teak is susceptible to various kinds of pests and diseases. Generally, defoliators and skeletonizer cause the greatest problems. Mohd Farid *et al.*, (2005), reported that diseases such as root rot, stem canker and foliage diseases, mainly leaf rust and yellowing are common. Ukoima *et al.*, (2013), reported that root rot of teak which is becoming endemic in Nigeria is caused by *Rigidoporus lignosus*. Sometimes, young seedling are destroyed by damping off parasites particularly soon after seed germination. Damping off may also through root infection kill a tree after its stem had become woody (Nwoboshi, 1982 cited in Ukoima *et al.*, 2013).

Recently, monthly and annual returns at the forestry headquarters, Awka showed that Mamu River Forest Reserve and Achalla Forest Reserve are depleted of forest trees due to excessive logging which aggravates deforestation. This makes Anambra State a wood-deficit state. Seedling diseases have been reported at the nurseries of the Anambra State Forestry reserves of the Ministry of Environment. However, little is known about the severity of the disease at the nursery. There are no empirical records on the fungal pathogens which affect seedlings in Anambra state nurseries and very little pathological research has been done on the diseases affecting these valuable forest species.

Okigbo and Odurukwe (2009); Okigbo *et al.*, (2015) reported that the use of chemicals has helped in the control of plant diseases. But due to some identifiable problems such as chemical residues, biodegradation, phytotoxicity, pollution, development of resistance in target organisms, high cost, a times non

availability and hazard to man and his environment, renders them either slow to adopt or farmers have totally failed to adopt them for one cultural reason or the other hence alternative control methods are employed. Recently Okigbo *et al.*, (2015) reported that considerable efforts are directed at exploring the potentials of plant extracts as alternative to synthetic chemical. These plant extracts are readily available, affordable, non-phytotoxic and are biodegradable fungicides, thus being friendly to man and his environment.

The aim of this research work is to investigate fungal pathogens which affect *G. arborea* and *T. grandis* seedlings in Anambra state nurseries and evaluate the effect of extracts of *Allium sativum* Linn., *Carica papaya* Linn. and *Zingiber officinale* Rosc. on the fungal isolates.

The specific objectives of this research study include;

1. To conduct a survey of the diseases affecting *G. arborea* and *T. grandis* in the forest reserves
2. To isolate and identify fungi associated with *G. arborea* and *T. grandis* from the leaves, stem, root and soil (rhizosphere)
3. To carry out pathogenicity test
4. To control fungal pathogens associated with *G. arborea* and *T. grandis* using plant extracts of *A. sativum*, *C. papaya* and *Z. officinale* *in vitro*

## **Materials and Methods**

### ***Collection and Identification of Materials***

Seeds for planting were collected from *T. grandis* L.f. and *G. arborea* Roxb plantations from Mamu River forest Reserve in Orumba North Local Government Area and Achalla forest Reserve in Awka North Local Government Area of Anambra State, Nigeria. Diseased plant parts comprising of the roots, stems and leaves of teak and gmelina were also collected from the two locations. All diseased plant parts were identified by Mr. J.A. Onuorah, Director of Forestry, Anambra state Ministry of Environment, Awka and authenticated by Prof. C.U. Okeke of Botany Department, Nnamdi Azikiwe University, Awka. Soil samples were collected from the rhizosphere of the plantation from the two locations. Bulbs of *A. sativum*, *C. papaya* leaves and rhizomes of *Z. officinale* were used as test plants were collected from the Ginger programme and Horticulture Unit of the National Root Crops Research Institute, Umudike. The botanical identities of the plants were authenticated by the Horticulture Unit of the Institute. All Laboratory research work was carried out in the Plant Pathology Laboratory, Soil Science Laboratory and Green

House of the National Root Crops Research Institute (NRCRI) Umudike, Abia State, Nigeria.

***Survey of diseases in gmelina and teak plant stands.***

This study was conducted in two localities, Mamu river forest reserve and Achalla forest reserve in Anambra state, Nigeria. Surveys were carried out to determine the frequency of occurrence of diseased plant parts. At each site, twenty plant stands were sampled randomly in five compartments. Each plant stands was examined for various disease symptoms. A visual assessment technique described by Derso (1999); Okigbo and Osuinde (2003) were used with which many plantations can be evaluated in a relatively short time. Diseased leaves, stems, roots and soil were collected and brought to the Laboratory for isolation and identification of pathogens.

***Sterilization of equipment used:***

All equipment and glass wares used were sterilized according to methods described by Cheesbrough (2000); Jawetz *et al.*, (2004). Glass wares were washed with detergent and thoroughly rinsed with water. They were placed in racks to dry and were packed into the autoclave for sterilization at a temperature of 121°C for 15minutes at 15psi.

***Preparation of culture media***

The culture media used for fungal growth and maintenance was potato dextrose agar (PDA). The PDA was prepared according to manufacturer's recommendation by dissolving thirty nine grams (39g) of PDA powder in one litre of distilled water in a one thousand mililitres (1000ml) of round bottom flask. It was swirled and boiled to melt in a heater. It was sterilized with an autoclave at a temperature of 121°C for 15minutes. The medium was allowed to cool to 47°C and one mililire (1ml) of acetic acid was added to inhibit the growth of any bacteria and eliminate contaminants on the cultures (Green, 1994; Okigbo *et al.*, 2015). The sterile media was aseptically dispensed into sterile petri dishes in the laminar airflow hood chamber in the inoculation room and was allowed to gel.

***Isolation of fungi associated with T. grandis and G. arborea seedlings.***

Isolations were made from diseased plant parts (leaf, stem and root) and rhizosphere (soil). Isolation from diseased plant parts was carried out using the

method of Richter and Dallwitz (2000); Umana *et al.*, (2015). Pieces of diseased plant parts were cut with a sterile scalpel and placed separately. These were washed several times with distilled water and sterilized with 95% ethanol and finally rinsed thrice with distilled water. Sterile inoculating needle was used to pick sterile plant parts and placed on sterile paper towels in a laminar airflow hood chamber for 10 minutes to dry and then placed on PDA. The plates were incubated at 28±2°C for seven days.

Isolation from soils was carried out by the serial dilution plate method as described by Akachuku and Amakiri (1992) cited in Umana *et al.*, (2015). Twenty grams (20g) of soil from the two locations were collected in polyethylene bags. This was thoroughly mixed to ensure that all soils aggregates were broken down. Ten grams (10g) of each soil was suspended in ninety millilitres (90ml) of distilled water. Ten-fold dilution series was made and one millilitre (1ml) of each was incorporated into PDA in petri dishes (9mm). The plates were incubated at 28±2°C for seven days and fungal counts made from 10<sup>-1</sup> dilution plates and recorded as mean of triplicate analysis in percentages. Percentage occurrence of each fungus was recorded.

#### ***Sub-culturing/ purification and Identification of fungal isolates***

When growth has established, sub-culture were prepared using inoculum from the different organisms in the mixed cultures to obtain a pure culture. Pure cultures of each isolate was prepared by aseptically transferring the mycelia to newly prepared PDA and incubated at 28±2°C for seven days. The procedure was repeatedly carried out until pure cultures were obtained. The resulting pure cultures were used for characterization and identification of the fungi isolates. Fungi identification was carried out by microscopic studies of the isolates. Identification of the isolates were based on morphological characteristics described by Barnett and Hunter (1998); Dugan (2006); Ellis *et al.*, (2007). Frequency of occurrence of isolated fungi associated with different parts of *T. grandis* and *G. arborea* were determined. Number of times each fungus was encountered was recorded. Percentage frequency of occurrence was calculated using the formula described by Ebele (2011) thus:

$$\frac{\text{Number of times a fungus was encountered} \times 100}{\text{Total fungal isolations}}$$

#### ***Pathogenicity Test***

To confirm pathogenicity of fungal isolates obtained from leaf, stem and root, a seven-day old culture of pure isolates of *Aspergillus niger*, *Rhizopus*

*stolonifer*, and 10-day old pycnidium of *Botryodiplodia theobromae* was used for pathogenicity test. Healthy seedlings growing in the screen house were inoculated with the fungal isolates. To prepare fungal inoculum, spores were harvested from colonies with sterile forceps, which were then swirled in 5ml sterile water to release spores. Spore suspensions were centrifuged at 3000 rands for ten minutes. Supernatant was discarded and 5ml sterile water was added to the tube. This collection step was repeated three times. Spores were then counted with a hemacytometer and spore suspension was adjusted to a desired concentration (Hsieh and Huang, 2001).

Leaves, stem and roots were surface sterilized by dipping entire leaves, root and stem in 0.1% sodium hypochlorite three times for three minutes, rinsed in three changes of sterile water and blotted dry on sterile paper towels. Leaf disks were cut from surface disinfected leaves. The disks (eight pieces per dish) were placed adaxial surface down in petri dishes containing a 60mm diameter piece of sterilized filter paper. Two millilitres of sterile water was added to the filter paper to maintain moisture. A spore suspension prepared earlier were applied to leaf disks and incubated at 24°C for 14 days and percent infected area per leaf disk was recorded. Sterile distilled water was used as control. The procedure was repeated for roots and stems. Pathogenicity tests of each isolate were replicated three times. On appearance of symptoms, the area of infection was measured using a metre rule (mm) and the mean percentage infection (disease severity) was calculated using the formula cited in Umana *et al.*, (2015) thus:

$$\text{Disease severity (Area)} = \frac{\text{Area of plant tissue affected} \times 100}{\text{Total area}}$$

### ***Preparation of Plant Extracts (Botanical)***

Bulbs of *A. sativum*, leaves of *C. papaya* and rhizomes of *Z. officinale* were thoroughly washed with tap water and rinsed with distilled water. They were sliced and placed on Laboratory tray and air dried for five days, at a point they were dry enough for milling. Dried samples were separately grinded in a Laboratory mill (Thomas Wiley model ED-5 made in USA) after which the ground samples were sieved to obtain powdered processed sample used for extraction. The method described by Okigbo *et al.*, (2015) was used to prepare the plant extracts. 25g, 50g, 75g and 100g portion of each processed sample were mixed with 100ml of each solvent (aqueous and ethanol) separately in a conical flask to produce 2.5%, 5.0%, 7.5% and 10.0% extract concentrations, respectively. The extracts were sieved through four layers of sterile cheese cloth and the filtrate was evaporated over a water bath until a pasty extract was

obtained. Extracts were stored in sterile conical flasks in the refrigerator and used for the control of the fungal isolates.

#### ***Control of fungal pathogens associated with T. grandis and G. arborea***

Effect of plant extracts on mycelia growth of three test fungi, *Aspergillus niger*, *Botryodiplodia theobromae* and *Rhizopus stolonifer* was studied using the food poisoning technique of antimicrobial assay described by Sangoyomi (2004) cited in Okigbo *et al.*, (2015). One millilitre of each plant extract concentration (2.5%, 5.0%, 7.5% and 10.0%) was dispensed per petri dishes and 9ml of the molten media (PDA) was added to each of the petri dishes containing extract and carefully spread evenly over the plate. This gave rise to PDA-extract mixture with corresponding 2.5%, 5.0%, 7.5% and 10.0% extract concentration. The plates were gently rotated to ensure even dispersion of extracts. Agar-extract mixture was allowed to solidify and then inoculated at the centre with a 4mm diameter mycelia disc obtained from the colony edge of 7-day old pure cultures of each of the three test fungi. Each treatment consists of three replicates.

Commercial fungicide, Grisovid served as positive control. Petri dishes were dispensed with molten PDA and 1ml of Grisovid solution (0.5g in 100ml of sterilized distilled water) inoculated with each test fungus. A negative control set up consists of blank agar plates (no extracts) and sterile distilled water inoculated with the test fungi as described above. All the plates were incubated at 28±2°C for 5 to 7 days and examined daily for growth and presence of inhibition. Colony diameter was taken as the mean growth along two directions on two pre-drawn perpendicular lines on the reverse side of the plates. The effectiveness of the extract was recorded in terms of percentage inhibition described by Whipps (1987); Okigbo *et al.*, (2015) thus;

$$\text{Percentage inhibition} = \frac{R_1 - R_2}{R_1} \times \frac{100}{1}$$

where  $R_1$  = Growth of pathogen in control plate.

while  $R_2$  = Growth of pathogen in test plate.

#### ***Experimental Design***

The Experimental design used was Complete Randomized Design (CRD) with three replicates (Wahua, 1999). Data collected were subjected to statistical analysis using Analysis of Variance (ANOVA) to test for variance using Statistical Analysis System (SAS) of Version 9.2. Means were separated using Fisher's Least Significance Difference (FLSD) at 0.05 Probability Level, via General Linear Model (GLM) procedure (SAS, 1999).



## Results

### *Survey of fungal diseases in G. arborea and T. grandis plant stands.*

A total of four (4) fungal diseases were identified in the field during the survey. The diseases are stem canker, leaf spot, butt and root rot and damping-off (Table 1). Results show that *G. arborea* in the two locations sampled had 11.67 stands being infected by stem canker in the Mamu forest reserve while 12.67 stands were infected in Achalla forest reserve which represented 58.35% and 63.35% infection respectively. *T. grandis* in Mamu had 7 stands being infected by stem canker and 16 stands were infected in Achalla, representing 35% and 80% infection respectively (Table 1). Damping-off disease was observed in 12 plant stands (60%) of *G. arborea* and 12.33 plant stands (61.55%) of *T. grandis* in Mamu forest reserve. While 10 plant stands (50%) of *G. arborea* and 12.67 stands (63.35%) of *T. grandis* were infected with damping-off disease in Achalla Forest Reserve (Table 1).

Leaf spot was the most significant disease observed in both *G. arborea* and *T. grandis* stands in the two locations with a total of 12.67 stands (63.35%) of *G. arborea* and 10.67 stands (53.33%) of *T. grandis* in Mamu. In Achalla forest reserve, 15.33 stands (76.65%) of *G. arborea* and 14.67 stands (73.35%) of *T. grandis* were infected with leaf spot disease (Table 1). Butt and root rot disease were mostly observed in Achalla forest reserve with 14 stands (70%) of *G. arborea* and 16.33 (81.65%) of *T. grandis* infected. The results showed that all the diseases were more prevalent in Achalla Forest Reserve than Mamu forest reserve (Table 1).

### *Fungal isolates associated with different parts of G. arborea and T. grandis*

A total number of ten (10) fungi were isolated from different parts (root, stem, and leaf) of *G. arborea* and *T. grandis* as well as the rhizosphere (soil). A breakdown of the fungal isolates is presented in (Table 2). The result clearly shows the part of the plant that is constantly associated with the fungus. *Aspergillus flavus* was isolated from infected root and stem, while *Aspergillus niger* was isolated from infected root, stem, leaf and soil. *Botryodiplodia theobromae* was isolated from infected root, stem and leaf. *Fusarium oxysporum*, *Penicillium* sp and *Rhizoctonia solani*, were isolated only from infected soil (rhizosphere). *Geotrichium* sp was isolated from infected stem, leaf and soil. *Rhizopus stolonifer* was isolated from infected root, stem, leaf and

soil. *Sclerotium rolfsii* was isolated from infected root, while *Trichoderma viride* was isolated from infected root, stem and soil.

***Frequency of occurrence of fungi Isolates from G. arborea and T. grandis.***

There was remarkable variation in the frequency of occurrence of the various fungi associated with the root, stem, leaf and rhizosphere (soil). The most frequently occurred were *Rhizopus stolonifer* (23.73%), *Botryodiplodia theobromae* (22.03%), *Aspergillus niger* (15.25%) and *Trichoderma viride* (10.17%). *Rhizoctonia solani* (1.69%) and *Sclerotium rolfsii* (1.69%) were the least occurred fungi (Table 3).

***Mean percentage frequency of occurrence of fungal pathogens***

The most prominent among the fungi isolated from infected root were *R. stolonifer* with mean percentage frequency of  $11.87 \pm 1.514\%$ . *B. theobromae* had mean percentage frequency of  $11.20 \pm 0.40\%$ , followed by *T. viride* with mean percentage frequency of  $10.13 \pm 0.833\%$ . *F. oxysporum*, *R. solani*, *Pencillium* sp and *Geotrichium* sp were not present in the infected root of *G. arborea* and *T. grandis* (Table 4).

The most prominent fungi isolated from infected stem of *G. arborea* and *T. grandis* were *B. theobromae* with mean percentage frequency of  $12.27 \pm 0.611\%$ , followed by *R. stolonifer* and *A. niger* with mean percentage frequencies of  $10.80 \pm 0.400\%$  and  $9.07 \pm 0.611\%$  respectively. However, the following fungi, *F. oxysporum*, *R. solani*, *Pencillium* sp and *S. rolfsii* were absent from the stem. *B. theobromae*, *R. stolonifer* and *Geotrichium* sp had the highest mean percentage frequencies of  $12.80 \pm 0.20\%$ ,  $11.27 \pm 0.97\%$  and  $10.87 \pm 0.31\%$  respectively from the leaf of *G. arborea* and *T. grandis*. This was followed by *A. niger* with mean percentage frequency of  $8.37 \pm 0.40\%$ . *A. flavus*, *F. oxysporum*, *R. solani*, *Penicillium* sp, *S. rolfsii* and *T. viride* were not present on the leaf (Table 4).

*R. stolonifer* had the highest mean percentage frequency of  $12.33 \pm 0.70\%$  from the rhizosphere (soil). This was followed by these fungi with the following frequencies: *F. oxysporum* ( $10.33 \pm 1.815\%$ ), *Geotrichium* sp ( $9.93 \pm 0.416\%$ ), *Aspergillus niger* ( $9.53 \pm 0.305\%$ ), *R. solani* ( $8.73 \pm 0.76\%$ ) and *T. viride* ( $8.00 \pm 0.200\%$ ). *Penicillium* sp had the least mean percentage frequency of ( $7.20 \pm 0.40\%$ ). The fungi that were absent from the rhizosphere (soil) of *G. arborea* and *T. grandis* were *A. flavus*, *B. theobromae* and *S. rolfsii*. Fungal isolates that most frequently occurred were found in the rhizosphere (soil), followed by the root and stem. However, most of the fungi were absent in the leaf (Table 4).

### ***Pathogenicity test***

The Pathogenicity of the different fungi isolated from the root, leaf and stem showed significant differences ( $P < 0.05$ ) based on the fungi applied and the time allowed for the pathogenicity test (i.e. after 14 days inoculation). Of all the ten (10) fungi isolated from the root, stem, leaf and rhizosphere (soil) of *G. arborea* and *T. grandis*, the three most pathogenic ones were *A. niger*, *B. theobromae* and *R. stolonifer*, and they showed significant pathogenicity (at  $P < 0.005$ ). Results from the study showed that *A. niger* and *B. theobromae* showed significant pathogenicity of  $52.00 \pm 2.00\%$  and  $50.00 \pm 2.00\%$  respectively on the root (Table 5).

*B. theobromae* was significantly ( $P < 0.05$ ) pathogenic to the leaf ( $59.33 \pm 3.06\%$ ) of *G. arborea* and *T. grandis*. The next most pathogenic fungi that affected the leaf of *G. arborea* and *T. grandis* were *A. niger* ( $49.00 \pm 1.00\%$ ) and *R. stolonifer* ( $39.67 \pm 2.08\%$ ) but to a lesser extent. *R. stolonifer* showed significant pathogenicity ( $59.33 \pm 3.06\%$ ) on the stem of *G. arborea* and *T. grandis*. *A. niger* was significantly pathogenic ( $50.00 \pm 2.00\%$ ) on the stem, followed by *B. theobromae* ( $41.67 \pm 4.04\%$ ) (Table 5).

### ***Effects of plant extracts on the growth of three test fungi in vitro.***

The inhibitory effects of aqueous and ethanolic extracts of *A. sativum*, *C. papaya* and *Z. officinale* showed varying degrees of inhibition on the test fungi, *A. niger*, *B. theobromae* and *R. stolonifer*. This was dependent on the concentration of the extract and was significant ( $P < 0.05$ ). The interaction of extraction medium and concentration of extract was also significant ( $P < 0.05$ ) on the inhibition of all three test fungi (Table 6-11).

Aqueous extracts of *A. sativum* at 10.0% extract concentration gave the highest inhibitory effect of  $87.91 \pm 0.453\%$  on *A. niger*. This was significantly higher than other interactions, while the least inhibitory effect ( $0.92 \pm 0.022\%$ ) was recorded by *Carica papaya* at 2.5% extract concentration (Table 6). The ethanolic extract of *A. sativum* gave the highest zone of inhibition on *A. niger* ( $89.80 \pm 0.212\%$ ) at 10.0% extract concentration, which was significantly ( $P < 0.05$ ) greater than the inhibitory effect of ( $77.14 \pm 0.170\%$ ) recorded by *Z. officinale* at 10.0% concentration, while *C. papaya* had the least inhibitory effect of ( $7.73 \pm 0.163\%$ ) at 2.5% extract concentration on the test organism (Table 7).

For *Botryodiplodia theobromae*, the aqueous extracts of *A. sativum* had the highest inhibitory effect of  $78.04 \pm 0.544\%$ , followed by *C. papaya* with inhibition zone of  $68.86 \pm 0.049\%$  at 10.0% extract concentration. These were significantly better than other interactions with the exception of the positive control ( $87.33 \pm 1.315\%$ ). The least inhibitory effect was recorded by *Z. officinale* ( $25.27 \pm 0.785\%$ ) at 2.5% extract concentration (Table 8).

The ethanol extracts of *A. sativum* with percentage inhibition of  $84.31 \pm 0.438\%$  had the highest inhibitory effect on *B. theobromae* at 10.0% extract concentration. This was significantly different from other interactions with the exception of grisoid which showed an effective inhibition ( $90.25 \pm 1.174\%$ ) on the growth of the fungus, *B. theobromae*, while the negative control showed uninhibited growth of *B. theobromae*. The least inhibitory effect was observed in *C. papaya* with percentage inhibition of  $48.33 \pm 0.999\%$  at 2.5% extract concentration (Table 9). Aqueous extract of *A. sativum* gave the highest inhibitory effect of  $77.70 \pm 0.318\%$  against the mycelia growth of *R. stolonifer*, followed by *Z. officinale* with inhibitory effect of  $70.15 \pm 0.064\%$  at 10.0% extract concentration. These were significantly better than other interactions with the exception of the positive control ( $81.08 \pm 0.856\%$ ). *C. papaya* recorded the lowest inhibitory effect of  $17.43 \pm 0.764\%$  at 2.5% extract concentration (Table 10).

Ethanol extracts of *Z. officinale* and *A. sativum* with percentage inhibition of  $82.68 \pm 0.311\%$  and  $79.36 \pm 0.085\%$  respectively at 10.0% extract concentration had the highest inhibitory effect on *R. stolonifer*. The value ( $92.22 \pm 0.085\%$ ) observed from the positive control was significantly greater than all other interactions (Table 11).

**Table 1:** Survey of fungal diseases in *Gmelina arborea* and *Tectona grandis* plant stands.

Diseases	No. of Plant stands	Mamu River Forest Reserve		Achalla Forest Reserve	
		<i>Gmelina arborea</i>	<i>Tectona grandis</i>	<i>Gmelina arborea</i>	<i>Tectona grandis</i>
Stem canker	20	11.67±3.512 <sup>a</sup>	7.00±2.00 <sup>a</sup>	12.67±3.055 <sup>a</sup>	16.00±2.00 <sup>d</sup>
Leaf spot	20	12.67±3.055 <sup>c</sup>	10.67±3.055 <sup>b</sup>	15.33±3.055 <sup>d</sup>	14.67±5.033 <sup>b</sup>
Butt and root rot	20	-	-	14.00±2.000 <sup>c</sup>	16.33±1.528 <sup>c</sup>
Damping-off	20	12.00±2.000 <sup>b</sup>	12.33±3.512 <sup>c</sup>	10.00±2.000 <sup>a</sup>	12.67±1.155 <sup>a</sup>
P-value	-	0.000	0.000	0.000	0.000

Note: Results are in means of triplicate analysis ± standard deviation

Means followed by the same superscript in a column are not significantly different.

**Table 2:** Fungal isolates associated with different parts of *G. arborea* and *T. grandis* and rhizosphere (Soil).

S/N	Fungal Isolates	Source of Inoculum			
		Root	Stem	Leaf	Soil
1	<i>Aspergillus flavus</i> Link ex Grey	✓	✓	-	-
2	<i>Aspergillus niger</i> Van Tieghem	✓	✓	✓	✓
3	<i>Botryodiplodia theobromae</i> Sacc	✓	✓	✓	-
4	<i>Fusarium oxysporum</i> Schlecht	-	-	-	✓
5	<i>Geotrichium</i> sp Link	-	✓	✓	✓
6	<i>Penicillium</i> sp Link: Fries	-	-	-	✓
7	<i>Rhizoctonia solani</i> D.C.	-	-	-	✓
8	<i>Rhizopus stolonifer</i> Ehrenberg ex Corda.	✓	✓	✓	✓
9	<i>Sclerotium rolfsii</i> Tode	✓	-	-	-
10	<i>Trichoderma viride</i> Persoon ex Grey	✓	✓	-	✓

**Table 3:** Percentage frequency of occurrence of fungi isolated from *G. arborea* and *T. grandis*

S/N	Fungal Isolates	Percentage (%) frequency of occurrence
1	<i>Aspergillus flavus</i> Link ex Grey	5.08
2	<i>Aspergillus niger</i> van Tieghem	15.25
3	<i>Botryodiplodia theobromae</i> Sacc.	22.03
4	<i>Fusarium oxysporum</i> Schlecht	6.78
5	<i>Geotrichium</i> sp Link	6.78
6	<i>Penicillium</i> sp Link: Fries	6.78
7	<i>Rhizoctonia solani</i> D.C.	1.69
8	<i>Rhizopus stolonifer</i> Ehrenberg excorda.	23.73
9	<i>Sclerotium rolfsii</i> Tode	1.69
10	<i>Trichoderma viride</i> Persoon ex Grey	10.17

**Table 4:** Mean percentage frequency of occurrence of fungal isolates from different parts of *G. arborea* and *T. grandis*

S/N	Fungal Isolates	Source of Inoculum			
		Root	Stem	Leaf	Soil
1	<i>Aspergillus flavus</i>	7.43±0.862 <sup>a</sup>	7.53±0.416 <sup>b</sup>	-	-
2	<i>Aspergillus niger</i>	10.53±0.3055 <sup>c</sup>	9.07±0.611 <sup>d</sup>	8.37±0.0404 <sup>a</sup>	9.53±0.3055 <sup>d</sup>
3	<i>Botryodiplodia theobromae</i>	11.20±0.400 <sup>d</sup>	12.27±0.611 <sup>f</sup>	12.80±0.200 <sup>d</sup>	-
4	<i>Fusarium oxysporum</i>	-	-	-	10.33±1.815 <sup>e</sup>
5	<i>Geotrichium</i> sp	-	5.60±0.200 <sup>a</sup>	10.87±0.3055 <sup>b</sup>	9.93±0.416 <sup>d</sup>
6	<i>Penicillium</i> sp	-	-	-	7.20±0.400 <sup>a</sup>
7	<i>Rhizoctonia solani</i>	-	-	-	8.73±0.757 <sup>c</sup>
8	<i>Rhizopus stolonifer</i>	11.87±1.5144 <sup>e</sup>	10.80±0.400 <sup>e</sup>	11.27±0.987 <sup>c</sup>	12.33±0.7024 <sup>f</sup>
9	<i>Sclerotium rolfsii</i>	8.67±0.3055 <sup>b</sup>	-	-	-
10	<i>Trichoderma viride</i>	10.13±0.833 <sup>c</sup>	8.20±0.200 <sup>c</sup>	-	8.00±0.200 <sup>b</sup>
	P-value	0.000	0.000	0.000	0.000

Note: Results are in means of triplicate analysis ± standard deviation; - Means no occurrence;

Means followed by the same superscript in a column are not significantly different;

FLSD<sub>0.05</sub> for comparing means = 0.4325;

FLSD<sub>0.05</sub> for comparing the interaction means of fungi X source of inoculums = 0.8649

**Table 5:** Pathogenicity test (mean percentage rot) of fungal from healthy parts of *G. arborea* and *T. grandis* Seedlings

Fungal Isolates	Percentage	Infection of	
	(%) Root	Parts Leaf	Stem
<i>Aspergillus niger</i>	52.00±2.00 <sup>c</sup>	49.00±1.00 <sup>b</sup>	50.00±2.00 <sup>b</sup>
<i>Botryodiplodia theobromae</i>	50.00±2.00 <sup>b</sup>	59.33±3.06 <sup>c</sup>	41.67±4.04 <sup>a</sup>
<i>Rhizopus stolonifer</i>	37.00±1.00 <sup>a</sup>	39.67±2.08 <sup>a</sup>	59.33±3.06 <sup>c</sup>
P-value	0.000	0.000	0.000

Note: Results are in means of triplicate analysis ± standard deviation.

Means followed by the same superscript in a column are not significantly different.

FLSD<sub>0.05</sub> for comparing means = 2.1898

FLSD<sub>0.05</sub> for comparing the interaction means of fungi X source of inoculums = 3.793

**Table 6:** Inhibitory Effects of Aqueous Plant Extracts at Different Concentrations on the Growth of *Aspergillus niger*

Extracts from Botanicals	Concentrations(%)				X Botanicals
	2.5	5.0	7.5	10.0	
Grisovid (+ve control)	83.36±1.754 <sup>e</sup>	83.36±1.754 <sup>e</sup>	83.36±1.754 <sup>e</sup>	83.36±1.754 <sup>d</sup>	
<i>Allium sativum</i>	48.50±0.369 <sup>d</sup>	60.12±0.169 <sup>d</sup>	72.66±0.262 <sup>d</sup>	87.91±0.453 <sup>e</sup>	67.30
<i>Carica papaya</i>	0.92±0.022 <sup>b</sup>	20.56±0.191 <sup>b</sup>	25.68±0.347 <sup>b</sup>	41.44±0.361 <sup>b</sup>	22.15
<i>Zingiber officinale</i>	1.79±0.042 <sup>c</sup>	25.27±0.785 <sup>c</sup>	37.91±0.092 <sup>c</sup>	42.38±0.106 <sup>c</sup>	26.84
Distilled water (-ve control)	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	
$\bar{X}$ concentration	17.07	35.32	45.42	52.24	
P-value	0.000	0.000	0.000	0.000	0.000

Note: Results are in means of triplicate analysis ± standard deviation.

Means followed by the same superscript in a column are not significantly different

FLSD<sub>0.05</sub> for comparing means of botanicals = 0.2604

FLSD<sub>0.05</sub> for comparing the means of concentration = 0.2604

FLSD<sub>0.05</sub> for comparing means of botanicals X concentration interaction means = 0.5208

**Table 7:** Inhibitory Effects of Ethanol Plant Extracts at Different Concentrations on the Growth of *Aspergillus niger*

Extracts from Botanicals	Concentrations(%)				X Botanicals
	2.5	5.0	7.5	10.0	
Grisovid (+ve control)	84.72±0.134 <sup>e</sup>	84.75±0.134 <sup>e</sup>	84.75±0.134 <sup>e</sup>	84.75±0.134 <sup>d</sup>	
<i>Allium sativum</i>	55.38±0.346 <sup>d</sup>	72.46±0.283 <sup>d</sup>	76.87±0.0778 <sup>d</sup>	89.80±0.212 <sup>e</sup>	73.63
<i>Carica papaya</i>	7.73±0.163 <sup>b</sup>	26.31±0.134 <sup>b</sup>	32.48±0.481 <sup>b</sup>	43.84±1.216 <sup>b</sup>	27.59
<i>Zingiber officinale</i>	34.60±0.318 <sup>c</sup>	47.90±0.742 <sup>c</sup>	66.22±0.495 <sup>c</sup>	77.14±0.170 <sup>c</sup>	56.47
Distilled water (-ve control)	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	
$\bar{X}$ concentration	32.57	48.89	58.52	70.26	
P-value	0.000	0.000	0.000	0.000	0.000

Note: Results are in means of triplicate analysis  $\pm$  standard deviation.

Means followed by the same superscript in a column are not significantly different

FLSD<sub>0.05</sub> for comparing means of botanicals = 0.4511

FLSD<sub>0.05</sub> for comparing the means of concentration = 0.4511

FLSD<sub>0.05</sub> for comparing means of botanicals X concentration interaction means = 0.9022

**Table 8:** Inhibitory Effects of Aqueous Plant Extracts at Different Concentrations on the Growth of *Botryodiplodia theobromae*

Extracts from Botanicals	Concentrations(%)				X Botanicals
	2.5	5.0	7.5	10.0	
Grisovid (+ve control)	87.33±1.315 <sup>d</sup>	87.33±1.315 <sup>e</sup>	87.33±1.315 <sup>e</sup>	87.33±1.315 <sup>e</sup>	
<i>Allium sativum</i>	52.69±0.354 <sup>c</sup>	58.72±0.148 <sup>d</sup>	63.89±0.460 <sup>d</sup>	78.04±0.544 <sup>d</sup>	63.34
<i>Carica papaya</i>	25.68±0.283 <sup>b</sup>	41.52±0.339 <sup>c</sup>	59.20±0.304 <sup>c</sup>	68.86±0.049 <sup>c</sup>	48.82
<i>Zingiber officinale</i>	25.27±0.785 <sup>b</sup>	39.11±0.410 <sup>b</sup>	41.55±0.523 <sup>b</sup>	52.86±0.113 <sup>b</sup>	39.70
Distilled water (-ve control)	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	
$\bar{X}$ concentration	34.55	46.45	54.88	66.59	
P-value	0.000	0.000	0.000	0.000	0.000

Note: Results are in means of triplicate analysis  $\pm$  standard deviation.

Means followed by the same superscript in a column are not significantly different

FLSD<sub>0.05</sub> for comparing means of botanicals = 0.248; FLSD<sub>0.05</sub> for comparing the means of concentration = 0.248

FLSD<sub>0.05</sub> for comparing means of botanicals X concentration interaction means = 0.496



**Table 9:** Inhibitory Effects of Ethanol Plant Extracts at Different Concentrations on the Growth of *Botryodiplodia theobromae*

Extracts from Botanicals	Concentrations(%)				X Botanicals
	2.5	5.0	7.5	10.0	
Grisovid (+ve control)	90.25±1.174 <sup>e</sup>	90.25±1.174 <sup>e</sup>	90.25±1.174 <sup>e</sup>	90.25±1.174 <sup>e</sup>	
<i>Allium sativum</i>	63.61±0.035 <sup>d</sup>	77.54±0.163 <sup>d</sup>	79.10±0.290 <sup>d</sup>	84.31±0.438 <sup>d</sup>	76.12
<i>Carica papaya</i>	48.33±0.099 <sup>b</sup>	71.28±0.205 <sup>c</sup>	74.61±0.127 <sup>c</sup>	82.64±0.028 <sup>c</sup>	69.22
<i>Zingiber officinale</i>	58.44±0.325 <sup>c</sup>	62.16±0.368 <sup>b</sup>	71.97±0.106 <sup>b</sup>	80.53±0.163 <sup>b</sup>	68.28
Distilled water (-ve control)	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	
$\bar{X}$ concentration	56.76	70.33	75.23	82.49	
P-value	0.000	0.000	0.000	0.000	0.000

Note: Results are in means of triplicate analysis  $\pm$  standard deviation.

Means followed by the same superscript in a column are not significantly different

FLSD<sub>0.05</sub> for comparing means of botanicals = 0.4295; FLSD<sub>0.05</sub> for comparing the means of concentration = 0.4295

FLSD<sub>0.05</sub> for comparing means of botanicals X concentration interaction means = 0.859

**Table 10:** Inhibitory Effects of Aqueous Plant Extracts at Different Concentrations on the Growth of *Rhizopus stolonifer*

Extracts from Botanicals	Concentrations(%)				X Botanicals
	2.5	5.0	7.5	10.0	
Grisovid (+ve control)	81.08±0.856 <sup>c</sup>	81.08±0.856 <sup>c</sup>	81.08±0.856 <sup>c</sup>	81.08±0.856 <sup>c</sup>	
<i>Allium sativum</i>	55.70±0.078 <sup>d</sup>	62.15±0.361 <sup>d</sup>	67.19±0.382 <sup>d</sup>	77.70±0.318 <sup>d</sup>	65.69
<i>Carica papaya</i>	17.43±0.764 <sup>b</sup>	23.65±0.318 <sup>b</sup>	39.36±0.177 <sup>b</sup>	52.30±0.233 <sup>b</sup>	33.19
<i>Zingiber officinale</i>	46.08±0.134 <sup>c</sup>	53.49±0.488 <sup>c</sup>	64.59±0.530 <sup>c</sup>	70.15±0.064 <sup>c</sup>	58.58
Distilled water (-ve control)	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	
$\bar{X}$ concentration	39.74	46.43	57.05	66.72	
P-value	0.000	0.000	0.000	0.000	0.000

Note: Results are in means of triplicate analysis  $\pm$  standard deviation.

Means followed by the same superscript in a column are not significantly different

FLSD<sub>0.05</sub> for comparing means of botanicals = 0.1542; FLSD<sub>0.05</sub> for comparing the means of concentration = 0.1542

FLSD<sub>0.05</sub> for comparing means of botanicals X concentration interaction means = 0.3084

**Table 11:** Inhibitory Effects of Ethanol Plant Extracts at Different Concentrations on the Growth of *Rhizopus stolonifer*

Extracts from Botanicals	Concentrations(%)				$\bar{X}$ Botanicals
	2.5	5.0	7.5	10.0	
Grisovid (+ve control)	92.22±0.085 <sup>e</sup>	92.22±0.082 <sup>e</sup>	92.22±0.085 <sup>e</sup>	92.22±0.085 <sup>e</sup>	
<i>Allium sativum</i>	55.48±0.064 <sup>d</sup>	65.89±0.127 <sup>d</sup>	67.51±0.035 <sup>c</sup>	79.36±0.085 <sup>c</sup>	67.06
<i>Carica papaya</i>	15.57±0.276 <sup>b</sup>	33.98±0.007 <sup>b</sup>	44.67±0.092 <sup>b</sup>	58.49±0.304 <sup>b</sup>	38.18
<i>Zingiber officinale</i>	51.88±0.552 <sup>c</sup>	62.24±0.226 <sup>c</sup>	68.49±0.262 <sup>d</sup>	82.68±0.311 <sup>d</sup>	66.32
Distilled water (-ve control)	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	
$\bar{X}$ concentration	40.98	54.04	60.22	73.51	
P-value	0.000	0.000	0.000	0.000	0.000

Note: Results are in means of triplicate analysis ± standard deviation.

Means followed by the same superscript in a column are not significantly different

FLSD<sub>0.05</sub> for comparing means of botanicals = 0.2671

FLSD<sub>0.05</sub> for comparing the means of concentration = 0.2671

FLSD<sub>0.05</sub> for comparing means of botanicals X concentration interaction means = 0.5342

## Discussion

In this research study, some major fungal diseases were observed in the field during the survey of forest reserves for the incidence of fungal diseases in *Gmelina arborea* and *Tectona grandis* plant stands. The diseases were stem canker, leaf spot, butt and root rot and damping-off. These findings agree with the works of Nair and Sumardi (2000); Orwa *et al.*, (2009); Umana *et al.*, (2015). These researchers reported wilting in 1-2 month old seedlings; damping-off disease which caused high seedling mortality, root-collar disease on 4 month-old seedlings and Anthracnose disease in nurseries. These researchers equally observed plantation diseases such as leaf spot, vascular necrosis and chlorosis, heart rot and root rot, stem and branch canker (Machete disease) and a bark disease (worm disease) that girdle the base of the tree and cause die-back of branches in 2-year-old plantations.

Fungal pathogens associated with various parts of *G. arborea* and *T. grandis* were investigated. In this study, ten (10) pathogenic fungi were constantly associated with and isolated from the root, stem, leaf and rhizosphere (soil surrounding plant parts). The fungi were: *A. flavus* isolated from infected root and stem, while *A. niger* was isolated from infected root, stem, leaf and soil. *B. theobromae* was isolated from infected root, stem and leaf. *F. oxysporum*, *Penicillium* sp and *R. solani* were isolated only from infected soil. *Geotrichum* sp was isolated from infected stem, leaf and soil. *R. stolonifer* was isolated from infected root, stem, leaf and soil. *S. rolfsii* was isolated from infected root only, while *T. viride* was isolated from infected root, stem and soil. This is in tandem with reports of some scientists Anukwuorji *et al.*, (2013); Anukwuorji *et al.*, (2016). The following fungi; *A. flavus*, *A. niger*, *B. theobromae*, *R. stolonifer*, *S. rolfsii* and *T. viride* that were isolated from the root of *G. arborea* and *T. grandis* agreed with the report of Inyang (1990) and Umana *et al.*, (2015). However, some fungi such as *F. oxysporum*, *Geotrichum* sp and *Penicillium* sp were not isolated from infected root of *G. arborea* and *T. grandis* in this study as they were isolated from earlier studies. As pointed out by Tsao (1983) in the report of Umana *et al.*, (2015), some of these isolated fungi were isolated from the root of rubber plant (*Hevea brasiliensis*) and were pathogenic to the plant.

The following fungi, *A. flavus*, *A. niger*, *B. theobromae*, *Geotrichum* sp, *R. stolonifer* and *T. viride* isolated from the stem of *G. arborea* and *T. grandis* and which was found to be highly pathogenic to the plant especially at the seedling stage agreed with the findings of Inyang (1990) as cited in Umana *et al.*, (2015). Although *F. oxysporum* was absent from the stem in this study. *A. niger*, *B. theobromae*, *Geotrichum* sp and *R. stolonifer* isolated from the leaf of

*G. arborea* and *T. grandis* agrees with the findings of Duke (2002). *B. theobromae*, *F. oxysporum*, *Geotrichium* sp, *Penicillium* sp, *R. solani*, *R. stolonifer* and *T. viride* were isolated from infected soil (rhizosphere surrounding the tree). This finding did not conform to the observations of Inyang (1990); Umana *et al.*, (2015) who isolated only four fungi from the soil. However, reports of Ukoima *et al.*, (2013) is in tandem with this research findings, where the researchers isolated more fungal species from nursery soils. Of all fungal isolates, *A. niger* and *R. stolonifer* occurred in all parts of the plant and soil, while others occurred in one or two plant parts. However, most of the fungi were isolated from infected soil than other plant parts.

The most prevalent fungi were *R. stolonifer* (23.73%), followed by *B. theobromae* (22.03%) and *A. niger* (15.25%). This did not completely agree with the results of Anukwuorji *et al.*, (2016); Okigbo *et al.*, (2009) who reported that *A. niger* and *B. theobromae* were the most prevalent fungi in their studies respectively. The mean percentage frequency of occurrence of fungal pathogens isolated from different parts of *G. arborea* and *T. grandis* and soil revealed the most prominent fungi from the various plant parts and the distribution relative to the positions (i.e. the niche of the fungi).

Pathogenicity study revealed that all the test pathogens were pathogenic on healthy *G. arborea* and *T. grandis* seedling (*in vitro*) with different degrees of virulence. The most virulent was *B. theobromae* (59.33%) from the leaf, followed by *R. Stolonifer* (59.33%) from the stem, while the least virulent was *R. stolonifer* (37.00%) from the root. This finding agrees with the report of Okigbo and Emeka (2010) who reported that *B. theobromae* was the most virulent. However, reports of Umana *et al.*, (2015) differed from the present finding where the most pathogenic fungus was *F. oxysporum* isolated from diseased root.

Some workers (Okigbo *et al.*, 2009; Anukwuorji *et al.*, 2013; Ukoima *et al.*, 2013; Anukwuorji *et al.*, 2016) have reported the antifungal potential of some plant materials in controlling different organisms pathogenic to food and tree crops. The inference of this research work depicted that the radial growth (*in vitro*) of the entire test organisms were greatly inhibited by all the plant extracts of *A. sativum*, *C. papaya* and *Z. officinale* tested at varying concentrations. This is an indication that fungitoxic compounds abounds in these plant materials (botanicals). This observation is in consonance with the earlier reports of Amienyo and Ataga (2007), Anukwuorji *et al.*, (2013); Umana *et al.*, (2015); Anukwuorji *et al.*, (2016). However, the efficacy of the extracts differed with the plant material, concentration, solvent of extraction and with each test fungus.

Ethanol plant extracts were more effective than aqueous plant extracts. This suggests that water used in the extraction process was probably not able to dissolve all the principle compounds present in the plants, which were contained in the ethanol extract. The ethanol extract gave higher yields in all plants. This agrees with the report of Ekwenye and Elegalam (2005) on *Z. officinale* (ginger) and *A. sativum* (garlic), who attributed this to the fact that ethanol is an organic solvent and dissolved organic compounds better hence, liberated the active compounds (phytochemical) required for antifungal activity. The difference in the fungitoxic activity between the extraction medium can also be as a result of the different susceptibility of each of the test isolates to different concentrations of the extracts. This observation also agrees with the findings of Anukwuorji *et al.*, (2013).

This research study showed that *A. sativum* was highly effective against mycelia growth of almost all the test fungi with inhibition zone ranging from (48.50%) to (89.90%). This was followed by *Z. officinale* extract with inhibition zone ranging from (1.79%) to (82.68%). While the extracts of *C. papaya* had inhibition zone which ranged from slightly to moderately effective inhibition (0.92%) to (82.64%). This is similar to the results obtained by Sangoyomi (2004) on yam rot and Ukoima *et al.*, (2013) on *T. grandis* seedlings. The commercial fungicide (grisovid) showed a very significant effective inhibition on the radial mycelia growth of the fungi tested (81.08%) to (92.22%).

There was a similar trend in the fungitoxic effect of all the plant extracts with respect to concentration. 10.0% extract concentration proved to be the most fungitoxic on all the test organisms, followed by 7.5% extract concentration, while the least inhibitory effect was observed at 2.5% extract concentration. Results obtained from the effects of various plant materials showed that *A. sativum* at 10.0% concentration was a better inhibitor than *Z. officinale* and *C. papaya* at 7.5%, 5.0% or 2.5% extract concentration. This agrees with the observation of Suleiman (2010) who stated a significant difference between the mycelia growth value recorded on the various plant extract concentration. This suggests that there is a difference in the solvent and soluble antifungal elements in the respective extracts as reported by Sofowara (1997) and Iwu (2003). Okigbo *et al.*, (2009); Anukwuorji *et al.*, (2016), elucidated that the differences in the inhibition ability can be linked to the differences in the nature, quantity and quality of their biological active ingredients. Thus, suggesting the potentials of plant materials (or botanicals) as alternative to synthetic chemicals.

## Conclusion and Recommendation

In conclusion, results of this research study has shown that the botanical (plant extracts) of *A. sativum*, *C. papaya* and *Z. officinale* possess potential inhibitory activity against fungal pathogens of *G. arborea* and *T. grandis* at varying concentrations.

The demonstrated antifungal potential of these plant materials (extracts) recommends their use as natural fungicides. Studies should be carried out to further screen these plants of various phytochemicals and their evaluation as fungicide. It is also recommended that further investigations should be done on the chemical nature of the active principles of the plants. Also further investigations can combine the plant extracts for possible synergistic effect.

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