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(RESEARCH ARTICLE)

Effect of antifungal botanical extracts on seed-borne fungi prevalence and germination of *Terminalia ivorensis* seeds in Ghana

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Abstract

The use of synthetic fungicides for the management of seed-borne pathogens are detrimental to human health and the environment. In view of this research was conducted to assess the effect of antifungal botanical extracts (garlic, neem and *Senna alata*) on seed-borne fungi prevalence and seed germination of *Terminalia ivorensis* seeds. Two major seed-borne fungi of two genera namely *Aspergillus niger* and *Colletotrichum sp.* were isolated from the seeds. There was a hundred percent incidence of seed-borne fungi infection of *Terminalia ivorensis* seeds. *Aspergillus niger* was predominant with an incidence of 91.7% while *Colletotrichum sp.* had an incidence of 8.3%. There were significant differences between treatments with respect to total seed-borne fungi prevalence. Seeds treated with *Senna alata* aqueous extract showed the highest 100.0% prevalence of total seed-borne fungi followed by seeds treated with neem (75.0%) whilst seeds treated with aqueous garlic extract recorded the least (8.3%) total seed-borne fungi prevalence. The highest (100.0%) prevalence of *Aspergillus niger* was recorded by seeds treated with *Senna alata* whilst the least (8.3%) recorded in seeds treated with garlic. *Colletotrichum sp.* was only prevalent on seed treated with Mancozeb 80% WP at 8.3%. Significant variations were observed between treatments regarding germination percentages of *Terminalia ivorensis* seeds. Seeds treated with garlic extract recorded the highest (50.0%) germination percentage whilst the lowest (21.7%) recorded by *Senna alata.* Treating forest tree seeds with garlic extract before sowing could inhibit the growth of fungal pathogens and therefore improve germination.

Keywords: Antifungal; Botanical; Seed-borne; Germination; Fungi

1. Introduction

Terminalia ivorensis (T. ivorensis)(A. Chev.) is a tree species belonging to the family *Combretaceae.* It is widely harvested from the wild and has been introduced into many other tropical countries as a promising timber plantation species. *T. ivorensis* is commonly present in Ghana, Ivory Coast, Liberia, Cameroon, Nigeria and Sierra Leone. It is locally known in Ghana as "Emire" and is one of the principal timber tree species of West Africa. *T. ivorensis* is listed as vulnerable on the International Union for Conservation of Nature (IUCN) Red List. The species is threatened by habitat loss due to its existence in lesser populations, retarded regeneration as well as over exploitation [1].

T. ivorensis have been introduced to plantations of many tropical nations to obtain high-quality timber for construction and other woodworks [2]. It is also used for fuel; firewood and charcoal, provide shade and also contributes significantly to soil fertility by decomposing fallen leaves and adding nutrients to the soil [3].

Seed-borne pathogenic fungi of numerous agricultural crops in Ghana and other nations have been reported by many researchers [4][5]. Nonetheless, existing information on seed-borne fungi of forest trees such as *T. ivorensis* in Ghana is scanty. Seed-borne fungi may either be pathogenic or saprophytic. Seeds could be infected by seed-borne pathogenic

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fungi in the field or storage and negatively impact seed vigor, initial plant growth and subsequently cause field disease epidemics [6][7]. Saprophytic seed-borne fungi infect seed at storage and lead to seeds discoloration, weight of seed reduction as well as decreased seeds germination [8]. Pathogenic fungi could cause seed infections internally and damage the endosperm and embryo or infect the seeds and negatively affect seed germination and seedling development.

Over reliance on synthetic fungicides by foresters and farmers for the management of seed-borne fungi is worrisome nationally and globally. Researches indicate that the use of synthetic fungicides and other chemicals have harmful effects on the health of foresters and farmers and also leads to environmental pollution and degradation mostly in underdeveloped nations [9]. Studies have moreover shown that essential oils, resins and other extracts of many plants have inhibition capability on plant pathogenic fungi *in vitro* and *in vivo,* hence could serve as bio-fungicidal product [10][11]. Generally, extracts from plants exhibit low field persistence, reduced shelf life and less harmful to mankind as well as the environment as compared to chemical fungicides [12]. Botanicals such as Moringa as well as Neem extracts have been confirmed by many researchers for the management of seed-borne fungi [13][11]. The control of seed-borne pathogenic fungi by the application of suitable seed treatments lead to the decrease in seedling losses [14]. Fungal disease is a serious problem in reforestation and sometimes can cause severe seedling mortality in nurseries and field of planting. Several fungal pathogens are transported through seeds into forest nurseries and become well established on seedlings. When such poor seedlings are used as planting stocks for reforestation, they further spread the disease to plantations and forests, leading to severe damage.

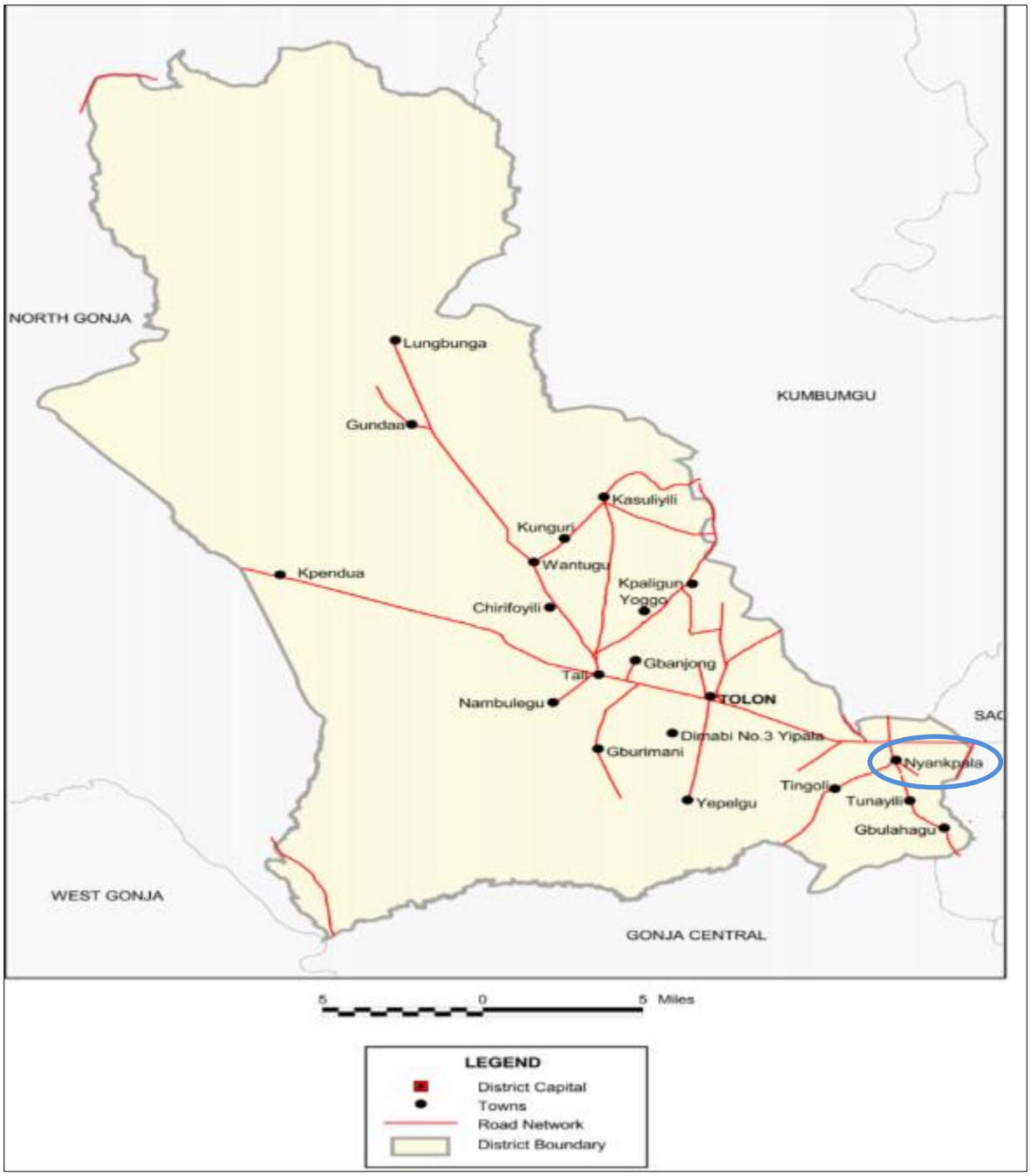
Foresters and Private Plantation managers are faced with the challenge of good quality seed, leading to poor seed germination and plant stand establishment. Germination is a significant function of a seed as it is an indicator of its viability and growth [15]. Reports indicate that *T. ivorensis* is greatly under threat due to difficulty in germination, destruction of habitat and reduced generational ability, and efforts to restoring *T*. *ivorensis* through plantations had failed due to many diseases including diebacks [16]. *T*. *ivorensis* fruits are often attacked by fungi and insects [17]. Seed-borne pathogenic fungi are significant stressors impacting seeds and seedlings health of forest trees.

In order to ensure good germination and regeneration of *T. ivorensis* in Ghana, proper and adequate knowledge of seed-borne fungi pathogens identification and management of *T*. *ivorensis* seeds are major contributing factors to improving *T. ivorensis* production in Ghana. Treating seeds with synthetic fungicides have been shown to have high effectiveness in seed-borne fungal disease reduction. However, use of synthetic fungicides are harmful and disrupts the environment. Numerous studies have been done with synthetic or chemical fungicide for the management of seed-borne fungi. However, research on the use of extracts from plants for managing seed-borne fungi of forest trees seeds in Ghana is inadequate. Hence, this study was conducted to assess the effect of antifungal botanical extracts (garlic (*Allium sativum*), neem (*Azadirachta indica*) and *Senna alata* on seed-borne fungi prevalence and germination of *Terminalia ivorensis* seeds in Ghana.

2. Material and methods

2.1. Study area

This research was carried out at the University for Development Studies, Nyankpala Campus located in the Tolon district of the Northern region of Ghana. The district is located between latitudes 9° 15'` and 10°0 02` North and Longitudes 0° 53'and 1° 25' West and shares boundaries to the North with Kumbungu, the West with North Gonja, the South with Central Gonja and Sagnarigu districts to the East. There is a single rainy season within the district, which begins in the late April with small amount of rainfall and rises to its peak in July to August and declines sharply and stops completely in October to November. The dry season begins in November and ends in March with day temperatures ranging from 33 °C to 39 °C, whereas mean night temperature range from 20 °C to 26 °C. The district has a mean yearly rainfall ranging from 950 mm to 1,200 mm and experiences intermittent storms, which often leads to soil erosion subject to the frequency and intensity especially towards the end of the dry season. The Guinea savannah woods are distinguished by drought-resistant trees such as the acacia (*Acacia longifolia*), mango (*Mangifera*), baobab (*Adansonia digitata Linn*), shea trees (*Vitellaria paradoxa*), dawadawa, and neem (*Azadirachta indica*), are interspersed with grasslands, which make up the majority of the vegetation in the district. Shea, dawadawa, and mango are the principal tree species, and they are important sources of income for many people. There is also annual bush fires which affects the vegetation.



NB: Study area circled in blue on the map; Source: [18]

Figure 1 Map of Tolon District

2.2. Source of seeds

Terminalia ivorensis seeds were sourced from the Forestry Research Institute of Ghana (FORIG), Fumesua-Kumasi of the Ashanti region of Ghana. Seeds were stored in plain polythene bag and transported to Tamale for laboratory and nursery experimental studies.

2.3. Laboratory experiment

2.3.1. Media preparation

Potato Dextrose Agar (PDA) of 39 g was weighed using KERN electronic balance made by KERN and Sohn GmbH in Germany into a conical flask containing 500ml of distilled water. Chloramphenicol sulphate (250 mg) was added to the suspension to suppress bacterial growth. The suspension was topped up with 500ml of distilled water to attain a 1 litre

suspension. The resultant suspension was stirred thoroughly with sterile glass rod. The conical flask containing the suspension was stoppered with non-absorbent cotton wool and autoclaved at 121 °C, 0.98 kg/cm^2 pressure for 15 min. The suspension after autoclaving was allowed to cool to about 45 °C and then poured into sterilized Petri dishes at 15 ml per plate. The plates were allowed to solidify under sterile condition in the lamina flow hood.

2.3.2. Seed treatments

The experiment comprised of the five treatments below:

- T_0 = Untreated seeds (control)
- T_1 = Seeds treated with garlic (*Allium sativum*) aqueous extract
- T_2 = Seeds treated with *Senna alata* aqueous extract
- T_3 = Seeds treated with neem (*Azadirachta indica*) aqueous extract
- T_4 = Mancozeb 80% WP synthetic fungicide (Standard check)

2.3.3. Preparation and application of seed treatments

Garlic, *Senna alata* and neem extracts were used in the research for inhibiting the growth of seed-borne fungi of *Terminalia ivorensis*. A chemical fungicide (Mancozeb 80 % WP) was involved as a positive control or standard check. Plant extracts and chemical fungicide suspension preparation as well as seed treatment were carried out at the UDS-Spanish Laboratory, Tamale, Ghana.

2.3.4. Control treatment

Terminalia ivorensis seeds were not subjected to chemical or botanical extract treatments. Seeds were soaked in sterile distilled water for three days under normal room conditions in the laboratory.

2.3.5. Preparation of aqueous garlic extract

Garlic bulbs were purchased from the Tamale main market, cleaned adequately with flowing water. Peeled bulbs were properly grinded to fine paste using Toni electronic blender at 3000 rpm for 15 mins. 70 g of the blended fine garlic was weighed into a flask with 100ml of distilled water and carefully stirred to obtain homogenous suspension using sterilised glass rod. Suspension was sieved with muslin cloth to obtain a 70% (w/v) aqueous extract.

2.3.6. Preparation of aqueous Senna alata and neem leaf extracts

Fresh leaves of *Senna alata and Neem* were collected from the Nyankpala community. The leaves were washed thoroughly with flowing water to get rid of all dirt. The leaves were properly grinded to fine paste using Toni electronic blender at 4000 rpm for 15 minutes. 70 g of each blended fine paste was weighed into a flask with 100ml of distilled water and carefully stirred to obtain a homogenous suspension using sterilised glass rod. Suspensions were sieved with muslin cloth to obtain 70% (w/v) aqueous extract.

2.3.7. Mancozeb (80% WP) suspension preparation

Mancozeb (80% WP) was bought from Ganorma Agrochemical shop in Tamale Metropolis. The suspension was prepared as recommended by the manufacturer. 7.5g of Mancozeb (80% WP) fungicide powder was weighed and dissolved in 1 L distilled water. An aliquot of 200 ml was taken for use as a standard check in the experiment.

2.3.8. Treatment of Terminalia ivorensis seeds with prepared aqueous botanical extracts and mancozeb (80% WP) suspension

Two hundred physically pure seeds were soaked in each of the botanical extracts and the Mancozeb (80% WP) suspension prepared. The soaked seeds were arranged on a bench in the Spanish Laboratory-UDS and stored for 72 hours.

Figure 2 Treated seeds stored for 72 hours

2.4. Isolation and purification of fungal pathogens

Isolation of fungal pathogens from *Terminalia ivorensis* were done at the Spanish Laboratory of the Faculty of Agriculture, University for Development Studies (UDS). Seeds were surface sterilized with 1% sodium hypochlorite solution for 2 minutes and rinsed three times in changes of sterile distilled water and allowed to dry on a two-ply tissue paper in the laminar flow hood for 30 mins. Seeds were then inoculated on PDA in 90mm diameter sterilized petri plates and incubated at ambient temperature (28 ± 2 °C) under alternating cycles of 12/12 hours of near ultra-violet light and darkness for seven (7) days. Four seeds were plated in each petri dish and replicated three times. After 7 days, the plates were observed for the growth of fungi pathogens. Mycelia grown were sub-cultured using fresh PDA media to attain pure isolates of the pathogens. The experimental design used was completely randomized design (CRD) with three replications. Data were recorded on the frequency of fungi occurrences on the *Terminalia ivorensis* seeds soaked in all the treatments and was expressed as percentage of control of the fungi by the aqueous botanical extracts and mancozeb 80% WP using the formula; $\frac{f.c-f.t}{\mathbf{f.c}} \times 100$ %, Where **f.c** is the occurrence of the fungus after treatment with the control (water) and **f.t** is the occurrence of fungus after treatment with the botanical extract and mancozeb 80% WP

2.5. Identification of fungal pathogens

Slides of 8-day-old mycelia or colony from pure cultures of fungal growth were prepared. Identification of fungal isolates were done using their morphological and cultural characteristics such as shapes, colour of mycelia, conidia or spore shape and others. Compound light microscope (Leica, Wetzlar GmbH, Germany) was used for the identification with the help of fungi descriptive manuals developed by [19] and [20].

2.6. Nursery experiment

2.6.1. Experimental design and layout

The experiment comprised five seed treatments laid out in Complete Randomized Blocks in three replications.

2.6.2. Germination percentage

The assessment was conducted at the Nursery of the Department of Forestry and Forest Resources Management, Faculty of Natural Resources and Environment, University for Development Studies, Tamale, Ghana. 150 seeds were randomly selected from each of the treated and untreated (control) seed lots. 50 seeds of each seed lot was replicated three times in three blocks. The selected seeds were sown using the poly pots at one (1) seed per pot. Forest topsoil was used to fill the poly pots before seed sowing. Thirty-one (31) days after sowing, the number of seedlings emerged in each pot was counted and recorded. Number of seedlings emerged from the 150 seeds of each treatment were determined. The germination capacity was expressed in percentage based on total seeds used in the experiment according to [21].

Data was calculated in percentage based on the total number of seeds sown using the following formula [21];

$$\% \text{ Germination} = \frac{X_1}{X} \times 100$$

Where,
X = Total number of seeds sown in all the poly pots of each treatment
X_1 = Number of seedlings in all the poly pots of each treatment

2.7. Data presentation and analysis

Data analysis was done using GenStat 12th Edition statistical package. Differences in treatment means were compared for significance using Least Significance Difference at 5% probability level ($p \leq 0.05$). Percent fungi incidence data were transformed using Square Root transformation ($\sqrt{x} + 0.5$) prior to the analysis. Species of fungi identified from the tested seeds were also recorded.

3. Results

3.1. Seed-borne fungi isolated and their incidences on *Terminalia ivorensis* seeds

Two major seed-borne fungi of two genera namely *Aspergillus niger* and *Colletotrichum sp.* were isolated from the seeds of *Terminalia ivorensis*. There were a hundred percent occurrence of seed-borne fungi infection of *Terminalia ivorensis* seeds. *Aspergilus niger* was predominant with an incidence of 91.7% while *Colletotrichum sp.* had an incidence of 8.3% (figure 3).

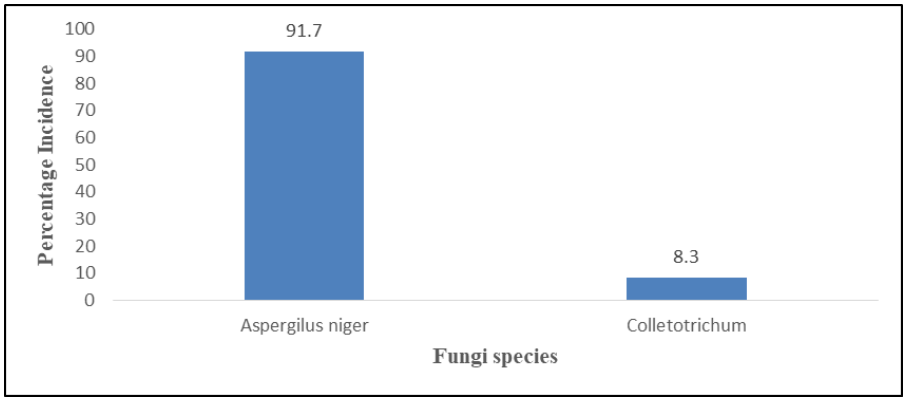


Figure 3 Fungi species and their incidences on *Terminalia ivorensis seeds*

Figure 4 Untreated (control) seeds of *Terminalia ivorensis* showing 100% seed-borne fungi infections on PDA

3.2. Seed-borne fungi prevalence on *Terminalia ivorensis* seeds after treatment with antifungal botanical extracts

There were significant differences between treatments with respect to total seed-borne fungi prevalence (Table 1). Seeds treated with *Senna alata* aqueous extract and control showed the highest 100.0% prevalence of total seed-borne fungi followed by seeds treated with neem (75.0%) whilst seeds treated with aqueous garlic extract recorded the least total seed-borne fungi prevalence. Seeds treated with Mancozeb 80%WP as a standard check recorded a total seed-borne fungi prevalence of 25.0% (Table 1). The prevalence of *Aspergillus niger* showed significant differences between treatments. The highest (100.0%) prevalence of *Aspergillus niger* were recorded by untreated seeds and seeds treated with *Senna alata*. The least (8.3%) prevalence of *Aspergillus niger* was recorded in seeds treated with Garlic whilst seeds treated with Mancozeb 80% WP as standard check recorded 16.7% (Table 1). *Colletotrichum* sp. was only prevalent on seed treated with Mancozeb 80% WP at 8.3% and therefore showed no significant differences between treatments (Table 1).

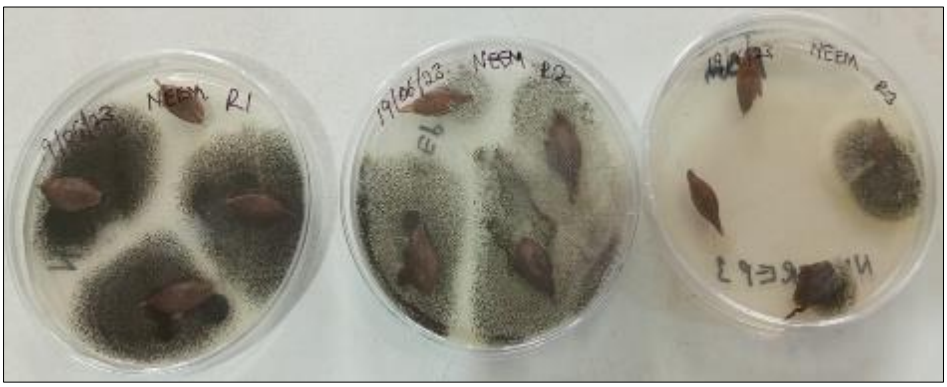


Figure 5 Effect of Neem extract on seed-borne fungi of *Terminalia ivorensis* seeds

Figure 6 Effect of garlic extract on seed-borne fungi of *Terminalia ivorensis* seeds

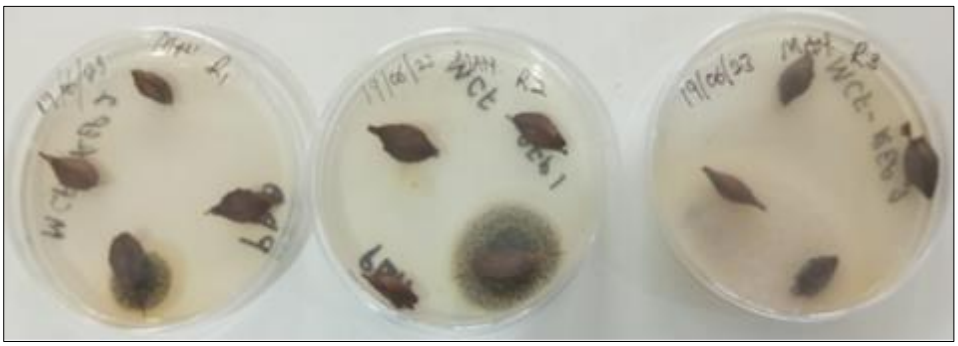


Figure 7 Effect of Mancozeb 80% WP on seed-borne fungi of *Terminalia ivorensis* seeds

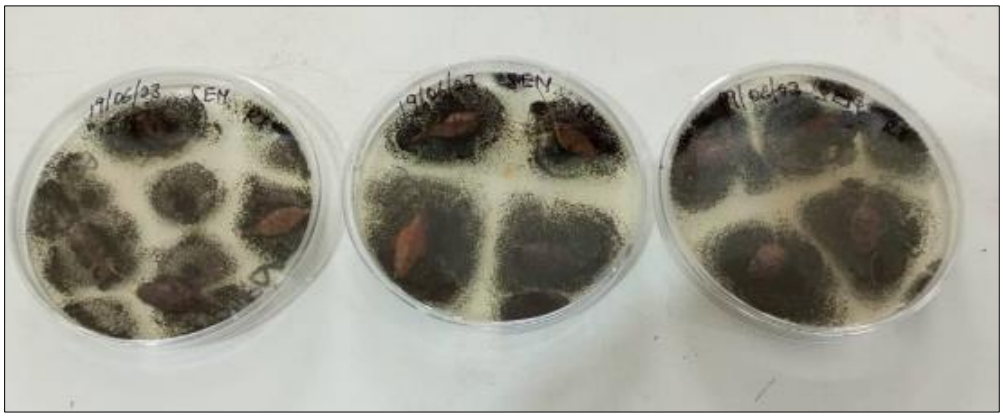


Figure 8 Effect of (*Senna alata*) on seeds of *Terminalia ivorensis* showing 100% seed-borne fungi infections on PDA

3.3. Germination percentage of *Terminalia ivorensis* seeds after treatment with antifungal botanical aqueous extracts

There were significant variation between treatments regarding germination percentages of *Terminalia ivorensis* seeds (Figure 9). Seeds treated with Mancozeb 80%WP recorded the highest percentage (53.3%) of germination followed by seeds treated with garlic extract (50.0%) whilst untreated seeds (control) recorded the least percentage of seed germination (Figure 9).

Table 1 Seed-borne fungi Prevalence on *Terminalia ivorensis* Seeds after Treatment with Antifungal Botanical Extracts

Treatments	**Prevalence of fungi infection on *Terminalia ivorensis* seeds**		
	Total fungi prevalence	***Aspergillus niger***	***Colletotrichum* sp**
T_0 Control	100a	100a	0a
T_1 Garlic	8.3b	8.3b	0a
T_2 *Senna alata*	100a	100a	0a
T_3 Neem	75a	75a	0a
T_4 Mancozeb 80% WP	25b	16.7b	3.3a
LSD	23.5	26.3	11.7
CV	20.9	24.1	387.3

Means in a column followed by same letter are not significantly different (P= <0.05)

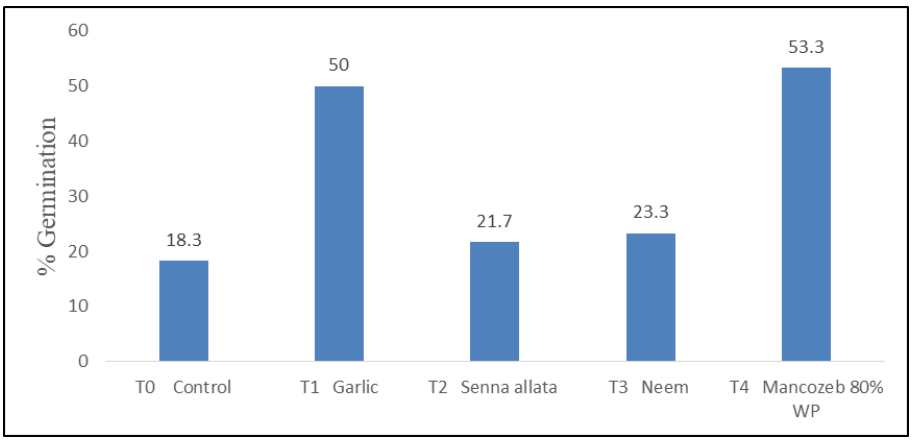


Figure 9 Germination Percentage of *Terminalia ivorensis* seeds after treatment

4. Discussion

4.1. Seed-borne fungi isolated and their incidences on *Terminalia ivorensis* seeds

The major seed-borne fungi namely *Aspergillus niger*, and *Colletotrichum* sp. isolated from *Terminalia ivorensis* seeds is an indication that forest tree seeds are highly vulnerable to the infection by seed-borne pathogenic fungi. This result is in agreement with [22] whose study revealed the infection of *Terminalia brownii* with seed-borne fungi namely *Fusarium equiseti, Pestalotia* sp., *Alternaria alternate* and *Penicillium* sp. Similar studies by [23] also revealed the infection of *Albezia lebbeck* seeds by *Pythium* sp., *Fusarium oxysporum, Cladosporium* sp., *Ascochyta* sp. and *Sclerotinia sclerotiorum.* Li [24] isolated *Fusarium, Penicillium,* and *Aspergillus* from *Pinus massoniana* seeds in China. Gomes, [25] discovered a varied spectrum of fungi linked with the seeds of Brazilian savanna tree species, including *Colletotrichum sp.*, *Fusarium sp.* and *Penicillium*.

The hundred percent incidence of seed-borne fungi infection of *Terminalia ivorensis* seeds could be attributed to factors such as poor seed collection, handling and storage [26]. Majority of forest seed collectors are unaware of seed-borne fungi and therefore collect seeds from the forest floor or mix collected seeds of different parent trees together for storage [27]. Different parent trees may have been infected with diverse fungi pathogens and mixing such seeds could proliferate fungi infection in seeds. Sutherland et al. [28] moreover confirms that one of the major causes of seeds infection with seed-borne fungi is the contact of seeds or fruits with contaminated soil.

4.2. Effect of antifungal botanical extracts on seed-borne fungi prevalence on *Terminalia ivorensis* seeds

Seeds treated with garlic aqueous extract showed the highest (91.3%) inhibition of total seed-borne fungi followed by seeds treated with Mancozeb 80% WP (75.0%) . Seeds treated with garlic aqueous extract moreover showed the highest 91.3% significant inhibition of *Aspergillus niger* followed by seeds treated with Mancozeb 80%WP as a standard check which also inhibited the growth of *Aspergillus niger* by (83.3%). This study reveals that garlic is an effective antifungal botanical that could have a promising control of seed-borne fungi of forest tree seeds especially *Aspergillus niger*. This results is in agreement with Gyasi et al. [29] who reported that aqueous garlic extract at (60% (w/v)) was effective in controlling seed-borne fungi *Aspergillus niger* and *A.flavus* on pepper seeds. Neem had a less inhibition of (25.0%) *Aspergillus niger*. This results is further in agreement with Gyasi et al. [29] who asserted that Neem leaf extract was found to have fungicidal effect in controlling *Aspergillus niger* and *A. flavus* on seeds of pepper. Seeds treated with aqueous *Senna alata* extract recorded no inhibition of total seed-borne fungi. This results contradicts the assertion by some researchers [30] that *Senna alata* has antifungal potentiality against seed-borne fungi. *Senna alata* in this study having no fungicidal effect on seed-borne fungi could be attributed to low concentration of the extract or not having fungicidal effect on *Aspergillus niger and Colletotrichum sp. Colletotrichum sp.* only observed in seeds treated with Mancoozeb 80%WP indicates that Mancozeb 80%WP is not effective in controlling *Colletotrichum sp.*

4.3. Germination percentage of *Terminalia ivorensis* seeds after treatment with antifungal botanical aqueous extracts

Generally, seed germination was very low in this study, however, there were significant variation between treatments regarding germination percentages of *Terminalia ivorensis* seeds. Seeds treated with Mancozeb 80%WP recording the highest percentage (53.3%) of germination followed by seeds treated with garlic extract (50.0%) could greatly be attributed to their high effectiveness in controlling seed-borne fungi. This results is in line with Islam [31] who studied the effects of extracts of garlic, allamanda and neem to control seed-borne fungi of okra. Among all the plant extracts, garlic exhibited the best performance in increasing seed germination (95.5 %) and reduction of fungal flora (53.3 %). Seed-borne fungi have been reported by several researchers to have negative impact on seed germination and seedling health and hence seed-borne fungi is a threat to seed germination. Poudel et al. [32] discovered that *Fusarium oxysporum*-infected seeds had lower germination rates and generated weaker seedlings. Similarly, Sultana et al. [33] discovered that seeds infected with *Colletotrichum sp.* had lower percentages of germination.

5. Conclusion

There were a hundred percent (100%) incidence of seed-borne fungi infection of *Terminalia ivorensis* seeds. *Terminalia ivorensis* seeds are highly susceptible to *Aspergillus niger.* This study reveals that garlic is an effective antifungal botanical that could have a promising control of seed-borne fungi of forest tree seeds especially *Aspergillus niger* and improve germination of seeds. Generally, seed germination was very low in this study, however, there were significant variations between treatments. It is therefore recommended that laboratory analysis on seed-borne fungal pathogens

be conducted to ensure only healthy seeds are stored for future use and also prior to sowing, seeds be treatment with garlic extract to enhance high germination.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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Appendix

Pure Culture and Conidia of Identified Fungi

Figure 10 Pure culture of *Aspergillus niger*

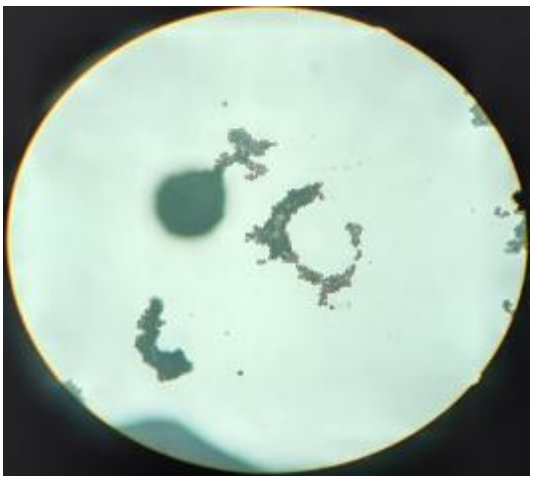

Figure 11 Conidia of *Aspergillus niger*

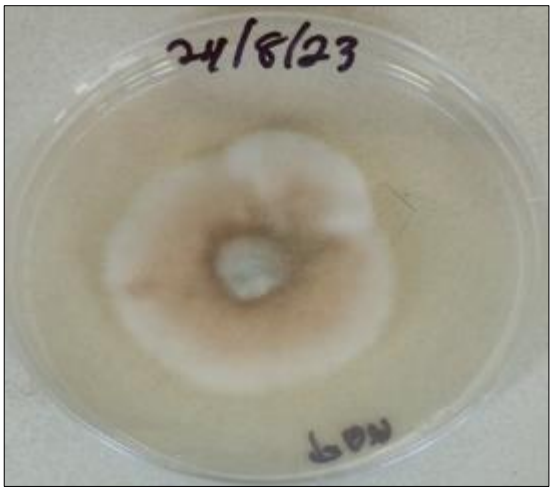

Figure 12 Pure culture of *Colletotrichum* sp

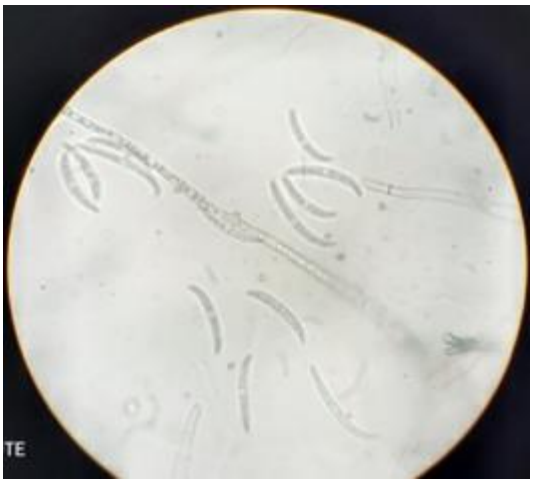

Figure 13 Conidia of *Colletotrichum* sp