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Effect of provenance on seed-borne Mycoflora, germination and seedling disease incidence on *Khaya Senegalensis* seeds in Ghana

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Abstract

Seed provenance is an important component determining seed quality in forest restorations. Collection of seeds from diseased-free agro-ecological zones for reforestation programs in Ghana is key. To contribute to knowledge, a research was conducted to assess the effect of provenance on seed-borne mycoflora prevalence, germination and initial seedling disease incidence of *Khaya Senegalensis* seeds in three agro-ecological zones of Ghana. Four seed-borne fungi in three genera namely *Aspergillus niger*, *A. flavus*, *Colletotrichum* sp. and *Penicillium* sp. were isolated from *Khaya Senegalensis* seeds collected from the three different provenances namely Deciduous forest, Transitional and Guinea Savannah zones. Total seed-borne mycoflora infections on *Khaya Senegalensis* varied significantly ($p<0.05$) between provenances. Transitional and Deciduous zones recorded equal highest incidence of 100.0% total seed-borne mycoflora infections whilst seeds from the Guinea Savannah zone recorded the least total seed-borne mycoflora infection of 58.3%. Significant differences ($p<0.05$) were observed between provenances with respect to seed germination. Seeds from Guinea Savannah zone recorded the highest (73.7%) whilst the least percentage (16.3%) germination was observed in seeds sourced from the Deciduous forest zone. There were no significant difference ($p<0.05$) for percent seedling disease incidence amongst provenances. However, the highest (20.0 %) seedling disease incidence was recorded in seeds sourced from Transitional zone whilst the least (12.5%) recorded in seeds from the Deciduous Forest zone. Provenance has significant effect on seed-borne mycoflora prevalence and seed germination. It is recommended that seeds be collected from provenances with low incidence of seed-borne mycoflora to enhance high seed germination for reforestation programs.

Keywords: Provenance; Seed-borne; Mycoflora; Disease incidence; Germination

1. Introduction

Khaya Senegalensis (A. Juss) commonly known as Mahogany is a member of the Meliaceae family. The tree can grow up to 35 m high on fertile soils with 10-16m clean bole. Its trunk is very thick with a generally short and stocky appearance, up to 2 m in diameter [1]. Its wood is hard, dense, attractive reddish colour and highly resistant to biodegradation [2]. The wood has straight grain orientation and a high lustre. The tree is used for high-class cabinetwork, furniture, joinery, building and construction purposes and in the production of decorative veneers [2]. The species has also high traditional medicinal values and also used as an ornamental tree for gardens and avenues [2]. Forest trees are predominantly propagated by seeds and these seeds are mostly affected by a number of pathogenic fungi or mycoflora which include *Aspergillus niger, Rhizoctonia spp.*, *Fusarium spp.*, *Colletotrichum spp*, *Cercospora spp*, *Phytophthora spp.* [3].

Location of seed production is one important factor for occurrence of seed borne pathogens [3]. *Kaya senegalensis* is primarily propagated by seeds. A healthy seed is the basis of a healthy plant for forest restoration [4]. Seeds are common

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carriers of plant pathogens to new fields where they are not present [5]. Forest-tree seed diseases and diseases related to seed-borne pathogens are often caused by fungi [6]. Seed-borne pathogenic fungi or mycoflora can greatly affect seed quality and cause diseases that impact negatively seedling production in nurseries and plantations. Losses to seed-borne pathogens include reduced seed germination, increased damping-off, and mortality of older seedlings in nursery beds [6]. In forest restoration efforts, seed provenance has been discovered to be a crucial element determining seed quality, seedling growth, and survival [7]. *Khaya Senegalensis* seeds and other forest tree seeds have been shown to have different mycoflora among different ecological zones of the Ghanaian forest, provenance affected the diversity and incidence of fungi in forest tree seeds of Ghana [8]. Germination is highly critical in forest regeneration and establishment. Seed germination can be influenced by various factors, including seed provenance. Location of seed production is one important factor for occurrence of seed borne pathogens that negatively impact seed germination and seedling health. The agro-ecological conditions comprising of edaphic and environmental factors have more than one effect on the performance of the seed apart from its genetic makeup. Seed-borne mycoflora like *Aspergillus niger* and *A. flavus* manifests seed discolouration, seed rotting, loss in germination capacity and toxification in seeds [9]. Quality seed plays a very important role for the production of healthy tree. Healthy and pathogen free seeds are among the basic requirements for disease free plants [3]. Fungal diseases are high threats to reforestation and afforestation success by causing severe seedling mortality in nurseries and plantation fields. Several fungal pathogens are disseminated through seeds into forest nurseries and become well established on seedlings. When such unhealthy seedlings are used as planting stocks for reforestation, they further spread the disease to plantations and forests, leading to severe damage. Since seeds are the prime sources of planting stock of most forest trees and provenance has great impact on the health of tree seeds, it is highly essential to examine the health of important forest tree seeds such as *Khaya Senegalensis* in order to identify the location of seeds of healthy seeds. Enhanced understanding of the location of healthy seeds for afforestation and reforestation programs is essential. Hence this study was conducted to assess the effect of provenance on seed-borne mycoflora prevalence, germination and initial seedling disease incidence of *Khaya Senegalensis* seeds in three agro-ecological zones of Ghana.

2. Material and methods

2.1. Study area

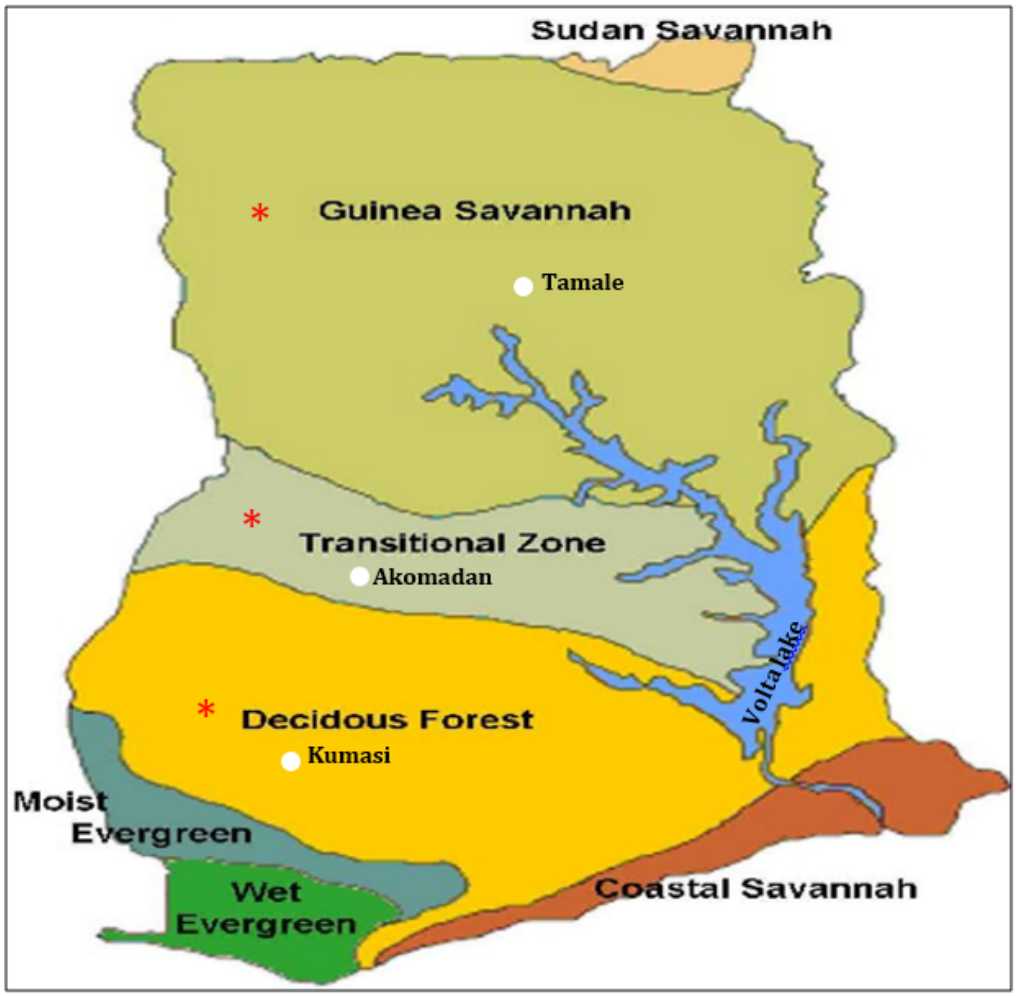
The study was conducted at the UDS-Nyankpala Campus located in the Tolon District of the Northern Region of Ghana. The District lies between latitudes 9° 15'` and 10°0 02` North and Longitudes 0° 53'and 1° 25' West. It shares boundaries to the North with Kumbungu, North Gonja to the West, Central Gonja to the South, and Sagnarigu Districts to the East. The district is characterized by a single rainy season, which starts in late April with little rainfall, rising to its peak in July-August and declining sharply and coming to a complete halt in October-November. The dry season starts from November to March with day temperatures ranging from 33 °C to 39 °C, while mean night temperature range from 20 °C to 26 °C. The Mean annual rainfall ranges between 950mm - 1,200mm. The area experiences occasional storms, which have implications for base soil erosion depending on its frequency and intensity especially when they occur at the end of the dry season. The situation also has an implication as staple crop farming for instance is highly restricted by the short rainfall duration. The main vegetation is grassland, interspersed with guinea savannah woodland, characterized by drought-resistant trees such as acacia, (*Acacia longifolia*), mango (*Mangifera*), baobab (*Adansonia digitata* Linn), shea (*Vitellaria paradoxa), Parkia biglobosa, and neem (Azadirachta indica)*. The vegetation is also annually affected by bush fires, which sweep across the savannah woodland.

Source: [10].

Figure 1 Map of Tolon District (study area indicated in blue)

2.2. Sources of seeds

Khaya Senegalensis seeds were sourced from Kumasi (Forestry Research Institute of Ghana (FORIG)) in the Deciduous forest zone, Akomadan (Offinso Forest District) in the Transitional zone and Tamale (Tamale Forest District) in the Guinea Savannah zone. Seeds were stored in plain polythene bags and transported to the University for Development Studies, Nyankpala, Tamale for laboratory and nursery experimental studies.



Source: [11].

Figure 2 Vegetation Map of Ghana (areas indicated by * show the provenances of seed collection)

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 three different provenances as seed treatments:

- Pt = seeds from Transitional zone (Akomadan)
- Pf = seeds from Deciduous forest zone (Kumasi)
- Ps= seeds from Guinea Savannah zone (Tamale)

2.3.3. Isolation and purification of fungal pathogens

Isolation of fungal pathogens from *Khaya Senegalensis* 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 a laminar flow hood for 30 minutes. The seeds were then plated on PDA in 90 mm diameter sterilized Petri dishes 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. Five seeds were plated in each petri dish and the experimental design used was completely randomized design (CRD) with three replications. After 7 days, the plates were inspected to observe the growth of seed-borne mycoflora or pathogens on the media. Mycelium emerging from the seeds were sub-cultured on fresh PDA media with the help of a sterile loop to obtain pure isolates of the pathogens. Mycelium were checked and transferred on to new PDA media weekly and kept at room temperature (21-25 ^{o}C) to maintain pure cultures at all times for correct identification of fungal pathogens. Occurrence of fungi on *Khaya Senegalensis* seeds was expressed in percentage based on total seeds plated using the formula recommended by [12].

$$\% \text{ Infection of seed-borne fungi on seeds} = \frac{Sum\ of\ infected\ seeds}{\text{Total number of seeds tested}} \times 100$$

2.3.4. Identification of seed-borne mycoflora/ fungal pathogens

Slides of 8-day-old mycelia or colony from pure cultures of fungal growth were prepared. The fungal isolates were identified based on their cultural and morphological characteristics including shapes of spores or conidia, mycelial colour amongst others. The fungal identification was carried out using a compound light microscope (Leica, Wetzlar GmbH, Germany) with the aid of fungi descriptive manuals developed by [13] and [14]

2.4. Nursery experiment

2.4.1. Experimental design and layout

The experiment comprised three seed treatments representing three provenances and was laid out in Complete Randomized Blocks with three replications.

2.4.2. 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. Three hundred pure seeds were randomly selected from each of the three provenances seed lots. 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. Ten days after sowing, the number of seedlings emerged in each pot was recorded. Number of seedlings emerged from the three hundred seeds of each provenance were determined. The germination capacity was expressed in percentage based on total seeds used in the test according to [15]. The data as calculated in percentage based on the total number of seeds sown was done using the following formula [15];

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

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

2.5. Data presentation and analysis

Data on seed-borne mycoflora prevalence or infection and germination percentage were transformed using Square Root transformation before analysis and the results presented in tables and graphs. GenStat 12th Edition statistical package was used to analyze the data. Differences in treatment means were compared for significance using Least Significance Difference at 5% probability level ($p \leq 0.05$). Fungal species identified from the tested seeds were also recorded.

3. Results and discussion

3.1. Effect of provenance on seed-borne mycoflora and their incidences on *Khaya Senegalensis* seeds

Four seed-borne fungi in three genera were isolated from *Khaya Senegalensis* seeds collected from the three different provenances. The identified fungi were *Aspergillus niger*, *A. flavus*, *Colletotrichum* sp. and *Penicillium* sp.

The total seed-borne fungal infections on *Khaya Senegalensis* varied significantly ($p<0.05$) between provenances (Table 1). Transitional and Deciduous zones recorded highest equal incidence of 100.0% total seed-borne fungal infections whilst seeds from the Guinea Savannah zone recorded the least total seed-borne fungal infection of 58.3% (Table 1). There were significant variation ($p<0.05$) in *Aspergillus niger* incidence on seeds between provenances. Seeds from the Deciduous Forest Zone recorded the highest (100.0%) incidence of *Aspergillus niger* whilst seeds from the Transitional Zone recorded the least (16.7%) (Table 1). *Aspergillus flavus* incidence showed no significant difference ($p<0.05$) between provenances. However, seeds from the Transitional Zone recorded the highest (25.0%) incidence of *Aspergillus flavus* whilst seeds from Deciduous Forest zone recorded no incidence (Table 1). *Colletotrichum* sp. incidence had no significant difference between provenances, however, seeds from the Transitional zone showed the highest (50.0%) whilst seeds from the Deciduous zone recorded no incidences of *Colletotrichum* sp. There were no significant variation ($p<0.05$) in *Penicillium sp.* incidence on seeds between provenances, however, only seeds from the Transitional Zone recorded (8.3%) incidence whilst seeds from both the Deciduous and Guinea Savannah zones recorded no incidence of *Penicillium* sp. (Table 1).

Table 1 Seed-borne mycoflora/ fungi isolated and their incidences on *Khaya Senegalensis* seeds

Provenance	**Incidence of fungi infection on *Khaya Senegalensis* seeds/provenance**				
	Total fungi incidence	***Aspergillus niger***	***Aspergillus flavus*,**	***Colletotrichum* sp.**	***Penicillium* sp.**
Guinea Savanna zone	58.3b	33.3b	16.7a	8.3a	0.0a
Transitional zone	100.0a	16.7b	25.0a	50.0a	8.3a
Deciduous Forest zone	100.0a	100.0a	0.0	0.0	0.0
LSD	16.7	23.5	33.3	60.0	16.6
CV	9.7	23.6	120.0	154.5	300

Means followed by the same letter within a column are not significantly different at 5% probability level.

Four seed-borne fungi namely *Aspergillus niger*, *A. flavus*, *Colletotrichum* sp. and *Penicillium* sp. isolated from *Khaya Senegalensis* seeds from the three different provenances is an indication that forest tree seeds are highly vulnerable to the attack and infection by seed-borne pathogenic fungi or mycoflora. This result is in agreement with [16] 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 [17] also revealed the infection of *Albezia lebbeck* seeds by *Pythium* sp., *Fusarium oxysporum, Cladosporium* sp., *Ascochyta* sp. and *Sclerotinia sclerotiorum.* The total seed-borne fungal infections on *Khaya Senegalensis* varied significantly between provenances. Transitional and Deciduous forest zones recorded equal incidence of 100.0% total seed-borne fungal infections whilst seeds from the Guinea Savannah zone recorded the least total seed-borne fungal infection.

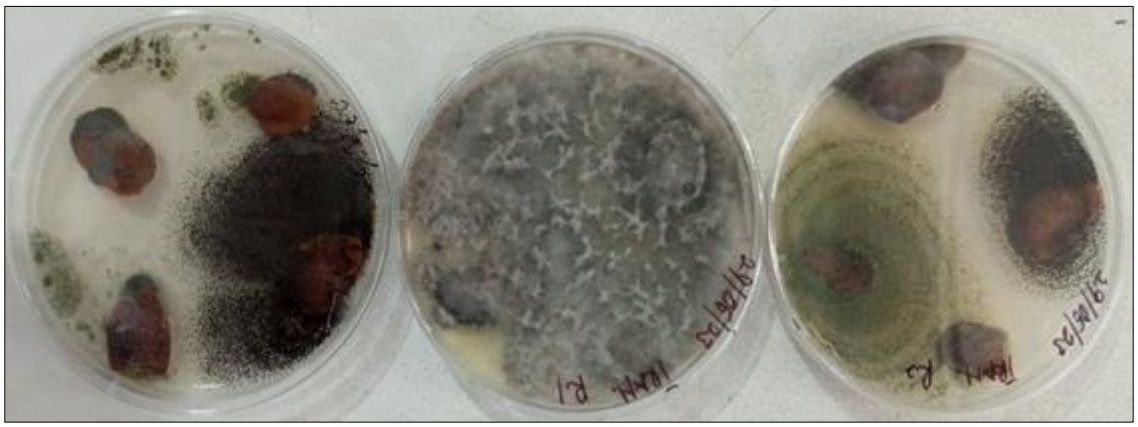


Figure 3 Seeds from Transitional zone showing seed-borne fungi infections

This could be attributed to the variation in the humidity or moisture in the environment. Moisture or humidity is higher in the forest zones and reduces through the Transitional zone and least in the Savannah zones as the amount of rainfall pattern increases in the Forest zone(1900mm) and reduces in the Savannah zone (800mm-900mm) [18]. The high humidity in the forest zones may likely increase the moisture content of prevailing seeds of forest plants. High moisture content of seeds increases seed-borne fungi infections [19].

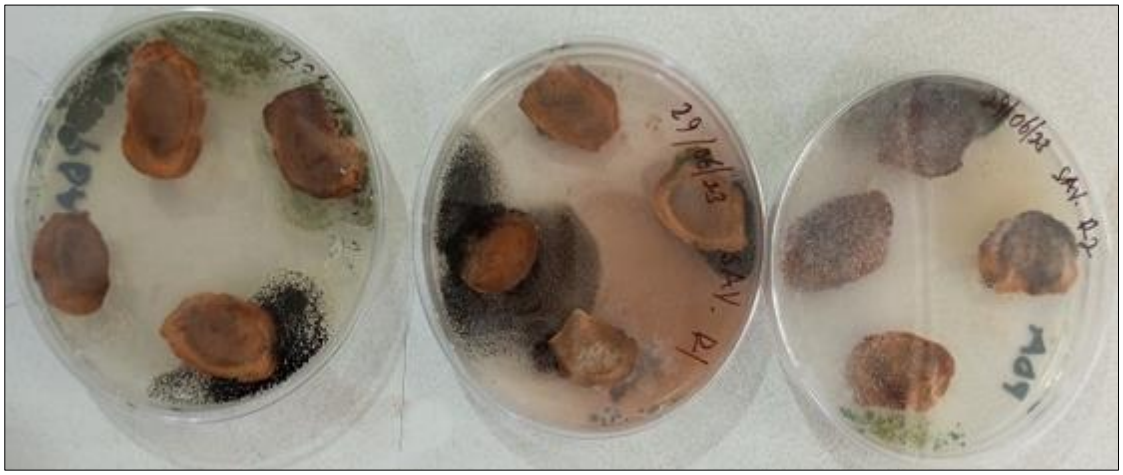


Figure 4 Seeds from Guinea Savannah zone showing seed-borne fungi infections

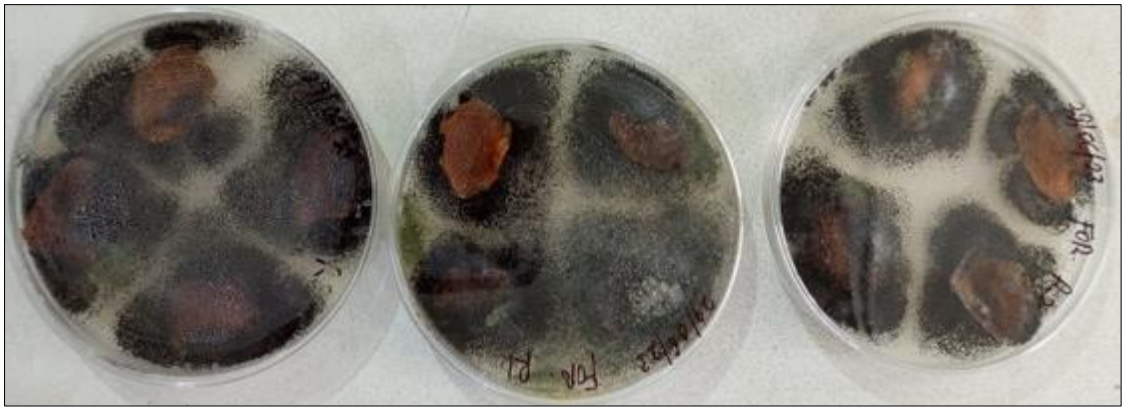


Figure 5 Seeds from Deciduous forest zone showing seed-borne fungi infections

3.2. Effect of provenance on germination percentage of *Khaya Senegalensis* seeds from different provenances

Significant differences ($p<0.05$) were observed between provenances with respect to seed germination. Seeds from the Guinea Savannah zone recorded the highest (73.7%) whilst the least percentage (16.3%) of germination was observed in seeds sourced from the Deciduous Forest zone (Figure 6)

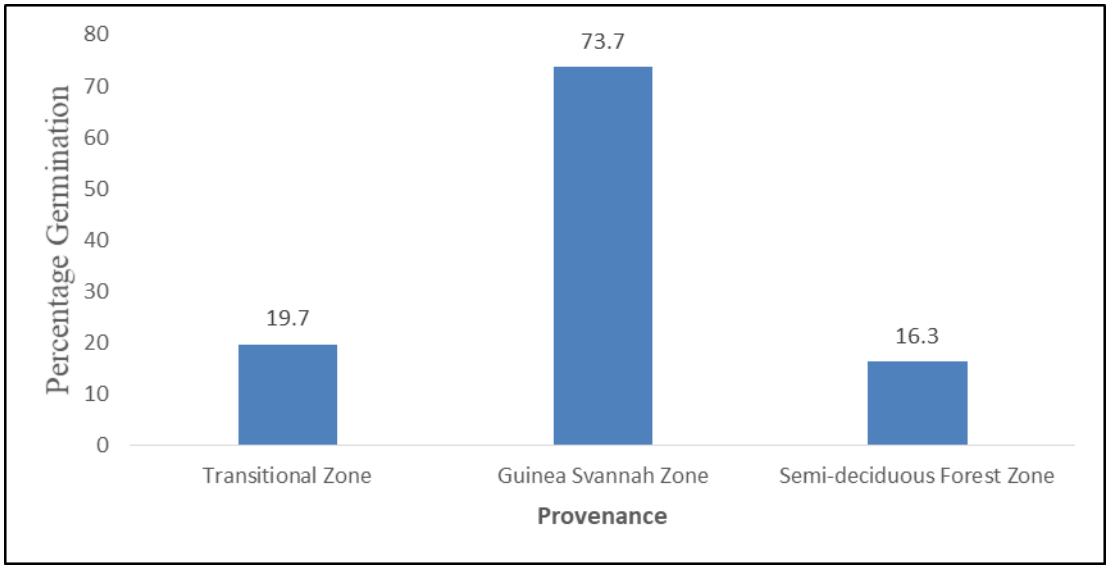


Figure 6 Germination percentage of *Khaya Senegalensis* seeds from different provenances

The significant effect of provenances on germination rate found in the study could be related to the trees genetic background which determined the quality and the germination of the seeds [20]. Also ecological factors such as temperature or rainfall prevailing during the fructification could be involved in poor or high percentage of germination as suggested by [21]. Seeds collected from different sources with varying environmental conditions differ in the ease with which they can germinate [22]. Seeds from the Guinea Savannah zone recorded the highest (73.7%) germination percentage whilst the least percentage (16.3%) of germination was observed in seeds from the Deciduous Forest zone. This could be due to peculiar health conditions of seeds. Seeds from the savannah had least seed-borne fungi infection (58.3%) whilst forest zone recorded seed-borne fungi infection of 100%. Seed-borne fungi cause death of seed embryos resulting poor and failure of germination of seeds [23].

3.3. Effect of provenance on seedling disease incidence among seeds obtained from different provenances

There were no significant difference ($p<0.05$) for percent seedling disease incidence amongst provenances. However the highest (20.0 %) seedling disease incidence was recorded in seeds sourced from Transitional zone whilst the least (12.5%) recorded in seeds from the Deciduous Forest zone (Figure 7). Major disease identified was leaf blight and damping-off.

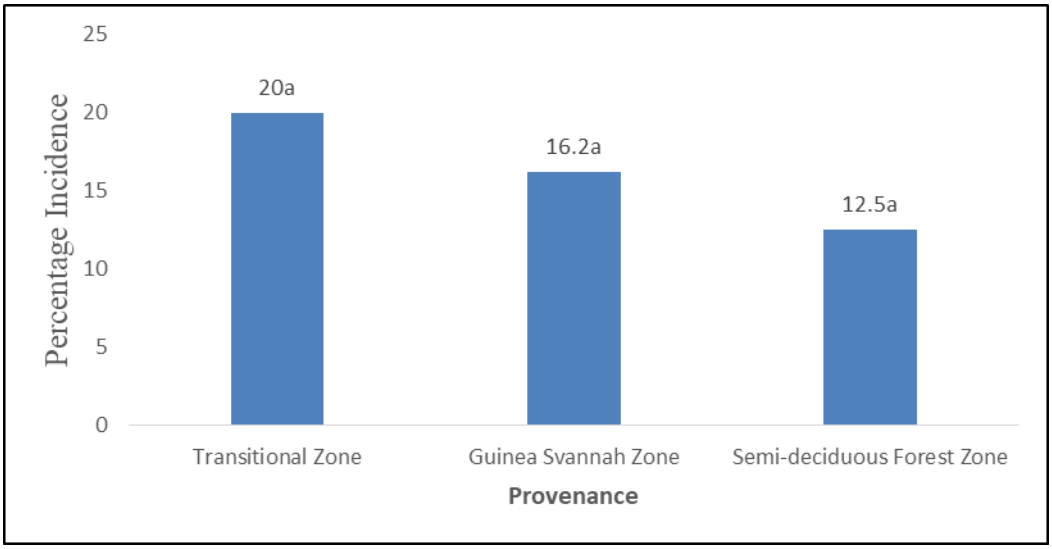


Figure 7 Percentage seedling disease incidence of *Khaya Senegalensis* seeds from different provenances

Significant variation which were not observed among provenances for percent seedling disease incidence in the study could be attributed to high failure of germination of seeds from forest zone and Transitional zone. Adequate number of seedlings are required for monitoring for a period of time for disease incidence. High failure of germination of seeds from forest zone and Transitional zone greatly inhibited the effect of provenance on the seedling disease incidence of *Khaya Senegalensis* seeds sourced from the different provenances.

4. Conclusion

Aspergillus niger, *A. flavus*, *Colletotrichum* sp. and *Penicillium* sp. isolated from *Khaya Senegalensis* seeds from the three different provenances is an indication that *Khaya Senegalensis* seeds are highly vulnerable to the attack and infection by seed-borne pathogenic fungi or mycoflora.

Seeds from all the three provenances were seriously infected with seed-borne fungi and negatively impacted seed germination which is a great threat to healthy seedlings for afforestation programs

Provenance has significant effect on seed-borne fungi prevalence and seed germination

It is therefore recommended that organizations and individuals in afforestation and reforestation programs collect seeds from provenances of healthy trees seeds to enhance high germination for reforestation programs and also conduct laboratory analysis on seed-borne fungal pathogens from different provenances before storage and usage for afforestation and reforestation purposes.

Compliance with ethical standards

Disclosure of conflict of interest

Authors declare no conflict of interest.

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Appendix

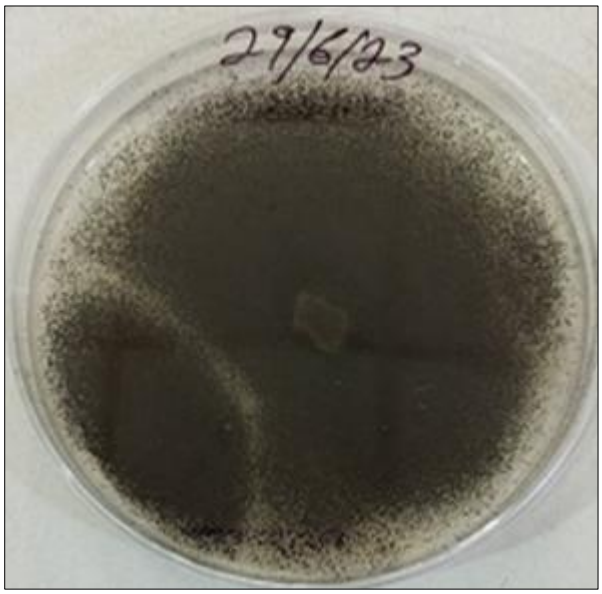

Figure 8 Pure culture of *Aspergillus niger*

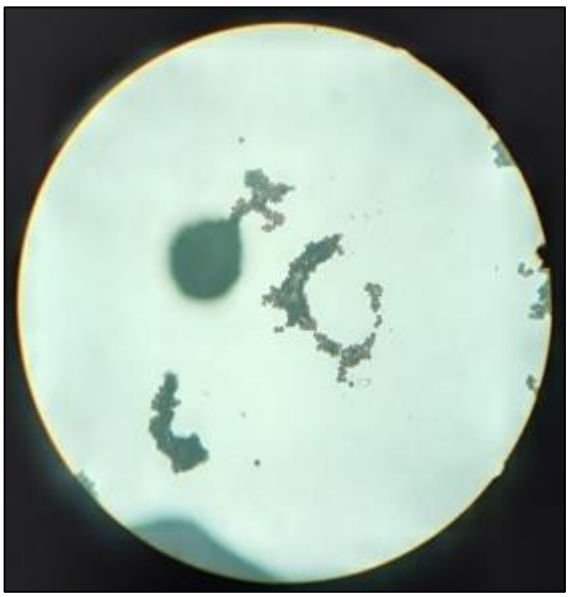

Figure 9 Conidia of *Aspergillus niger*

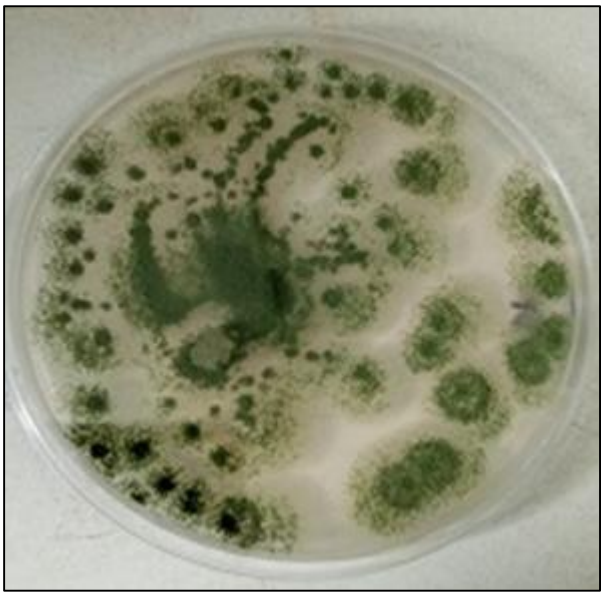

Figure 10 Pure Aspergillus flavus

Figure 11 Conidia of Aspergillus flavus

Figure 12 Pure culture of *Colletotrichum* sp.

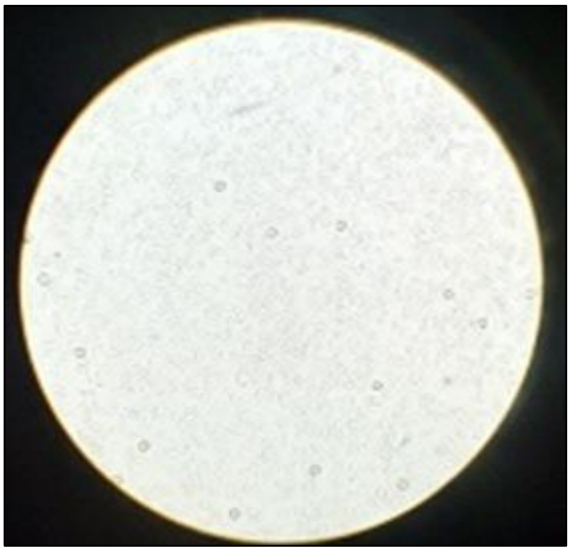

Figure13 Conidia of *Colletotrichum* sp

Figure 14 Pure Penicillium sp.

Figure 15 Conidia of Penicillium