(RESEARCH ARTICLE)

Incidence and severity of fungal seedling diseases in Tamale central forest nursery, Ghana

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Abstract

Fungal diseases cause serious seedling mortality in nurseries and threaten future seedling production for forest regeneration. To abate this menace, knowledge about them is key. Therefore, this study aimed at determining the incidence and severity of fungal diseases of forest seedlings in the Central Forest Nursery of Tamale, Ghana. *Khaya senegalensis, Tectona grandis* and *Polyalthia longifolia* in the nursery were infected with eight fungal pathogens and an unidentified fungus causing leaf blight, wilt, and dumping-off diseases. The pathogens included *Rhizoctonia sp.*, *Fusarium sp.*, *Corrynespora sp.*, *Colletotrichum sp.*, *Cercospora sp.*, *Phytophthora sp.*, *Chaetomium sp.,* and *Macrophomina sp. Rhizoctonia* was the dominant pathogen isolated from all the leaves of the seedling species. *Colletotrichum* and *Chaetomium* were isolated from the leaves of *Polyalthia longifolia. Cercospora* and an unidentified spp. were found on the stems of *Tectona grandis. Fusarium* and *Phytophthora* were respectively found on *Polyalthia longifolia* and *Khaya senegalensis* stems*. Chaetomium* was identified on both *Polyalthia longifolia* and *Khaya senegalensis* stems*. Cercospora* and *Fusarium* were isolated from the roots of *Tectona grandis* and *Polyalthia longifolia respectively* while *Macrophomina* was associated with both *Tectona grandis and Khaya senegalensis roots.* Leaf blight recorded the highest disease incidence (76.7%) and affected all the three seedling species. Wilt disease showed low incidence (10.2-15.8 %) among the different seedling species. Dumping-off disease incidence (10.4%) was only recorded in *Khaya senegalensis.* The severities of the diseases identified were low (1-2) score except leaf blight of *Tectona grandis* and *Polyalthia longifolia* recording a moderate (3.0) severity score. It is recommended that appropriate disease management strategies be employed to prevent high incidences and severities.

Keywords: Fungal Pathogens; Leaf Blight; Disease Incidence; Dumping-Off; Pure Culture

1. Introduction

Nursery is a designated area of raising seedlings, cuttings, and grafts with intensive care before transplanting [1]. Apart from few trees and shrubs, seedlings are the bases of all healthy forest and terrestrial ecosystems restoration. Forest and land degradation is a global disaster and necessitates diverse approaches to mitigate [2, 3], nearly all of which call for some level of seedling establishment in the nursery. Projected effects of global climate change propose the future need for forest and land restoration will rise [4]. In recent times, global leaders have pledged to restore millions of hectares of deforested and degraded lands. Global Forest Goal 1 calls for reversing the loss of forest cover worldwide through sustainable forest management, including protection, restoration, afforestation, and reforestation, and increase efforts to prevent forest degradation and contribute to the global effort of addressing climate change [5]. In Ghana, the Green Ghana initiative seeks to create a collective action towards restoration of degraded landscape in the country, mitigate climate change, and inculcate in the youth the values of planting and nurturing trees and their associated benefits [6]. The global Bonn Challenge likewise pursued to restore 150 million hectares of deforested and degraded land worldwide by 2020 and 350 million hectares by 2030 [7].

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In assistance to meeting these exceptional and ambitious pledges to forest and landscape restoration, availability of healthy nursery seedlings is a vital consideration [8, 9]. Healthy nursery seedlings are often a critical requirement for successfully implementing forest and landscape restoration plans to create healthy, functional, sustainable, and resilient forest and ecosystems which will in turn provide multiple ecological, social, and economic benefits. Nursery which is a prime factor of raising good-quality seedlings to meeting the set targets of global forest and land restoration is faced with some challenges of which infection of seedlings with fungal diseases is not an exception [10].

Fungal diseases are a serious problem in reforestation and sometimes can cause severe seedling mortality in nurseries. Many fungal pathogens are transported through seeds into forest nurseries and become well established on seedlings. Aside seed-borne fungal pathogens, soil-borne fungal pathogens have also been shown to be destructive by invading seedlings in forest nurseries [10]. These seedlings are mostly susceptible to numerous diseases due to their tender tissues. When such poor seedlings are used as planting stocks for reforestation, they further spread the disease to plantations and forests, leading to severe damage. Since seedlings grown in forest nurseries are the primary sources of planting stock, it is highly essential to examine the seedling diseases in order to deduce management strategies either before sowing the seeds or at the seedling stage. Enhanced understanding of the incidence and severity of these microorganisms and the disease they cause in order to suggest appropriate management strategies is also prime [10].

2. Materials and methods

2.1. Study Area
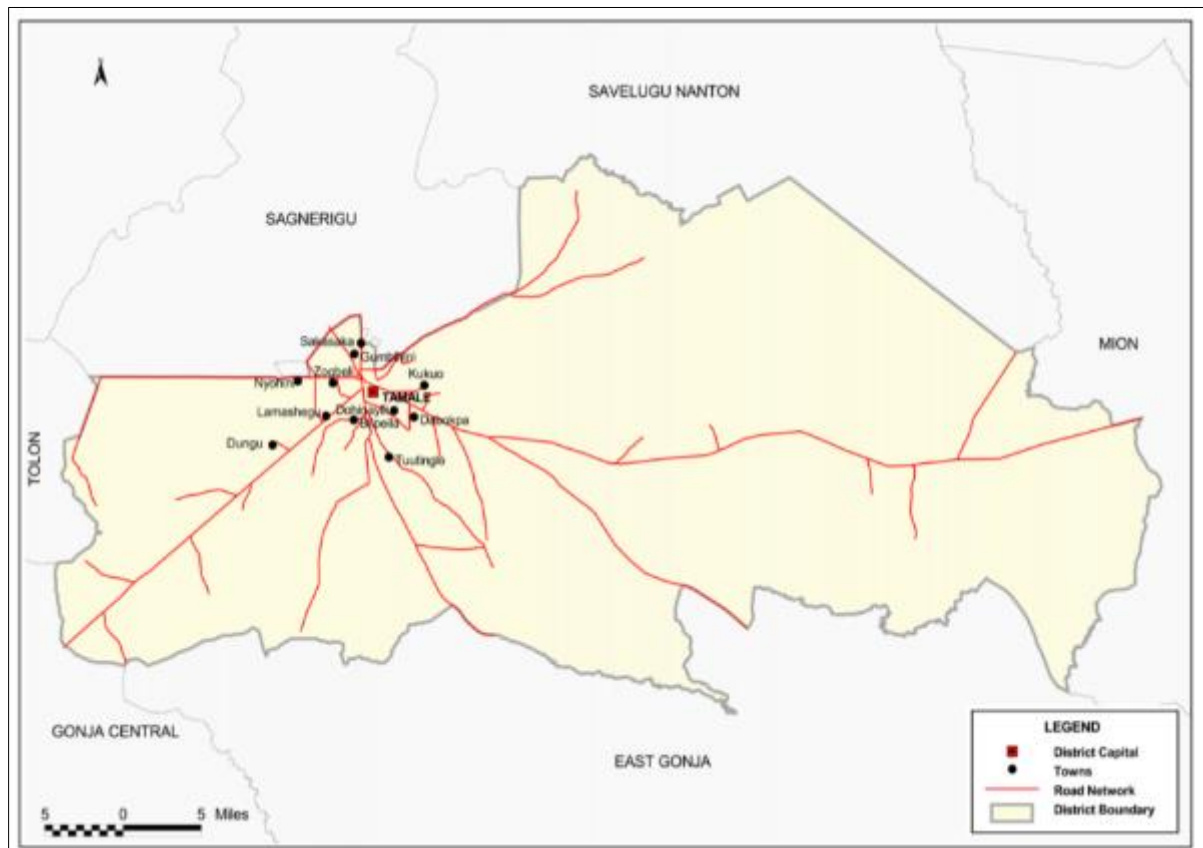
Source: [11]

Figure 1 Map of the Tamale Metropolis

The study was conducted in the Tamale Metropolis in the Northern Region of Ghana. It is located in the central part of the Region and shares boundaries with the Sagnarigu District to the west and north, Mion District to the east, East Gonja to the south and Central Gonja to the south-west. The Metropolis has a total estimated land size of 646.90180sq. km [11]. Geographically, the Metropolis lies between latitude 9º16 and 9º 34 North and longitudes 0º 36 and 0º 57 west. Generally, the Tamale Metropolis is about 180 meters above sea level. The land is generally undulating with a few isolated hills. The Metropolis receives only one rainfall season (May – September). On average, September is the wettest

month with 231 mm of precipitation in a year and this has affected effective agricultural production in the area. Daily temperature in the Metropolis varies from season to season. During the rainy season there is high humidity, slight sunshine with heavy thunder storms, compared to the dry season (January, February, November, and December) which is characterized by dry Harmattan winds from November-February and high sunshine from March-May. The Metropolis lies within the savannah woodland zone in the country. The trees in this zone and for that matter the Metropolis has short scattered wood lots in nature. Major tree types in the Metropolis are *Parkia biglobosa, Adzadiracta indica*, *Senna siamea*, *Khaya senegalensis, Adansonia digitata* among others. The Metropolis is endowed with naturally grown tall grasses during the rainy season which are used to make the local mats popularly called "Zanamat" Besides, the only economic tree is the Shea tree which has gained international recognition. Cashew is also widely grown in the Metropolis. The main soil types in the Metropolis are sandstone, gravel, mudstone, and shale that have weathered into sand, clay, and laterite ochrosols [11]. Figure one shows the Map of the Tamale Metropolis of Ghana.

2.2. Field Assessment of Seedling Disease Incidence and Severity

2.2.1. Sampling Method

Field assessment was undertaken in May, 2022 on 4-months old *Tectona grandis* (L.f.)*,* 3-months old *Khaya senegalensis* (Desr) A. Juss and 4-months old *Polyalthia longifolia* (Sonn) seedlings which were predominant and showed disease symptoms in the nursery during a survey to examine the incidence and severity of fungal diseases. Disease symptoms were restricted mainly to the aerial parts since the nursery is a central and commercial one and many seedlings need not be uprooted for underground symptoms detection. Five sampling plots each measuring 60 cm × 40 cm and comprising of 35 potted seedlings were randomly established in the nursery

2.2.2. Collection of Diseased Seedling Samples

Diseased samples were collected and sorted into closed paper envelopes according to the different species. The envelopes were identified (i.e., numbered serially) and the species names were clearly written on each envelope.

2.2.3. Disease Incidence

Tectona grandis, Khaya senegalensis and *Polyalthia longifolia* seedlings were each scored for the presence or absence of disease symptoms, according to Abang et al. [12] scoring method. The disease incidence (%) was calculated using the formula:

$$\text{Disease incidence } = \frac{\text{Number of diseased plants}}{\text{Total number of plants examined}} \; x100\% \; [13].$$

2.2.4. Disease Severity

A modified disease severity scale (1 – 5) by CSIR-Crops Research Institute (2011) was used to score the diseases as follows:

- None (*No disease symptoms*)
- Minor (*Disease symptoms covering* less than 25% of total shoots)
- Moderate (*Disease symptoms covering* between 25-50% of total shoots)
- Severe (*Disease symptoms covering* between 50-75% of total shoots)
- Very severe (*Disease symptoms covering* between 75-100% of total shoots)

The mean disease severity for each sample plot was estimated by summing severity scores > 1 divided by the total number of symptomatic plants [14].

The disease severity was calculated using the formula:

$$\text{Disease Severity } = \frac{\Sigma(\text{Number of seedlings scored for each rating } \times \text{ the rating value})}{\text{Total seedlings scored per plot}}$$

2.2.5. 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 500 ml of distilled water. Chloramphenicol sulphate (250 mg) was added to the suspension to suppress bacterial growth. The suspension was topped up with 500 ml 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/cm² 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.

2.2.6. Isolation and Purification of Fungal Pathogens

Isolation of fungal pathogens from diseased seedlings were done at the Spanish Laboratory of the Faculty of Agriculture, University for Development Studies (UDS). Diseased seedling samples (leaves, stems, and roots) of the different species were washed separately with tap water to remove debris, excised with a sterilized razor blade at the point of progression of disease symptom (1 cm fragment), surface sterilized with 1% sodium hypochlorite solution for 2 minutes, 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 20 minutes. The pieces were then plated on PDA in 90 mm diameter sterilized Petri dishes and incubated at ambient temperature (28 ± 2 °C) for seven (7) days. A total of 18 petri dishes of PDA media were plated for the diseased sample leaf, stem, and root tissues (i.e., two petri dishes each for leaf, stem, and root tissues for the three seedling species).

After 7 days, the plates were inspected to observe the growth of pathogens on the media. Mycelium emerging from the tissues were sub cultured on fresh PDA media with the help of a sterile loop to obtain pure isolates of the pathogens. They were checked and transferred on to new PDA media weekly and kept at room temperature (21-25 ºC) to always maintain pure cultures for correct identification of fungal pathogens.

2.2.7. Identification of 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 Barnett and Hunter [15] and Watanabe [16].

2.3. Data Presentation and Analysis

Calculations were done on data for each disease incidence and severity and were summarized for their mean percentages per seedling species and the results presented in Tables. Data on percent disease incidence and severity were analysed statistically for analysis of variance (ANOVA) using GenStat 12th Edition statistical package. Percent disease incidence and severity data were transformed using Square Root transformation ($\sqrt{x + 0.5}$) prior to the analysis. The mean disease incidence and severity differences among the seedling species were tested using Least Significant Difference (LSD) at the 5% probability level. Fungal species identified from the diseased seedlings were also recorded.

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3. Results

3.1. Major Fungal Diseases Identified in the Tamale Central Forest Nursery

Three major diseases namely leaf blight, wilt, and dumping-off were identified on the seedlings of *Khaya senegalensis, Tectona grandis and Polyalthia longifolia* in the nursery. The symptoms of diseased seedlings identified are as follows:

3.1.1. Leaf Blight

The infected plants showed grayish brown patches that enlarge rapidly and cover a large part or the entire lamina. The infected leaves dry up and are eventually shed. The disease spreads laterally in the nursery through overlapping foliage of the adjoining seedlings often resulting in group blighting of seedlings.

3.1.2. Wilt

The infected seedlings showed drooping leaves, stunting, slow growth, sparse, and distorted foliage

3.1.3. Dumping-off

Rotting stems, toppling, or drying of stems, sudden deaths, and decay of stems at or near the soil line

3.2. Fungal Pathogens Isolated from Diseased Seedlings

Eight and unidentified fungal pathogens were isolated from the diseased samples. Table 1 reports the fungal pathogens isolated from diseased seedlings sampled. *Rhizoctonia solani* was the dominant fungal pathogen isolated from all the

leaves of the seedling species. *Corrynespora* sp. was only isolated from the leaves of *Tectona grandis* while *Colletotrichum gloeosporiodes* and *Chaetomium* sp. were found to be associated with the leaves of *Polyalthia longifolia* leaves. With regards to seedlings stems, *Cercospora* sp. and an unidentified sp. were found on *Tectona grandis*, *Fusarium* and *Phytophthora* spp were respectively found on *Polyalthia longifolia* and *Khaya senegalensis* while *Chaetomium* sp. was found to be associated with both *Polyalthia longifolia* and *Khaya senegalensis*. *Cercospora* and *Fusarium* spp. were isolated from only the roots of *Tectona grandis* and *Polyalthia longifolia* while *Macrophomina* was associated with both *Tectona grandis* and *Khaya senegalensis* roots (Table 1).

Table 1 Fungal Pathogens Isolated from Diseased *Tectona grandis*, *Khaya senegalensis* and *Polyalthia longifolia* Seedlings in Tamale Central Forest Nursery

Seedling species	Pathogens		
	Leaf	**Stem**	**Roots**
Tectona grandis	*Rhizoctonia solani* *Corrynespora sp.*	*Cercospora sp.* Unidentified sp	*Cercospora sp.* *Macrophomina phaseolina*
Polyalthia longifolia	*Rhizoctonia solani* *Colletotrichum gloeosporiodes* *Chaetomium erectum*	*Fusarium* *Chaetomium sp.*	*Fusarium sp.*
Khaya senegalensis	*Rhizoctonia solani*	*Chaetomium sp.* *Phytophthora sp.*	*Macrophomina phaseolina*

Source: Field survey, 2022

3.3. Incidence of Fungal Diseases Identified

Major diseases identified on the three seedling species namely *Tectona grandis, Polyalthia longifolia, and Khaya senegalensis* were leaf blight, wilt, and dumping-off. Leaf blight recorded the highest percentages (20.6 -76.7%) of disease incidence affecting all the three seedling species whilst dumping off recorded the least percentage (10.4%) of disease incidence affecting only *Khaya senegalensis* (Table 2). Leaf blight disease incidence varied significantly ($p < 0.05$) from (20.6 -76.7%) among the different seedling species. *Polyalthia longifolia* recorded the highest (76.6%) leaf blight disease incidence whilst least (20.6%) was observed in *Khaya senegalensis*. Wilt disease showed no significant difference in percent incidence among the different seedling species but was highly (15.8%) observed in *Khaya senegalensis* whilst least (10.2%) in *Tectona grandis* (Table 2). With respect to Dumping off disease, only *Khaya senegalensis* recorded an incidence of 10.4% (Table 2).

Table 2 Incidence of major fungal diseases of *Tectona grandis*, *Khaya senegalensis* and *Polyalthia longifolia* seedlings in Tamale Central Forest Nursery

Species of seedlings	Disease incidence (%)		
	Leaf blight	**wilting**	**Dumping-off**
Khaya senegalensis	20.6c	15.8a	10.4a
Tectona grandis	67.4b	10.2a	0.0b
Polyalthia longifolia	76.7a	10.6a	0.0b
LSD(0.05)	0.2	0.7	0.2
CV (%)	1.3	3.3	5.6
Means followed by the same letter within a column are not significantly different at 5% probability level.			

Source: Field survey, 2022

3.4. Severity of Fungal Diseases Identified

The average severities of all the fungal diseases of *Tectona grandis, Khaya senegalensis, and Polyalthia longifolia* identified in the nursery were low. Leaf blight of *Tectona grandis* and *Polyalthia longifolia* recorded the moderate (3.0) severity score (Table 3).

Table 3 Severity of Major Fungal Diseases of *Tectona grandis, Khaya senegalensis* and *Polyalthia longifolia* Seedlings in Tamale Central Forest Nursery

Species of seedlings	Disease Severity		
	Leaf blight	**Wilting**	**Dumping-off**
Khaya senegalensis	2.0b	2.0a	1.5a
Tectona grandis	3.0a	2.0a	0.0b
Polyalthia longifolia	3.0a	1.5b	0.0b
LSD(0.05)	0.4	0.5	0.5
CV (%)	3.9	5.8	10.4
Means followed by the same letter within a column are not significantly different at 5% probability level. *Scores representing means of severity measured on 1-5 scale are defined below: 1 = None (No disease symptoms); 2 = Minor (Disease symptoms covering less than 25% of total shoots); 3 = Moderate (Disease symptoms covering between 25-50% of total shoots); 4 = Severe (Disease symptoms covering between 50-75% of total shoots; 5 = Very severe (Disease symptoms covering between 75-100% of total shoots)			

4. Discussion

4.1. Fungal Disease Pathogens Identified in the Tamale Central Forest Nursery

Leaf blight, wilt, and dumping-off diseases identified on the seedlings of *Khaya senegalensis, Tectona grandis, and Polyalthia longifolia* in the nursery are common seedling diseases in the nursery. This results agree with Ondieki et al. [17] who identified dumping-off and wilt diseases in tree nurseries and plantations in Kimondi forest of Nandi County, Kenya. Different fungal pathogens isolated from each of leaf, stem, and root tissues of the different seedling species is an indication that the disease symptoms observed are not caused by a single pathogen and therefore disease complexes could occur in the nursery if effective disease management strategies are not undertaken. *Rhizoctonia* is a soil-borne pathogen but was the dominant fungal pathogen isolated from all the leaves of the seedling species and therefore could be inferred that it is the major pathogen contributing to the blight disease in the nursery [18]. This inference is in agreement with TSS [19] and Shivanna [20] who identified Rhizoctonia leaf blight of *Cassia fistula* and *Bauhinia variegata*. *Colletotrichum gloeosporiodes, Corynespora, Chaetomium* spp., and *Cercospora* identified on the diseased seedling tissues have the potential of causing leaf spot disease in the nursery [20][21]). Wilt and dumping off diseases observed in the nursery could be attributed to the Fusarium spp. identified on the tissues of the diseased seedlings as asserted by Agrios [23] and James [24] that *Fusarium* generally causes both pre- and post-emergence damping off and vascular wilts of herbaceous perennial ornamentals and plantation crops. *Macrophomina* spp. identified in the nursery is also a potential pathogen for causing diseases such as stem and root rot, charcoal rot, and seedling blight [25] if effective management tactics are not ensured.

4.2. Incidence and Severity of Fungal Diseases

Three fungal diseases namely leaf blight, wilt, and dumping-off identified on the three seedling species namely *Tectona grandis, Polyalthia longifolia* and *Khaya senegalensis* had varied incidence. Leaf blight recording the highest percentages (20.6 -76.7%) incidence and affecting all the three seedling species, which is an indication that leaf blight is a common nursery disease and could infect a variety of nursery seedlings. This assertion is in agreement with TSS [19] and Shivanna [20]. Moreover, the period of the study (May) could be a contributing factor for the high incidence of the leaf blight disease as asserted by TSS [19] that leaf blight disease is caused by *Rhizoctonia solani* and is often serious in nurseries during April-May when warm humid conditions prevail. *Polyalthia longifolia* recording the highest (76.6%) leaf blight disease incidence, an indication that it is susceptible to the pathogens causing the disease. Wilt and dumping off diseases showed low incidence among the different seedling species. This could be inferred that the pathogens causing these diseases were less prevalent, experienced unfavourable environmental condition or absent.

The average severities of all the fungal diseases of *Tectona grandis, Khaya senegalensis, and Polyalthia longifolia* identified in the nursery were low except leaf blight of *Tectona grandis* and *Polyalthia longifolia* which were moderate. The low average severity of the diseases could be attributed to early developmental stages of the disease or unfavourable environmental conditions on the pathogens [20].

5. Conclusion

Leaf blight, wilt, and dumping-off are diseases of *Khaya senegalensis, Tectona grandis* and *Polyalthia longifolia* seedlings. *Rhizoctonia solani, Fusarium sp., Corynespora sp., Colletotrichum gloesporoides, Cercospora sp., Phytophthora sp., Chaetomium sp.* and *Macrophomina phaseolina* were the fungal pathogens identified on diseased samples of *Khaya senegalensis, Tectona grandis*, and *Polyalthia longifolia* seedlings. Different fungal pathogens were isolated from the same disease sample tissues which is a condition for disease complexes. *Rhizoctonia solani* was identified on all the leaves of *Khaya senegalensis*, *Tectona grandis*, and *Polyalthia longifolia* signifying that it is a major pathogen in the forest nursery. *Tectona grandis* and *Polyalthia longifolia* had high incidence of leaf blight disease. Averagely, incidences and severities of all the fungal diseases of *Tectona grandis, Khaya senegalensis,* and *Polyalthia longifolia* identified in the nursery were minor to moderate. Even though this research reveals minor to moderate disease incidence and severity, they are major grounds for serious disease outbreak of high incidences and severity, therefore appropriate fungicides or sanitary measures should be employed to prevent such high incidences and severities. Majority of the fungal pathogens identified such as *Rhizoctonia solani, Fusarium sp., Phytophthora sp. Macrophomina phaseolina,* and *Corynespora sp.* are soil-borne pathogens and therefore the soils used for raising seedlings should be sterilised or fumigated.

Compliance with ethical standards

Disclosure of conflict of interest

Authors declare no conflict of interest.

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Appendix

Figure 2 Pure culture of *Rhizoctonia solani*　　**Figure 3** Sclerotia of *Rhizoctonia solani*

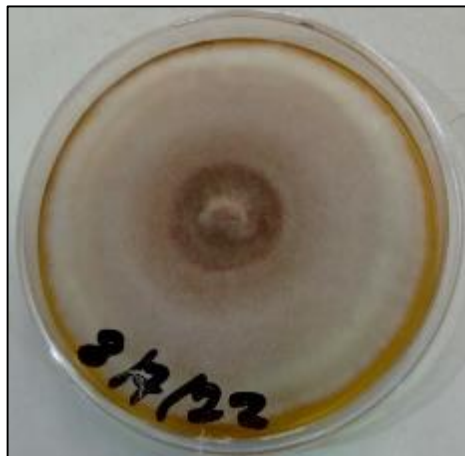**Figure 4** Pure culture of *Fusarium sp*

Figure 5 Conidia of *Fusarium sp*

Figure 6 Pure culture of *Corrynespora sp.*

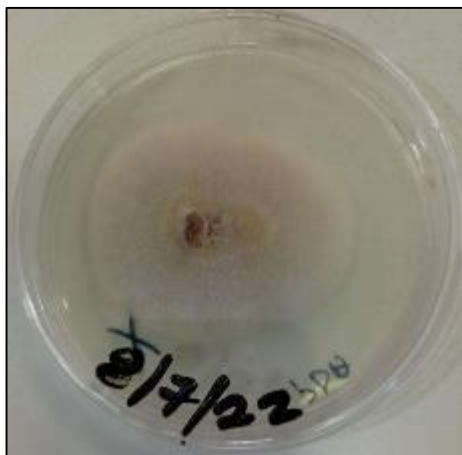**Figure 7** Conidia of *Corrynespora sp*

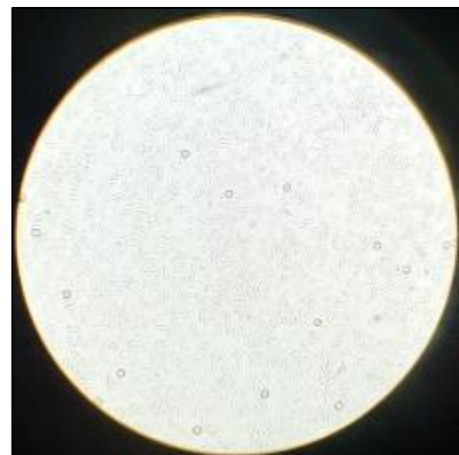**Figure 8** Pure culture of *C. gloeosporiodes*

Figure 9 Conidia of *C. gloeosporiodes sp*

Figure 10 Pure culture of *Chaetomium sp*

Figure 11 *Conidia of Chaetomium sp*

Figure 12 Pure culture of *Cercospora sp*

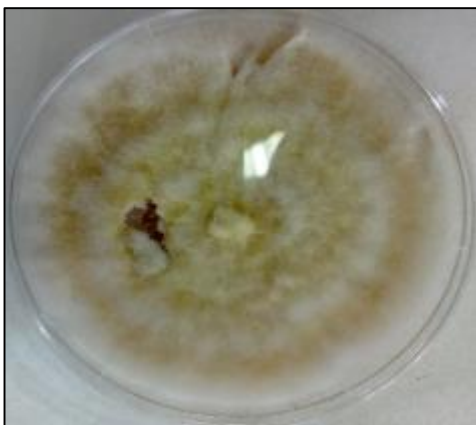
Figure 13 Conidiophores of *Cercospora sp*

Figure 14 Pure culture of *Phytophthora* sp

Figure 15 Conidia of *Phytophthora* sp

Figure 16 Pure culture of unidentified sp   **Figure 17** Mycelia of unidentified sp