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DIVERSITY OF PATHOGENIC FUNGI ON PLANTATION FORESTS OF NORTH AND NORTH-WEST ETHIOPIA

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A B S T R A C T

Forest plantations in Ethiopia are mainly exotic genera of *Eucalyptus, Cupressus, Casuarina, Pinus* and native *Juniperus* species. Plantations species have suffered at varying degrees of attack by disease causing agents, particularly Amhara region is among regions with plantation forests that have in recent years been subjected to attack by diseases in Ethiopia. Plantation trees in commercial stands, farmlands and woodlots were surveyed for diseases symptoms in 20 selected areas of Amhara and Tigray from May to June 2016.Leaf blight, leaf spot, tip blight and stem canker were the most common symptoms appeared during the survey period with leaf spot and stem canker the most prevalent. Tree samples showing clear disease symptoms were collected, surface sterilized, cultured and morphologically characterized for pathogen identification. A total of 42 isolates of fungi colonies were identified from samples collected of 20 localities. Morphological characterization of fungal isolates reveals, six fungal genera belonging to *Alternaria*, *Dioplodia*, *Pestalotiopsis*, *Curvularia*, *Phoma,* and *Penicillium* were the cause of the symptoms of the symptoms of the observed disease. Among the isolates 14 (33.3%) were *Alternaria* species, 15(37.7%) were *Phoma* species, and the remaining 13 isolates were *Diplodia*3(7.2%), *Pestalopsis*7(16.7%), *Curvularia*2(4.7%) and *Penicillium*1 (2. 4%).Based on the findings of the study *Phoma lingam, Phoma glomerata, Alternaria alternata,* genera of *Curvularia, Pestalotiopsis, Penicillium,* and *Diplodia* were found to be the cause of diseases of the tree plantations. *Phoma* and *Alternaria* species were the most prevalent isolates, showing a majority of symptoms observed on plantations were due to their co-infection. The pathogenicity test result of the research also confirms fungal isolates were the cause of the symptoms of the disease observed. The findings of this research enable to study and design appropriate management options for the future prevention and control of the diseases especially when there is prolonged environmental stress in the country.

Keywords: *A. alternata*, leaf spot, *Phoma* spp., *Pestalotiopsis* spp., *Curvularia* spp.

INTRODUCTION

Plantation forests are cultivated forest ecosystems established by planting or seeding using introduced or indigenous species in the process of afforestation and reforestation, primarily for wood biomass production, it coversabout5% of the global forest with *Pinus* and *Eucalyptus* species the most commonly used in the world (FAO, 2001).

The total area of plantation forests in Ethiopia is estimated to be 972,000-190400 ha for the supply of largest volume of wood products used in the construction sector, the biomass fuel consumed in the country, satisfy household demands for wood and additional household incomes (Lemenih and Kassa, 2014).

Yirdaw (2002) showed that forest plantations in Ethiopia are mainly exotic genera of *Eucalyptus, Cupressus, Casuarina, Pinus,* and native *Juniperus* species. Majority of forest plantation are located in Oromia, Amhara, the Southern Nations and Nationalities Peoples Region, and Tigray region (Bekele, 2011). According to the study of Jenbere *et al*. (2011) high rate of plantations compared to other farm, enterprises leads to the extent of conversion croplands and grazing fields to *Eucalyptus* woodlots in Amhara region. An income from plantations like Eucalyptus tree sales contributes on average up to 25% of total household annual cash income and for poor households up to 72% of the total annual cash income which is the largest non-agricultural source of household income in the country (Jegger, 2003).

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Successful forest plantations require species well matched with sites, improvement of genetic stock, control and related operations that enhance tree quality and stand growth. Currently, plantations are more at risk from diseases due to pathogens than natural forests (Evans, 2000). Among the wide variety of pathogens that cause plant diseases, fungi are important stressors that affect tree health. The magnitude of fungal diversity is estimated to be 1.5 million species, with only 5% of species were described. Available evidence also indicates that fungal diversity in the tropics is richer than others (Berrin *et al*., 2012). Large numbers of fungi species are plant pathogens causing about 70% of plant disease. Fungal plant pathogens, if not controlled one way or the other, can have a devastating effect on biodiversity, forest structure and dynamics, commercial plantations, agroforestry and urban environments.

The impacts of fungal pathogens on plantation forestry species can be more severe with increased movement of humans and plant products around the world as it facilitates distribution of diseases causing agents to new areas of the world (Chimwamurombe, 2016).

Forest plantations of Ethiopia are at varying degrees of attack by disease causing agents, particularly that of Amhara region are among that have in recent years been subjected to attack by diseases. Study of Gbadegesin *et al*. (1999) reveals that leaf disease of *Eucalyptus,* cypress, shoot blight and dieback of *Pine* are common diseases in the region, with most of the diseases observed are expected to be from nursery sites. Fungi species can associate a host plant as phylloplane, saprobes, endophytes, mycorrhizal, parasites or commensals (Tang, 2003). Plant disease complexes can also involve association of more than one pathogenic fungus in a host as in brown apical necrosis of walnut fruit where numerous plant pathogenic fungi *Fusarium*, *Alternaria*, *Cladosporium*, *Colletotrichum, Pestalotiopsis* and *Phomopsis* involved (Lamichhane and Venturi, 2015; Lee, 2003).

Most fungal species that cause diseases such as leaf spot, leaf blight and stem canker show overlapping symptoms of small, scattered, circular to oval dead areas in the leaves; usually tan, dark brown, yellow, gray, purple, or black with some spots rose, shiny, and coal black forming ragged holes with marked light and dark concentric zones (Figure 1).

The objective of this study was to investigate type, diversity, and distribution of pathogenic fungi associated with plantation forest trees showing diseases symptoms in Amhara and Tigray region, North and North West of Ethiopia.

Material and methods

Study areas, Sampling and Sampling techniques

Study areas: Amhara Region is located between 8°45'N and 13°45'N latitude and 35°46'E and 40°25'E longitude in North West Ethiopia with annual mean minimum and maximum temperatures between 15°C and 21°C and the average annual rainfall of 1194 in mm. Tigray forms the northernmost reaches of Ethiopia, and is located between 36 degrees and 40 degrees east longitude, north-south extent spans12 and a half degrees to 15 degrees north with average annual rainfall between 450-980 in mm, and the annual minimum and maximum mean temperature of the region is between 9.86°C and 24.9°C (Bewket, 2009; Taye *et al.,* 2013; Ayalew *et al*., 2012).

Sampling and Sampling techniques: Plantation trees in commercial stands, farmlands and woodlots were surveyed in the selected areas of Amhara and Tigray regions in moist season from May to June 2016 for disease symptoms. Random sampling was used in the collection of samples from 20 plantation sites, based on Gbadegesin *et al*. (1999). The severity of the problems in the areas as pointed out by Regional, Zonal and District Forestry and protection staffs.

Identification and morphological characterization of fungal pathogens: Leaves, pieces of bark, twigs and segments of stems showing disease symptoms were collected and processed for identification of causal fungi from plant tissues exhibiting clear symptoms. Infected tissues aseptically cut into small pieces (2–5 mm squares) along with adjacent small unaffected tissue were surface sterilized transferring to sterile Petri dishes containing tap water,90% ethanol solution and distilled water for 30-60s. The sterilized pieces were transferred to Petri dishes containing potato dextrose agar (PDA) in a hood and incubated at room temperature (25-30°C) for 5-7 days and examined daily for the growth. Morphological studies of cultures isolate on PDA were conducted following the methods described by (Boerema *et al.,* 2004). Micromorphological descriptions for 42 fungal culture were carried out from mature conidiomata and conidia using slides mounted in water (Aveskamp *et al*., 2010; Chen *et al*., 2015). Slides were prepared to make detailed observations of the morphological features, size, and shape, colors of conidiomata, pyinida, conidia and patterns of fungal

growth in *vitro* using a compound microscope. Colony colors on the surface and reverse of inoculated Petri plates were assessed according to the color charts of (Rayner 1970). Fungal cultures were identified at genus and species level based on observed macroscopic and microscopic characteristics (Khan *et al*., 2015; Ngobisa *et al*., 2015; Raymond *et al*., 2000; Saju *et al*., 2011).

Pathogenicity trials: Pathogenicity of fungal isolates was tested using seedlings of the respective host plants raised in the nursery sites of Central Ethiopian Environmental and forest research center(CEE-FRC). As it is not practical to test all the fungi isolated to their respective host plants to establish the pathogenic status, only selected fungi (rarely encountered ones and weak pathogens) were screened. Pathogenicity of the fungal isolates to the respective hosts was tested by using 3-6 month-old seedlings and spraying conidial suspension (2 x 103 conidia/ml of sterile water) of the respective fungus. Three to five leaves of seedlings of the respective host plants were inoculated and incubated in a humidity chamber (>90% R.H, 26 ± 2 ^{0}C with 12h dark and light period). Disease symptoms developed were recorded and fungi were re-isolated from the diseased host tissues and pathogenicity of the respective fungal species confirmed (Xue *et al.,* 2004).

Data Analysis: The collected data were summarized, ranked and expressed using simple descriptive statistics such as percentages and graphs. Survey data of Morpho-cultural characters. The relative prevalence of each pathogenic fungal species with respect to localization and others were analyzed using SAS Ver. 9 procedures at probability level, p=0.05 (SAS Institute Inc., 2002).

RESULTS

Symptomology of disease: Symptoms of several diseases were observed on *Eucalyptus*, *Cupressus*, *and Juniperus and Chordia* species at study sites. Most of the study sites were dominated by *Eucalyptus* plantations, particularly *Eucalyptus globulus* in the high land areas. The most common symptoms appearing during the survey were leaf blight, leaf spot and stem canker typical of those caused by *Phoma, Alternaria, Curvularia* and *Pestalotiopsis* sp. on *Eucalyptus* and *Chordia* spp. and tip blight symptoms similar to infection by the *Diplodia* sp. (Figure 1) on *Juniperus procera* and *Cupressus lusitanica* of plantations. Leaves and stems were associated with brown to black spots, round to irregular-shaped. Leaf spots were circular or irregular in shape separated or aggregated and often located at the margins with brown, pale brown to grey coloration while stem canker is observed with elongated, greyish, hell brown to the dark brown border between discolored tissues lesions typically on *Eucalyptus globules* as shown in figure 1.

Figure 1. Plantation samples showing stems canker, leaves spot and tip blight symptoms.

Morphological characterization of fungal isolates: A total of 42 isolates of fungal colonies were identified from 20 localities (Table 1). Colony textures of the isolates on PDA appeared as appressed with sparse aerial mycelium with raised and slightly dense aerial mycelium, or floccose with raised and dense aerial mycelium. Colony colors of the isolates were observed white, gray, and black, brown, green to dark green and pink (Figure 2). Mean colony diameter of the fungal isolates on the PDA were found insignificant with ranges from 4.48cm to 6.25cm on 9cm Petri plate within seven days of incubation at 25-30°C (Table 2).

Table 1. Survey sites and key fungal isolates from diseased Tree plantations in Ethiopia.

Region and Zones	Locality	Altitude	Tree species	Genera, species of Fungi isolated
South Gondar, Amhara	Lomi Dur	2705	*E. globulus*	*Phoma glomerata, Alternaria Alternata*
South Gonder Amhara,	Atrik	2975	*C. lusitanica*	*Penicillium, Diplodia* spp.
South Wollo, Amhara	Jeme	2359	*E. globulus*	*A. Alternata, P.glomerata, Pestalotiopsis* spp.
South Wollo, Amhara	Harbu	1495	*Cordia Africana*	*A. Alternata , Phoma lingam*
South Wolo	Lomiye	1812	*C. Africana*	*A. Alternata, P. lingam*
South Gondar, Amhara	Gelaye	3095	*E. globulus*	*A. Alternata, P. lingam, Pestalotiopsis* spp.
South Gondar, Amhara	Atrik	2975	*E. globulus*	*A. Alternata, P. glomerata, Pestalotiopsis* spp.
West Gojjam, Amhara	Yinesa	1824	*E.camaldulensis*	*Pestalotiopsis*spp.
Bahir Dar Zuria, Amhara	Kimbeba	1945	*E.camaldulensis*	*A. Alternata, Pestalotiopsis* spp.
South Wollo, Amhara	Harego	2320	*J. procera*	*Diplodia* spp.
North Tigray	Desea	2203	*J. procera*	*Diplodia* spp.
Central Tigray	Mekele	2014	*Acacia saligna*	*A. Alternata, P. glomerata*
Central Tigray	Adi mesino	2635	*E. globulus*	*P. lingam, A. Alternata*
SouthEast Tigray	May Keyhe	2312	*E. globulus*	*P. lingam, A. Alternata*
South Gondar, Amhara	Kosso Mado	3206	*E. globulus*	*P. lingam, Pestalotiopsis* spp.
South Tigray	KoremZuria	2579	*E. globulus*	*P. glomerata, A. Alternata*
South Wollo, Amhara	Sulula	2290	*E. globulus*	*P. lingam,Pestalotiopsis*, *Curvularia* spp.
North Showa, Amhara	Tarma ber	2215	*E. globulus*	*A. Alternata, P. glomerata, Curvularia* spp.
North Showa, Amhara	Keyit	2880	*E. globulus*	*P. glomerata, A. Alternata*
North Showa, Amhara	Elu	2891	*E. globulus*	*P. lingam, A. Alternaria*

Table 2. Colony growth diameter of Fungal isolates on PDA during seven days of incubation.

Fungal isolates	Mean colony growth Diameter of major fungal isolates on PDA in centimeter (cm)							
	48hrs	72hrs	96hrs	120hrs	144hrs	168hrs	Mean	SE
Phoma glomerata	3.5	4.6	6	7	7.8	8.6	6.25	0.79
Alternaria alternata	4	5	7	8	9	9	7	0.8
Diplodia spp.	3	3.5	5	6.7	7	7.5	5.45	0.78
Pestalotiopsis spp.	2.5	3.5	4.5	5.6	6.4	6.9	4.9	0.69
Phoma lingam	3.7	4.3	5.8	6.8	7	8	5.93	0.67
Curvularia spp.	2.7	2.8	3.4	5	6	7	4.48	0.73

Morphological feature of fungal isolates reveals, six fungal genera belonging to *Alternaria*, *Diplodia*, *Pestalotiopsis*, *Curvularia*, *Phoma,* and *Penicillium* were the cause for the diseases symptom observed in the study areas (Figure 2). Among the isolates 14 (33.3%) were *Alternaria* species, identified as *A. alternata*, 15 (37.7%) were *Phoma* species, identified as *Phoma lingam* and *Phoma glomerata* while remaining 13 isolates belongs to genera *Diplodia* (7.2%), *Pestalotiopsis* (16.7%), *Curvularia* (4.7%) and *Penicillium* (2.4%). Results of the study also show, species of *Phoma* and *Alternaria* were the most frequently isolated and found to be pathogens for the majority of stem canker and leaf spot diseases symptoms observed in the survey areas (Figure 3). Pathogenicity result of fungal pathogens revealed typical symptoms of the disease appeared after 10 days of inoculation on host plants confirming the causal agents for the diseases on the plantations.

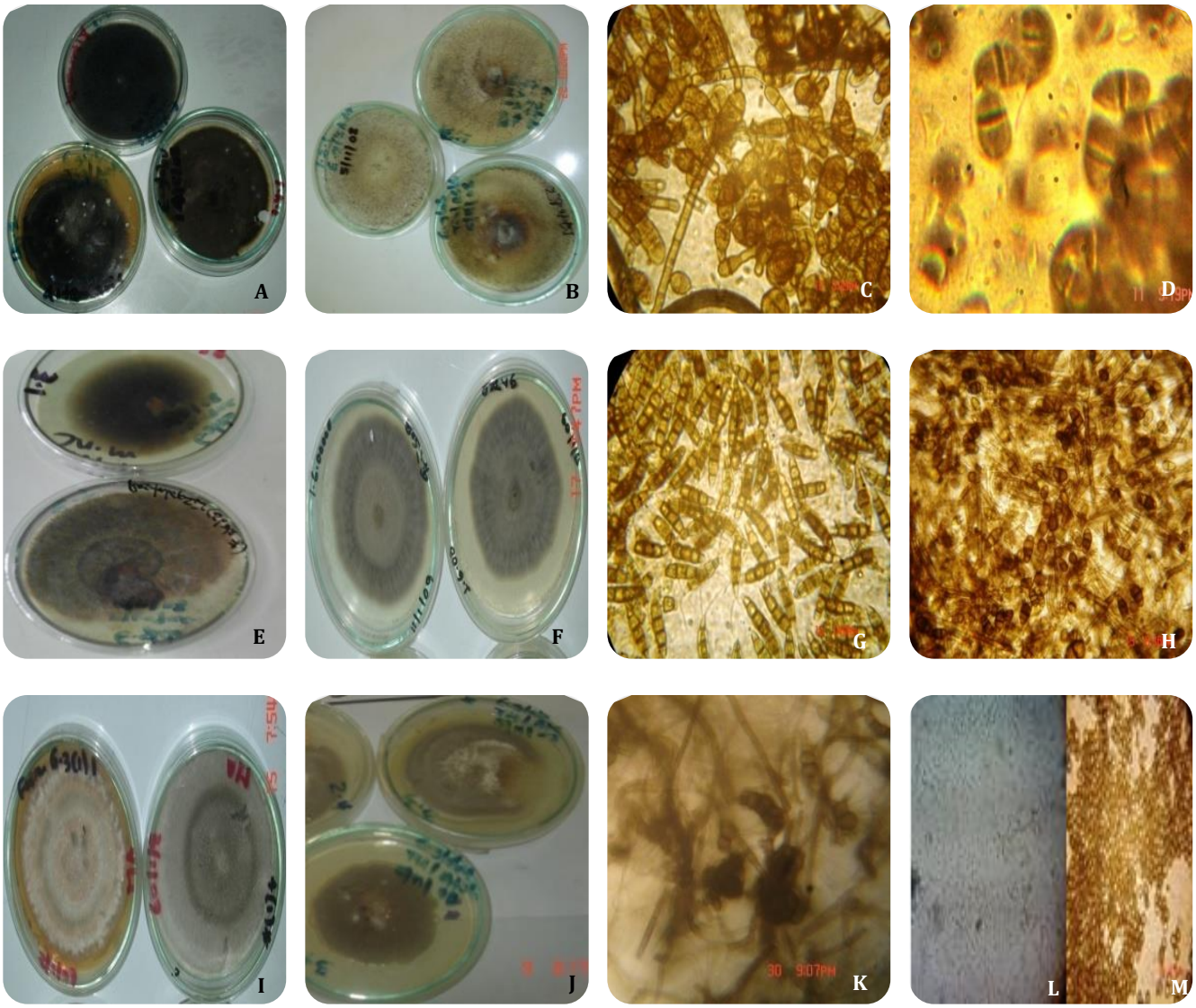

Figure 2. Macroscopic and microscopic morphologies of fungal isolates from stems and leaves showing: *Alternaria* (A, G), *Dioplodia*(B, H), *Pestalotiopsis* (C, I), *Curvularia* (D, J), *Phoma* (E, K, L) and *Penicillium* (F, M) species.

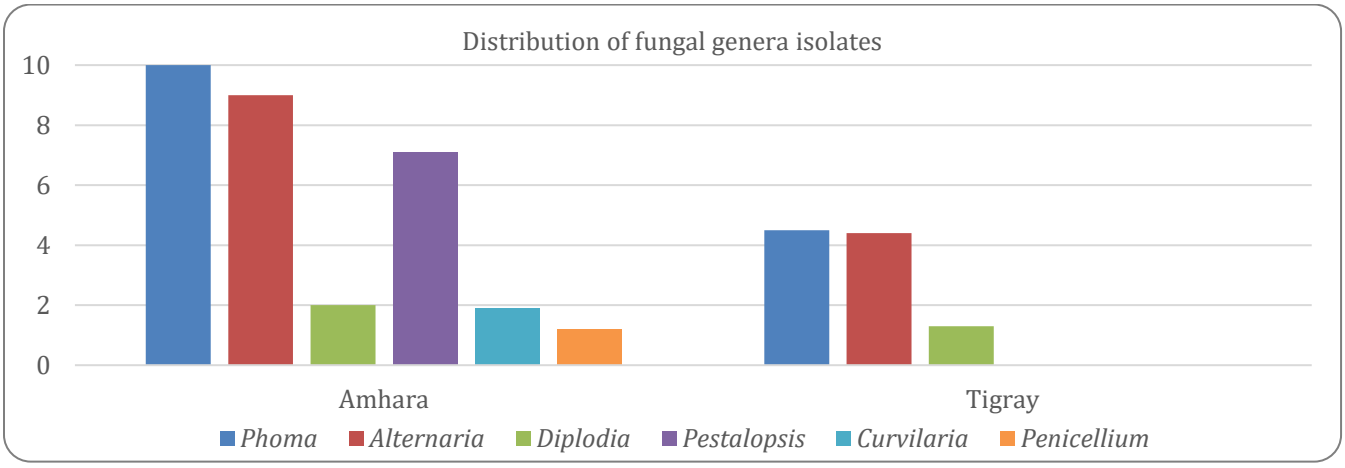

Figure 3. Distribution of fungal genera isolates in Amhara and Tigray regions of Ethiopia.

DISCUSSION

Morphological characterization result of fungal isolates indicates, six fungal genera belonging to *Alternaria*, *Diplodia*, *Pestalotiopsis*, *Curvularia*, *Phoma,* and *Penicillium* were identified. Tang (2003) stated that several genera of fungi, notably *Pestalotiopsis*, *Diplodia*, *Alternaria,* and *Phoma* are responsible for infections on plants; they are latent opportunists (endophytes) that colonize and cause asymptomatic infections in healthy plant tissues. In the current survey, it was found that *Phoma* and *Alternaria* were the most abundant. They were found in 29out of 42 fungal isolates studied. According to the findings of Zimowska (2011) Fungi of the genus, *Phoma* are at present cosmopolitan in respect of geography consisting of a large number of species in varied ecological niches with the majority are pathogenic species known infecting plant that is economically important. Boerema *et al*. (2004) also indicated that these species are found in association with symptoms of blight, leaf spots; fruit rot and stem canker throughout the world. The study of Aveskamp *et al*. (2008) also strengthen this idea in that, *Phoma* diseases are most prevalent in cool, wet weather, light and frequent rains, fog or heavy dews, high humidity, and crowded or shady plantings with infection occurring any time from June to August following temporary periods of cool, wet weather. Regarding *Alternaria* and *Pestalotiopsis* species, Fernández *et al*. (2015) reported that they are aerial plant pathogens, infecting plant tissues usually facilitated by injuries and simultaneous isolation of the two species from symptomatic leaves, branches, and a fruit is very common. According to Keith *et al.* (2006), different species of *Pestalopsis* caused leaf spots, needle blight, tip blight, and a gray blight on a range of hardy ornamentals plants. *Diplodia* species are widely distributed opportunistic pathogens of conifers like *Pinus* spp. worldwide (Bihon *et al*., 2011; Hanso and Drenkhan, 2009). *Curvularia*spp.is among fungal plant pathogens that cause leaf spot diseases associated with dark brown pinpoints in most parts of the leaves forming dark brown lesions surrounded by yellowish halos, which finally became diffused leaf blight (Sunpapao *et al*.,2014). *Penicillium* is among fast-colonizing opportunistic fungi characterized by the fast establishment on wounds or susceptible regions of leaf, stem and other parts of plants (Tang, 2003). According to Ezekiel *et al.* (2008), *Aspergillus* species*, Penicillium* species*, Curvularia* species *Alternaria* species*,* and *Phoma* species are seed fungal pathogens*,* this indicates they can possibly cause diseases at nursery level and further at plantation site if there is no appropriate fungal pathogen management system for seeds at storage and seedling at nursery level. This study helped to have overall pictures of fungal diversity on plantation forests dominated by Eucalyptus species in Amhara and Tigray regions, North and North-West Ethiopia with associated symptoms. In the study areas, exotic host species are found more vulnerable to fungal pathogens than indigenous one. Leaf spot and stem canker are the most prevalent symptoms on plantations. *Phoma lingam, Phoma glomerata, Alternaria Alternata* and genera of *Curvularia, Pestalotiopsis, and Penicillium* including *Diplodia* were found to be the cause for symptoms observed on plantations. Among the isolates *Phoma* and *Alternaria* genera were observed the most prevalent, showing a majority of leaf spot and stem canker symptoms on plantation trees were due to these fungal pathogens, which is also an indication for leaf spot diseases to be due to co-infection of more than one fungal species on a host.

CONCLUSION

The results of Survey of Plantation Forests Plantation trees in commercial stands, farmlands, and woodlots of Amhara and Tigray regions indicates existing diversities of fungal species which can possibly cause diseases when there is prolonged environmental stress. Based on the findings of the study *Phoma lingam, Phoma glomerata, Alternaria alternata,* genera of *Curvularia, Pestalotiopsis, Penicillium,* and *Diplodia* were found to be the cause for diseases symptoms observed on the tree plantations. *Phoma* and *Alternaria* species were the most prevalent isolates, showing a majority of symptoms observed on plantations were due to their co-infection.

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