

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

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ABSTRACT

Plantation forests are cultivated forest ecosystems established, by planting introduced or indigenous species. Forest plantations in Ethiopia are mainly exotic genera of Eucalyptus, Cupressus, Casuarina, Pinus, and native Juniperus species. Plantations species have suffered in varying degrees of attack by disease causing agents, particularly the Amhara region is among the regions, with plantation forests that have in recent years been subjected to attack by diseases in Ethiopia. Plantation trees in commercial stands, farm lands and woodlots were surveyed in 20 selected areas of Amhara and Tigray, from May to June 2016 for disease symptoms. Tree samples showing clear disease symptoms, were collected and processed for identification. Leaf blight, leaf spot, tip blight and stem canker were the most common symptoms appeared during the survey period. A total of 42 isolates of fungi colonies were identified from 20 localities. A morphological feature of fungal isolates reveals, six fungal genera belonging to Alternaria, Diplodia, Pestalotiopsis, Curvularia, Phoma and Penicillium were the cause of the disease symptoms. 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 Penicillium1 (2.4%). The result of the study shows leaf spot and stem canker is the most prevalent symptoms. Phoma lingam, Phoma glomerata, Alternaria Alternata, genera of Curvularia, Pestalotiopsis, and Penicillium including Diplodia were found to be, the cause of diseases of the trees. Pharma and Alternaria species were the most prevalent isolates, showing the majority of symptoms observed on plantations, were due to their co-infection. The pathogenicity result confirms, fungal isolates were the cause of the diseases. Fungal pathogens observed in this study need attentions, as they can cause severe damages to plantations during favorable environmental conditions. Most of the isolates were found seed pathogens, seeds need to be tested for quarantine fungi and sterilized before using for seedling, plantation at the nursery sites has to be assessed for disease symptoms and treated well before distribution to reforestation sites help prevent and control the spread of these fungal pathogens.

KEYWORDS: Leaf Spot, Phoma Soups, Pestalotiopsis Spp, A. Alternate, Curvularia Spp

INTRODUCTION

Plantation forests are cultivated forest ecosystems, established by planting or seeding, using introduced or indigenous species in the process of a forestation and reforestation, primarily for wood biomass production, it covers about 5% of the global forest with Pinus and Eucalyptus species, the most commonly used plantations in the world (FAO, 2001).

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

Forest plantations in Ethiopia are mainly exotic genera of Eucalyptus, Cupressus, Casuarina, Pinus, and native Juniperus species (Yirdaw, 2002). Majority of forest plantation is located in Oromia, Amhara, the Southern Nations and Nationalities Peoples Region, and Tigray region (Bekele, 2011). In the Amhara region, high rate of plantations, compared to other farm enterprises, leads to the extent of conversion croplands and grazing fields, to Eucalyptus woodlots (genre et al., 2011). 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).

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 a wide variety of pathogens that cause plant diseases, fungi are important stresses that affect tree health. The magnitude of fungal diversity is estimated to be 1.5 million species, with only 5% of species been 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, agro forestry 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 facilitate 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 in recent years, subjected to attack by diseases. A leaf disease of Eucalyptus, cypress, shoot blight and dieback of Pine is a common disease in the region. Most of the diseases observed are expected to be from nursery sites (Gbadegesin et al., 1999).

Fungi species can associate a host plant as full-blown, 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 disease 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 raised, shiny, and coal black forming ragged holes with marker light and dark concentric zones (Figure 1).

The objective of this study was to investigate the diversity and distribution of pathogenic fungi associated with plantation forest trees 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 spans 12 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, farm lands 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 the severity of the problems in the areas as pointed out by the Regional, Zonal and District Forestry and Crop 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). Micro morphological descriptions for 42 fungal spp 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 the reverse of inoculated Petri plates were assessed according to the colour 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).

Pathogen city Trials

Pathogen city of fungal isolates were tested using seedlings of the respective host plants raised in the nursery sites in 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. Pathogen city of the fungal isolates to the respective hosts was tested by using 3-6 month-old seedlings and spraying conidial suspension (2×10^3 conidia/ml of sterile water) of the respective fungus. Three to five seedlings of the respective host plants were inoculated and the inoculated seedlings were incubated in a humidity chamber (>90% are. h, 26 ± 2 0C with 12h dark and light period). Disease symptoms developed in the host plants were recorded and fungus was re-isolated from the diseased host tissues and pathogen city 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 SPSS version 16.0 and SAS procedure (SAS, Ver. 9).

RESULTS

Symptom

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 highland 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 shops on Eucalyptus and Cody spas' and tip blight symptoms, similar to infection by the Diplodia spp (Fig 1), on Juniperus procera and Cupressus lusitanica of plantation forests, in Amhara and Tigray regions of Ethiopia, from May to June, 2016.

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 grayer coloration while stem canker is observed with elongated, grayish, hell brown to dark brown border between discolored tissue lesions typically on Eucalyptus globulus.



Figure 1: Plantation Samples Showing Stems Canker, Leaves Spot and Tip Blight Symptoms

Morphological Characterization of Fungal Isolates

A total of 42 isolates of fungi colonies were identified from 20 localities (Table 1). Colony textures of the isolates on PDA were appeared as impressed with sparse aerial mycelium, flocculose 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 (Fig 2).

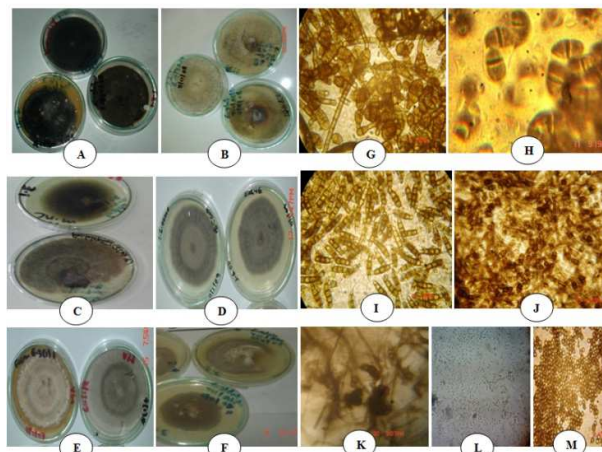


Figure 2: .Macroscopic and Microscopic Morphologies of Fungal Isolates from Stems and Leaves Showing: Alternaria (A, G), Diplodia (B, H), Pestalopsis (C, I),Curvularia (D,J), Phoma (E, K, L) and Penicillum (F, M) Species

A morphological feature of fungal isolates reveals, six fungal genera belonging to Alternaria, Diplodia, Pestalotiopsis, Curvularia, Phoma and Penicillium were the cause of the disease symptom observed in the study areas (Figure 2). Among the isolates, 14 (33.3%) were Alternaria species, identified as A. Alternate, 15 (37.7%) were Phoma species, identified as Phoma lingam and Phoma glomerata while remaining 13 isolates belonging to genera Diplodia 3 (7.2%), Pestalopsis 7 (16.7%), Curvularia 2 (4.7%) and Penicillium1 (2.4%).

Table 1: Fungi Isolated from Diseased Tree Plantations in Ethiopia

Region And Zones	Locality	Altitude (Masl)	Tree Species	Genera, Species Of Fungi Isolated
South Gondar, Amhara	Lomi Dur	2705	E. globulus	Phoma glomerata, Alternaria Alternata
South Gonder Amglobulus	Atrik	2975	C. lusitanica	Penicillium, Diplodia spps
South Wollo, Amhara	Jeme	2359	E. globulus	A. Alternata, P.glomerata, Pestalotiopsis spps
South globulusmhara	Harbu	1495	Cordia Africana	A. Alternate, Phoma lingam
South Wolo	Lomiye	1812	C. Africans	A. Alternate, P. lingam
South Gondar, Amhara	Gelaye	3095	E. globulus	A. Alternate, P. lingam, Pestalotiopsis shops
South Goglobulusara	Atrik	2975	E. globulus	A. Alternata, P.glomerata, Pestalotiopsis spps
West Gglobulusmhara	Yinesa	1824	E. camaldulensis	Pestalotiopsis spp
Bahir Dar Zuria, Amhara	Kimbeba	1945	E. camaldulensis	A. Alternate, 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
South East Tigray	May Keyhe	2312	E. globulus	P. lingam, A. Alternata
South Gondar, Amhara	Kosso Mado	3206	E. globulus	P. lingam, Pestalotiopsis spp

Table 1 Condt				
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

Results of the study also shows, 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 (Fig 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.

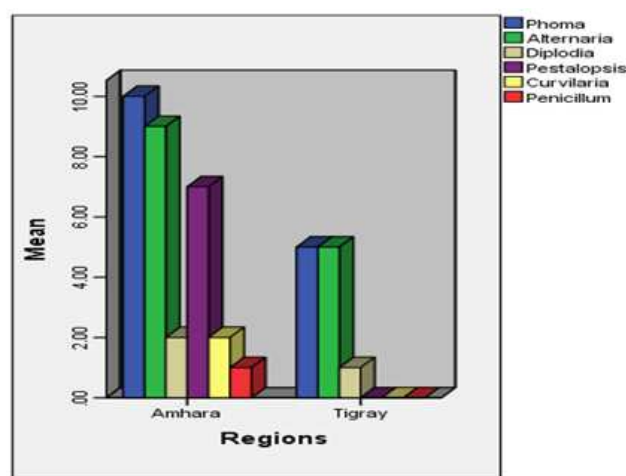


Figure 3: Distribution of Fungal Genera in Amhara and Tigray Regions of Ethiopia

DISCUSSIONS

Morphological characterization result of fungal isolates indicates, six fungal genera belonging to Alternaria, Diplodia, Pestalotiopsis, Curvularia, Phoma and Penicillium were identified. 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 (Tang,2003). In the current survey, it was found that Phoma and Alternaria were the most abundant. They were found in 29 out of 42 isolates studied. Fungi of the genus Phoma are at present cosmopolitan in respect of geography consisting of a large number of species in varied ecological niches. From among 3000 taxa described so far, majority are pathogenic species known infecting plant that are economically important (Zimowska, 2011). It is frequently found in association with symptoms of blight, leaf spots; fruit rot and stem canker throughout the world (Boerema et al. 2004). The disease is most prevalent in cool, wet weather, light and frequent rains, fog or heavy dews, high humidity, and crowded or shady plantings but infection can occur any time from June to August following temporary periods of cool, wet weather (Aveskamp et al., 2008). Alternaria and Pestalotiopsis species have been reported aerial plant pathogens, infecting plant tissues usually facilitated by injuries,

simultaneous isolation of the two species from symptomatic leaves, branches and a fruit is common. (Fernández et al., 2015). According to Keith et al. (2006) different species of *Pestalopsis* caused leaf spots, needle blight, tip blight, and 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 are among fungal plant pathogens that cause leaf spot diseases associated with dark brown pin points 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 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.

CONCLUSIONS

This study helped to have over all 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 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.

RECOMMENDATIONS

Fungal pathogens observed in the study need due attentions as they can cause severe damages to plantations when there is favorable environmental conditions. Most fungal isolates were observed seed pathogens, seeds need to be tested for quarantine fungi and sterilized before using for seedling, further plantation at the nursery sites has to be assessed for diseases symptoms and treated well before delivery to reforestation sites help prevent and control spread of these fungal pathogens. Similar study needs to be carried out in the other regions for overall picture of pathogenic fungi diversity on plantation forests in the country help to formulate critical measures for prevention and control of diseases at national level.

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