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Fungi Associated with Decline of Kikar (*Acacia nilotica*) and Red River Gum (*Eucalyptus camaldulensis*) in Faisalabad

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***Abstract*—**During this research, a comprehensive survey of tree growing areas of Faisalabad district of Pakistan was conducted to observe the symptoms, spectrum, occurrence and severity of *A. nilotica* and *E. camaldulensis* decline. Objective of current research was to investigate specific fungal pathogens involved in decline of *A. nilotica* and *E. camaldulensis*. For this purpose, infected roots, bark, neck portion, stem, branches, leaves and infected soils were collected to identify associated fungi. Potato dextrose agar (PDA) and Czepak dox agar media were used for isolations. Identification of isolated fungi was done microscopically and different fungi were identified. During survey of urban locations of Faisalabad, disease incidence on Kikar and Eucalyptus was recorded as 3.9-7.9% and 2.6-7.1% respectively. Survey of Agroforest zones of Faisalabad revealed decline incidence on kikar 7.5% from Sargodha road while on Satiana and Jhang road it was not planted. In eucalyptus trees, 4%, 8% and 0% disease incidence was observed on Jhang road, Sargodha road and Satiana road respectively. The maximum fungus isolated from the kikar tree was *Drechslera australiensis* (5.00%) from the stem part. *Aspergillus flavus* also gave the maximum value of (3.05%) from the bark. *Alternaria alternata* gave the maximum value of (2.05%) from leaves. *Rhizopus* and *Mucor* spp. were recorded minimum as compared to the *Drechslera, Alternaria* and *Aspergillus*. The maximum fungus isolated from the Eucalyptus tree was *Armillaria luteobubalina* (5.00%) from the stem part. The other fungi isolated were *Macrophamina phaseolina* and *A. niger*.

***Keywords*—**Decline, frequency of mycoflora, *A. nilotica, E. camaldulensis, Drechslera australiensis*, *Armillaria luteobubalina*.

I. Introduction

KIKAR *(Acacia nilotica)* is commonly found in the plain of Punjab and due to its medicinal and economical values cultivated as an important agroforestry tree. *A. nilotica* is a medium size evergreen tree with bright yellow flowers which grow in clusters. The tree is native to Egypt, India, Pakistan, Arabia and different parts of Africa. *A. nilotica* has been reported to have arabinose, catechol, galactan, galactose, saponin and tannin. Seeds of *A. nilotica* have crude protein 18.6%, ether extract 4.4%, fiber 10.1%, nitrogen extract 16.2%, ash 5.7%, phosphorus 0.29% and calcium 0.90% [13].

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Red river gum (*E. camaldulensis*) also called as sufaida is widely grown in different regions of Pakistan and it is the third largest agroforestry tree species after *Dalbergia sissoo* and *A. nilotica*. It can tolerate pH up to 8.8-11 in loamy, clayey and sandy soils; thus the tree is suitable to all soil types and climates of Pakistan. It does not compete with other field crops because of its conical crown, less shade, pronounced tap root system, and never becomes a host for any major pest. As a timber, it is straight, tall with medium to high density, and its growth is quite desirable to farmers; as it is grown for short rotation. *A. luteobubalina* is a local root rot fungus that is a cause of decline and dieback of eucalyptus in inner Victoria and south-western Australia [8], [12]. Decline in these forest trees has been reported in almost all forest growing areas. The common symptoms produced during decline are stunted growth, shortened internodes, root necrosis, early leaf fall, change in color and ultimately loss of foliage, drying of twigs and branches. Generally, it starts from the upper side of crown and disturbs the tree growth which leads to death^. Fungal pathogens are major cause of decline. Different fungi have been isolated form declined trees including *Fusarium solani, F. moniliforme, F. equiseti, F. oxysporum, F. semitectum, Rhizoctonia solani, Alternaria alternate, Curvularia lunata, A. niger* and few species of *Penicillium* [2]-[4], [11], [14].

From the previous researches given above, it was observed that Kikar and Eucalyptus have severely been victimized by decline syndrome. These are multipurpose tree species, and hence selected for present studies to investigate the decline syndrome problem. During this study, a survey of urban and agro-forest growing regions in Faisalabad was conducted to determine the extent of decline. Furthermore, diseased samples from decline trees (collected during this survey) were studied to examine the fungal pathogens associated with decline. The present studies were therefore designed with following objectives:

- To examine the extent of decline incidence on two major forest tree species in Faisalabad
- To identify different fungi associated with these declining trees

II. Material and Methods

A. Extent of Damage in Agro-Forest Plantations

A survey of Kikar and Eucalyptus trees growing areas of Faisalabad including Jinnah Garden, Getwala Park, PFRI (Punjab Forestry Research Institute) and University of

Agriculture, Faisalabad was conducted. Survey of different agro-forest zones of Faisalabad including Jhang road, Sargodha road and Satiana road was also done to collect the data of disease incidence. Kikar and Eucalyptus showing specific signs of decline were considered as affected. Diseased samples included roots, bark from neck portion, twigs, leaves, stem and branches were collected for the isolation of the associated fungal pathogens. Equipment included Cutters and Knives, Axes, Polythene bags, Markers for labeling, Spirit and automizer were used for the collection of diseased samples.

B. Isolation and Identification of Associated Mycoflora

Cutters and knives used for taking samples were sterilized with 2.5% sodium hypochlorite; it is surface disinfectant and used to avoid the contamination. Roots were digged up to 30-45 cm, whereas aerial parts were harvested depending upon the size of tree and diseased portion. Samples were collected in sterilized polythene bags, labeled, and stored in a refrigerator at 4 °C until processed [10].

Stored samples (infected roots, bark, neck portion, stem, branches and leaves) were cut into small pieces and treated with 1% $HgCl_2$ (mercuric chloride) solution and then with distilled water. After this, samples were inoculated in laminar air flow cabinet on media with the help of forecep.

C. Media Used for Isolation of Mycoflora

1. PDA Media Recipe

- Distilled water = 1 L
- Potato = 200 g
- Dextrose = 20 g
- Agar = 20 g

Procedure of PDA Media

Sliced and un-peeled potatoes were boiled in distilled sterilized water for 30 minutes. The material was then filtered through cheese cloth and effluent was saved. Glucose (dextrose) and agar was added to potato effluent. This material was heated gently to mix all the ingredients. The medium was then placed in autoclave for 15 minutes at 121 °C and 15 lbs for sterilization. After that, the prepared PDA was poured in petri-plates in laminar flow hood and was allowed to solidify.

2. Czapek Dox Agar Recipe

Czapek dox broth is a semi-synthetic medium used for general cultivation of fungi.

- Sucrose = 30 gm/Litre
- Sodium nitrate = 3 gm/Litre
- Dipotassium phosphate = 1 gm/Litre
- Magnesium sulphate = 0.5 gm/Litre
- Potassium chloride = 0.5 gm/Litre
- Ferrous sulphate = 0.01 gm/Litre
- Final pH = 7+- 0.2

Procedure of Czapek Dox Agar Media

All the ingredients were mixed in 1000 ml distilled sterilized water and were heated to dissolve the medium completely. The medium was then placed in autoclave at 15 lbs pressure and 121 °C temperature for 15 minutes for sterilization. After that, the medium was poured in petri-dishes in laminar flow hood and were allowed to solidify.

D. Characterization of Associated Mycoflora

Samples collected from different locations were placed on petri-dishes having Czapek dox agar medium after giving two washings in 1% $HgCl_2$. When the fungal colonies were formed on culture medium, the fungi were recognized through compound microscope on the basis of colony characteristics and conidial morphology using standard keys [5], [7], [9]. Organisms were maintained on PDA medium for further studies.

E. Analysis of Data

The data were analyzed using statistical software package, Meet Minitab 15 by Minitab Inc. U.S.A. Analysis of variance (ANOVA), and comparison of means were made through least significant difference test (LSD at $P<0.05$).

III. Results

A. Survey of Urban Locations in Faisalabad

The data on disease incidence were recorded at various locations of Faisalabad on Kikar and Eucalyptus and it was 3.9-7.9% and 2.6-7.1%, respectively. In Faisalabad, organic matter was 0.69%-0.80% while water holding capacity was 37-39%. Soil structure was loamy in all the areas under study on different locations (Jinnah Garden, Getwala Park, PFRI and U.A.F). pH was 8.1-8.5% in almost all the areas. The percentage of disease incidence from each of the above site is given separately in Table I.

TABLE I
PERCENTAGE OF DISEASE INCIDENCE AT URBAN AREAS OF FAISALABAD

Sites	Kiakar %age	Red River Gum %age
Jinnah garden	7.9	1.9
Gatwala Park	3.9	2.8
PFRI	7.4	7.1
U.A.F	5.9	2.6

B. Survey of Different Agroforest Zones of Faisalabad

Jhang road, Sargodha road and Satiana road were surveyed to record the decline incidence of selected tree species. Kikar and Eucalyptus plantations were present on road sides. Decline incidence of kikar was found to be 7.5% on Sargodha road while on Satiana and Jhang road it was not planted. Disease incidence in Eucalyptus was observed 4% on Jhang road, 8% on Sargodha road and 0% on Satiana road. Maximum Eucalyptus incidence was observed on Sargodha road while minimum was observed on Satiana road (Table II).

TABLE II
PERCENTAGE OF DISEASE INCIDENCE AT DIFFERENT AGROFOREST ZONES OF FAISALABAD

Sites	Kiakar %age	Red River Gum %age
Jhang Road	Not present	3.9
Sargodha Raoad	7.5	7.9
Satiana Road	Not present	0.0

C. Isolation of Fungi from Kikar Plant Samples

The frequencies of fungi recorded from different plant parts were statistically different from each other. The maximum (5.00%) fungi were isolated from the stem followed by roots (2.07%) and the minimum (1.05%) from the twigs (Table III).

From Kikar trees maximum fungus isolated was *D. australiensis* (5.00%) from the stem part. From roots, twigs, leaves and bark, *D. australiensis* was recorded 2.07%, 1.05%, 1.16% and 1.91% respectively. *A. flavus* also gave the maximum value of 3.05% from the bark while it gave the minimum value of 0.40% from the root portion. From the stem, twigs and leaves of kikar, *A. flavus* was recorded 0.03%, 0.06% and 0.17%, respectively. *A. alternata* gave the maximum value of 2.05% from leaves and minimum (0.09%) from the stem. *A. alternata* was recorded 1.06% from the roots, 1.33% from the twigs and 0.75% from bark. *Rhizopus* and *Mucor* spp. were recorded minimum (Table III).

TABLE III
AVERAGE FREQUENCY OF FUNGI ISOLATED FROM KIKAR SAMPLES

Fungi associated	Stem	Roots	Twigs	Leaves	Bark
***Rhizopus* spp.**	2.1 b	1.33 b	0.50 d	0.25 d	0.34 d
D. austaliensis	5.00 a	2.07 a	1.05 b	1.16 b	1.91 b
A. alternata	0.09 c	1.06 c	1.33 a	2.05 a	0.75 c
A. flavus	0.03 e	0.40 e	0.06 c	0.17 e	3.05 a
***Mucor* spp.**	0.05 d	0.75 d	0.01 e	0.33 c	0.01 e

LSD value 2.099

TABLE IV
AVERAGE FREQUENCY OF FUNGI ISOLATED FROM RED RIVER GUM SAMPLES

Fungi associated	Stem	Roots	Twigs	Leaves	Bark
M. phaesolina	1.00 b	0.08 c	0.75 a	0.21 b	0.01 c
A. luteobubalina	5.00 a	1.93 a	0.03 b	0.66 a	1.85 b
A. niger	0.03 c	0.40 b	0.01 c	0.17 c	2.45 a

LSD value 2.167

D. Isolation of Fungi from Eucalyptus Plant Samples

ANOVA indicates highly significant difference among the frequencies of fungi isolated from different plant parts collected from different locations. The frequencies of fungi recorded from different plant parts were statistically different from each other.

A. luteobubalina was the maximum fungus isolated from the stem parts of Eucalyptus trees (5.00%). *A. luteobubalina* was recorded 0.66% from leaves and 1.85% from the bark portion of collected samples. *A. niger* was recorded 0.03% from stem, 0.04% from roots, 0.01% from twigs, 0.17% from leaves and 2.45% from the bark of Eucalyptus. Maximum value of *A. niger* was recorded from the bark portion while minimum value was recorded from the twigs. *M. phaesolina* fungus was recorded minimum from the collected samples (Table IV).

IV. DISCUSSION

The survey was carried out in different areas of Faisalabad city, Pakistan. These different sites such as road sides, canal sides and agro-forest plantations were selected in order to estimate the extent of damage on each site, separately. Maximum disease incidence 7.5 and 7.9% on Kikar was recorded from Sargodha road and Jinnah garden, respectively. While, on Eucalyptus, maximum disease incidences 7.1 and 7.9 were recorded from PFRI and Sargodha Road, respectively. The prevalence of disease was not noticed to be influenced by age of trees. The symptoms were more severe under the areas of water stress and water logging conditions as compared to the regular watered trees. Similar studies were conducted in Nepal on selected sites such as river side, degraded forest, natural stands canal side, road side and enrichment plantations and almost similar results were obtained [1].

During the isolation studies from Kikar samples collected from different sites, a number of fungi viz., *D. australiensis, Rhizopus* spp., *A. alternata, A. flavus* and *Mucar* spp., were isolated from roots, bark, twigs, leaves and stem with varying percentage from each site. *Drechslera* spp., *A. flavus* and *A. alternata* were relatively more abundant in the collected samples of Kikar from Sargodha road. The natural regeneration/recruitment of *A. nilotica* subsp. *indica* is quite low in India [6]. During four years of field study (2005-2008), it was recorded that population of *A. nilotica* subsp. *hemispherica* is decreasing with an alarming rate.

Decline and dieback of Eucalyptus is a complex disorder involving exotic pathogen, various native organisms, climatic factors and agricultural or urban pollution. Among these, any biotic or abiotic factor cannot be excluded. The retrospective investigations on Eucalyptus trees in eastern and temperate Australia have shown that these factors are vital for Eucalyptus decline and dieback [12].

During our studies of isolation of Eucalyptus samples collected from UAF (Nursery of Forestry), Punjab Forestry Research Institute (PFRI), Gatwala Park, Jinnah Garden, Jhang road and Sargodha road, *M. phaesolina, A. luteobubalina* and *A. niger* were isolated. *A. luteobubalina* was mostly found from root samples whereas *M. phaesolina* was relatively more abundant in twig samples. *A. luteobubalina* is a root rot fungus that was involved in decline and dieback of Eucalyptus in central Victoria and south-western Australia. Previous researches have shown that logging increases the level of *A. luteobubalina* inoculum by increasing the food supply provided by dead stumps and roots, and is assumed that this triggers disease in healthy trees [8], [12].

Intensive survey based on aboveground symptoms of *Armillaria* root disease underestimated true levels of disease by at least 20% and sometimes by up to 40% in high-quality karri regrowth stands. The results challenge the reliability of surveys based on above-ground disease symptoms. While most disease was established within the subdominant stratum, a very high proportion (30–60%) of the dominant trees was also infected. Within the study area, 15 distinct genotypes of *A. luteobubalina* were identified. Individual genotypes existed as clones; with 2–3 clones per hectare. These factors needed to be considered in stand management planning and yield predictions. A broader study, including lower-quality sites, is

needed to determine whether these findings apply to all types of karri regrowth [12].

V. Conclusion

From the current research, it is evident that mycoflora including *Drechslera australiensis*, *Aspergillus flavus*, *Rhizopus and Alternaria alternata* are contributing towards decline of these trees. In forest nursery establishment healthy and disease free seeds should be used for healthy future generations.

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