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QUALITATIVE AND QUANTITATIVE ASSESSMENT OF SEED MYCOFLORA DIVERSITY IN *EUCALYPTUS TERETICORNIS* AND *E. CAMALDULENSIS*

R. Y. Giri[1], T. Dinaker Chinna[2], T. Shankar[3], S. M. Reddy[4] and Cherkupally Rama Raju[5]*

[1]Associate Professor of Botany, Government Degree College, Hayath Nagar, Dist: Ranga Reddy, Osmania University, Telangana, India.

[2]Assistant Professor of Botany, Government Arts & Science College (A) Kamareddy, Dist: Kamareddy, Telangana University.

[3]Associate Professor of Botany, Government Degree College, Sircilla, Dist: Rajanna Sircilla, Satavahana University, Telangana.

[4]Professor (Retd), Department of Botany, Kakatiya University, Warangal, Telangana, India.

[5*]Assistant Professor of Botany, Government Degree College, Badangpet, Dist: Ranga Reddy, Osmania University, Telangana, India.

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***Corresponding Author**
Cherkupally Rama Raju
Assistant Professor of Botany, Government Degree College, Badangpet, Dist: Ranga Reddy, Osmania University, Telangana, India.

ABSTRACT

The present study investigates the seed mycoflora diversity of two important agroforestry species, *Eucalyptus tereticornis* and *Eucalyptus camaldulensis*. Using blotter and dilution plate techniques, a comprehensive assessment was conducted to identify the seed-borne fungi and their incidence, frequency, and abundance. A total of 31 fungal species belonging to 19 genera were isolated from *E. tereticornis* seeds, while 21 species representing 13 genera were detected from *E. camaldulensis*. *Aspergillus flavus* and *A. niger* were the most dominant fungi across both species, showing high incidence and frequency. Pathogenic fungi such as *Fusarium* spp., *Sclerotium rolfsii*, *Colletotrichum gloeosporioides*, and *Curvularia lunata* were also detected, highlighting potential risks to seedling health. The study underlines the variation in fungal detection based on isolation techniques and emphasizes the importance of both methods for a

complete evaluation of seed mycoflora. The findings provide critical insights into the role of seed-borne fungi in forest tree health, germination, and disease transmission, especially in seed storage and nursery practices.

KEYWORDS: Seed mycoflora, fungal diversity, blotter technique, dilution plate method, seed pathology, agroforestry.

1. INTRODUCTION

Seeds are of paramount importance for the propagation of plants. A sustained supply of good quality seeds is necessary for afforestation and reforestation programmes. The requirement of seeds has increased tremendously in recent years in view of a large number of tree species being grown under social forestry, agro forestry, and farm forestry to meet the dwindling forests and increasing needs of the ever-growing human population. The seeds are also in great demand for their valuable oils and other products by seed-based industry under the programmes of utilization of forest minor products.

Seeds of forest trees, like those of agricultural and horticultural crops, carry a wide variety of microorganisms like bacteria, fungi, and viruses either externally or internally. Seeds may be attacked by pathogens while still on the plants in the field, during storage, and subsequent handling prior to sowing. Deterioration of seeds during storage is governed by the moisture content of the seed and storage conditions such as temperature and humidity. Unhealthy seeds have the potential of introducing dangerous diseases to new areas. The introduction of seeds from foreign countries is another source of introducing pathogens. The fungi which are merely associated and had no role in seed health in their native country may turn out to be virulent in an introduced country (Wingfield et al., 2001).

Almost all groups of fungi have been reported to be associated with seeds of forest tree species. Seed-borne infections are known to behave differently depending on the nature of the pathogen and on sowing and growing conditions in the field. In severely infested seeds, the fungi attack the embryo and such seeds fail to germinate, resulting in a huge loss in terms of crop productivity. On the other hand, the slightly infested seeds germinate and the pathogen also gets activated along with the seed and may cause seedling disease. Unhealthy seeds are fraught with the risks of introducing new pathogens and pests with potential disaster to crops.

The importance of seed pathology of forest trees has increased in view of the rising demand for quality seeds. The study in seed pathology will help in checking the loss of seeds in storage, seed transmissible diseases in the nursery both at pre-emergence and post-emergence stages, and the spread of diseases from the nursery to the plantation. Realizing this factor, extensive researches have been undertaken on seed mycoflora of different crop plants in relation to crop diseases (Ilyas & Manohara, 2023; Panwar; Gupta & Kumar, 2020; Narayanasamy, 2017). However, very limited information is available on seed-borne fungi of forest plants. Therefore, an opportunity was seized to investigate the seed mycoflora of two species of Eucalyptus in relation to diseases affecting this important agro forestry plant.

2. MATERIALS AND METHODS

A periodical survey of *Eucalyptus* plantations in Telangana state was made and the diseased material (seedlings/leaves/ stem/root of eucalyptus) were collected separately in a sterilized polythene bags. The severity of disease was assessed at the field level. The symptoms were recorded carefully, while the foliage and infected material were green and fresh. Completely infected material was avoided for isolation as they contained several secondary pathogens. These diseased specimens were collected from different nurseries and plantations labeled and brought to the laboratory for isolation of the pathogen. If fruit- bodies were present on the infected portions, slides were prepared by cutting of very thin sections as well as scraping of infected portions and the pathogen was identified.

The isolations were carried out on Potato Dextrose Agar- PDA (Potatos 250g; Dextrose 20g; Agar agar 20g; and distilled water 1 litre) or Asthana and Hawkers medium A (Glucose 5g; KNO3 3.5g; KH2PO4 1.75g; MgSO4 0.75g; Agar agar 15g; and distilled water 1 litre). Isolations were made from the junction of healthy and infected regions after surface sterilization by chlorox solution or 90% alcohol. Surface sterilized bits were kept on moist blotters which were kept in the petri dishes or humid chambers. Some were placed on the surface of PDA or Asthana and Hawkers medium A. These slants were incubated at 25±2°C. After two or three days the hyphal threads coming out of the diseased tissue were picked up and transferred to another fresh slant. The fungi were identified with the help of standard monographs (Barnett and Hunter, 1972; Sutton, 1980). Mycoflora associated with different samples of *Eucalyptus tereticornis* and *E. camaldulensis* was studied.

1. Blotter technique

Three layers of blotter (size equivalent to the petri dish) soaked in sterilized distilled water were kept in sterilized petri plates. Excess water was drained and a fixed number of seeds (20-25) were placed at a equidistance in each petri plate. They were incubated at 27±2°C for 7 days. Nearly 300- 400 seeds were employed for qualitative and quantitative estimation of spermosphere. The fungi appearing on the seeds were isolated and identified with the help of standard monographs (Jameel Akhtar, 2014; Benny, 2008) after recording morphological features. Percentage of incidence, frequency and abundance of individual fungus was calculated by the following formulae.

$$\text{Percentage of incidence} = \frac{\text{Number of seeds on which a species appeared}}{\text{Ttal number of seeds appeared}} \times 100$$

$$\text{Percentage of frequency} = \frac{\text{Number of observations in which a species appeared}}{\text{Total number of observations}} \times 100$$

$$\text{Percentage of abundance} = \frac{\text{Total Number of colonies of a species in all observations}}{\text{Total Number of colonies in all observations}} \times 100$$

2. Dilution plate technique (Waksman, 1922)

Ten grams of each sample was taken in 250ml conical flask containing 100ml of sterilized water and subjected to horizontal shaking for 30 minutes. From this solution, dilutions were made as desired. 0.5ml of this solution was poured aseptically into sterilized petri plates and sterilized and cooled Asthana and Hawker's medium A (Glucose - 5gr; KNO3 - 3.5gr; KH2PO4 1.75gr; MgSO4 - 0.75gr; Agar agar - 15gr; distilled water 1 litre) was poured by making gentle rotational movements of petri plates so as to ensure uniform spreading of the sample. Petri plates thus prepared were incubated in an inverted position at 27±2°C. To suppress the bacterial growth and to restrict the fungal colonies streptomycin and rose bengal was added. The fungi thus developed in the petri plates were isolated and identified from third day onwards. The percentage of incidence of individual fungus was calculated by the formula.

$$\text{Percentage of incidence} = \frac{\text{Number of colonies of a species in all plates}}{\text{Total number of colonies of all species in all plates}} \times 100$$

The percentage of frequency and abundance of individual fungus was calculated as described earlier.

3. RESULTS AND DISCUSSION

The seed mycoflora of *Eucalyptus tereticornis* was investigated, and the results are précised in Table 1. It is evident that the seeds harbored a variety of fungi, including both saprophytes and pathogens. The mycoflora was qualitatively and quantitatively diverse. In total, 31 species representing 19 genera were isolated. *Aspergillus flavus*, followed by *A. niger*, were dominant, while *A. ustus* and *Fusarium solani* showed the least percentage of incidence. The incidence of *Alternaria alternata, Aspergillus fumigatus, F. chlamydosporum, Rhizopus stolonifer*, and *Lasiodiplodia theobromae* was also considerable. Other fungi showed varying percentages of incidence. *Aspergillus flavus, A. niger,* and *Chaetomium globosum* recorded the highest frequency, while *Phoma sp., F. solani, F. chlamydosporum, Curvularia pallescens,* and *A. japonicus* had the least. *R. stolonifer, C. lunata*, and *A. alternata* had moderate frequency. In terms of abundance, *A. flavus* and *A. niger* were highest, while *Phoma sp., F. solani, C. pallescens*, and *A. japonicus* were least.

The dilution plate method revealed 24 fungal species representing 16 genera. *A. flavus* and *A. niger* had the highest incidence, followed by *Trichoderma viride* and *A. fumigatus*. The incidence of *F. solani, A. alternata, Cylindrocladium clavatum, F. oxysporum, Scytalidium* state of *Hendersonula toruloides*, and *L. theobromae* was lowest. Frequency-wise, *A. flavus, A. niger, A. ustus,* and *A. japonicus* ranked highest, followed by *C. lunata*, *Penicillium* sp., and *A. fumigatus. A. flavus* and *A. niger* showed highest abundance, while *Alternaria sp., F. solani, Pestalotiopsis versicolor,* and *Phoma sp*. had the lowest.

There is a difference in mycoflora based on the isolation method used. *Cladosporium cladosporioides, Humicola grisea,* and *T. viride* were not detected by the blotter technique, while *Myrothethecium roridum* is not detected by dilution plate technique. With few exceptions, a correlation was noted among incidence, frequency, and abundance. Differences in mycoflora composition may be attributed to spore characteristics or the nutritional environment (Silva & Asiegbu, 2023).

The mycoflora of *E. camaldulensis* significantly differed from *E. tereticornis*. A total of 25 species representing 18 genera were recorded. A. niger, followed by *A. flavus*, showed the highest incidence, while *Sclerotium rolfsii, T. roseum, L. theobromae*, and *D. spicifer* had the lowest. *A. flavus, A. niger, R. stolonifer*, and *C. gloeosporioides* showed highest frequency and abundance, while *S. rolfsii* and *L. theobromae* showed the least.

Some pathogenic fungi such as *S. rolfsii, Myrothecium roridum, P. versicolor, L. theobromae, Fusarium sp., D. spicifer, C. lunata*, and *A. alternata* occurred with notable incidence and may be associated with poor health in forest species. *C. clavatum*, a serious pathogen of *Eucalyptus*, and *C. gloeosporioides*, responsible for anthracnose, were detected in both species but by different techniques. *C. lunata, F. oxysporum*, and *L. theobromae* are also known to cause diseases in Eucalyptus. Hence, spermosphere fungi may influence disease dynamics, with *Fusarium* sp., *S. rolfsii, C. clavatum*, and *A. alternata* known to cause seedling blight and being common components.

The seed mycoflora study uncovered a rich diversity and high concentration of fungi associated with both *Eucalyptus tereticornis* and *E. camaldulensis*. Among them, *Aspergillus* was most dominant, particularly A. flavus, with 23.75% incidence and 23.77% abundance in *E. tereticornis*, and even higher in *E. camaldulensis* at 27.33% and 26.77%, respectively. *A. niger* and *A. fumigatus* were also significant, and *A. flavus* and *A. niger* showed 100% detection in dilution plating.

Genera like *Alternaria, Cladosporium, Curvularia*, and *Penicillium* were more often isolated using the blotter method, indicating possible surface-level associations. *Chaetomium globosum*, *Colletotrichum gloeosporioides*, and *Fusarium spp*. were exclusive to *E. camaldulensis*, possibly due to host specificity or environmental factors. The dilution plate method favored high-abundance, fast-growing fungi like *Aspergillus* and *Trichoderma*, while the blotter method captured slow-growing or less abundant fungi. *E. camaldulensis* showed slightly higher fungal load and diversity, possibly influenced by seed traits or collection environment.

The dominance of *Aspergillus flavus* poses ecological and pathological concerns, particularly due to its aflatoxin production, which can affect seed viability and germination (Mangwende et al., 2021). Its consistent detection aligns with studies on other tree seeds where *Aspergillus* spp. causes pre- or post-emergence damping-off (Lacey, 1995). This calls for improved seed health strategies, especially for *E. camaldulensis*, which may be more susceptible (Poole et al., 2017).

The detection of other genera, particularly by the blotter method, supports their identification as superficial colonizers or weak pathogens (Martín et al., 2022). The exclusive detection of some fungi in *E. camaldulensis* highlights the role of environmental variation or host

specificity (Yamazaki et al., 2009). The need for both methods is emphasized, as each detects unique fungal profiles: dilution plating for sporulating fungi and blotter for broad-spectrum slow growers (Batzer et al., 2024; Cleary et al., 2019).

Table 1: Seed mycoflora of species of *Eucalyptus*.

1. Name of the fungus	*E. tereticornis*						*E.canaidulencis*					
	Blotter technique			Dilution plate method			Blotter technique			Dilution plate method		
	Incidence	Abundance	Frequency	Incidence	Abundance	Frequency	Incidence	Abundance	Frequency	Incidence	Abundance	Frequency
Alternaria alternata	4.37	5.73	66.66	3.82	1.66	33.33	3.88	4.18	40.00	6.45	2.75	40.0
Alternaria	2.5	1.63	33.33	3.27	0.66	16.66	-	-	-	-	-	-
Aspergillus flavus	12.08	23.77	100.0	23.75	21.33	100.0	9.66	24.26	100.0	27.33	26.77	100.0
Aspergillus fumigatus	7.91	5.19	33.33	10.79	7.00	66.66	3.33	1.67	20.0	8.74	5.51	60
Aspergillus japonicus	2.5	0.82	16.66	4.08	3.33	56.56	4.15	2.09	20	6.12	1.18	20
Aspergillus niger	11.94	23.49	100.0	20.44	18.00	100.0	13.33	33.47	100.0	2991	2952	100
Aspergillus terreus	2.49	1.63	33.33	7.85	2.66	50	3.33	1.67	40.0	11.43	4.33	40.0
Aspergillus casteus	1.66	1.09	33.33	4.66	3.33	66.66	2.50	1.25	40.0	4.76	1.18	26.0
Chaetomium globosum	2.91	5.73	100	6.14	4	50	2.91	2.92	60	-	-	-
Cladosporium cladosporioides	-	-	-	5.94	3.66	50	-	-	-	5.4	4.33	80
Colletotrichum gloeosporioides	2.77	2.73	50	-	-	-	-	-	-	4.76	0.78	20
Curvularia lunata	2.91	3.82	65.66	6.61	4.33	83.33	4.16	2.92	40	5.63	2.75	40
Curvularia pallescens	2.5	0.81	16.66	-	-	-	-	-	-	-	-	-
Cylindrocladium clavatum	2.49	3.55	66.66	3.53	2.00	16.66	3.33	1.67	20.0	4.08	0.78	20
Drechslera spicifera	2.21	1.09	50	-	-	-	1.66	0.84	20	-	-	-
Fusarium clamydosporum	4.16	1.36	16.66	5.23	1	16.66	-	-	-	-	-	-
Fusarium equiseti	-	-	-	-	-	-	2.49	2.09	20	4.25	0.78	20
Fusarium moniliforme	-	-	-	-	-	-	2.91	2.92	40	4.76	1.18	20
Fusarium oxysporum	2.5	1.63	33.33	3.21	1.33	50	3.33	1.67	40	2.83	1.18	40
Fusarium solani	1.66	0.82	16.66	1.81	0.33	16.66	-	-	-	-	-	-
Scytalidium sp.	2.91	1.91	33.33	3.48	2	50	-	-	-	-	-	-
Hemicolum griseum	-	-	-	4.42	2.66	50	-	-	-	4.25	0.78	20
Lasiodiplodia theobromae	3.33	2.18	33.33	2.49	1	33.33	1.66	0.83	20	-	-	-
Penicillium sp.	2.21	2.18	50	8.02	5.33	66.66	4.16	1.67	40	8.76	5.51	60.0
Pestalotiopsis versicolor	2.08	1.36	33.33	4.1	0.66	16.66	2.5	1.25	20	-	-	-
Phoma sp.	2.5	0.82	16.66	3.63	0.66	16.66	-	-	-	-	-	-
Rhizopus stolonifer	4.33	7.1	83.33	6.83	3.00	33.33	3.74	5.86	60.0	12.76	2.38	20.0
clerotium rolfsii	-	-	-	-	-	-	1.66	0.83	20.0	4.21	1.57	40.0
Trichoderma viride	-	-	-	11.37	4.56	50	-	-	-	9.66	5.9	50
Trichoderma roseum	-	-	-	3.7	2.66	33.33	1.66	1.25	20	3.17	0.78	20
Myrothecium roridum	-	-	-	-	-	-	2.5	1.25	20	-	-	-

4. CONCLUSION

The qualitative and quantitative assessment of seed mycoflora in *Eucalyptus tereticornis* and *E. camaldulensis* revealed a rich diversity of associated fungi, with *Aspergillus flavus* and *A. niger* being the most dominant across both species. The study highlights a significant variation in fungal detection based on the method employed, with the blotter method revealing slow-growing and surface-associated fungi, and the dilution plate method capturing fast-growing and sporulating species. The presence of known pathogens such as *Fusarium* spp., *Curvularia lunata*, and *Colletotrichum gloeosporioides* indicates the potential for seed-borne disease transmission, which may compromise seedling emergence and plantation health. *E. camaldulensis* showed slightly higher fungal diversity and incidence, possibly due to differences in seed characteristics or environmental conditions at the time of collection. These results emphasize the need for regular seed health monitoring, improved storage practices, and preventive seed treatment protocols to safeguard forest nurseries and afforestation efforts. The findings also call for more extensive research in forest seed pathology in India, especially in view of increasing demands for disease-free, high-quality planting material in forestry and agroforestry systems.

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