



## Detection of Fungal Pathogens Associated with Forest Tree Seeds

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### ABSTRACT

Seeds are regarded as highly effective means for transporting plant pathogens over long distance. Compared to seed borne disease problems of agricultural crops, research has been limited on seed borne pathogens of forest tree species and its management practices. Various fungal species (*Alternaria*, *Botryodiplodia*, *Aspergillus*, *Trichoderma*, *Curvularia*, *Fusarium* etc.) are commonly associated with seeds of many forest tree species. Most of seed borne pathogens of trees affect nursery seedlings and reduces seed germination and seedling vigor, also cause deformation, decay or destroy viability of seeds. The present study was carried during 2012 and 2013 at Department Plant Pathology, ASPPE College of Horticulture and Forestry, N.A.U., Navsari to investigate the fungal microorganisms associated with forest tree seeds. Seeds from different forest trees like Teak (*Tectona grandis*), Subabul (*Leucaena leucocephala* (Lam.) de Wit.), Gulmohar (*Delonix regia* (Boj.) Raf.), Mangium (*Acacia mangium*), Ratangunj (*Adenanthera pavonina* Linn) and Garmalo (*Cassia fistula* Linn) were collected from ACHF College, NAU, Navsari. Two methods viz., standard blotter technique and agar plate method (ISTA) were employed for both sterilized and non-sterilized seeds. The results revealed that maximum number of microorganisms and their per cent incidence were higher in non-surface sterilized seeds (NS) than surface sterilized (SS). The microorganisms like, *Alternaria*, *Aspergillus* sp., *Fusarium* sp. and *Trichoderma* sp. were found to be most frequently occurring fungus. Moreover, the occurrence of different fungi was more on blotter method in all the tested seeds of forest trees.

### Keywords:

Pathogens, forest trees, seeds, standard blotter, agar plate method

### INTRODUCTION

Forest tree species are known to be attacked by various pathogens in forest nurseries, plantations and also in natural forests. The quality

of planted seeds has a critical influence on the ability of crops to become established and to realize their full potential yield. Seed borne diseases of forest plants are incited by different

causal agents such as fungi, bacteria and viruses. Among these, the impact of fungi is considerable and many of them are serious pathogens of maturing seeds and reduce the yield and impair the quality (Mohan et al. 2001). Other fungi include saprophytes and weak pathogens which lower the quality of seeds by causing discoloration and reducing germination (Anonymous 1993). The forest tree seeds are vehicles and victims of numerous pathogens. Seeds are regarded as highly effective means for transporting plant pathogens over long distance. Compared to seed borne disease problems of agricultural crops, research has been limited on seed borne pathogens of forest tree species and in the development of disease control practices. Various fungal species (*Alternaria*, *Botryodiplodia*, *Aspergillus*, *Trichoderma*, *Curvularia*, *Fusarium* etc.) are commonly associated with seeds of many forest tree species (Javaid et al. 2010). Most of seed borne pathogens of trees affect nursery seedlings and reduce seed germination and seedling vigor, also cause deformation, decay or destroy viability of seeds. The present study is carried out to investigate the fungal microorganisms associated with forest tree seeds.

#### MATERIALS AND METHODS

Seeds from different forest trees like (Teak) *Tectona grandis*, Subabul (*Leucaena leucocephala* (Lam.) de Wit.), Gulmohar (*Delonix regia* (Boj.) Raf.), Mangium (*Acacia mangium*), Ratangunj (*Adenanthera pavonina* Linn.) and Garmalo (*Cassia fistula* Linn.) were collected from ACHF College, NAU, Navsari. Two methods viz., standard blotter technique and agar plate method (ISTA) were employed for both sterilized and non-sterilized seeds. Seeds were surface

sterilized with 0.1% HgCl<sub>2</sub> or 2% sodium hypochlorite solution for two minutes followed by several washing with sterilized distilled water. In blotter method ten seeds of each forest tree species were placed on a sterilize filter paper bed in sterilized Petri plates. Distilled water was added to each Petri plates. In agar plate method ten surface sterilized seeds and then washing with the sterilized distilled water were placed on PDA medium in Petri dishes and incubated for 7 to 10 days. The two set ups were repeated for five times. The same method was followed for the non-sterilized seeds. Fungi found associated with seeds were carefully examined under microscope. Based on the count of different fungi per cent infection was calculated by using the following formula.

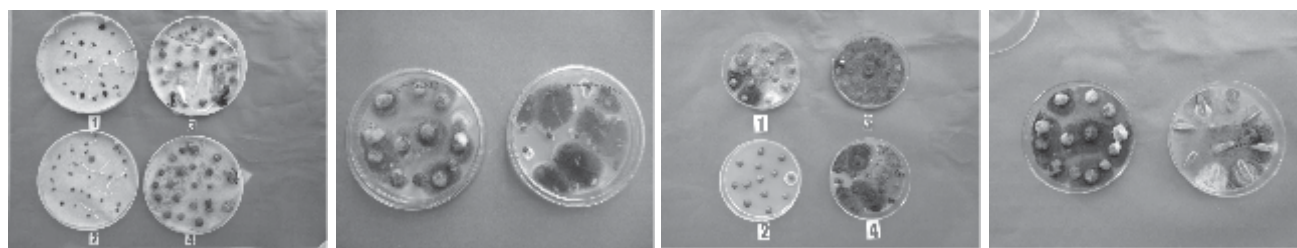
% infection of fungus =

$$\frac{\text{No. of seed associated with fungus}}{\text{X 100 Total no. of seeds observed}}$$

#### RESULTS AND DISCUSSION

**Teak:** Three years data are presented in table-1 indicated that total of six fungal genera viz., *Alternaria*, *Aspergillus*, *Fusarium*, *Trichoderma*, *Penicillium* and *Botryodiplodia* were recorded from the seeds of teak in blotter method. The data also revealed that, in case of sterilized seeds (SS) the fungi load was less as compared to non sterilized (NS) seeds. *Aspergillus* sp., *Fusarium* sp. and *Trichoderma* sp. were found most frequently. Highest occurrence of *Trichoderma* sp. was recorded (35.16%) in the year 2014 in non sterilized seeds. Moreover the occurrence of different fungi was more on blotter method in all the tested seeds of forest trees (Photo 1).

Three years pooled mean data revealed that in the blotter method *Trichoderma* sp. was found



**Photo:1.** Blotter and Agar plat method

most frequently occurring fungi in sterilized seeds (7.39%) and (26.39%) in SS and NS seeds of teak, respectively.

In agar plate method, *Aspergillus* sp., *Fusarium* sp. and *Trichoderma* sp. were recorded as most frequently occurring fungi in three years of experimentation. The three years pooled mean

indicated that *Fusarium* sp. was found with maximum occurrence (3.78% and 7.94%, respectively) in SS and NS seeds of teak (Table-1). The results of present investigation are in confirmation with those reported by Sharma and Mohan (1997).

Table 1. Micro organisms detected on seeds of *Tectona grandis* (Teak)

Microorganisms	Blotter method						Av. SS	Av. NS
	2012-13		2013-14		2014-15			
	SS	NS	SS	NS	SS	NS		
<i>Alternaria</i> sp.	0.00	2.00	0.00	0.00	0.00	0.00	0.00	0.67
<i>Aspergillus</i> sp.	2.00	16.00	8.00	32.00	8.33	32.50	6.11	26.83
<i>Fusarium</i> sp.	4.00	20.00	8.00	16.00	7.66	15.50	6.55	17.17
<i>Trichoderma</i> sp.	4.00	12.00	8.00	32.00	10.16	35.16	7.39	26.39
<i>Botryodiplodia</i> sp.	0.00	2.00	0.00	4.00	4.33	5.33	1.44	3.78
<i>Bacteria</i> sp.	3.00	9.00	0.00	0.00	0.00	0.00	1.00	3.00
Microorganisms	Agar plate method						Av. SS	Av. NS
	2012-13		2013-14		2014-15			
	SS	NS	SS	NS	SS	NS		
<i>Alternaria</i> sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Aspergillus</i> sp.	2.00	4.00	4.00	8.00	4.33	6.83	3.44	6.28
<i>Fusarium</i> sp.	3.00	10.00	4.00	8.00	4.33	5.83	3.78	7.94
<i>Trichoderma</i> sp.	1.00	3.00	0.00	4.00	4.33	7.16	1.78	4.72
<i>Botryodiplodia</i> sp.	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.33
<i>Bacteria</i> sp.	0.00	5.00	0.00	0.00	0.00	0.00	0.00	1.67

**Subabul:** Three years individual and pooled data are presented in the table-2 showed that *Alternaria*, *Aspergillus*, *Fusarium*, *Trichoderma* and *Botryodiplodia* were recorded from the seeds of subabul in blotter method. In the first and second year, *Fusarium* sp. and *Aspergillus* sp. were found with maximum occurrence in both SS and NS seeds of subabul. While in the third year, *Trichoderma* sp. was recorded with maximum occurrence 1.33 and 2.16, respectively.

Three years pooled mean data revealed that in the blotter method, *Fusarium* sp. was found most frequently occurring fungus i.e. (6.72%) and (13.83%) in SS and NS seeds of subabul, respectively.

In agar plate method, *Fusarium* sp. and *Aspergillus* sp. were recorded as most frequently

occurring fungi in the first and second years of experimentation. However, in the third year, *Aspergillus* sp. was recorded with maximum occurrence.

Three years pooled mean indicated that *Aspergillus* sp. was found with maximum occurrence (4.28% and 5.72%), respectively in SS and NS seeds of subabul (Table 2).

**Gulmohar:** In blotter plate method the data presented in table-3 revealed that, *Alternaria*, *Aspergillus*, *Fusarium*, *Trichoderma*, and *Penicillium* were recorded from seeds of gulmohar. Among them *Fusarium* and *Aspergillus* were observed with maximum occurrence in both SS and NS treated seeds. Whereas in the third year only *Aspergillus* sp. was recorded with maximum occurrence.

**Table 2.** Micro organisms detected on seeds of *Leucaena leucocephala* (Subabul)

Microorganisms	Blotter method						Av. SS	Av.NS
	2012 -13		2013 -14		2014 -15			
	SS	NS	SS	NS	SS	NS		
<i>Alternaria</i> sp.	1.00	3.00	0.00	0.00	0.00	0.00	0.33	1.00
<i>Aspergillus</i> sp.	4.00	12.00	8.00	12.00	7.16	12.50	6.39	12.17
<i>Fusarium</i> sp.	5.00	14.00	8.00	16.00	7.16	11.50	6.72	13.83
<i>Trichoderma</i> sp.	2.00	9.00	4.00	12.00	7.33	12.60	4.44	11.20
<i>Botryodiplodia</i> sp.	0.00	0.00	0.00	0.00	1.33	0.00	0.44	0.00
Microorganisms	Agar plate method						Av. SS	Av.NS
	2012 -13		2013 -14		2014 -15			
	SS	NS	SS	NS	SS	NS		
<i>Alternaria</i> sp.	2.00	8.00	0.00	0.00	0.00	0.00	0.67	2.67
<i>Aspergillus</i> sp.	8.00	11.00	4.00	4.00	0.83	2.16	4.28	5.72
<i>Fusarium</i> sp.	9.00	12.00	0.00	0.00	1.33	1.00	3.44	4.33
<i>Trichoderma</i> sp.	4.00	8.00	0.00	0.00	1.33	2.16	1.78	3.39
<i>Botryodiplodia</i> sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

The three years pooled mean data revealed that in the blotter method *Fusarium* and *Aspergillus* were found most frequently occurring fungus (8.11%) and (16.17%) in SS and NS seeds of gulmohar, respectively.

In agar plate method, *Alternaria*, *Fusarium* sp. and *Aspergillus* sp. were recorded as most frequently occurring fungus in all the three years of experimentation.

The three years pooled mean indicated that *Alternaria* and *Aspergillus* sp. were found with maximum occurrence (3.39% and 8.72%),

respectively in SS and NS seeds of gulmohar (Table 3).

**Mangium:** The three years individual and pooled data are presented in the table 4. *Alternaria*, *Aspergillus*, *Fusarium*, *Trichoderma* and *botryodiplodia* were recorded from the seeds of mangium in blotter method. In the first year, *Fusarium* sp. was found with maximum occurrence in both SS and NS seeds of mangium. Whereas in the second and third year *Trichoderma* sp. and *Fusarium* were recorded with maximum occurrence (Photo 2).

**Table 3.** Micro organisms detected on seeds of *Delonex regia* (Gulmohar)

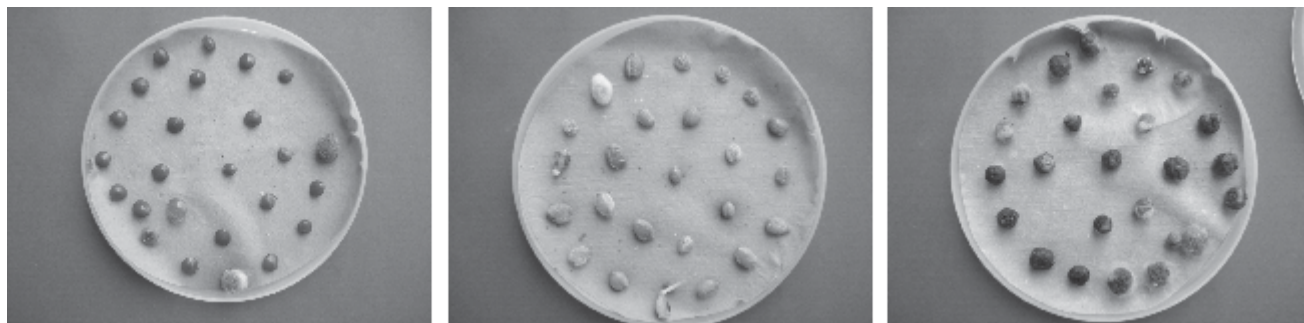
Microorganisms	Blotter method						Av. SS	Av.NS
	2012 -13		2013 -14		2014 -15			
	SS	NS	SS	NS	SS	NS		
<i>Alternaria</i> sp.	4.00	10.00	4.00	12.00	4.33	12.66	4.11	11.55
<i>Aspergillus</i> sp.	5.00	17.00	8.00	16.00	8.50	15.50	7.17	16.17
<i>Fusarium</i> sp.	8.00	12.00	8.00	12.00	8.33	12.16	8.11	12.05
<i>Trichoderma</i> sp.	4.00	8.00	4.00	8.00	4.00	8.33	4.00	8.11
<i>Penicillium</i> sp.	2.00	9.00	4.00	8.00	4.16	9.16	3.39	8.72
Microorganisms	Agar plate method						Av. SS	Av.NS
	2012 -13		2013 -14		2014 -15			
	SS	NS	SS	NS	SS	NS		
<i>Alternaria</i> sp.	2.00	9.00	4.00	4.00	4.16	4.00	3.39	5.67
<i>Aspergillus</i> sp.	1.00	10.00	0.00	8.00	5.50	8.16	2.17	8.72
<i>Fusarium</i> sp.	2.00	7.00	4.00	4.00	0.00	0.00	2.00	3.67
<i>Trichoderma</i> sp.	2.00	6.00	0.00	0.00	0.00	3.83	0.67	3.28
<i>Penicillium</i> sp.	0.00	4.00	0.00	4.00	0.00	3.33	0.00	3.78



The three years pooled mean data revealed that in the blotter method *Trichoderma* and *Fusarium* were found most frequently occurring fungus (8.67%) and (20.61%) in SS and NS seeds of mangium, respectively.

In agar plate method, *Fusarium* sp. and *Trichoderma* were recorded as most frequently occurring fungus in all the three years of experimentation.

The three years pooled mean indicated that



**Photo 2.** Blotter method

*Fusarium* and *Trichoderma* sp. were found with maximum occurrence (8.17% and 16.86%), respectively in SS and NS seeds of mangium (Table 4).

**Ratangunj:** The three years individual and pooled data are presented in the table 5. *Alternaria*, *Aspergillus*, *Fusarium*, *Trichoderma* and

*Rhizopus* were recorded from the seeds of ratangunj in blotter method. In the first year, *Aspergillus* sp. was found with maximum occurrence in both SS and NS treated seeds of ratangunj. Whereas in the second and third year *Trichoderma* sp. was recorded with maximum occurrence.

**Table 4.** Micro organisms detected on seeds of *Acacia mangium* (Mangium)

Microorganisms	Blotter method						Av. SS	Av. NS
	2012-13		2013-14		2014-15			
	SS	NS	SS	NS	SS	NS		
<i>Alternaria</i> sp.	9.00	13.00	4.00	8.00	4.33	8.33	5.78	9.78
<i>Aspergillus</i> sp.	10.00	12.00	4.00	8.00	4.33	8.83	6.11	9.61
<i>Fusarium</i> sp.	15.00	21.00	4.00	20.00	3.83	20.83	7.61	20.61
<i>Trichoderma</i> sp.	10.00	19.00	8.00	12.00	8.00	12.50	8.67	14.50
<i>Botryodiplodia</i> sp.	0.00	2.00	0.00	0.00	0.00	0.00	0.00	0.67
Microorganisms	Agar plate method						Av. SS	Av. NS
	2012-13		2013-14		2014-15			
	SS	NS	SS	NS	SS	NS		
<i>Alternaria</i> sp.	4.00	11.00	4.00	12.00	3.83	11.83	3.94	11.61
<i>Aspergillus</i> sp.	4.00	9.00	4.00	8.00	4.00	6.75	4.00	7.92
<i>Fusarium</i> sp.	9.00	13.00	8.00	12.00	7.50	12.00	8.17	12.33
<i>Trichoderma</i> sp.	8.00	12.00	8.00	20.00	7.16	18.58	7.72	16.86
<i>Botryodiplodia</i> sp.	0.00	4.00	0.00	0.00	0.00	0.00	0.00	1.33

The three year pooled mean data revealed that in the blotter method *Aspergillus* and *Trichoderma* were found most frequently occurring fungus (7.17%) and (17.22%) in SS and NS seeds of ratangunj, respectively.

In agar plate method, *Aspergillus*, *Fusarium* sp and *Trichoderma* were recorded as most

frequently occurring fungus in all the three years of experimentation.

The three years pooled mean indicated that *Fusarium* and *Trichoderma* sp. were found with maximum occurrence (8.03% and 12.78%), respectively in SS and NS seeds of ratangunj (Table 5).

**Garmalo:** The three years individual and pooled data are presented in the table 6. *Alternaria*, *Aspergillus*, *Fusarium*, *Trichoderma* and *Botryodiplodia* were recorded from the seeds of

garmalo in blotter method. In all the three years of experimentation, *Trichoderma* sp. was found with maximum occurrence in both SS and NS treated seeds of garmalo.

**Table 5.** Micro organisms detected on seeds of *Adenanthera pavonina* (Ratangunj)

Microorganisms	Blotter method						Av. SS	Av.NS
	2012 - 13		2013 - 14		2014 - 15			
	SS	NS	SS	NS	SS	NS		
<i>Alternaria</i> sp.	10.00	17.00	0.00	0.00	0.00	0.00	3.33	5.67
<i>Aspergillus</i> sp.	13.00	24.00	4.00	8.00	4.50	8.00	7.17	13.33
<i>Fusarium</i> sp.	11.00	18.00	4.00	12.00	4.66	12.83	6.55	14.28
<i>Trichoderma</i> sp.	9.00	15.00	8.00	16.00	4.00	20.66	7.00	17.22
<i>Rhizopus</i> sp.	1.00	4.00	0.00	4.00	7.25	4.66	2.75	4.22

Microorganisms	Agar plate method						Av. SS	Av.NS
	2012 - 13		2013 - 14		2014 - 15			
	SS	NS	SS	NS	SS	NS		
<i>Alternaria</i> sp.	0.00	9.00	0.00	0.00	0.00	0.00	0.00	3.00
<i>Aspergillus</i> sp.	10.00	14.00	4.00	12.00	4.41	12.16	6.14	12.72
<i>Fusarium</i> sp.	8.00	12.00	8.00	8.00	8.08	7.42	8.03	9.14
<i>Trichoderma</i> sp.	9.00	14.00	4.00	12.00	4.33	12.33	5.78	12.78
<i>Rhizopus</i> sp.	0.00	1.00	0.00	0.00	0.00	2.50	0.00	1.17

The three year pooled mean data revealed that in the blotter method *Trichoderma* was found most frequently occurring fungus (9.14%) and (24.33%) in SS and NS seeds of garmalo, respectively.

In agar plate method, *Fusarium* sp. and *Trichoderma* were recorded as most frequently occurring fungus in all the three years of experimentation.

The three years pooled mean indicated that, *Trichoderma* sp. was found with maximum

occurrence (5.03% and 14.50%), respectively in SS and NS seeds of garmalo (Table 6) (Photo 3 and 4).

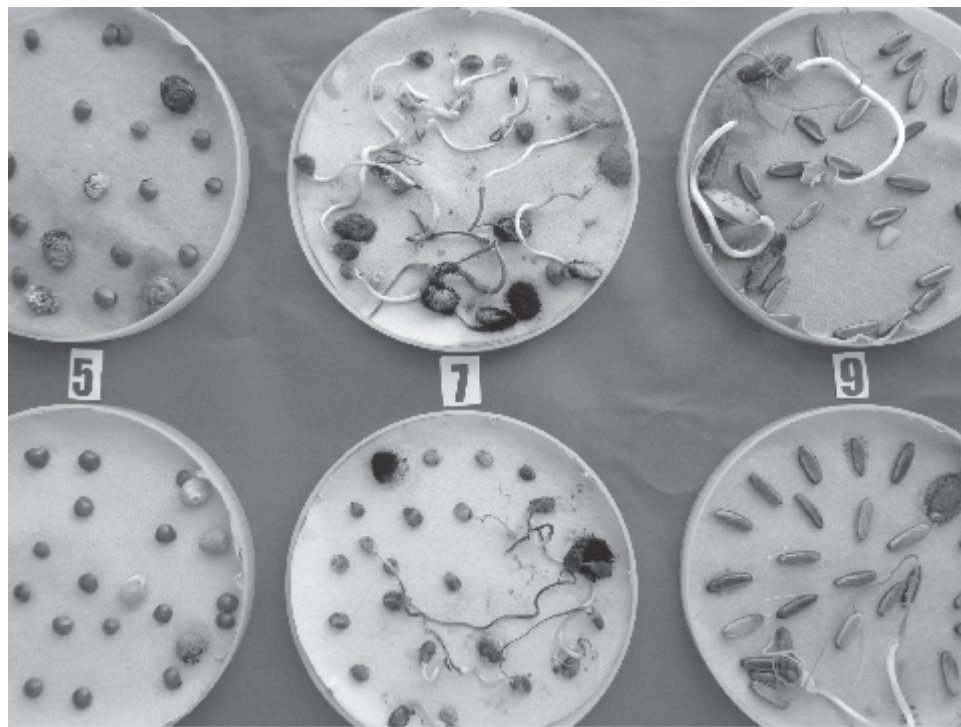
In past, More than fifty seed borne pathogens are associated with seeds of different forest trees. Important seed borne fungal diseases are caused by species of *Alternaria*, *Aspergillus*, *Botrytis*, *Cephalosporium*, *Chaetomium*, *Cladosporium*, *Colletotrichum*, *Curvularia*, *Fusarium*, *Penicillium*, *Phoma* and *Phomopsis* of different forest trees (Anderson 1986).

**Table 6.** Micro organisms detected on seeds of *Cassia fistula* (Garmalo)

Microorganisms	Blotter method						Av. SS	Av.NS
	2012-13		2013-14		2014-15			
	SS	NS	SS	NS	SS	NS		
<i>Alternaria</i> sp.	0.00	0.00	0.00	4.00	1.91	4.25	0.64	2.75
<i>Aspergillus</i> sp.	0.00	18.00	0.00	16.00	2.16	16.66	0.72	16.89
<i>Fusarium</i> sp.	2.00	21.00	8.00	20.00	7.50	15.75	5.83	18.92
<i>Trichoderma</i> sp.	4.00	26.00	12.00	24.00	11.41	23.00	9.14	24.33
<i>Botyodiplodia</i> sp.	0.00	5.00	0.00	0.00	0.00	0.00	0.00	1.67
<i>Curvularia</i> sp.	0.00	3.00	0.00	0.00	0.00	0.00	0.00	1.00

Microorganisms	Agar plate method						Av. SS	Av.NS
	2012-13		2013-14		2014-15			
	SS	NS	SS	NS	SS	NS		
<i>Alternaria</i> sp.	0.00	1.00	0.00	4.00	0.00	4.16	0.00	3.05
<i>Aspergillus</i> sp.	2.00	11.00	4.00	12.00	3.83	12.66	3.28	11.89
<i>Fusarium</i> sp.	3.00	12.00	4.00	12.00	4.00	12.58	3.67	12.19
<i>Trichoderma</i> sp.	7.00	11.00	4.00	16.00	4.08	16.50	5.03	14.50
<i>Botyodiplodia</i> sp.	0.00	2.00	0.00	0.00	0.00	0.00	0.00	0.67
<i>Curvularia</i> sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

**Photo 3.** Blotter method**Photo 4.** Pure isolated culture of *Aspergillus* sp., *Fusarium* sp. and *Trichoderma* sp.

**CONCLUSION**

The maximum number of microorganisms and their per cent incidence were higher in non-surface sterilized seeds (NS) than surface sterilized (SS). The fungi like, *Alternaria*, *Aspergillus* sp. *Fusarium* sp. *Trichoderma* sp. were found to be most frequently occurring fungus. Moreover the occurrence of different fungi was more on blotter method in all the tested seeds of forest trees.

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