STUDIES ON SEED PATHOLOGY AND SEEDLING DISEASES OF SOME IMPORTANT INDIGENOUS TREE SPECIES OF KERALA

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By

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DEDICATED
TO THE FOND MEMORY OF
MY BELOVED FATHER

DECLARATION

I hereby declare that this thesis entitled **'STUDIES ON SEED PATHOLOGY AND SEEDLING DISEASES OF SOME IMPORTANT INDIGENOUS TREE SPECIES OF KERALA'** has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

Peechi 680 653
February, 1993

(M.I. MOHAMED ALI)

CERTIFICATE

This is to certify that the thesis entitled "**STUDIES ON SEED PATHOLOGY AND SEEDLING DISEASES OF SOME IMPORTANT INDIGENOUS TREE SPECIES OF KERALA**" is the bonafide record of the work carried out by **Mr. M.I. MOHAMED ALI**, under my guidance and supervision and that no part thereof has been presented for the award of any other Degree.

15.2.93

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CONTENTS

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1. INTRODUCTION

1.INTRODUCTION

Kerala State, situated between latitudes 8° 18'and 12°48' North and longitudes 74°52' and 77°22' East, is bounded in the east by the Western Ghats and in the west by the Arabian Sea. Kerala has an area of 38355 km^2 which is about 1.03% of the geographical area of India. The State has typical tropical climate with average annual rain fall varying from 750-4000 mm, mean monthly temperature ranging from 17.5 to 35°C and mean relative humidity varying from 75-90 percent. The effective forest area of the State is about 9400 Km^2 which is 1.26% of the total forest area of India and 24% of geographical area of the State (KSLUB, 1989). The forests of Kerala are distributed in three distinct altitudinal zones. The lower zone consists of undulating narrow belt up to ca. 100 m m.s.l. comprising mainly bamboo forests and tropical moist deciduous forests. The intermediate zone reaching up to 1500 m consists of tropical semi-evergreen and wet-evergreen forests. The high altitude zone comprise of subclimax Savanna of high ranges and most of the non-refractory areas of these grasslands have recently been afforested with eucalypts, wattles, tropical pines, etc. The forest areas in Kerala can be broadly categorised as follows:

1. Tropical moist evergreen and semi-evergreen - 3450 km^2

2. Tropical moist deciduous forests - 4010 "

3. Tropical dry deciduous forests - 100

4. Grasslands - 134

5. Forest plantations - 1604

From the above figure it is obvious that nearly 42.9% of the total forest area of Kerala is covered by tropical moist deciduous forests (KSLUB, 1989) which is the abode of many valuable indigenous tree species of vast plantation potential. Plantation forestry is mainly focused on monoculture of a few species, mainly aimed at producing wood for industrial purposes and nearly 85% of the total forest plantations comprise teak, eucalypts and other soft wood and miscellaneous tree species (Evans, 1982). The percentage of area under plantations in Kerala has increased steadily from 3.62 in 1956-57 to 13.73 during 1987-88. The main tree crops grown in plantation are teak (50.97%), eucalypts (22.05%), soft wood (6.9%),and others (20.05%) which include cashew, wattle, *Ailanthus, Albizia,* balsa, bamboo, reed, etc. (Jayaraman and Krishnankutty, 1990).

In fact one of the main reasons why exotic species were preferred for afforestation programmes was the availability of adequate research and experimental background to grow them

successfully. Lack of such documented information in indigenous species is one of the major constraints for their less utilisation in plantation programmes. An indigenous species is one that grows naturally in the country concerned though not necessarily in all parts and not certainly suited to all sites. In addition, indigenous species have some important biological advantages over exotics such as i. they are well adapted to local environment; ii. even in monoculture they are more suited ecologically; and iii. their timber uses are well known to local consumers.

In India, particularly in Kerala, no organised effort has been made so far to evaluate the plantation potential of indigenous tree species, teak (*Tectona grandis* L.f.) being an exception. However, before evaluating their plantation potential, it is essential to understand their pathological problems, as high rainfall combined with tropical warm-humid climate provide conducive environment for the development and spread of several diseases especially when the host is also susceptible. Exotic tree species such as eucalypts are prone to serious diseases such as *Cylindrocladium* leaf blight and pink disease caused by *Corticium salmonicolor* Berk. & Br., which drastically affected the productivity of plantations

(Sharma *et al.*, 1985; Sharma and Mohanan, 1991). However indigenous species raised in monoculture are seldom affected seriously with indigenous pathogens. But when they suffer, they suffer seriously, the known example being that of rubber in Brazil where a native leaf blight pathogen *Dothidiella ulei* P.Henn. wiped out rubber plantations. So, before taking up any plantation programme with indigenous tree species it is imperative to have a good knowledge of pests and disease problems of tree species selected for such programmes.

In forestry, availability of seeds is an important factor for raising planting stock on a large scale Germinability of seeds greatly depends upon seed health and storage conditions. Like seeds of agricultural and horticultural crops, seeds of tree species are also liable to be affected by micro-organisms during storage (Mittal, 1979; Sharma and Mohanan, 1980; Mittal and Sharma, 1981; Mittal, 1986; Vijayan, 1988). The various ways by which seed-borne micro-organisms affect the quality of seeds are i. reduced germination; ii. introduction of seed-borne diseases into newly sown crops/areas and iii. reduction of viability during storage. Moreover, availability of healthy stock of seedlings is intrinsic for raising plantations and to meet this, control of

nursery diseases by appropriate chemicals is of prime importance. However, in the case of indigenous tree species, information on microbial deterioration of seeds, seedling diseases and their control measures is either completely absent or meagre.

With a view to select appropriate tree species with fewer manageable disease problem(s) for use in future plantation programmes, seed pathology, seedling diseases and their management were studied, in respect of four indigenous tree species such as,

1. *Albizia odoratissima* (L.f.) Benth. (Mimosaceae)
2. *Lagerstroemia microcarpa* Wt. (Lythraceae)
3. *Pterocarpus marsupium* Roxb. (Papilionaceae) and
4. *Xylia xylocarpa* (Roxb.) Taub. (Mimosaceae).

Importance of the present investigation

Seed pathology is an integral part of seed technology. However, forest seed pathology has not developed to the extent of seed pathology of agricultural and horticultural crops. Production either of Agriculture or Forestry depends to a great extent on the quality of seeds used. Revolution in agriculture was possible to a large extent due to the use of

quality seeds. In the same way it could be possible to increase the productivity of our forest lands by the use of quality seeds.

Seed health testing forms the first and foremost procedure in pre and post-entry quarantine. Seed testing procedures depend invariably on the importance of the pathogen on the seed and the disease potential assigned to the pathogen in a given situation (Neergaard, 1977). Even though quite a number of methods have been developed to test the seed health in agriculture and horticulture crops in forestry very few methods have been standardised; standard blotter and agar plate method being the exceptions. A particular micro-organism whether pathogenic or saprophytic has specific requirements for its occurrence and subsequent growth on the seed. It is unlikely that all the micro-organisms present on a seed will be recorded by a particular method. Hence in the present investigation, an attempt was made to evaluate various seed health testing procedures for forest tree crops to find out the best method for the expression of most of the seed-borne micro-organisms.

Several fungi have been found associated with the seeds but only a few of them may be pathogenic causing various types of disorders. Poor germination of seeds also could be caused

by seed-borne pathogens. However, literature on the seed microflora and its significance, especially of tropical forest seeds is scanty and an attempt has been made to bridge the information gap. Storage of agricultural seeds is a common feature, as adequate storage facilities are available . Though seed of forestry species are not stored for a long time as in the case of agricultural seeds , in certain cases it is imperative to store them for later use . Appropriate methods of storage under humid tropical conditions have not been standardised. Search of literature revealed that effect of seed microflora on the storage of forestry seeds has not even been attempted. Recent advances in storage practices have also unveiled the fact that the seeds stored at low temperature under dehumidified conditions and with fungicides are viable for a longer period and they showed reduced incidence of microbial attack (Christensen and Kaufmann,1974; Morneo and Vidal, 1981; Morneo *et al.*, 1985; Soman and Seethalakshmi, 1989). Hence, a detailed investigation was also carried out to find out the effects of storage of forestry seeds under different storage conditions and fungicidal application, on seed microflora, seed germination and seedling growth.

Hot water and fungicidal seed treatments are commonly used to control the seed-borne pathogens (Venkatasubbaiah *et al.*, 1984; Donald and Lundquist, 1984). The use of fungicides

as dust, slurry and soaking have been used not only to remove the inoculum from the seed but also to protect the seedlings from diseases while they are in the nursery (Munjal and Sharma, 1976 ; Mittal and Sharma, 1982 abcd; Mittal,1983 ab; Shukla *et al.*, 1990). Since no detailed investigations have been carried out on the above line in indigenous tree crops, management of seed microflora with hot water and chemical treatment was attempted.

With the increasing demand for wood, forestry has gained importance and intensive forest management practices are practiced in order to achieve higher productivity of the plantations. Diseases , especially in nursery, began to appear due to these intensive management practices. In this situation availability of healthy stock of seedlings for planting and their disease free condition in the field became an important aspect of forest management. To minimise the disease hazards or control them is the most important aspect of this challenge. Before taking up any nursery disease control measures, it is imperative that the recognition of the causal organism of the disease through symptoms is attempted first. Later, the incidence of the disease can be monitored for a period of time to understand its level of severity so that chemical control measures can be worked out economically.

While considerable attention is being paid in preserving the natural forests, no attempt has been made to study diseases of seedlings of indigenous tree species. Under conducive macro and micro climatic conditions seedlings of exotics/indigenous tree species are liable to be affected from one or more serious diseases during their entire nursery period. Fungal pathogens cause heavy loss in forest nurseries and even though excellent literature is available on diseases of seedlings of some economically important exotic tree crops (Sharma *et al.*, 1985) no information on seedling disease of indigenous tree crops and their management is available and hence, studies were taken up to identify serious disease problems in seedlings of indigenous tree species and work out the management strategy for economically potential ones.

2. REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Plants are normally propagated through seeds. The seeds of forest tree species like other seeds have the potential to harbour a wide variety of micro-organisms on or with them. Seed Pathology, recognised as one of the most important branches of Plant Pathology, has made remarkable progress in the past decades. Considerable amount of literature has accumulated on various aspects of seed pathology in agricultural and horticultural crops (Richardson, 1979). Recently, Mittal *et al.*(1990) published a check list of micro-organisms associated with the tree seeds in the world, and later Mohanan and Sharma (1991) reviewed the present status, practical problems and future prospects of seed pathology of forest tree species in India, but very little is known about the role of seed microflora of indigenous tree crops. Similarly information pertaining to seedling diseases of indigenous tree species as compared with exotics is very meagre. So an attempt is made to review the relevant literature available on seed pathological studies and diseases of young trees of some broad leaved tree species.

2.1. Seed microflora and their pathogenicity

2.1.1. Micro-organisms of broad-leaved tree crops

Mathur (1974) reported the occurrence of *Alternaria alternata*, *Fusarium moniliforme*, *Fusarium oxysporum* and *Trichothecium roseum* from the seeds of *Dalbergia sissoo* Roxb. He also recorded seven and five fungal species respectively from *Acrocarpus fraxinifolius* Wright and *Adenanthera microsperma* Teijsm. & Binn. from India. The same author reported thirteen fungal species from *Azadirachta indica* Juss.

From *Terminalia chebula* Retz., Nisha and Bhargawa (1976) isolated sixteen species using Martin's rose bengal agar while only six species viz., *Aspergillus flavus*, *A. niger*, *A. ochraceus*, *A. tamarii*, *Penicillium funiculosum* and *P. oxalicum* could be isolated from surface sterilised seeds.

Seed-borne fungi of nutmeg (*Myristica fragrans* Houtt.) from Malabar coast was studied by Varkey and Leelavathy (1978) and they isolated *Botryodiplodia* sp., *Curvularia* sp., *Colletotrichum gloeosporioides*, *Mucor* sp., *Pestalotiopsis* sp., *Phomopsis* sp. and *Fusarium moniliforme*.

Manoharachary *et al.*(1978) studied the seed rot of *Artocarpus integrifolia* Lam. and recorded *Botryodiplodia theobromae* from the affected seeds.

Seed mycoflora of *Carica papaya* L. was recorded by Srivastava and Lal (1978) and fourteen fungal species were recorded. Agmata (1979) studied the seed-borne micro-organisms in some forest trees viz., *Anthocephalus chinensis* Hassk., *Endospermum peltatum* Merr. *Pterocarpus indicus* Willd. *Swietenia macrophylla* King. and *Vitex parviflora* Juss.

While working on sugar maple seeds (*Acer oblongum* Wall.), Janerette (1979) recorded species of *Alternaria, Aureobasidium, Epicoccum, Penicillium* and *Rhizopus* and observed that when seedlings were grown in the presence of these fungi, symptoms were produced which included lesions, distorted leaves and stunted growth.

Decay of sal seeds due to *Aspergillus niger* was recorded by Sujan Singh *et al.* (1979). Seed mycoflora of tree species of *Albizia lebbeck* (Linn.) Benth., *Albizia lucida* (Boiv.) Benth., *Albizia procera* Benth., *Cassia fistula* L., *Cassia lacvigata, Cassia nodosa* Ham., *Eucalyptus* sp. *Polyalthia longifolia* (Sonner.)Thw. *Terminalia chebula* and *Terminalia myriocarpa* Heurck. & Muell. collected from the states of Assam and Meghalaya was studied by Tiwari and Sharma (1980). They observed that *Penicillium* and *Aspergillus* spp. were the most dominant ones followed by *Rhizopus* sp. and *Trichoderma* sp. Seed microflora were dominated by saprophytic ones and *Fusarium oxysporum* was the sole pathogenic fungus.

Sharma and Mohanan (1980) surveyed the spermoplane micro-flora of stored seeds of *Tectona grandis, Bombax ceiba* L., and *Eucalyptus* spp. in relation to germinability. They found that *Aspergillus* was the most dominant genus in all the tree species except *Bombax* wherein it was an actinomycete. In the blotter method, it was found that except *E. tereticornis*, seeds of other species showed low germination and this was directly correlated to percentage of seeds colonised by micro-organisms and frequency of seed microflora. They also observed that poor germination of teak seeds and incidence of seedling mortality was comparable in blotter and growing on method and mortality of seedlings was caused by species of *Fusarium, Alternaria, Curvularia* and bacteria.

Mittal and Sharma (1981 a) studied the seed mycoflora of *Cassia fistula* L., and isolated ten and five fungal species respectively from non-surface sterilised seeds involving moist blotter (SB) and PDA method. When surface sterilised seeds were used, only *Aspergillus fumigatus* grew in blotter method, while *Alternaria tenuis, Aspergillus flavus, A. fumigatus* and *Rhizopus oryzae* were detected in PDA method.

The same authors (Mittal and Sharma, 1981 b) while investigating the seed mycoflora of *Dalbergia sissoo* Roxb. isolated thirteen fungi belonging to eleven genera using SB and

PDA methods. They found that most of the fungi developed on the seed surface only; however, *Alternaria tenuis, Aspergillus flavus, A. niger* and *Fusarium* sp., besides infecting the seeds externally, also caused internal infections.

Archana and Mehrotra (1982) isolated forty two fungal species belonging to sixteen genera from the seeds of *Quercus, Sapium, Pyrus, Melia* and other spp., using seed washings and plating on Czapek's medium and PDA medium. They also found that the association of these fungi inhibited the seed germination in *Quercus*.

Seed mycoflora of *Albizia lebbeck* Benth. was studied by Mittal and Sharma (1982a) using both blotter and agar plate methods. *Rhizopus oryzae* was found to be the most dominant one, followed by *Penicillium canadense* and *Spicaria simplicissima*.

Cylindrocladium clavatum responsible for leaf blight and seedling diseases in *Eucalyptus* hybrid was found to be seed-borne by Rattan *et al.* (1983). They also recorded *Alternaria alternata, Curvularia pallescens* and *Drechslera* sp. from the seeds of above mentioned species.

Sujan Singh *et al.* (1983) reported a serious infection of *Fusarium semitectum* on seeds and pods of subabul *(Leucaena*

leucocephala). They observed that the infected seeds were darker in colour compared to healthy ones.

Seed-borne mycoflora of Red Sanders (*Pterocarpus santalinus* Linn.f.) from Andhra Pradesh was investigated by Reddy and Dayanand (1983). They found that *Aspergillus niger, A. flavus, Cladosporium cladosporioides* and *Fusarium* sp. were the ones causing seed infection.

Jamaludeen *et al.* (1983) studied the pod rot of *Pongamia pinnata*, and found that seeds were infected with *Colletotrichum* and *Macrophomina* spp. while inside the pods on the trees.

Seed mycoflora of tree different cultivars of Koo-babul (*Leucaena leucocephala*) were investigated using standard seed health testing procedures by Venkatasubbaiah *et al.* (1984). They observed seventeen fungal species and considerable reduction in seed mycoflora was achieved by hot water and fungicidal seed treatment.

Chalermpongse *et al.* (1984) from Thailand reported incidence of mycoflora from *Lagerstroemia calyculata* and *Xylia xylocarpa* var. Kern. From *X. xylocarpa* they recorded *Aspergillus flavus, A. niger, A. versicolor* and *Penicillium* sp. while from *L. calyculata, Aspergillus niger, A. restrictus, Curvularia lunata* and *Penicillium* sp. were recorded.

Seed-borne fungi of *Eucalyptus grandis* (Hill) Maiden., and *E. tereticornis* were studied by Saxena (1985) and he recorded sixteen internally seed-borne fungi and fourteen surface contaminants. The pathogenicity trials indicated that thirteen fungi produced disease symptoms in the form of inhibition in seed germination, seed rot, radicle necrosis, damping-off and wilting. *Curvularia lunata, Fusarium semitectum, Helminthosporium tetramera* and *Myrothecium roridum* caused maximum damage to young seedlings followed by *Drechslera australiensis, Trichothecium roseum, Fusarium oxysporum, F. moniliforme, F. equiseti, F.poae, Alternaria alternata, Macrophomina phaseolina,* and *Aspergillus niger.* Prasad (1985) observed species of *Aspergillus, Curvularia, Chaetomium, Fusarium* and *Penicillium* associated with the seeds of *Pongamia pinnata.*

Rhizopus nigricans was found to be the most common fungus associated with rotting of seeds and abnormal seedlings in *Cassia fistula.* In addition, *Aspergillus flavus, A. niger* and *Penicillium* sp. were also recorded from the rotting seeds (Randhawa *et al.*, 1986). Mittal (1986) studied the mycoflora associated with *Eucalyptus* hybrid and recorded fourteen fungal species belonging to ten genera. He also found that *A. niger, P. albicans, R. oryzae* and *Curvularia* sp. showed

differences in pathogenicity during seed germination and early seedling development.

Paul and Bharadwaj (1987) reported *Alternaria alternata, Fusarium solani, F. oxysporum* and *F. moniliforme*, from *Celtis australis* Linn., *Cassia siamea* Lamk., *Bauhinia variegata* Linn., and *Acacia catechu* .

Shukla *et al.* (1990) while studying the effect of seed dressing, reported that maximum number of seeds in *Leucaena leucocephala* (var. K.8) were colonised by *F. solani, Mucor* sp. *Spicaria divarigata* and *A. flavus*, while in variety K. 28 maximum fungal colonisation occurred due to *A. flavus, Curvularia lunata, F. solani, Penicillium prefaldianum* and *Mucor* sp.

Recently, Pongpanich (1990) reported various fungi associated with 60 samples of seeds of forest tree crops belonging to 15 families in Thailand using blotter and agar plate method. About 49 genera comprising of 92 species of fungi were identified, with saprophytic fungi as predominant. The various seed-borne pathogens causing seedling blight were *Alternaria longissima* on *Bambusa aurundinacea* (Retz.) Willd.; *Coryne-spora* sp. on *Cassia siamea* Britt.; *Colletotrichum gloeospo-rioides* on *Dalbergia cultrata* Grah.ex.Benth. and *Pterocarpus*

macrocarpus, Kurz. *Fusarium* sp. on *Dipterocarpus alatus* Roxb.; *Macrophomina* sp. on *Eucaluptus camaldulensis* Dehn. and *Botryodiplodia* sp. on *Melia azedarach* Linn. He further observed that parasitic fungi on forest seeds are not serious at present.

2.2. Seed health testing methods for forestry seeds

Standard blotter (SB) and agar plate methods widely recommended for the detection of number of fungi associated with seeds (ISTA. 1976,1985) are the most routinely and widely used methods for seeds of forestry importance. The SB method is the simplest and universally accepted seed health testing procedure, followed by sterile agar method for the detection of many seed-borne fungi(ISTA, 1976). Later, Musket and Malone (1941) developed "ulster" method for the detection of seed-borne fungi in flax, with malt extract agar. In most of the studies related to microflora of forest seeds, in addition to SB method, agar plate method has been used commonly. Mittal and Sharma (1982c) reported in the case of seeds of *Pinus roxburghii* that *Aspergillus niger*, *Rhizopus arrhizus* and *R. oryzae* developed better on PDA as compared with blotter method. However, the same authors (1982 b)reported from the seeds of *Shorea robusta*, 21 fungal species in blotter method, and only 6 fungal species were recorded on PDA. Later also, Mittal (1983b) recorded 26 fungal species in blotter method

and only 10 species on seeds of *Cedrus deodara* in PDA method. A similar trend was also reported by Vijayan (1988) on seeds of *Acacia catechu*, where he recorded 22 fungal species in SB method and only 17 fungal species in PDA method. He found that slow growing forms like *Penicillium*, *Trichothecium*, *Trichoderma* and *Fusarium* were better isolated in blotter method compared to agar method. He obtained similar results also with the seeds of *Cassia fistula*.

In order to enhance the growth of the pathogen, the seeds are usually killed or inactivated either by subjecting them to freezing temperatures or dipping blotters in 2,4-D (2,4- Dichlorophenoxy acetic acid). According to Limonard (1966) *Alternaria porri* grew well on seeds of *Allium cepa* Linn.,in deep freeze method (DF). Neergaard (1977) reported that DF method was favorable for various species of *Fusarium* and *Septoria* in cereals, while *Phoma lingam* on seeds of crucifers was detected easily by 2,4-D method. Shetty and Shetty (1988) while comparing six methods, viz., SB, 2,4-D, DF, PDA with guaicol agar and rice extract agar for the detection of seed-borne fungi in rice, reported that the rice extract agar was equally good in comparison with all the methods except PDA in ascertaining the incidence of *Trichoconis padwickii*. Since the efficacy of methods like deep freeze, 2,4-D and MEA has

not been evaluated so far for any of the forestry seeds, these were included in this study.

2.3. Management of seed-borne pathogens of forestry crops

Treating the seeds in hot water at 50 to 60°C for 15-30 min. helps to eradicate the surface-borne, as well as deep seated pathogens. Scarification of seeds using hot water has been practiced with the seeds of various species of *Albizia, Acacia, Bauhinia, Cassia, Delonix* and *Leucaena leucocephala* (Ram Prasad and Kandya, 1992) for improving only the seed germination. Venkatasubbaiah *et al.* (1984) reported that hot water treatment at 85°C for 5 min. reduced the incidence of various mycoflora and consequently higher percentage of seed germination was achieved in 3 cultivars of *L. leucocephala*. Similarly, Donald and Lundquist (1984) reported that hot water treatment of eucalypt seeds at 50°C for 5, 10 and 20 min. not only restricted fungal development but also enhanced the seed germination. Other than these reports no other literature pertaining to the effect of hot water treatment and fungal incidence in forestry seeds is available.

Fungicidal seed treatment is considered as one of the most effective and economic methods in controlling seed-borne fungi. Seed dressing with fungicides before sowing is known

to control the seed-borne infection on one hand and on the other hand protect seeds and seedlings from soil-borne plant pathogens (Kishore and Jotwani, 1983). Scanty reports are available on the management of seed-borne pathogens of forestry importance by seed dressing with fungicides. Ceresan was recommended for the control of most of fungi associated with teak seeds (Dabral, 1976). Munjal and Sharma (1976) have also recommended Ceresan (0.25%) to control the seed-borne fungi of *Pinus roxburghii* and *P. wallichiana*. Storage of sal seeds at 75% r.h. and 12% moisture with 3 ml of oil of eucalypt/100 cm^3 of storage space prevented *A. niger* attack (Sujan singh *et al.*, 1979). Ghosh *et al.* (1981) effectively controlled the damping-off caused by *Rhizoctonia* sp. using quintozene and mancozeb in pine nurseries through seed dressing. Mittal and Sharma (1981a) recommended quintozene, carbendazim and mancozeb against some commonly occurring seed-borne fungi, viz., *Aspergillus niger, A. sydowi, Cladosporium* spp., *Memnoniella echinata, Penicillium canadense, Rhizopus oryzae* and *Trichoderma viridae. Aspergillus niger* recorded on the seeds of *Shorea robusta* was controlled by carbendazim and quintozene applied @ 0.25% of seed weight (Mittal and Sharma, 1982b). Mittal (1983b) reported that RH 2161 (0.1%) a liquid fungicide and mancozeb were effective in controlling various seed-borne fungi of *Cedrus deodara*. Agallol (0.2%) was found

to be most effective fungicide against *Cylindrocladium clavatum* infection of *Eucalptus* hybrid seeds using poisoned food technique (Rattan *et al.*, 1983). Sujan singh *et al.*(1983) reported that subabul seeds infected with *F. semitectum* were effectively controlled by 0.1% solution of MEMC. Further more, Venkatasubbaiah *et al.*(1984) reported that carbendazim, benomyl and thiophanate methyl were very effective in reducing the fungal population in subabul seeds.

Vijayan (1988) reported that all the six fungicides, viz., Bavistin, Dithane M-45, captan, ziram, thiram and captafol tested have enhanced germination percentage of *Acacia catechu* and *Dalbergia sissoo* and later, root shoot lengths of seedlings. He also reported that complete control of mycoflora of *Cassia fistula* was achieved by dusting seeds with Dithane M-45 or Bavistin or captan @ 0.25% seed weight. Seeds of *Leucaena leucocephala* treated with Bavistin gave highest percentage of germination and complete protection against seed-borne fungi (Vijayan, 1988).

2.4. Seed storage and its influence on mycoflora and germination

The seeds are known to harbour many pathogenic fungi before they are stored. However, the saprophytic or storage fungi do not invade seeds before harvest and only during stor-

age period they colonise on the seeds. Although excellent work has been carried out on post-harvest microbial deterioration of seeds of different agricultural crops (Vidhyasekaran *et al.*, 1970 ; Agrawal, 1980), no work has been done on storage pathology of forestry seeds. A few classical and recent references pertaining to other crops are mentioned to indicate various factors affecting the seed and its mycoflora under storage conditions. The excellent review by Christensen and Kaufmann (1969) mentioned that moisture content of the seed plays an important role in the establishment of seed fungi. Moreover, the respiration by storage insects and fungi helps in building up moisture which finally lead to seed deterioration by seed-borne pathogens. Therefore, storage of seeds at low temperature and dehumidified conditions helps in improving the germinability (Dorworth and Christensen, 1968). Seed storage under air-tight condition helps in increasing the longevity of the stored seeds, as found in the case of *Helianthus annus* Linn. and *Brassica napus* Linn. (Poison *et al.*, 1980). *Colletotrichum gloeosporioides* causing tip blight of *Hibiscus cannabinus* Linn.,remained viable for 31 months, while on *Lupinus* sp. it survived for 18 months under storage (Weimur, 1952; Sy and Lo, 1958). *Alternaria alternata* was reported to be viable for 10 years in wheat and barley (Machacek and Wallace, 1952) and 6 years on cabbage seeds (Neergaard,

1969). According to Bilgrami *et al.* (1979), *A. tereus, R. nigricans,* and *M. phaseolina* were the commonly occurring fungi in summer, while *Chaetomium* sp., *Cladosporium oxysporum* and *Epicoccum* were recorded in winter months on seeds of mung, gram, masoor and paddy.

Morneo and Vidal (1981) and Morneo *et al.* (1985) reported that the viability of maize seeds could be improved by storing the seeds at less moisture content and treating them with fungicides. Recently Soman and Seethalakshmi (1989) also observed that a rapid loss of viability of seed of *Bambusa arundinacea* within two months of storage in plastic containers under laboratory conditions, while the seeds stored at low and room temperature over calcium chloride lost the viability gradually reaching 10% after 413 days of storage.

2.5. Seedling diseases and management

In India Bakshi (1967) initiated a systematic survey of forest diseases based on which intensive research was undertaken to tackle some important forest diseases, which was observed affecting mostly exotic tree species. Later Sharma *et al.*(1985) carried out an exhaustive forest disease survey in Kerala. But their survey also included mostly exotics except teak, *Bombax ceiba* and *Dalbergia latifolia*. However, as

far as indigenous tree species are concerned, no systematic work on diseases was carried out, and even a few references available, deal with only diseases of least significant importance (Mukerji and Jayanti Basin, 1987). Since there is no record of any seedling disease of the indigenous tree crops included in the study , diseases of young plants and trees recorded in natural stands in various parts of India are shown in Table 1-4.

Table 1. Diseases of *Albizia odoratissima* recorded in India

Diseases	Pathogen	Place of occurrence and Reference(s)
Seedling diseases	Nil	
Diseases in natural stands		
1. Anthracnose on leaf and pod	*Colletotrichum* sp.	Maharashtra Karnataka (Patel *et al.*, 1949)
2. Leaf spot	*Endodothella kanarensis* Ramakr. T.S. & Sund	Karnataka (Ramakrishnan, 1952)
3. Wood Canker	*Hypoxylon denstum* (Hoff. ex Fr.)	Assam (Agnihothrodu, 1964)
4. Sooty mould	*Meliola albizziae* Hansf.G Deighton	Assam (Agnihothrodu, 1960)
	Meliola albizziae var *odoratissima* Kapoor	Assam (Kapoor and Tandon, 1967)
5. Smut	*Microstroma albizziae* Syd.	Tamil Nadu (Ramakrishnan and Sreenivasan, 1950)
6. Leaf rust	*Ravenelia japonica* Diet & Syd.	Allahabad (Kapoor and Agarwal, 1972)
	Ravenelia odoratissimae Tyagi & Prasad	Rajastan (Tyagi and Prasad, 1978) (Barua *et al.*, 1982)

Table 2. Diseases of *Lagerstroemia microcarpa* recorded in India

Diseases	Pathogen	Place of occurrence and References
Seedling diseases	Nil	
Diseases in natural stands		
1. Black spot on leaves	*Rhytisma lagerstroemiae* Rabenh.	Karnataka Tamil Nadu (Rabenhorst, 1878)

Table 3. Diseases of *Pterocarpus marsupium* recorded in India

Diseases	Pathogen	Place of occurrence and References
Seedling diseases	Nil	
Diseases in natural stands		
1. Leaf spot	*Aldona minima* Muller & Patil	Maharashtra (Muller and Patil, 1973)
2. Leaf spot	*Cercospora canescens* Ell. & Mart.	Madhya Pradesh (Singh, 1971)
3. Stem infection	*Ciliochorella mangiferae* Syd.	Maharastra (Parndekar,1964)
4. Leaf rust	*Maravalia pterocarpi* (Thirum.)	Karnataka (Thirumalachar, 1947)
5. Leaf spot	*Neopericonia* sp.	India (Kamal *et al*,1983)
6. Trunk rot	*Polyporus gilvus* Schw.	Common (Llyod, 1898-1925 1904-1919)

Table 4. Diseases of *Xylia xylocarpa* recorded in India

Diseases	Pathogen	Place of occurrence and References
Seedling diseases	Nil	
Diseases in natural stands		
1. Stump rot	*Fomes fastuosus* (Lev.)Berk.	Orissa and Andhra Pradesh
	Polystictus steinheilianus Berk. &Lev.	Andhra Pradesh
	Trametes serpens Fr.	Orissa and Andhra Pradesh (Anon., 1950; Bose, 1919-28; Hennings, 1901; Lloyd, 1898-1925)
2. Leaf spot	*Colletotrichum gloeosporioides* (Penz.) Penz & Sacc.	Kerala (Sankaran *et al.*,1988)

It is quite obvious from the above tables, that most of the tree species have only very few disease problems that too of minor importance.

3. MATERIALS AND METHODS

3. MATERIALS AND METHODS

Four tree species indigenous to Kerala, viz., *Albizia odoratissima*, *Lagerstroemia microcarpa*, *Pterocarpus marsupium* and *Xylia xylocarpa* were selected for the seed pathological and seedling disease studies. The details pertaining to their distribution, habit and uses are given below.

3.1. *Albizia odoratissima* (l.f.) Benth.

Distributed throughout India, Sri Lanka, Myanmar and Malaysia. In Kerala it occurs in almost all forest divisions (Fig. 1). Deciduous trees with spreading crown, are usually seen in moist deciduous forests of Kerala up to an altitude of about 1200 m above m.s.l. Mature trees grow to a diameter of 90 cm and a height of 30 m with a straight clear bole up to 12 m length; bark black, leaves bipinnate with a sessile gland on the rachis, a little above its base; stipules cauducous; leaflets 8-15 pairs sessile; inflorescence axillary or terminal in umbellate or corymbose panicles; flowers sessile, cream coloured; pods subsessile, thin, flat, straight, seeds 5 to 15 per pod; flowers observed from March to June, often profuse during April-May and fruiting from July to January fruits mature during November-January (Troupe, 1983). No literature pertaining to seed disorders and seedling diseases of this species are available.

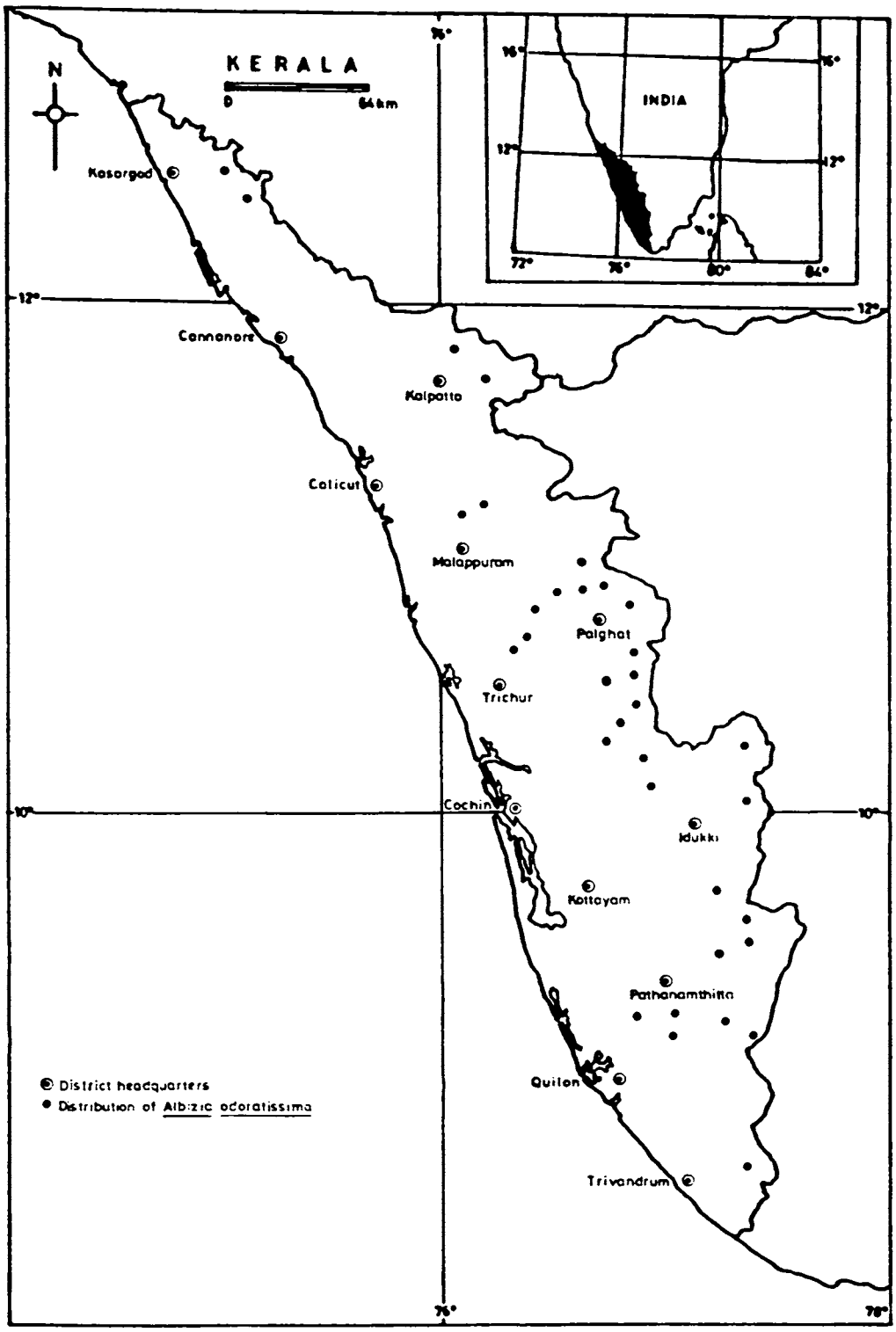


Fig.1. Distribution map of *A. odoratissima* in Kerala

3.2. *Lagerstroemia microcarpa* Wt.

This species is distributed in Tropical Asia and Australia and throughout Kerala (Fig. 2). Deciduous trees grow up to a height of 30 m and a diameter of 80 cm. The main bole is straight and branchless in most semi-evergreen forests and in more open areas, branching may be seen at lower levels. Bark smooth, pale white or ash peeling off as large thin stripes. Leaves simple, entire, petiolate; inflorescence in axillary or terminal racemes, flowers white with a rose tinge, capsules ellipsoid. Flowering from May to July; fruiting from June to December; mature in December. Ripend capsules are available from December-March (Troupe, 1983). As far as studies on seed pathology and seedling diseases of this species are concerned there is no literature available.

3.3. *Pterocarpus marsupium* Roxb.

Distributed in Peninsular India and Srilanka and in Kerala distributed in Trivandrum, Thenmala, Punalur, Konni, Ranni, Thekkady, Kottayam, Idukki, Munnar, Kothamangalam, Malayattoor, Trichur, Chalakudy, Vazhachal, Nemmara, Palghat, Parambikulam, Calicut, Nilambur and Wayanad Forest Divisions (Fig. 3). Semi-evergreen trees, grow from 10 to 25 m in height, corked bark, young leaves reddish, compound, alternate, leaflets obovate, broadly ovate; stipules small and deciduous;

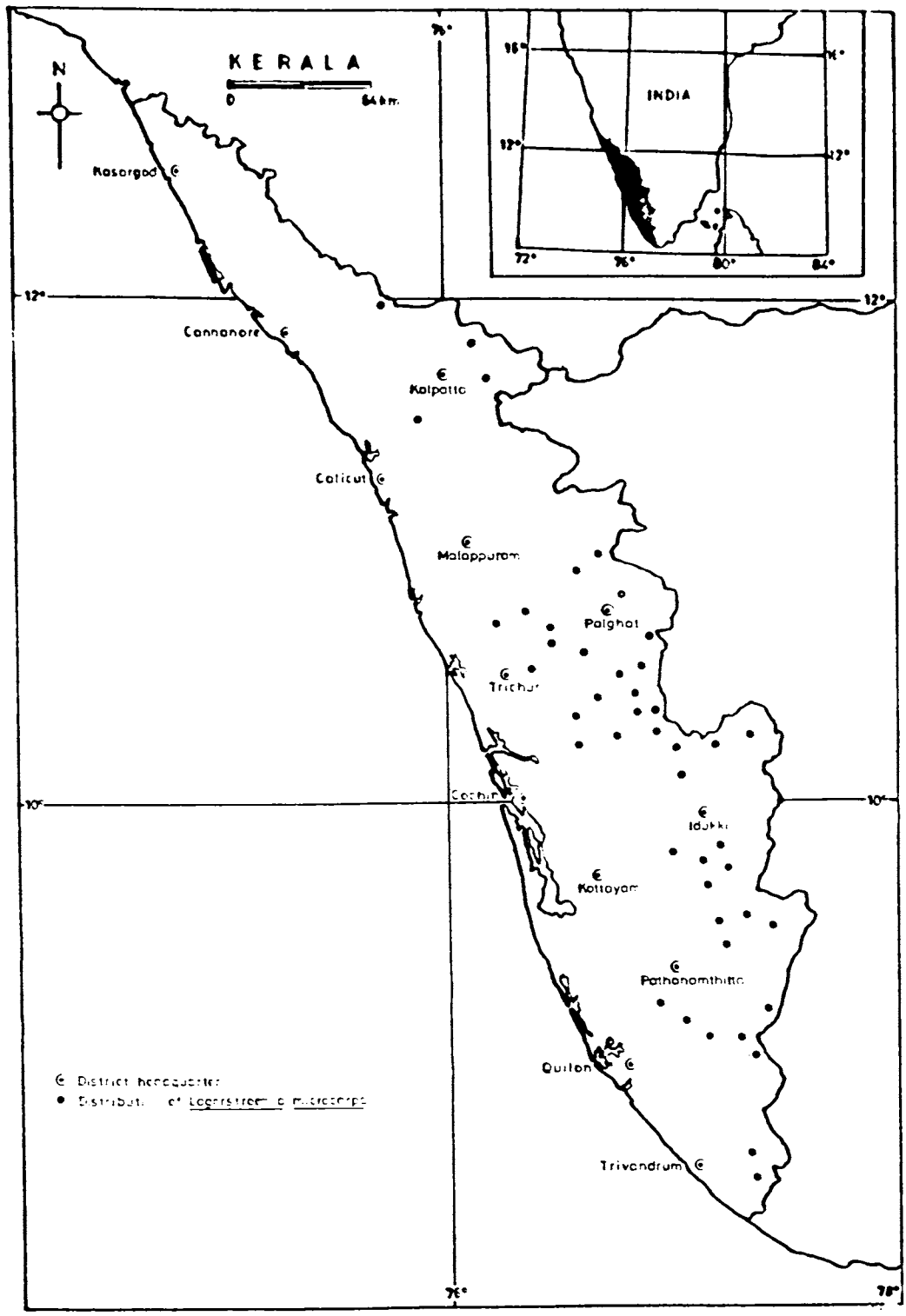


Fig.2. Distribution map of *L. microcarpa* in Kerala

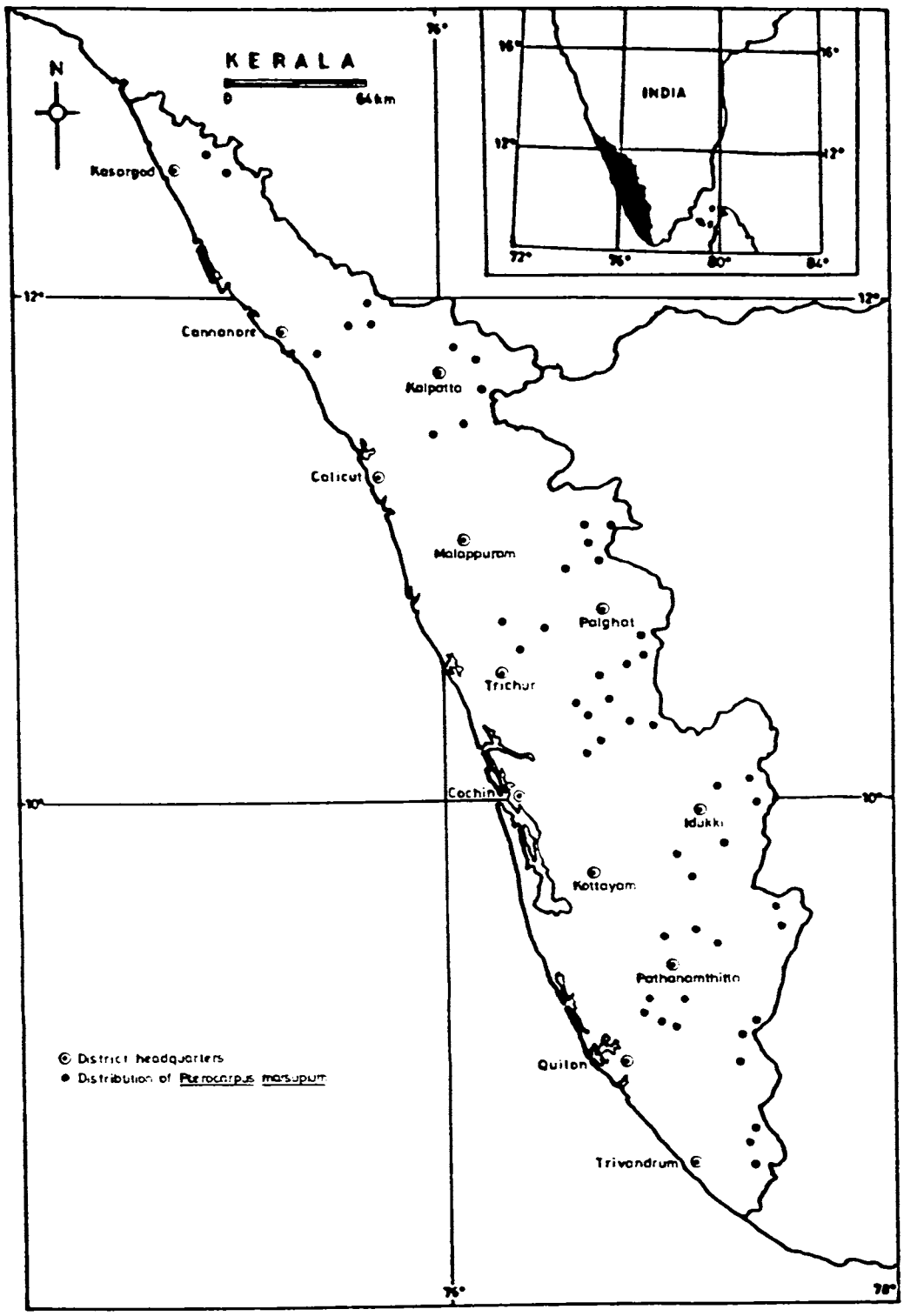


Fig.3. Distribution map of *P. marsupium* in Kerala

flowers yellow; pods winged, seeds one or very rarely two; trees usually densely foliated with often fissured bark exuding copious resin which dries into solid blocks. Trees are common in and around grasslands and rocky forests; flowering observed from May to October, maximum in October. Fruiting takes place from October to February (Troupe, 1983). No literature is available on seed pathology and seedling disease aspects of this species.

3.4. *Xylia xylocarpa* (Roxb.) Taub.

This tree species is distributed in Peninsular and Central India extending up to Orissa. In Kerala, this species is recorded from all forest divisions except Trivandrum (Fig. 4). Trees usually grow up to a height of 25 m and a diameter of 60 cm. The main bole is very rarely straight and cylindrical. Deciduous trees, bark reddish, tender leaves dull brown, leaves obovate; Inflorescence axillary, racemose; flowers creamy-white to light yellow; pods woody, oblong, septate between seeds; seeds 4-10 per fruit, smooth and polished. Flowering observed from March to May fruiting from May-December but maximum during June to December (Troupe, 1984), No literature pertaining to seed disorders and seedling diseases of this species is available.

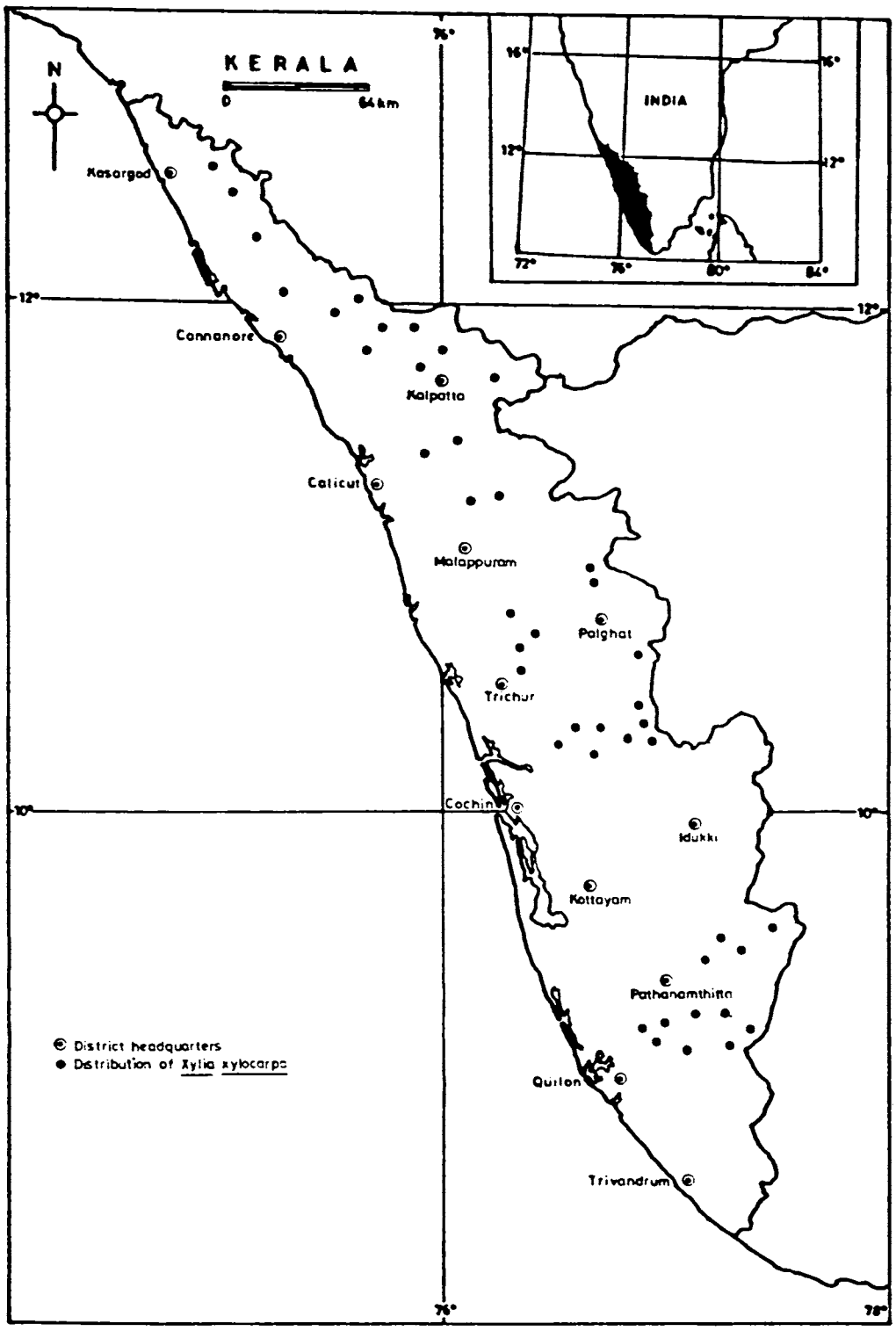


Fig.4. Distribution map of *X. xylocarpa* in Kerala

3.5. Seed collection and storage

Seeds of all the four tree species (Plate 1) were collected during 1988-1989 and 1990 seeding seasons from forest areas in Trichur and Nilambur Forest Divisions (Table 5). Initially, the seeds were collected from individual trees, and later composite samples were made by mixing the primary samples.

Soon after their collection, the composite samples were labeled, sun-dried, to reduce the moisture content to about 10-15% and stored separately in cloth bags at room temperature (30 $\pm$ 5°C). For chemical control studies, seeds were treated with appropriate chemicals and stored in wide mouthed tightly capped plastic containers at room temperature.

3.6. Seed pathological studies

3.6.1. Standardisation of seed health testing methods

To obtain maximum information on microflora harbouring seeds the following seed health testing methods, viz., standard blotter method, 2,4-D method, deep-freeze method, potato dextrose agar method and malt extract agar method were evaluated for their performances and standardised for each of the four forest tree species.

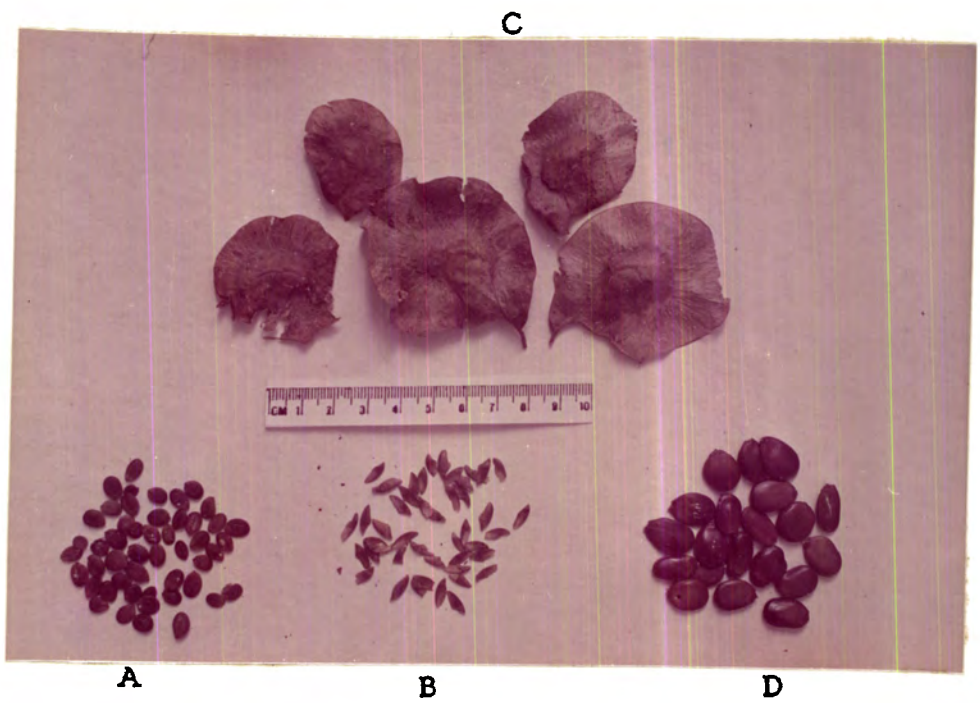


PLATE 1. Seeds of *Albizia odoratissima* (A), *Lagerstroemia microcarpa* (B), *Pterocarpus marsupium* (C), and *Xylia xylocarpa* (D).

Both surface sterilised and unsterilised seeds were used in all the methods. Surface sterlisation was carried out by treating the seeds with 0.1% mercuric chloride for 3-5 min, washed in 2-3 changes of sterile water. The seeds were dried in between the folds of sterile blotter before plating.

Various micro-organisms were tentatively identified by studying their cultural and morphological characters. Later, for authentic specific identification, the cultures were referred to the CAB International Mycological Institute, Kew, England.

Percent incidence of various spermoplane micro-organisms recorded from various tree species in various methods was subjected to one-way analysis of variance using Duncan's multiple range test (DMRT) at 5% level, after arc-sin transformation (Snedecor and Cochran, 1967).

3.6.1.1. Standard blotter (SB) method

SB Method as described by ISTA procedures (ISTA, 1976, 1985) was employed. A random sample of 400 seeds of *Albizia* and *Lagerstroemia* taken from the composite sample was tested. For *Pterocarpus* and *Xylia* where the seed size is large, respectively only 50 and 100 seeds were used Plastic petri plates measuring either 90 mm or 140 mm dia., lined with

four moistened blotter discs were used in the study depending upon the seed size. Excess water if any was drained from the blotter by inverting and briefly shaking the plates before plating the seeds. Seeds were placed one by one at equal distance to avoid inter seed contamination. The number of seeds incubated per plate varied with the size of the seeds. A petri plate of 140 mm accommodated either 5 seeds of *Pterocarpus*, 10 seeds of *Xylia* 25 seeds of each of *Lagerstroemia* and *Albizia* while a 90 mm petri plate accommodated 10 seeds each of *Albizia* and *Lagerstroemia*. The plated seeds were incubated at 25 $\pm$ 2°C in a BOD incubator adjusted with alternating cycles of fluorescent light and darkness for 6 days and the following day observation recorded using a stereomicroscope on microbial growth. Percent incidence of each micro-organism was calculated using the following formula.

$$\% \text{ incidence} = \frac{\text{No. of seeds recorded with an organism}}{\text{Total number of seeds examined}} \times 100$$

3.6.1.2. 2,4-D method (Neergaard and Saad, 1962)

This method was similar to SB method except that the blotter discs were soaked in 0.2% solution of 2,4-Dichlorophenoxy Acetic acid.

3.6.1.3. **Deep freeze (DF) method** (Limonard, 1966)

The seeds plated on blotter were initially incubated at 25 ± 2°C for 24 h as described in SB method. Later, they were incubated at - 20°C in total darkness for 24 h. Subsequently, these plates were incubated at 25 ± 2°C under 12/12 h alternating cycles of fluorescent light and darkness for five days.

3.6.1.4. **Potato dextrose agar (PDA) method** (ISTA, 1976)

Petri plates containing 15-20 ml of sterilised potato dextrose agar medium were plated with appropriate number of seeds depending upon the tree species. The plates were incubated at 25 ± 2°C for 4-5 days and observations recorded on the development of micro-organisms.

3.6.1.5. **Malt extract agar (MEA) method** (ISTA, 1976)

Petri plates containing 15-20 ml of MEA with 4% NaCl were plated with seeds of different species and incubated at 25 ± 2°C for 4-5 days and observations recorded on micro-organisms developing on the seeds.

3.6.2. **Seed microflora and its significance**

3.6.2.1. **Dry seed examination**

Seed samples drawn from composite samples were sorted into three different categories, viz., apparently healthy,

discoloured and deformed, based on their external appearance. The weight of 100 seeds each of the above categories was taken, percent of seeds in each category determined. The percent incidence of spermoplane microflora in each of these of categories of seeds was tested separately employing the Standard Blotter (SB) method (ISTA, 1976,1985).

Table 5. Details of seeds of various tree species included in the study

Species	Fruiting time	Locality of collection	No. of seeds kg^{-1}	Seed dimension lxb (mm) Mean ± SE
A. odoratissima	Jan-Mar	Peechi	20,000	6.6 ± 1.4 x 4.7 ± 0.8
L. microcarpa	Dec-Mar	Nilambur	2,50,000	8.8 ± 1.2 x 3.9 ± 1.0
P. marsupium	Feb-May	Peechi	1500-2000	49.5 ± 9.6 x 42.5 ± 5.9
X. xylocarpa	Jan-Mar	Peechi Nayatugundu	4000-5000	14.6 ± 1.7 x 9.7 ± 1.1

3.6.2.2. Pathogenicity studies

The spermoplane micro-organisms which caused damage like seed rot, decay and germination failure to seeds in different seed health testing methods were selected for the study. Single spore isolations were made and fungal isolates were cultured on PDA and bacteria on Nutrient Agar (NA).

Suspensions of all the test fungi and bacteria were prepared in sterile distilled water from 7-day-old cultures. The concentration of the inoculum was adjusted at 5 x 10^4 for fungal spores and 1 x 10^6 CFU/ml for bacteria. The surface sterilised seeds were soaked in the spore/bacterial suspension of the respective micro-organism for 18 h, air dried to remove excess water and sown in sterilised soil as described below.

3.6.2.3. Growing-on test

The sterilised garden soil (autoclaved at 20 psi for 30 min.) evenly spread in aluminium trays (30 x 30 x 5 cm) was sown with seeds of different species inoculated separately with respective micro-organisms. The surface sterilised seeds soaked in sterile water served as control. The trays were watered with sterile water as and when required. One hundred seeds were used for each micro-organism x host species combination.

Percent germination of seeds and shoot and root lengths of seedling were recorded. Vigour Index (VI) was calculated by using the following formula (Abdul-Baki and Anderson, 1973).

$$VI = (\text{Mean shoot length} \times \text{Percent germination} + \text{Mean root length})$$

The seedlings grown for 30 days were categorised into the following groups:

State of seed/seedlings		Seed/seedling symptoms
i. Normal apparently healthy seedlings	-	Seedlings of normal height and unblemished
ii. Delayed seedlings	-	Delayed germination and below normal height
iii. Distorted seedlings	-	Seedlings underdeveloped
iv. Blighted seedlings	-	Seeds germinated, but could not attain normal growth and blighted.
v. Ungerminated seeds	-	Seeds not germinated
vi. Decayed seeds	-	Seeds which are decayed

3.6.3. Management of seed microflora

3.6.3.1. Hot water treatment

The seeds of various tree species kept in a muslin cloth bag, were soaked in hot water maintained at 50° and 60°C in a thermostatically controlled water bath. At each temperature, the seeds were soaked separately for 15 and 30 minutes. The treated seeds were air dried on blotter sheets for 1-2 h at room temperature (30 $\pm$ 5°C). Later, the treated and control seeds were plated on blotter, incubated and examined for the development of micro-organisms as described under 3.6.1.1. The

percent incidence of various micro-organisms was subjected to one way analysis of variance after 'arc-sin' transformation. In addition, one hundred seeds in four replicates were also sown in sterilised garden soil evenly spread in aluminium trays (30x30x5cm). Observation on seed germination and shoot and root lengths were recorded after 15 days of sowing and vigour index was calculated as mentioned under 3.6.2.3. Data pertaining to percentage of seed germination were subjected to one way analysis of variance after 'arc-sin' transformation. The data on shoot and root lengths and vigour index were appropriately transformed using power transformation method (Montgomery and Peck, 1982) and the transformed values were subjected to one way analysis of variance. For convenience only the original values are presented in the tables.

3.6.3.2. Chemical treatment

The seeds of all the four tree species were treated separately with various fungicides listed in Table 6. Dusting of seeds was done by placing the seeds in polystyrene containers with the required dosage of fungicides and shaking them thoroughly for 10-15 min., so that the seeds were uniformly coated with the seed dresser. The treated seeds remained in polystyrene containers for a day at room temperature (30±5°) before utilising them in the experiment. The treated seeds

were subjected to 'SB' method, 24 h after treatment for studying the effect of seed dressers on seed microflora. The experimental details were the same as described 3.6.1.1.

One hundred treated seeds each, in four replicates were sown in aluminium trays (30 x 30x 5 cm) containing sterilised garden soil. Observations on seed germination, and shoot and root lengths were recorded as described under hot water treatment and vigour index was also calculated. The data were subjected to one way analysis of variance after appropriate transformation of values as described under 3.6.3.1.

3.6.4. Seed storage and its influence on microflora, seed germination and seedling development

Seed samples treated with different seed dressers (Table 6) were stored in wide mouthed air tight polystyrene containers at room temperature (25°-35°C) and ambient r.h. of $\leq$ 75%. In addition to these treatments, seed lots were also stored in cloth bags at i. room temperature; ii. room temperature under dehumidified condition (in a desiccator with calcium chloride) and iii. at 4°C in dehumidified condition. The "control" seeds were either stored in polystyrene containers or cloth bags at room temperature (25°- 35°C) and ambient r.h. of $\leq$ 75%. From each treatment, 100 seeds selected randomly were subjected to SB method for studying the spermoplane microflora as

Table 6. Seed dressers, their chemical name and dosage used in chemical control studies

Seed dressers (commercial name)	Chemical Name	Source	Dosage % a.i.
Bavistin-50 WP	Methyl-H-benzimidazole 2-yl-carbamate	BASF India Ltd, Bombay	0.2
Brassicol-75 WP	Pentachloro nitro-benzene	Dept. of Appl. Botany, Mysore University	0.3
Deltan-75 SD	N-trichloromethyl-thio-4 cyclohexene-1,2- dicarboximide	Coromandel Indag, Madras	0.3
Dithane- M-45 75 WP	Zinc ion+ manganese ethylene bis dithio-carbamate	Indofil Chemicals, Bombay	0.3
Emisan-6-WP	2-Methoxy ethyl mercuric chloride	Excel Industries, Bombay	0.0125
Foltaf-75 WP	Cis-N-(1,1,2,2, tetra-chloro ethyl thio) -4-cyclohexone-1,2-dicarboximide	Rallis India Ltd, Bombay	0.3
Thiride-75 SD	Tetramethyl thiurum disulphide	Sureksha Chemicals, Bombay	0.3
Vitavax-75 WP	5,6-dihydro-2-methyl-1,4-0xathin-3-carboxanilide	Hindustan Insecticides Ltd, New Delhi	0.3

described under 3.6.1.1. at an interval of 1-day, 90 days, 180 days and 365 days of storage. Moreover, 100 seeds of each of the tree species were sown in 4 replicates in garden soil in aluminium trays (30 x 30 x 5 cm). After 15-20 days of sowing, observations on seed germination, shoot and root lengths were recorded and vigour index calculated. Statistical analysis of data was carried out using the procedure as described under 3.6.3.1.

3.7. Diseases of seedlings

3.7.1. Experimental area

The major study where regular and intensive observations on incidence and severity of seedling diseases were recorded was a nursery maintained at Peechi in Trichur District of Central Kerala. Peechi, ca. 50 m above mean sea level, receives an annual rain fall of ca. 3000 mm or more. The area records high humidity throughout the year. The weather data for the year 1989 and 1990 is given in Table 7.

For recording the incidence of various seedling diseases, a nursery at Chandanathode in Wayanad District of northern Kerala was also selected. Chandanathode approximately 800 m above mean sea level, received a high annual rain fall of ca. 6000 mm, and a very high humidity prevails throughout

Table 7. Weather data for 1989 and 90 at Peechi
(Latitude 10°32' Longitude 76°20 E; altitude: 50 m)

Months	Mean Temp(°C) Max	Min	Mean r.h.(%) Max	Min	Monthly Rainfall (mm)	Daily Mean Wind velocity (Km/h)	Daily Mean bright sunshine (h)
1989							
Jan.	33.4	21.1	86	48	0(0)	9.0	8.4
Feb.	36.6	20.8	96	42	0(0)	6.5	10.2
Mar.	37.4	22.6	100	49	20(1)	4.8	9.8
Apr.	37.1	24.2	95	56	54(1)	4.2	8.5
May.	34.6	63.1	98	67	122(3)	3.5	7.6
Jun.	30.0	21.7	100	78	668(20)	5.7	3.4
Jul.	29.5	22.1	99	82	504(14)	NR	4.2
Aug.	30.1	22.5	98	76	298(10)	4.0	5.3
Sep.	31.3	22.7	100	76	186(6)	2.0	5.6
Oct.	32.3	22.4	100	76	329(9)	NR	6.0
Nov.	32.2	22.0	91	62	13(1)	NR	8.3
Dec.	32.3	21.7	93	61	24(1)	NR	9.5
1990							
Jan.	33.1	19.9	94	52	0(0)	NR	9.3
Feb.	35.6	21.5	80	43	0(0)	NR	10.4
Mar.	37.9	22.9	80	46	0(0)	NR	10.4
Apr.	37.4	24.0	88	52	229(1)	NR	5.5
May.	32.3	23.0	90	66	435(16)	NR	4.6
Jun.	29.8	22.5	96	76	890(14)	1.3	3.4
Jul.	29.3	21.7	100	80	759(22)	1.0	2.6
Aug.	26.9	22.2	100	77	357(10)	4.5	4.2
Sep.	31.4	22.7	100	71	78(3)	2.2	7.0
Oct.	32.3	22.6	100	69	330(9)	1.9	6.7
Nov.	31.8	21.8	87	63	91(9)	12.0	6.7
Dec.	32.0	22.0	90	59	2(0)	12.0	6.6

Note: NR: Not recorded, r.h.: Relative Humidity. The figures in parenthesis indicate the number of rainy days when rainfall was >10mm.

the year with mean minimum and maximum temperatures of 13°C and 32°C respectively. During the early years, the area was occupied by experimental eucalypt nurseries. In addition, incidence of seedling diseases of the four indigenous tree

species included in the study was also recorded from nurseries maintained by KFRI at Nilambur and by the Karnataka Forest Department in Haliyal Forest Division (Table 8).

3.7.2. Preparation of nursery beds at Peechi

The soil of the nursery site at Peechi was thoroughly worked and sixteen experimental beds of 12 m x 1.2 m x 0.3 m were prepared; all the sides of the bed were provided with a protective covering of bamboo reeds to prevent washing away of the edges of seed bed due to heavy rains and watering.

3.7.3. Shading

Shade over the seed beds was provided with coir mat of 7 mm mesh to protect the young seedlings from sun scorching. After a month of emergence of seedlings, shade was removed partially and removed completely when the seedlings were 45-60 days old.

3.7.4. Sowing and watering schedules

During the 1989 trials at Peechi, each standard bed was sown with seeds of different tree species separately (Table 9). After sowing the seeds were covered with 10 cm of thick layer of fine sieved soil to prevent the seeds from dislodging while watering and to provide moisture during germination.

Table 8. Forest nurseries surveyed during 1988-90 for the incidence of seedling diseases

Locality	Forest Range	Forest Dvn. (State)	Type of seedlings	Year of observation
Peechi	Peechi	Trichur (Kerala)	Seed beds and container seedlings	1988,1989, 1990
Chandanathode	Mananthody	Wayanad (Kerala)		1989
Begur				1989
Nilambur	Nilambur	Nilambur (Kerala)		1988,1989
Kurigadda	Haliyal	Haliyal (Karnataka)		1988
Karalkatta	Sambrani			
Bhagawathi	Bagawathi			
Kogilban	Dandeli			
Bailpar	Virnoli			
Nandigadda	Gund			
Gobral	Birchi			
Kilapani	Jagalpet			
Akrali				
Jalakath	Tinaighat			
Kodanad	Kodanad	Kodanad (Kerala)	Seed beds	1989
Erumapatty	Chalakudy	Chalakudy (Kerala)		1989

During the first two weeks after sowing, seed-beds were watered 2-3 times daily till emergence. Then the frequency of watering was gradually reduced to only once up to 60 days. Later, the watering frequency was adjusted according to prevailing weather conditions. The seedlings were maintained either in the mother beds or in polythene containers (18 x 12 cm) for recording the incidence of seedling diseases.

Table 9. Details of seeds of various tree species included in the study

Species	Quantity of seeds/ standard bed (1.2 X 12 m)	Pre treatment required for seed germination
A. odoratissima	1 - 2 kg	soaking over night
L. microcarpa	250 -500 g	nil
P. marsupium	4 - 5 kg	soaking over night
X. xylocarpa	1 - 2 kg	soaking over night

3.7.5. Recording observations on incidence of seedling diseases

Occurrence of seedling diseases if any, their symptoms and nature of damage were recorded in four standard beds assigned to each species. For post-emergence damping-off, total number of disease patches were counted and occurrence of

patches m^{-2} was calculated. For seedling blight or other foliar diseases, percent seedlings affected for a given density of seedlings in a seed bed was calculated (Sharma *et al.*, 1985).

3.7.6. Isolation and identification of causal organism

Appropriate parts of the diseased seedlings were collected for isolation and identification of the pathogens. Diseased specimens were taken in polythene bags to the laboratory under aseptic conditions. Generally potato dextrose agar (PDA) was used for isolation of fungal pathogens. Surface sterilisation of diseased specimens was done using 0.01% mercuric chloride followed by 2-3 changes of sterile water. Causal organism in pure culture was provisionally identified and identity confirmed through CAB International Mycological Institute, Kew, U.K.. The cultures were periodically subcultured and stored in a cold room at 25° $\pm$ 2°C.

3.7.7. Pathogenicity studies

For testing the pathogenicity of an isolate, a specially designed humidity chamber (Sharma *et al.*, 1985) fabricated locally was used. In the case of stem or root diseases of seedlings, the pathogenicity was tested on seedlings raised in aluminium trays (30 x 30 x 5 cm) with sterile garden soil. Initially, seedlings were transplanted to aluminium trays and

the seedlings were allowed to establish for a few days in the humidity chamber and then appropriately inoculated. For soil-borne diseases, the soil was infested with adequate quantity of inoculum of the test organism grown on corn meal sand medium dried and powdered (Sharma *et al.*, 1985). The trays were maintained in the humidity chamber for 10 to 20 days to observe the development of disease.

For inoculation of leaves a detached leaf culture technique was used. The detached leaves were floated on a solution of either 5-10 ppm of Benzimidazole or GA to prolong greenness (Sharma *et al.*, 1985).

3.7.8. Evaluation of fungicides for disease control

3.7.8.1. Poisoned food method(PFM)

According to the dosage, correct quantity of various fungicides was mixed thoroughly in sterilised PDA medium while it was luke warm. There were three -five petri plates for each concentration and each petri plate was inoculated at the centre with a mycelial disc of 4 mm taken from the margin of an actively growing colony. The inoculated plates were incubated at 25 $\pm$ 2°C till full radial growth was obtained in the control. Four diameter growth measurement were recorded in each petri plate. The percent inhibition of growth in each

treatment was calculated by the following equation (Vincent, 1927).

$$I = \frac{100(C - T)}{C}$$

where I = inhibition over control; C = growth in control and T = growth in treatment.

3.7.8.2. Soil fungicide screening method (SFSM)

The soil fungicide screening method (Zentmeyer, 1955; Cordon and Young, 1962) and modified by Sharma *et al.*(1985); and Sharma and Mohanan, (1991) was used to evaluate the efficacy of fungitoxicants against soil-borne micro-organisms, especially those producing sclerotia.

Sieved soil (3 mesh cm^{-2}) was autoclaved at 120°C for 30-45 min, and 10 g of this autoclaved soil was placed in a sterile glass vial of 80 x 30 mm. An 8 mm mycelial disc taken from the margin of an actively growing colony was kept over the soil. Another 10 g of sterile soil was placed over the mycelial disc. Appropriate quantity of fungicide solution (7-9 ml) of the desired concentration was gently poured over the soil surface and the mouth of the vial was closed with aluminium foil; each concentration of a fungicide had three replicate vials. All the vials were incubated at 25° $\pm$ 2°C for 24 h. The disc was gently removed with a sterile forceps, washed

in several changes of sterile water to remove the adhering soil particles and plated on PDA. Observations on the diameter growth were recorded till full radial growth was obtained in control. Diameter growth data of various treatments in PFM and SFSM were analysed using a two way analysis of variance.

3.7.9. Pilot scale nursery trials

The efficacy of the most effective fungicide identified in *in vitro* studies was evaluated in small scale field trials, conducted in a nursery area at Peechi of Trichur Forest Division. The soil of the area was thoroughly worked and experimental beds of 2m x 1m x 0.3 m were prepared at an espacement of 50 cm. The beds were provided with shade with coir mats which was removed partially when the seedlings were 30 days old and completely a month later.

Fungicides were applied as soil drench pre-sowing or as seed soaking or seed treatment . Schedule of fungicidal treatment, date of sowing, etc., is given in Table 10. Two to three days before sowing, fungicidal solution was applied @1.75-2 litre m^{-2} of the seed bed. After sowing, watering of beds was carried out 1-2 times daily till emergence and thereafter it was reduced or adjusted according to the prevailing weather conditions. Observations were recorded separately for post

emergence damping-off and other seedling diseases. For damping-off total number of active patches were counted and number of patches m^{-2} was calculated. For other seedling diseases, percent seedlings affected for a given density of seedlings in a seed bed were calculated. Since no seedling diseases were observed in *A. odoratissima*, no nursery trials were attempted.

Table 10. Schedule of fungicidal treatment, dosage (% a.i.) and type of application in 1990 nursery trial at Peechi

L. microcarpa	*P. marsupium*	*X. xylocarpa*
Carbendazim (0.2) Pre sowing soil drench	Carbendazim (0.1) Pre sowing soil drench	Carbendazim (0.1) Pre sowing soil drench
Thiram (0.2) Pre sowing soil drench	PCNB (0.2) Pre sowing soil drench	Thiram (0.2) Pre sowing soil drench
Carboxin(0.2) Pre sowing soil drench	Carboxin (0.2) Pre sowing soil drench	Carboxin (0.2) Pre sowing soil drench
MEMC (0.006) Pre sowing soil drench	MEMC (0.0125) Pre sowing soil drench	MEMC (0.006) Pre sowing soil drench
Copperoxychoride (0.2) Pre sowing soil drench	Thiram (0.2) Pre sowing soil drench	Thiram (0.3) Pre sowing soil drench
Captan (0.3) Dry seed treatment	Captan (0.3) Seed soaking overnight	Captan (0.3) Seed soaking overnight
Mancozeb (0.3) Dry seed treatment	Mancozeb (0.3) Seed soaking overnight	Mancozeb (0.3) Seed soaking overnight
Control No treatment	Control Seed soaking overnight	Control Seed soaking overnight

4. RESULTS

R E S U L T S

Results of various studies have been dealt with specieswise under the following heads.

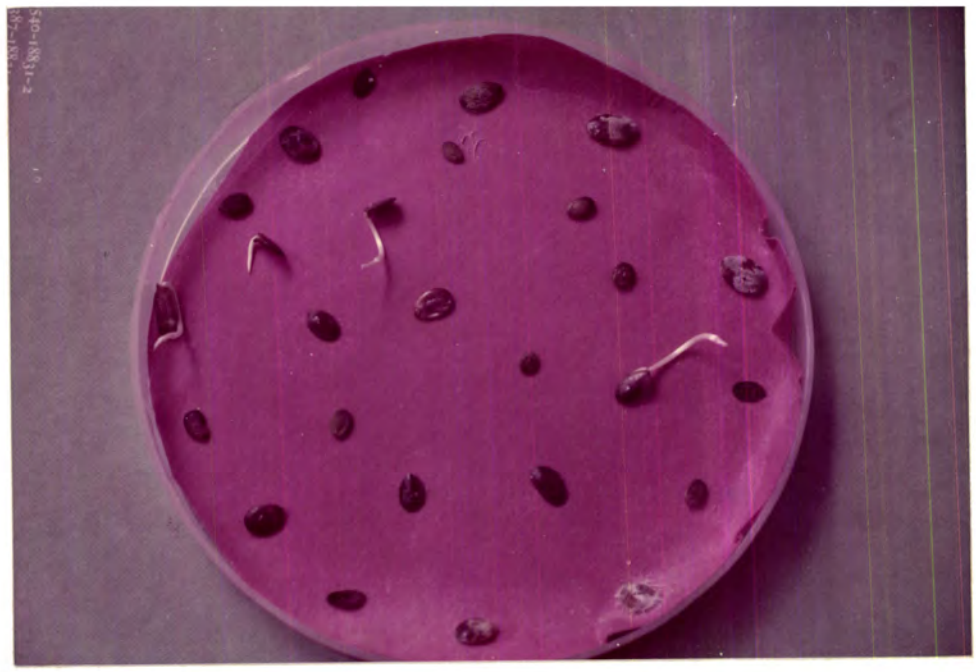
1. Evaluation of seed health testing methods
2. Seed microflora and their significance
3. Management of seed microflora
4. Seed storage and its influence on microflora, seed germination and seedling development and
5. Seedling diseases and their management

4A. *ALBIZIA ODORATISSIMA*

4A.1. Seed health testing methods

Of the fifteen micro-organisms recorded on non-surface sterilised seeds of *A. odoratissima* in different seed health testing methods, except actinomycetes, all were detected in SB method (Plate 2A; Table 11). Although actinomycetes grew well in 2,4-D and DF methods, surface sterilised seeds in all the methods did not harbour any actinomycetes. In non-surface sterilised seeds higher incidence of *Fusarium moniliforme* was recorded in SB method (Plate 2B), followed by other methods, while in surface sterilised seeds, its incidence was comparatively low in all the methods *Fusarium solani*, which made a rare appearance on a few seeds in SB method was not detected in other methods. *Colletotrichum gloeosporioides* was detected in all the methods employing non-surface sterilised seeds, while it was completely eliminated by surface sterilisation. A *Phomopsis* sp. not detected in the case of non-surface sterilised seeds, was found growing on surface sterilised seeds in PDA and MEA methods. Except 2,4,-D and DF methods, a Gram (-) bacterium was consistently recorded on both surface sterilised and non-surface sterilised seeds in other methods. In general, fungi like *Aspergillus flavus*, *A. niger*, *Cladosporium herbarum*, *Fusarium moniliforme*, *Penicillium citrinum* and *Rhizopus oryzae* recorded on non-surface sterilised seeds in all the methods, had lower incidence on surface sterilised seeds (Plate 3 & 4; Table 12).

A



B

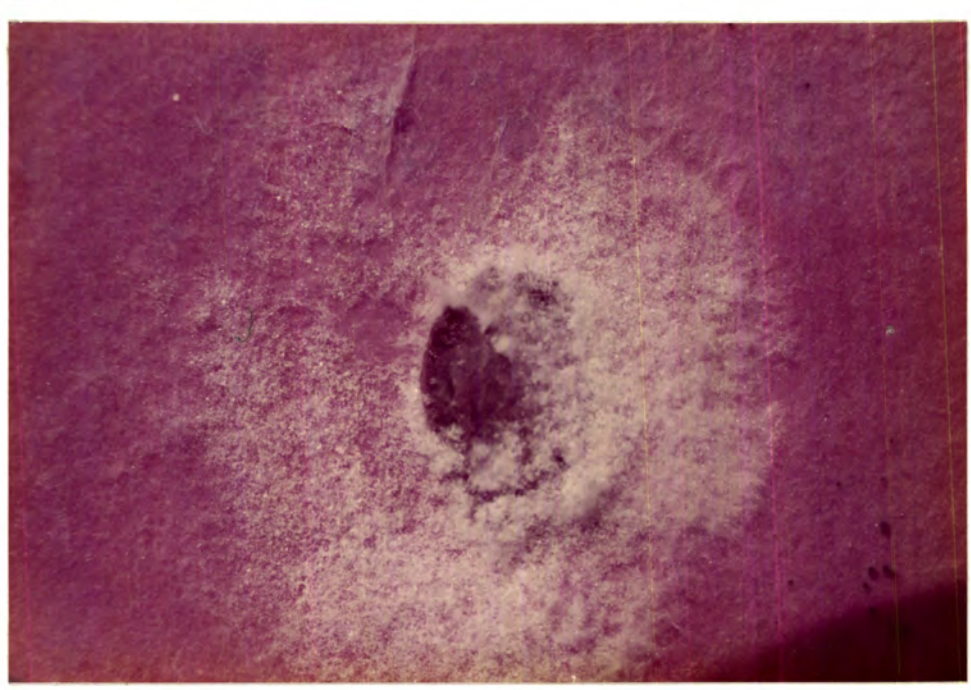
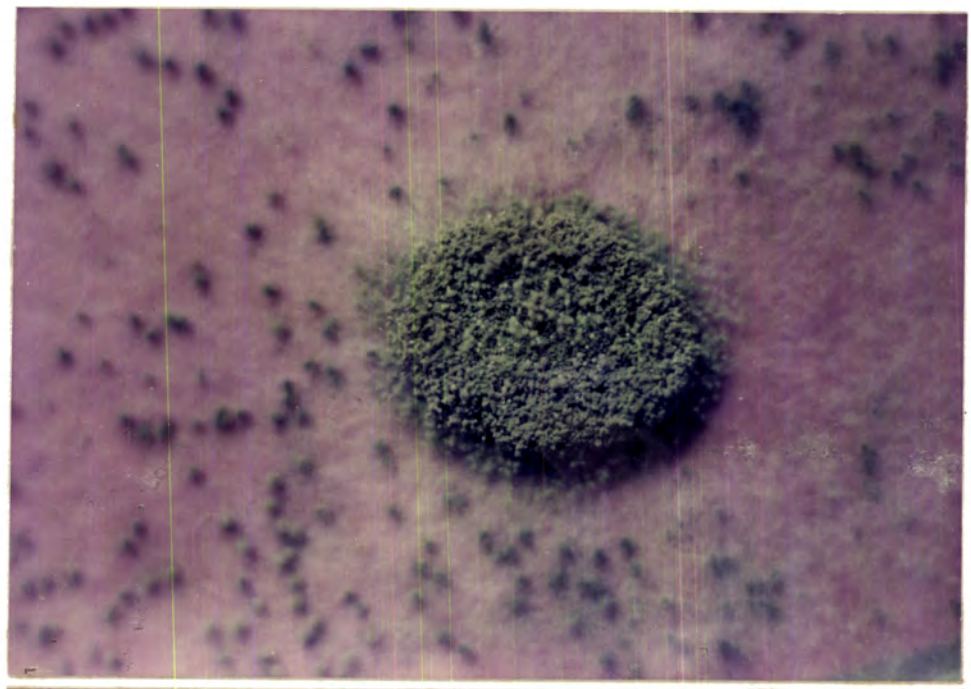


PLATE 2. *Albizia odoratissima*. A, Growth of various micro-organisms in blotter method; B, Profuse growth of *F. moniliforme* on seeds.

A



B

PLATE 3. *Albizia odoratissima*. A. Profuse growth of *Aspergillus flavus* on the seeds; B, Radicle rot symptoms caused by *A. flavus*.

A

B

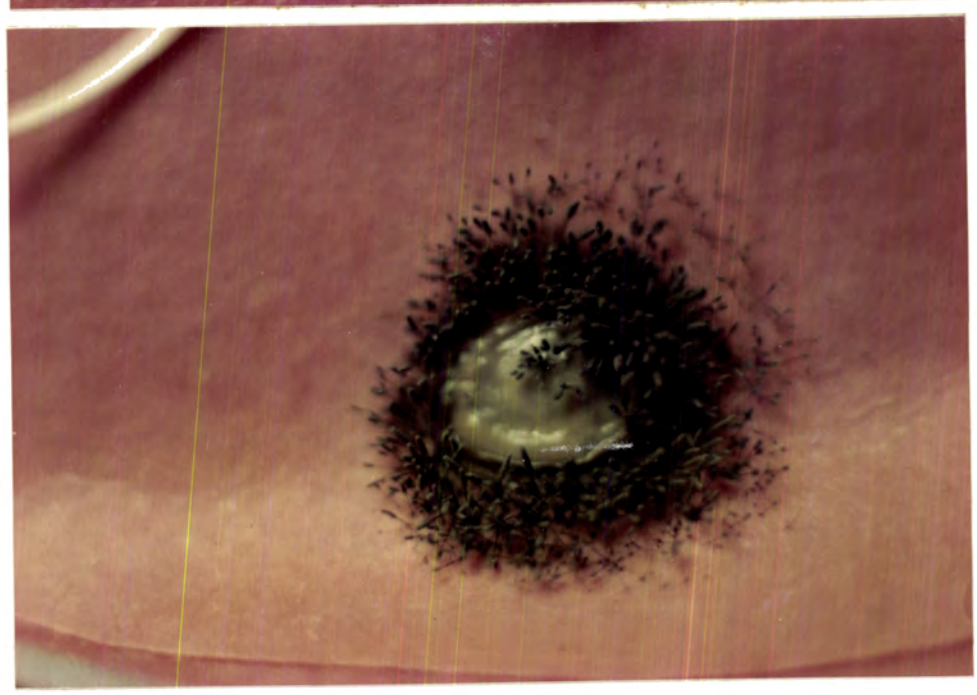


PLATE 4. A, Profuse ooze of a Gram (-) bacterium B, Growth of *Trichurus spiralis* and bacterium on the seeds of *A. odoratissima*.

Table 11. Percent incidence of spermoplane micro-organisms in different seed health testing methods on non-surface sterilised seeds of *A. odoratissima*

Micro-organism	Methods				
	SB	2,4-D	DF	PDA	MEA
Actinomycetes	0^{a*}	4.5^{b}	1.5^{a}	0^{a}	0^{a}
Aspergillus flavus Link.	3.5^{a}	8.5^{b}	3.5^{a}	4.0^{a}	4.5^{a}
A. niger van Tieghem	1.0^{a}	1.5^{a}	1.5^{a}	2.0^{a}	2.0^{a}
A. stellatus Curzi.	1.0^{b}	0^{a}	0^{a}	0^{a}	0^{a}
A. versicolor (vuill.) Tiraboschi	0.5^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Cladosporium herbarum (Pers.) Link. ex Gray	35.3^{b}	11.5^{a}	15.0^{a}	12.5^{a}	15.0^{a}
Colletotrichum gloeosporioides(Penz.)Penz & Sacc.	1.0^{a}	3.0^{b}	0.5^{a}	0.5^{a}	0.5^{a}
Fusarium moniliforme Sheld.	2.5^{b}	1.0^{ab}	1.0^{ab}	0.3^{a}	0.5^{a}
F. solani (Mart.) Sacc.	0.5^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Myrothecium roridum Tode: Fr	0.5^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Penicillium citrinum Thom.	16.5^{b}	21.0^{bc}	25.0^{c}	11.5^{a}	25.0^{c}
Rhizopus oryzae Went & Prinsen Geerligs	7.3^{b}	2.0^{a}	5.0^{a}	2.5^{a}	2.5^{a}
Trichurus spiralis Hasselbr.	0.5^{a}	0^{a}	0^{a}	0^{a}	0^{a}
sterile hyphae	0.5^{a}	0^{a}	0^{a}	0.5^{a}	0^{a}
Bacterium Gram (-)	4.5^{c}	0^{a}	0^{a}	3.5^{b}	2.5^{b}

* Mean values with the same superscript(s) do not differ significantly at p = 0.05 (Row-wise comparison)

Table 12. Percent incidence of spermoplane micro-organisms in different seed health testing methods on surface sterilised seeds of *A. odoratissima*

Micro-organism	Methods				
	SB	2,4-D	DF	PDA	MEA
Aspergillus flavus	5.0^{b*}	4.0^{b}	4.0^{b}	3.0^{b}	1.0^{a}
A. niger	1.0^{a}	1.5^{ab}	3.0^{b}	1.0^{a}	1.0^{a}
Cladosporium herbarum	3.0^{b}	2.0^{ab}	2.5^{b}	0^{a}	0^{a}
Fusarium moniliforme	1.0^{a}	1.5^{a}	0.5^{a}	0.5^{a}	0.5^{a}
Penicillium citrinum	3.0^{a}	6.0^{b}	6.0^{b}	2.5^{a}	6.0^{b}
Phomopsis sp.	0^{a}	0^{a}	0^{a}	1.0^{a}	1.0^{a}
Rhizopus oryzae	4.0^{a}	2.0^{a}	4.0^{a}	2.5^{a}	4.0^{a}
sterile hyphae	0.5^{a}	0^{a}	0.5^{a}	0^{a}	0^{a}
Bacterium Gram (-)	5.0^{b}	0^{a}	0^{a}	2.0^{a}	1.0^{a}

* Mean values with the same superscript(s) do not differ significantly at p=0.05 (Row- wise comparison)

4A.2. Seed microflora and their significance

4A.2.1. Dry seed examination

Macroscopic examination of seeds identified distinctly three categories of seeds, viz., apparently healthy, discoloured and deformed (Plate 5), the percentage being 43.8, 32 and 24.2 respectively; the average weight of 100 seeds for the

PLATE 5. Seeds of *A. odoratissima* showing apparently healthy (A), discolored (B) and deformed (C) categories.

three categories of seeds was 5.1, 3.5 and 1.6 g respectively, while the weight of 100 from the the pooled sample was 3.6 g.

4A.2.2. Incidence of micro-organisms in different categories of seeds

Apparently healthy seeds harboured less number of micro-organisms as compared with other categories of seeds. On non-surface sterilised seeds, *P. citrinum*, *C. herbarum*, *R. oryzae*, *A. flavus* and a bacterium were detected in higher percentage. However,their incidence was considerably reduced after surface sterilisation in all the categories of seeds . A total of 8, 11 and 12 microorganisms were detected in non-surface sterilised seeds of three categories respectively, which reduced to 5, 7 and 8 respectively after surface sterilisation. *Fusarium moniliforme* and *F. solani* were detected only in discoloured and deformed seeds. The germination percentage was also poor in deformed seeds, while discoloured and apparently healthy seeds had a better germination of 17% and 20.5% respectively, which increased to 18% and 24% after surface sterilisation (Figs.5 & 6)in SB method.

4A.2.3. Pathogenicity studies

Artificial inoculation with *Fusarium moniliforme*, *F. solani*, *Cladosporium herbarum*, *A. stellatus*, *T. spiralis* and *R. oryzae* affected the seed germination, growth and

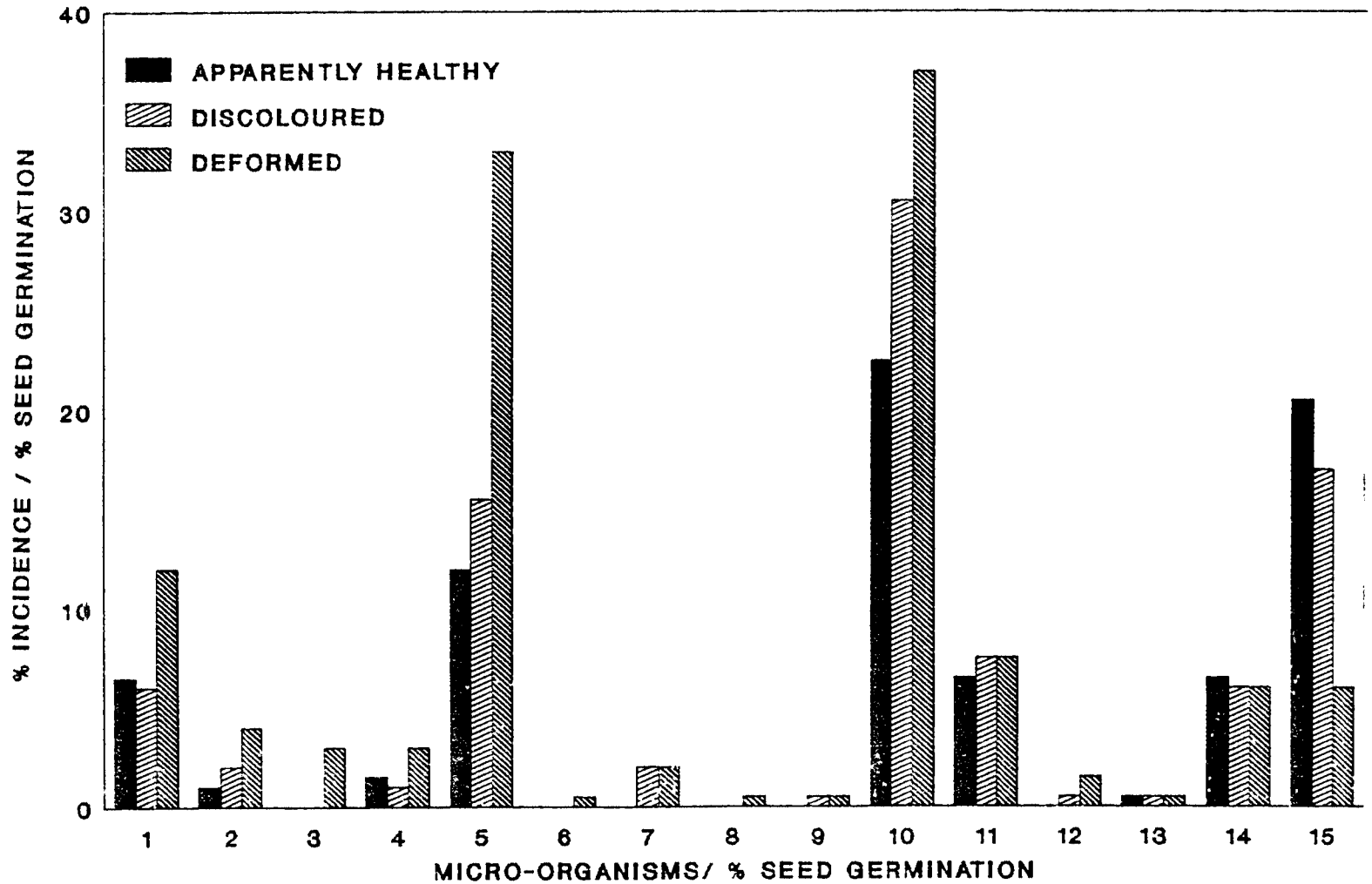


Fig.5. Percent incidence of spermoplane micro-organisms on non-surface sterilised seeds and % seed germination in different categories of seeds of *A. odoratissima*.

1. *Aspergillus flavus*, *A. niger*, 3. *A. stellatus*, 4. *versicolor*, *Cladosporium herbarum*, 6. *Colletotrichum gloeosporioides*, 7. *Fusarium moniliforme*, 8. *F. solani*, 9. *Myrothecium roridum*, 10. *Penicillium citrinum*, 11. *Rhizopus oryzae*, 12. *Trichurus spiralis*, 13. sterile hyphae. 14. Bacterium Gram (-), % seed germination.

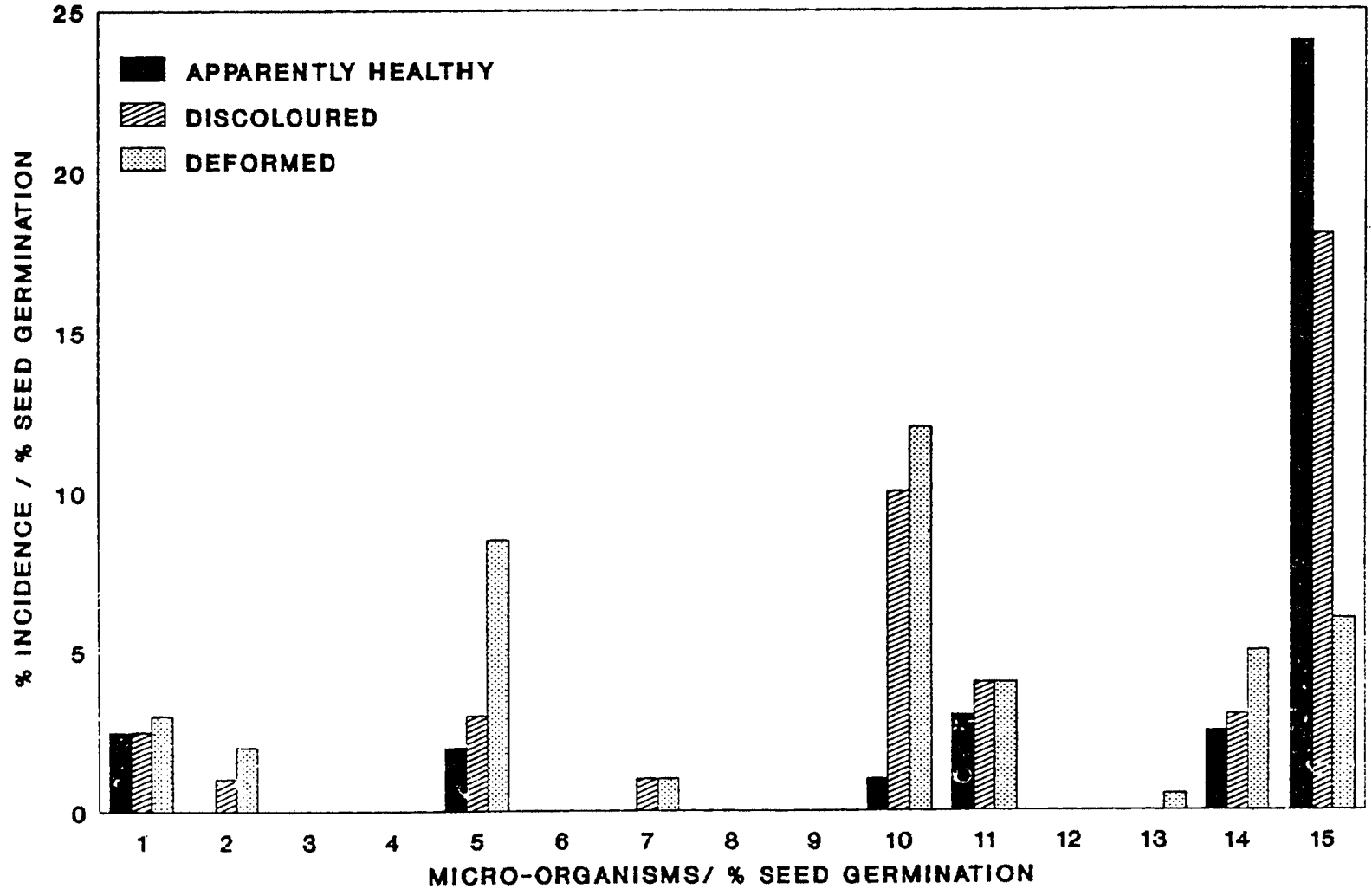


Fig.6. Percent incidence of spermoplane micro-organisms on surface sterilised seeds and % seed germination in different categories of seeds of *A. odoratissima*.

1. *Aspergillus flavus*, 2. *A. niger*, 3. *A. stellatus*, 4. *A. versicolor*, 5. *Cladosporium herbarum*, 6. *Colletotrichum gloeosporioides*, 7. *Fusarium moniliforme*, 8. *F. solani*, 9. *Myrothecium roridum*, 10. *Penicillium citrinum*, 11. *Rhizopus oryzae*, 12. *Trichurus spiralis*, 13. sterile hyphae, 14. Bacterium Gram (-), 15. % seed germination.

development of seedlings. *R. oryzae* and *T. spiralis* caused blight of seedlings and *A. stellatus, A. flavus, F. moniliforme, F. solani, C. herbarum , C. gloeosporioides* and *R. oryzae* caused decay of seeds. The percent seed germination was reduced considerably by *F. moniliforme, F. solani, A. stellatus, A. niger, C. herbarum, T. spiralis* and *R. oryzae* (Fig. 7). Mean shoot length was affected by *A. flavus, F. moniliforme, F. solani*, Bacterium and *T. spiralis*, while mean root length was reduced considerably by *T. spiralis*, and *F. moniliforme*. In other cases root length was higher as compared with control seeds. Curiously, seeds treated with *C. herbarum* recorded higher shoot and root lengths. Vigour index was the lowest in treatments of *Fusarium* spp. followed by *C. herbarum, A. flavus, A. stellatus*, Bacterium, *T. spiralis* and *R. oryzae* (Figs. 8A & B).

4A.3. Management of seed microflora

4A.3.1. Hot water treatment

Highest germination i.e., 36 % was recorded at 60°C-15 min. treatment (Table 13). The shoot length was not significantly reduced in any of the treatments as compared to control seedlings. The highest shoot and root lengths were recorded in treatments of 50°C-30 min. The root length of all

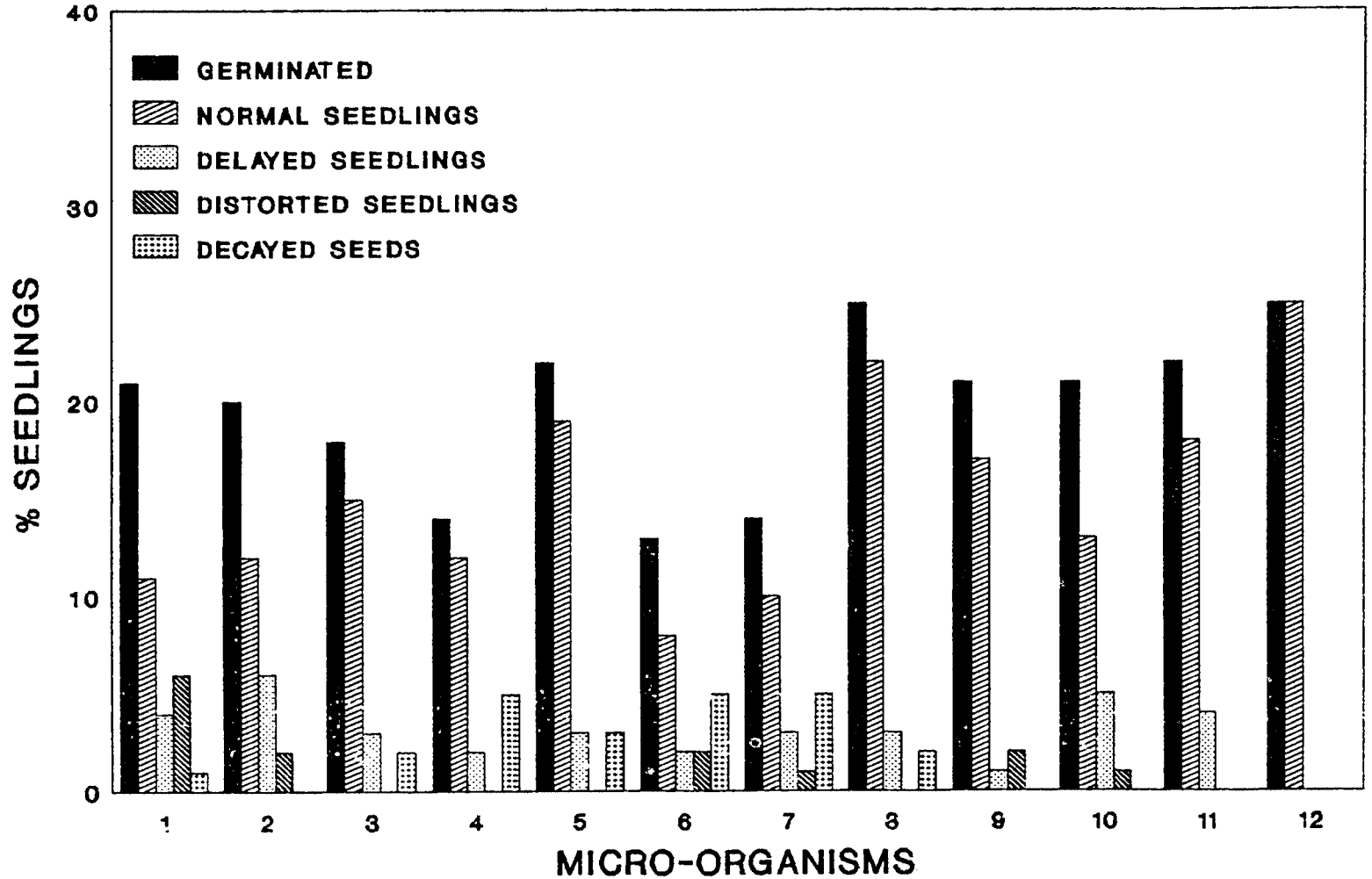
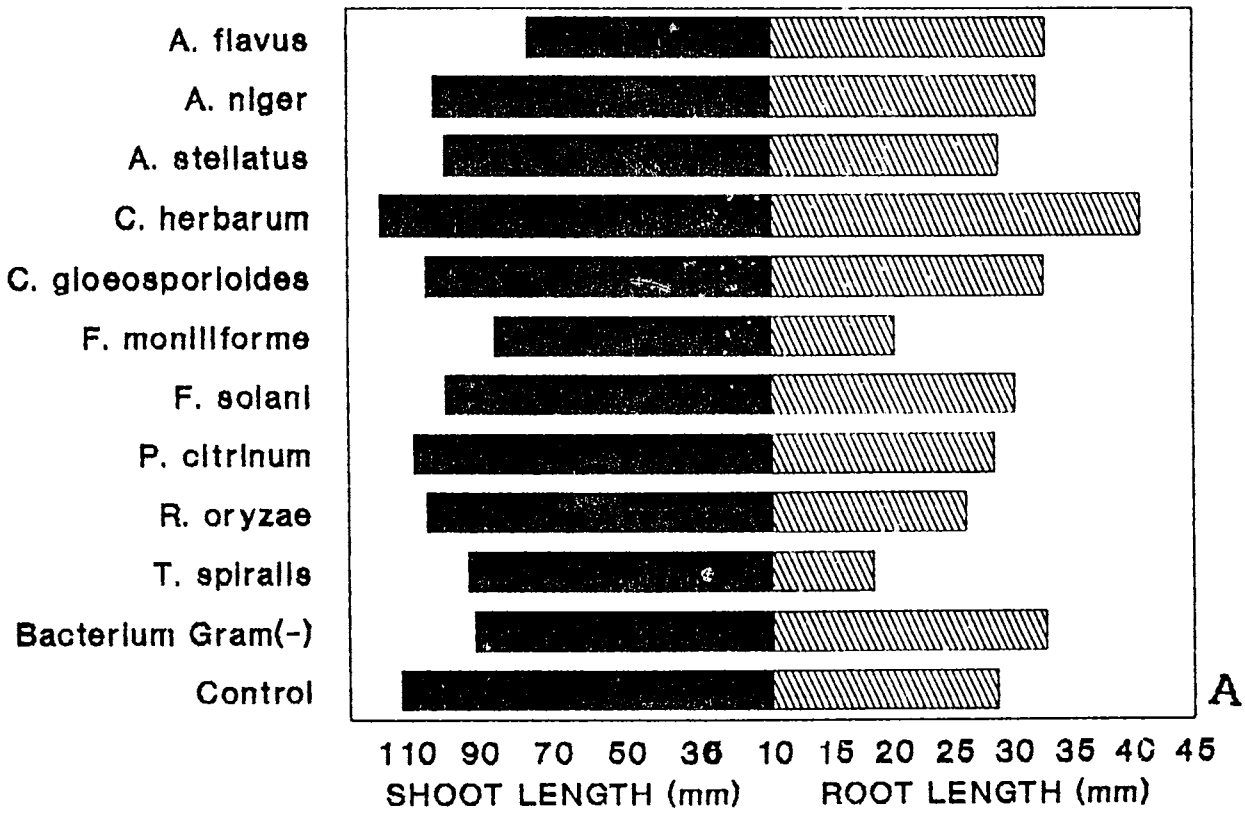


Fig.7. Effect of various micro-organisms on seed germination and seedling emergence of *A. odoratissima*.

1. *Aspergillus flavus*, 2. *A. niger*, 3. *A. stellitus*, 4. *Cladosporium herbarum*, 5. *Colletotrichum gloeosporioides*, 6. *Fusarium moniliforme*, 7. *F. solani*, 8. *Penicillium citrinum*, 9. *Rhizopus oryzae*, 10. *Trichurus spiralis*, 11. Bacterium Gram (-), 12. Control



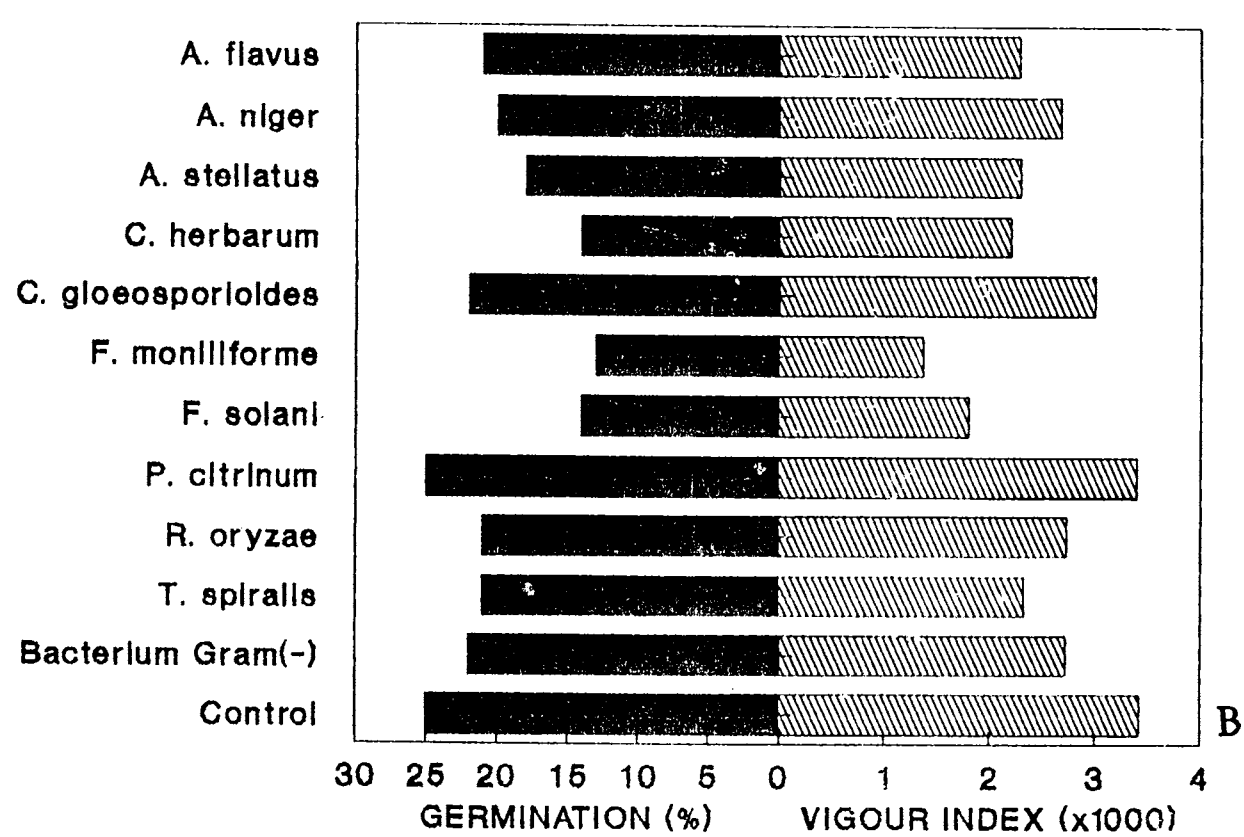


Fig.8. Effect of various micro-organisms on shoot and root length (A); seed germination and vigour index (B) of *A. odoratissima*.

Table 13. Effect of hot water treatment on seed germination and growth of seedlings of *A. odoratissima*

Observations	50°C		60°C		Control
	15 min.	30 min.	15 min.	30 min.	
Germination (%)	24^{a*}	26^{ab}	36^{b}	24^{a}	24^{a}
Shoot length(mm)	91.5^{ab}	96.9^{ab}	95.1^{ab}	82.8^{a}	81.6^{a}
Root length (mm)	35.9^{b}	37.2^{b}	36.2^{b}	34.7^{b}	26.7^{a}
Vigour index (VI)	3033.9^{ab}	3479.3^{ab}	3391.8^{ab}	4200.6^{b}	2580.0^{a}
No. of micro-organisms recorded	8	7	6	9	12

* Mean values superscribed by the same letter(s) do not differ significantly at p=0.05 (Row-wise comparison)

the treatments was significantly higher from untreated control. The vigour index of the treated seeds at 60°C- 30 min. was significantly higher as compared with other treatments. Hot water treatment did not induce sloughing - off the seed coat.

The number of micro-organisms developed in various hot water treatments ranged from 6-9, as compared with 12 in control seeds (Table 14). The incidence of Actinomycetes, *Aspergilllus niger, A. stellatus, Myrothecium roridum, Memnoniella echinata* and *T. spiralis* recorded on seeds treated

Table 14. Effect of hot water treatment on the % incidence of spermoplane micro-organisms of *A. odoratissima*

Micro-organism	50°C		60°C		Control
	15 min.	30 min.	15 min.	30 min.	
Actinomycetes	0^{a*}	0^{a}	0^{a}	0^{a}	1^{a}
Aspergillus flavus	9^{b}	0^{a}	4^{b}	5^{b}	11^{c}
A. niger	2^{a}	0^{a}	0^{a}	2^{a}	2^{a}
A. stellatus	0^{a}	1^{a}	0^{a}	9^{b}	3^{a}
Chaetomium globosum	0^{a}	0^{a}	22^{c}	3^{a}	0^{a}
Cladosporium herbarum	5^{a}	14^{b}	3^{a}	3^{a}	13^{b}
Fusarium moniliforme	12^{b}	2^{a}	12^{b}	5^{ab}	5^{ab}
F. solani	0^{a}	0^{a}	0^{a}	0^{a}	1^{a}
Myrothecium roridum	0^{a}	0^{a}	0^{a}	0^{a}	1^{a}
Memnoniella echinata	2^{a}	1^{a}	0^{a}	0^{a}	0^{a}
Penicillium citrinum	2^{a}	2^{a}	0^{a}	1^{a}	11^{b}
Rhizopus oryzae	0^{a}	0^{a}	0^{a}	0^{a}	2^{b}
Trichurus spiralis	4^{a}	3^{a}	6^{a}	3^{a}	11^{b}
Bacterium Gram (-)	9^{a}	15^{a}	12^{a}	6^{a}	10^{a}

*Mean values superscribed by the same letter(s) do not differ significantly at p = 0.05 (Row-wise comparison)

with hot water, was not significantly different from control, while *F. solani* was completely eliminated. *F. moniliforme* was

observed in all the treatments with the maximum incidence in 15 min. dip at 50°C and 60°C. The incidence of *Chaetomium globosum* was much higher at 60°C- 15 min. than in control and other treatments. *C. globosum* and *M. echinata* were recorded only in hot water treated seeds.

4A.3.2. Chemical Treatment

For high rate of seed germination and shoot length, captan was the best fungicide followed by carboxin, mancozeb, carbendazim and PCNB. Even though the treatments with thiram, MEMC and captafol reduced the germination, they were not significantly different from control. The shoot length of seedlings under various treatments did not differ significantly, while the root length differed (Table 15). Captafol reduced the root length significantly while higher root length was observed in carboxin, MEMC, carbendazim, mancozeb and thiram. Highest vigour index was recorded in seeds treated with captan, but it was not significantly different from carboxin, mancozeb, PCNB and carbendazim. In other treatments VI was not significantly different from control. Curiously the VI of treatments of MEMC, captafol and thiram was lower as compared with control.

Table 15. Effect of various seed dressers on seed germination and growth of seedlings of *A. odoratissima*

Treatment	Germination (%)	Mean shoot length (mm)	Mean root length(mm)	Vigour index	No.of micro-organism recorded
Captafol	18[a*]	57.5[a]	17.8[a]	1353.3[a]	1
Captan	35[c]	63.1[a]	29.0[bc]	3231.3[d]	2
Carbendazim	28[bc]	62.8[a]	35.4[c]	2744.6[cd]	1
Carboxin	30[bc]	58.0[a]	37.0[c]	2888.1[cd]	3
Mancozeb	30[bc]	57.5[a]	35.0[c]	2704.4[bcd]	1
MEMC	16[a]	60.8[a]	36.0[c]	1521.0[a]	1
PCNB	30[bc]	61.4[a]	30.7[c]	2675.6[bcd]	2
Thiram	20[ab]	58.0[a]	31.6[c]	1760.4[b]	3
Control	24[ab]	63.2[a]	20.6[ab]	1968.8[abc]	12

* Mean values in a column with the same superscript(s) do not differ significantly at p = 0.05

All the fungicides were effective in reducing the number of spermoplane micro-organisms (Table 16). Most of the storage micro-organisms, except *F. moniliforme* and a bacterium were more or less completely eliminated by fungicidal treatments. *F. moniliforme* was detected on seeds treated with thiram and carboxin, though at lower frequency than in control. However, its incidence in PCNB treated seeds was significantly higher as compared with all other treatments. The incidence of a

Table 16. Effect of various seed dressers on the % incidence of spermoplane micro-organisms of *A. odoratissima*

Micro-organism	Control	Captan	Captafol	Carbendazim	Carboxin	MEMC	Mancozeb	PCNB	Thiram
Aspergillus flavus	7^{b*}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	2^{a}
A. niger	3^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
A. stellatus	10^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Cladosporium herbarum	7^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	3^{b}	0^{a}
Colletotrichum gloeosporioides	1^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Fusarium moniliforme	10^{b}	0^{a}	0^{a}	0^{a}	6^{b}	0^{a}	0^{a}	29^{c}	5^{b}
F. solani	1^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Myrothecium roridum	1^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Penicillium citrinum	8^{b}	1^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Rhizopus oryzae	3^{b}	0^{a}	0^{a}	0^{a}	5^{b}	0^{a}	0^{a}	0^{a}	1^{a}
Trichurus spiralis	1^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Bacterium Gram (-)	6^{b}	9^{b}	9^{b}	24^{c}	11^{b}	18^{c}	22^{c}	0^{a}	0^{a}

*Mean values with the same superscript(s) do not differ significantly at p = 0.05 (Row-wise comparison)

bacterium which was controlled only by PCNB and thiram varied in other treatments and was significantly higher in treatments of mancozeb, carbendazim and MEMC.

4A.4. Seed storage and its influence on microflora, seed germination and seedling development.

4A.4.1. Incidence of micro-organisms

The micro-organisms observed in various treatments over a period of 1 year in storage (Table 17) indicated that most of the storage fungi observed initially continued to be recorded till the end of the storage. Infact storage of seeds under dehumidified conditions either at room temperature or low temperature decreased the number of micro-organisms. Most of the micro-organisms recorded in the above treatments were common storage fungi like *A. flavus, A. niger, P. citrinum, R. oryzae, C. herbarum, T. spiralis* etc. However, in addition to storage fungi, two new micro-organisms viz., *Memnoniella echinata* and *C. globosum* were recorded only on control seeds after 180 days of storage. *F. moniliforme* was recorded only up to 90 days , but *F. solani* continued its occurrence albeit in less frequency, till the end. However,in seeds stored under dehumidified condition, neither *F. moniliforme* nor *F. solani* was observed after 90 days of storage.

Table 17. Micro-organisms recorded on seeds of *A. odoratissima* stored for different periods under various treatments

Treatments	Day-1	Day-90	Day-180	Day-365
Captafol	14*	12,11	11	12
Captan	11,14	12	12	11,12
Carbendazim	14	2,12,14	2,14,12	1,2,14,15
Carboxin	7,12,14	7,11,14,15	7,11,14,15	1,7,14,15
Mancozeb	14	1,2,11	1,2	1,2,11
MEMC	14	12	12	12
PCNB	6,7	1,2,7,12,14	1,2,12,15	1,2,7,12,14
Thiram	1,7,12	1,7,11,15	1,11,15	1,6,11
Dehumidified cond. Room temperature	1,2,3,6,7, 8,9,10,11,14	1,2,3,4 11,12,15	1,2,3,4, 11,12,15	1,2,3,4, 11,12,15
Dehumidified cond. 4°C	2,3,5,6,7,10 11,12,14	1,6,8,10 11,12,13	1,2,6,11 12,13,15	1,2,5,6,11 12,13,14
Control (Plastic container)	1,2,3,5,6,7,8 9,10,11,12,14	1,2,3,4,6,7,8, 9,10,11,12,14	1,2,3,6,8,9,10, 11,12,14,16,17	2,3,4,5,8 10,11,12,13
Control (cloth bags)	1,2,3,5,6,7,8, 9,10,11,12,14	1,2,3,5,6 8,10,11,12	1,2,3,5,6,8,10 11,12,16,17	1,2,3,6,8,10, 11,12,15,16,17

*1. *Aspergillus flavus*, 2. *A. niger*, 3. *A. stellatus*, 4. *A. versicolor*, 5. *Colletotrichum gloeosporioides*, 6. *Cladosporium herbarum*, 7. *Fusarium moniliforme*, 8. *F. solani*, 9. *Myrothecium roridum*, 10. *Trichurus spiralis*, 11. *Penicillium citrinum*, 12. *Rhizopus oryzae*, 13. sterile hyphae, 14. Bacterium, 15. Actinomycetes, 16. *Chaetomium globosum*, 17. *Memnoniella echinata*

4A.4.2. Seed germination and seedling development

The percent seed germination in various treatments gradually decreased over the period of storage and only 4-6% of seeds germinated in most of the treatments except the seeds stored at 4°C under dehumidified conditions where the germination was 11% (Table 18). The vigour index also gradually decreased as the period of storage increased; under dehumidified condition (4°C) the VI was significantly higher from other treatments after 365-days of storage.

Analysis of variance of data on % seed germination and vigour index related to days of storage and treatment was found highly significant (Table 19).

Table 18. Effect of various seed dressers and storage conditions on % seed germination and vigour index of *A. odoratissima*

Treatment	Germination (%)				Vigour index (VI)			
	Day-1	Day-90	Day-180	Day-365	Day-1	Day-90	Day-180	Day-365
Captafol	18^{ab*}	16^{ab}	4^{a}	4^{a}	1353.3^{a}	1213.0^{a}	551.2^{ab}	398.1^{a}
Captan	35^{d}	26^{c}	6^{ab}	6^{a}	3231.3^{e}	2267.2^{bcd}	711.2^{ab}	728.2^{ab}
Carbendazim	28^{bcd}	20^{abc}	4^{a}	6^{a}	2744.6^{bcde}	2126.3^{bc}	709.9^{ab}	659.2^{ab}
Carboxin	30^{cd}	18^{ab}	8^{ab}	6^{a}	2888.1^{de}	1558.1^{ab}	533.5^{ab}	517.6^{ab}
Mancozeb	30^{cd}	20^{abc}	6^{ab}	4^{a}	2704.4^{bcde}	1705.4^{abc}	690.1^{ab}	601.0^{ab}
MEMC	16^{a}	13^{a}	8^{ab}	4^{a}	1521.0^{a}	1240.8^{a}	882.4^{abc}	651.1^{ab}
PCNB	30^{cd}	13^{a}	4^{a}	4^{a}	2675.6^{bcde}	1196.2^{a}	453.0^{a}	460.0^{ab}
Thiram	20^{abc}	12^{a}	8^{ab}	4^{a}	1760.4^{abc}	1077.2^{a}	988.2^{abc}	622.4^{ab}
Dehumidified cond. Room temp.	25^{abcd}	24^{bc}	10^{b}	6^{a}	2131.1^{abcd}	2458.0^{cd}	1148.9^{bc}	760.7^{ab}
Dehumidified cond.4°C	24^{abc}	26^{c}	17^{c}	11^{b}	1719.1^{ab}	3086.1^{d}	2078.1^{d}	1734.2^{c}
Control (plastic containers)	24^{abc}	18^{abc}	9^{b}	6^{a}	1968.8^{abcd}	1536.9^{ab}	1395.8^{cd}	786.5^{ab}
Control (cloth bags)	24^{abc}	19^{abc}	9^{b}	6^{a}	1929.5^{abcd}	1775.7^{abc}	1554.5^{cd}	766.5^{ab}

*Mean values in a column with the same superscript (s) do not differ significantly at p = 0.05

Table 19. Analysis of variance of germination and vigour index of seeds of *A. odoratissima* stored for 1 year

Sources	Germination			Vigour index		
	DF	MSS	F	DF	MSS	F
Day	3	2988.1	218.1**	3	3893.6	101.9**
Treatment	11	91.3	6.7**	11	314.4	8.2**
Day x treatment	33	29.6	2.2**	33	122.2	3.2**
Residual	144	13.7	-	139	38.2	-

** significant at p= 0.01

4A.5. Seedling diseases and their management

In all the nurseries surveyed, no seedling diseases were recorded either in seed beds or containers (Plate 6).

PLATE 6. A view of the nursery bed of *A. odoratissima* showing healthy seedlings.

4B. *LAGERSTROEMIA MICROCARPA*

4B.1. Seed health testing methods

Most of the field and storage micro-organisms were recorded in PDA, DF and SB methods. A few micro-organisms appeared in one or more methods such as *Alternaria alternata* was detected only by PDA and DF methods and *Phomopsis* sp. was recorded only in PDA and MEA methods (Table 20). Interestingly *Curvularia lunata* which appeared in varying intensities expressed poorly in MEA method. *Fusarium solani* was observed in all the methods and its incidence in SB and 2,4-D methods was higher than in other methods. Though a Gram (-) bacterium was observed in all the methods, its incidence was significantly higher in SB and 2,4-D methods. The surface sterilisation of seeds reduced the incidence of most of the field and storage micro-organisms and *Alternaria alternata* was completely eliminated (Table 21). For the growth of most micro-organisms, SB method was the best followed by DF and MEA methods (Plate 7).

4B.2. Seed microflora and their significance

4B.2.1. Dry seed examination

Seed examination showed the presence of apparently healthy, discoloured, and discoloured and broken seeds (Plate 8). The occurrence of healthy seeds was a meagre 10%, followed

Table 20. Percent incidence of spermoplane micro-organisms in different seed health testing methods on non-surface sterilised seeds of *L. microcarpa*

Micro-organism	Methods				
	SB	2,4-D	DF	PDA	MEA
Alternaria alternata(Fr.) Keissler	0^{a*}	0^{a}	0.8^{a}	2.8^{b}	0^{a}
Aspergillus flavus Link.	5.8^{b}	2.8^{ab}	2.8^{ab}	1.3^{a}	3.0^{ab}
A. niger van Tieghem	5.0^{ab}	7.0^{b}	1.3^{a}	3.5^{ab}	2.0^{a}
Curvularia lunata (Wakker)Bodijn	3.5^{ab}	4.3^{b}	3.0^{ab}	1.8^{a}	0.5^{a}
Fusarium solani(Mart.)Sacc.	4.8^{ab}	7.3^{b}	1.5^{a}	1.0^{a}	1.0^{a}
Memnoniella echinata (Riv.) Galloway	8.8^{c}	3.0^{a}	5.0^{ab}	1.0^{a}	0.8^{a}
Phomopsis sp.	0^{a}	0^{a}	0^{a}	10.5^{b}	2.8^{b}
Penicillium citrinum Thom.	11.5^{b}	1.8^{a}	5.0^{a}	2.0^{a}	2.0^{a}
Rhizopus oryzae Went & Prinsen Geerligs.	6.8^{b}	3.0^{a}	5.0^{b}	1.0^{a}	0.8^{a}
sterile hyphae (black)	2.8^{b}	0^{a}	0^{a}	0^{a}	0^{a}
sterile hyphae (white)	0^{a}	0^{a}	0.8^{a}	0^{a}	0^{a}
Bacterium Gram (-)	11.5^{c}	6.3^{c}	4.8^{ab}	2.0^{a}	2.0^{a}

* Mean values with the same superscript(s) do not differ significantly at p = 0.05 (Row-wise comparison)

Table 21. Percent incidence of spermoplane micro-organisms in different seed health testing methods on surface sterilised seeds of *L. microcarpa*

Micro-organism	Methods				
	SB	2,4-D	DF	PDA	MEA
Aspergillus flavus	3.3^{b*}	0^{a}	1.5^{ab}	0^{a}	0.5^{a}
A. niger	1.5^{a}	6.5^{b}	0.8^{a}	0.8^{a}	0.5^{a}
Curvularia lunata	1.8^{a}	2.5^{a}	1.0^{a}	0.5^{a}	0.5^{a}
Fusarium solani	1.8^{a}	7.0^{b}	0.8^{a}	2.0^{a}	0.8^{a}
Memnoniella echinata	5.3^{b}	0^{a}	1.5^{a}	0^{a}	0^{a}?
Phomopsis sp.	0^{a}	0^{a}	0^{a}	3.8^{b}	1.3^{a}
Penicillium citrinum	0.8^{a}	0^{a}	0^{a}	0.8^{a}	1.0^{a}
Rhizopus oryzae	2.8^{b}	2.8^{b}	2.0^{b}	0.8^{a}	0^{a}
sterile hyphae (black)	0.8^{a}	0^{a}	0^{a}	0^{a}	0^{a}
sterile hyphae (white)	1.0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Bacterium Gram (-)	6.3^{b}	5.5^{b}	0^{a}	0^{a}	0.5^{a}

* Mean values with the same superscript(s) do not differ significantly at p = 0.05 (Row-wise comparison)

by discoloured seeds (24%) and broken seeds (66%). However, the weight of 100 seeds of these three categories did not differ appreciably; the apparently healthy seeds weighed 325 mg, followed by 312.5 mg and 306.8 mg respectively for the discoloured and broken seeds. The average weight of 100 seeds of pooled sample was 320 mg.

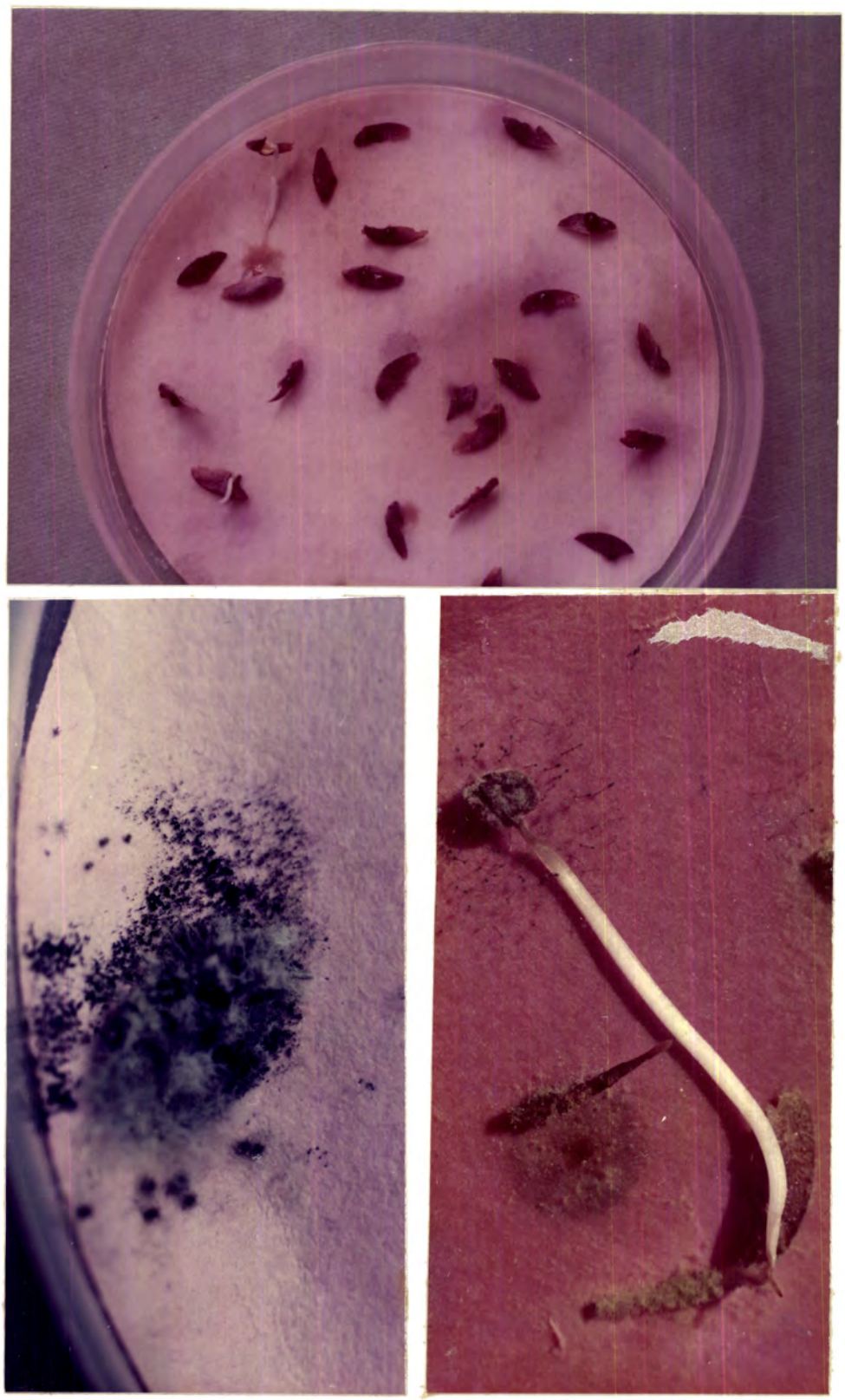


PLATE 7. *Lagerstroemia microcarpa*; A, Growth of various micro-organisms in blotter method; B, Profuse growth of *A. niger* and *F. solani*; C, *A. flavus* causing plumule rot.

A B C

PLATE 8. Seeds of *L. microcarpa* showing apparently healthy (A), discolored (B) and discolored and broken (C) categories.

4B.2.2. Incidence of micro-organism in different categories of seeds

The incidence of various micro-organisms in apparently healthy seeds was higher in non-surface sterilised seeds, as compared with the sterilised seeds. Surface sterilisation of seeds eliminated completely *C. lunata* and *F. solani*. The % germination of surface sterilised seeds was 11% as compared with 9% in non-surface sterilised seeds The percent incidence of micro-organisms in discoloured seeds was higher as compared with apparently healthy seeds. In this case also, surface sterilisation eliminated both *C. lunata* and *F. solani*. However, the germination % of seeds did not alter due to surface sterilisation. Eleven micro-organisms were recorded from non-surface sterilised seeds of discoloured and broken seed category. In surface sterilised seeds the incidence of micro-organisms was less in comparison to non-surface sterilised seeds. The germination was only 5% in the case of non-surface sterilised seeds of discoloured and broken category as compared with 8% in surface sterilised seeds (Figs.9 & 10).

4B.2.3. Pathogenicity studies

Delayed germination was noticed in treatments involving *A. flavus, C. lunata, Phomopsis* sp., *R. oryzae* and bacteria. No blighted and distorted seedlings were recorded in any of the treatments (Fig.11). *Fusarium solani* was pathogenic

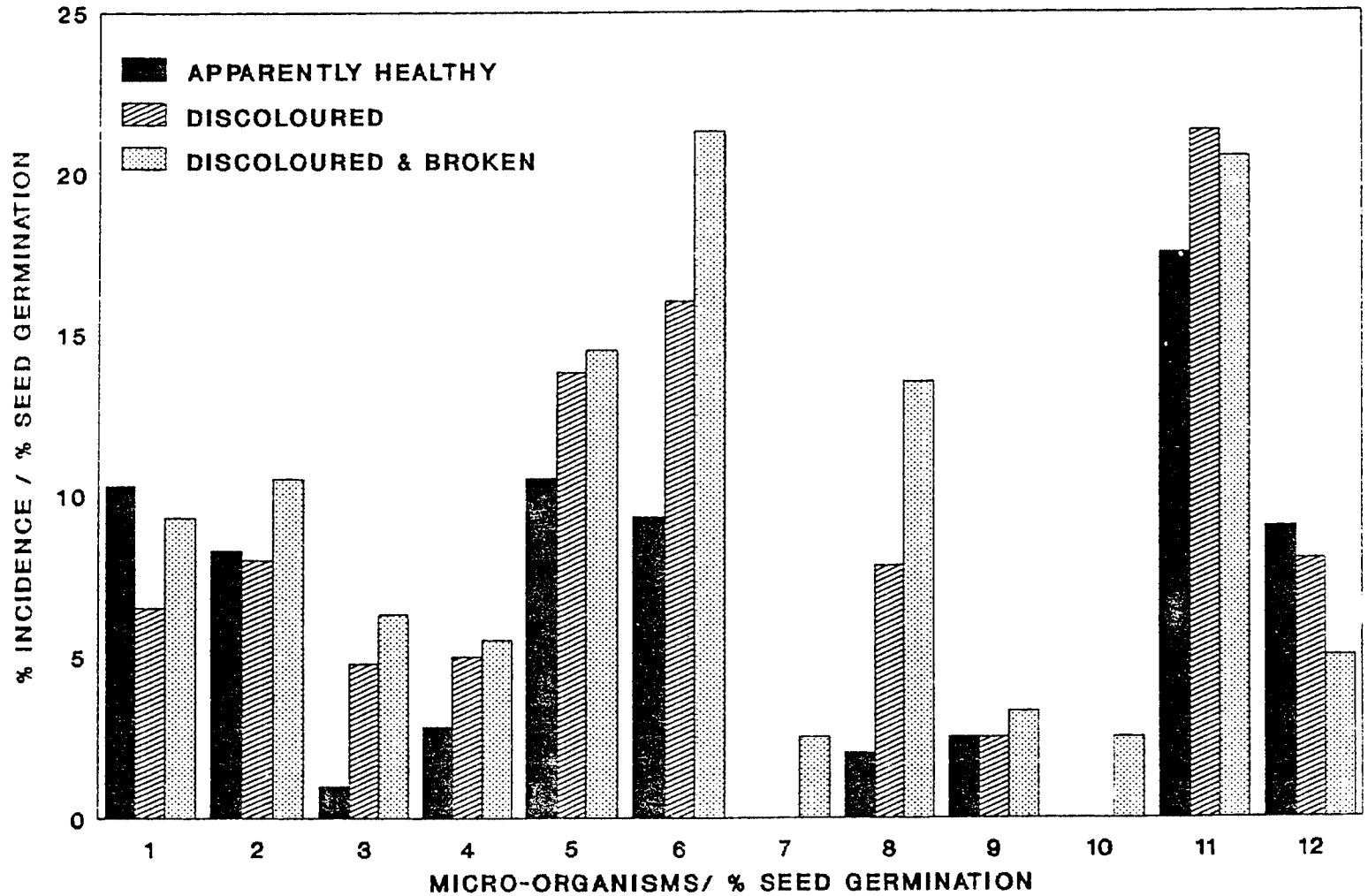


Fig.9. Percent incidence of spermoplane micro-organisms on non-surface sterilised seeds and % seed germination in different categories of seeds of *L. microcarpa*.

1. *Aspergillus flavus*, 2. *A. niger*, 3. *Curvularia lunata*, 4. *Fusarium solani*, *Memnoniella echinata*, 6. *Penicillium citrinum*, 7. *Penicillium* sp., 8. *Rhizopus oryzae*, 9. sterile hyphae (white), 10. sterile hyphae (black), 11. Bacterium Gram(-), 12. % seed germination

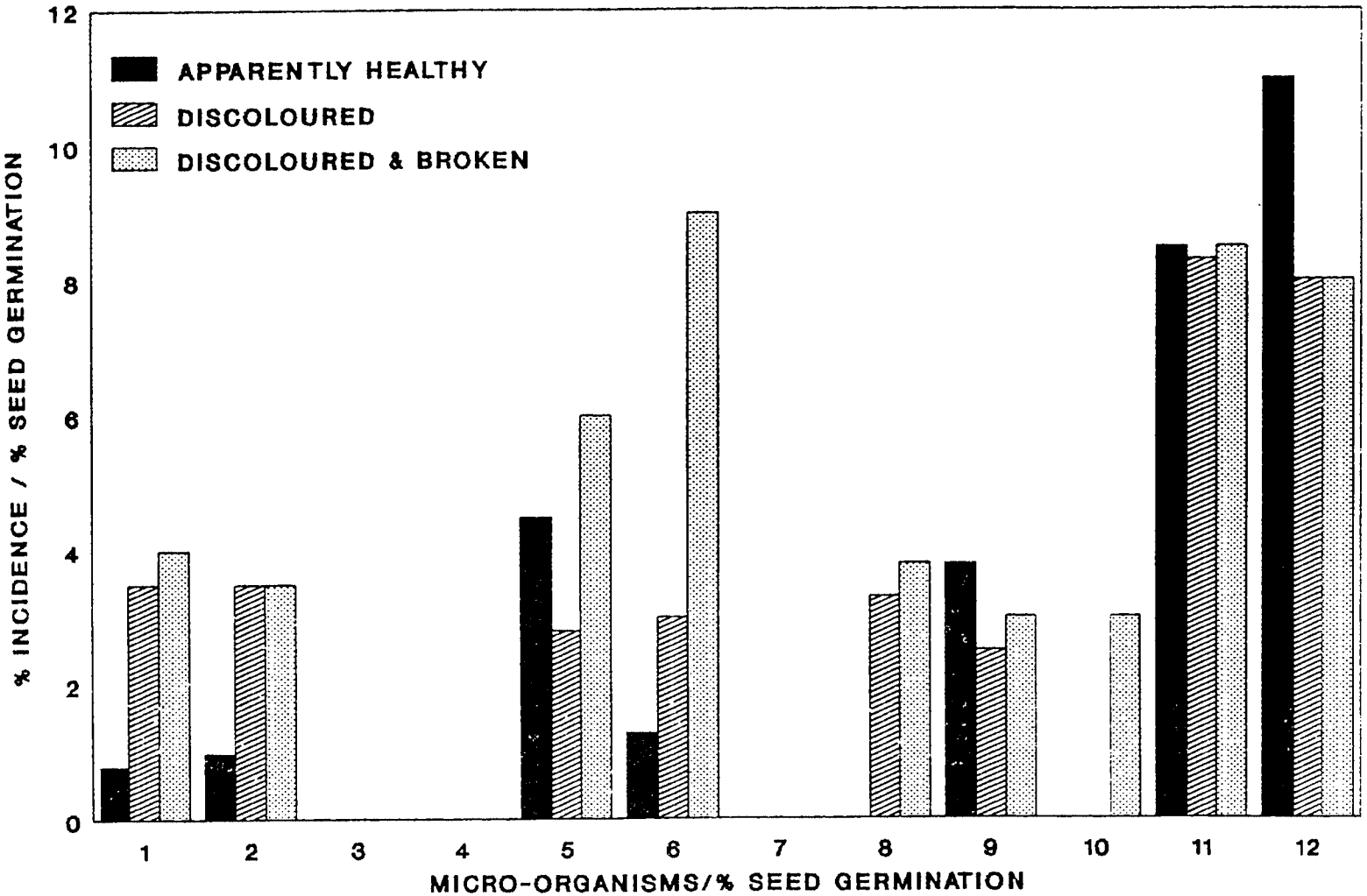


Fig.10. Percent incidence of spermoplane micro-organisms on surface sterilised seeds and % seed germination in different categories of seeds of *L. microcarpa*.

1. *Aspergillus flavus*, 2. *A. niger*, 3. *Curvularia lunata*, 4. *Fusarium solani*, 5. *Memnoniella echinata*, 6. *Penicillium citrinum*, 7. *Penicillium* sp., 8. *Rhizopus oryzae*, 9. sterile hyphae (white), 10. sterile hyphae (black), 11. Bacterium Gram(-), 12. % seed germination.

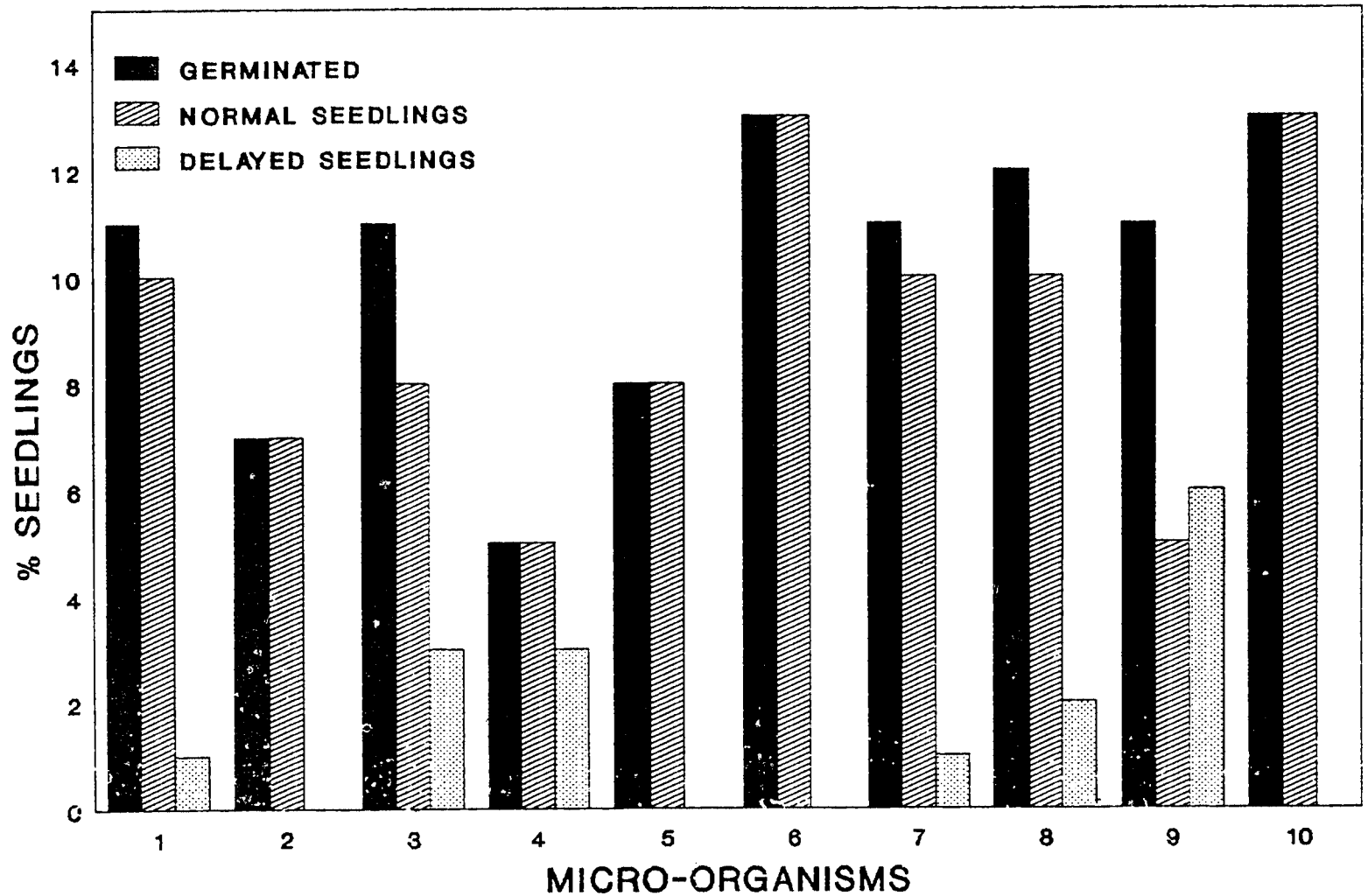


Fig.11. Effect of various micro-organisms on seed germination and seedling emergence of *L. microcarpa*.

1. *Aspergillus flavus*, 2. *A. niger*, 3. *Curvularia lunata*, 4. *Fusarium solani*, 5. *Memnoniella echinata*, 6. *Penicillium citrinum*, 7. *Phomopsis* sp., 8. *Rhizopus oryzae*, 9. Bacteria Gram (-), 10. Control.

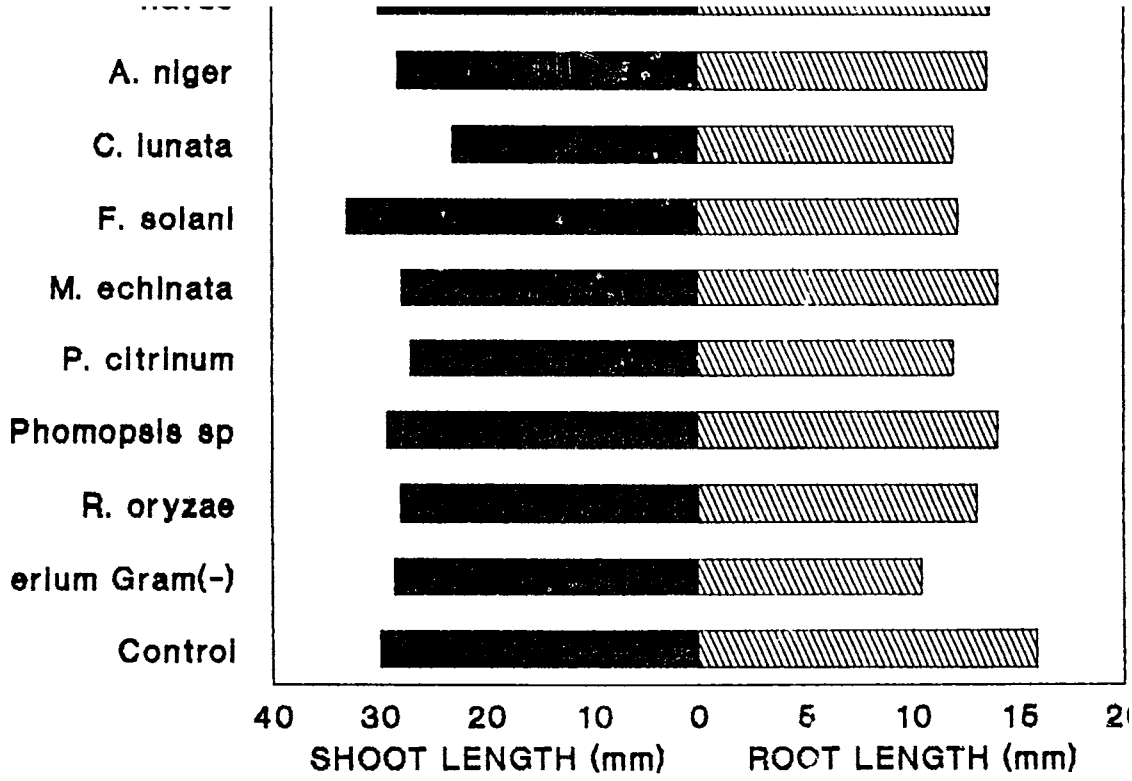
to seeds of *L. microcarpa*. The seeds treated with *F. solani* had a poor germination of 5% in comparison to 13% in control. The vigour index was 226, which was followed by 292 for *A. niger* and 335 for *M. echinata*. Other micro-organisms tested were not pathogenic. Generally the shoot and root lengths were not affected except for *C. lunata* and a bacterium respectively (Fig.12).

4B.3. Management of seed microflora

4B.3.1. Hot water treatment

Seed germination was affected significantly by hot water treatment. In 15 min. exposures at 50° and 60°C only 3 % of seeds germinated, while no seeds germinated in 30 min. exposures at both the temperatures. Shoot and root lengths did not show any significant reduction over control in all the treatments (Table 22).

Hot water treatment eliminated completely *Curvularia lunata* and *Cladosporium herbarum* and incidence of *F. solani* was reduced significantly in all the treatments. However, other common storage fungi were not controlled and they were recorded in different intensities. Interestingly the incidence of *Penicillium citrinum* was higher in the case of seeds treated with hot water than control (Table 23).



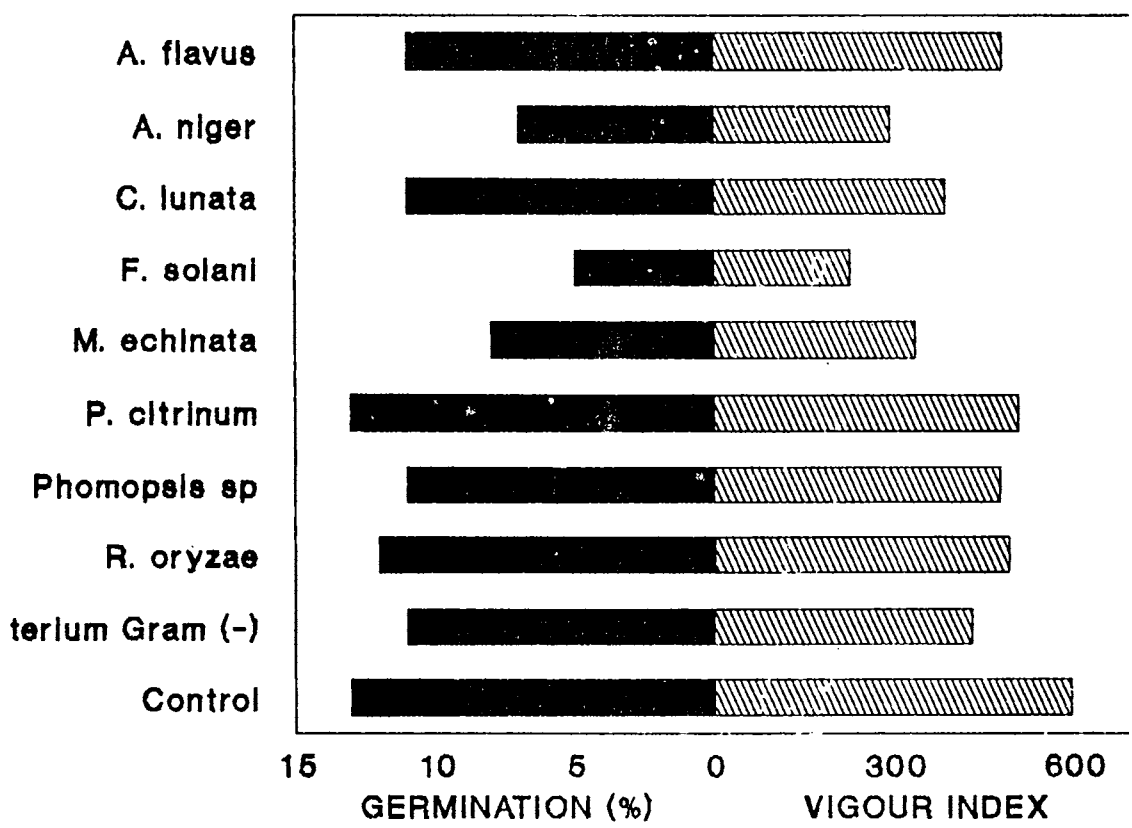


Fig.12. Effect of various micro-organisms on shoot and root length (A); seed germination and vigour index (B) of *L. microcarpa*.

Table 22. Effect of hot water treatment on % seed germination and growth of seedlings of *L. microcarpa*

Observations	50°C		60°C		Control
	15 min.	30 min.	15 min.	30 min.	
Germination (%)	3[a*]	0[a]	3[a]	0[a]	11[b]
Shoot length (mm)	22.0[b]	0[a]	17.8[b]	0[a]	23.1[b]
Root length (mm)	11.1[b]	0[a]	7.7[b]	0[a]	12.1[b]
Vigour Index (VI)	99.3[b]	0[a]	76.5[b]	0[a]	387.2[c]
No.of micro-organism recorded	5	6	7	6	10

* Means values superscribed by the same letter(s) do not differ significantly at p = 0.05 (Row-wise comparison).

4B.3.2. Chemical treatment

Mancozeb was the most effective fungicide in bringing about the highest vigour index followed by carboxin, MEMC, carbendazim and captan. Mancozeb recorded the highest shoot length; however, shoot and root lengths recorded in most of the treatments were not significantly different from the un-treated control (Table 24).

Table 23. Effect of hot water treatment on the % incidence of spermoplane micro-organisms of *L. microcarpa*

Micro-organism	50°C		60°C		Control
	15 min.	30 min.	15 min.	30 min.	
Aspergillus flavus	3^{a*}	2^{a}	2^{a}	0^{a}	1^{a}
A. niger	0^{a}	1^{a}	2^{a}	0^{a}	1^{a}
Chaetomium globosum	5^{b}	3^{a}	4^{a}	1^{a}	5^{b}
Cladosporium herbarum	0^{a}	0^{a}	0^{a}	0^{a}	1^{a}
Curvularia lunata	0^{a}	0^{a}	0^{a}	0^{a}	1^{a}
Fusarium solani	4^{a}	5^{a}	4^{a}	1^{a}	11^{b}
Memnoniella echinata	2^{a}	1^{a}	2^{a}	1^{a}	3^{a}
Penicillium citrinum	18^{a}	35^{b}	29^{ab}	27^{ab}	27^{ab}
Rhizopus oryzae	0^{a}	0^{a}	0^{a}	2^{a}	1^{a}
sterile hyphae	0^{a}	0^{a}	3^{b}	1^{a}	2^{a}

* Means values superscribed by the same letter(s) do not differ significantly at p = 0.05 (Row-wise comparison).

Table 24. Effect of various seed dressers on germination and growth of seedlings of *L. microcarpa*

Treatment	Germination (%)	Mean shoot length (mm)	Mean root length (mm)	Vigour index	No. of micro-organisms recorded
Captafol	8[a*]	27.2[a]	13.8[ab]	326.3[a]	3
Captan	13[a]	29.3[ab]	11.4[a]	517.8[ab]	1
Carbendazim	11[a]	30.0[ab]	15.1[ab]	518.5[ab]	5
Carboxin	11[a]	31.4[ab]	20.2[b]	577.2[b]	4
Mancozeb	11[a]	38.0[b]	15.3[ab]	581.0[b]	3
MEMC	11[a]	27.8[a]	19.6[b]	531.9[ab]	5
PCNB	10[a]	28.5[ab]	15.2[ab]	402.8[ab]	4
Thiram	11[a]	28.5[ab]	15.1[ab]	487.5[ab]	4
Control	10[a]	25.7[a]	15.0[ab]	441.5[ab]	9

*Mean values in a column with the same superscript(s) do not differ significantly at p = 0.05

The incidence of various micro-organisms after fungicidal treatment is given in Table 25. *Curvularia lunata* was arrested by captan, captafol, carboxin, thiram , MEMC, PCNB, mancozeb, while occurrence of *F. solani* was checked by captan. Other common storage fungi recorded in various intensities are also shown in Table 25. Captan was the best fungicide in eliminating all the micro-organisms except a bacterium followed by mancozeb and captafol.

Table 25. Effect of various seed dressers on the % incidence of spermoplane micro-organisms of *L. microcarpa*

Micro-organism	Control	Captan	Capta-fol	Carben-dazim	Carbo-xin	MEMC	Manco-zeb	PCNB	Thiram
Aspergillus flavus	7^{b*}	0^{a}	0^{a}	4^{b}	8^{b}	3^{a}	4^{b}	8^{b}	2^{a}
A. niger	9^{b}	0^{a}	0^{a}	2^{a}	2^{a}	2^{a}	0^{a}	3^{a}	0^{a}
Chaetomium globosum	2^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Curvularia lunata	7^{b}	0^{a}	0^{a}	5^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Fusarium solani	9^{c}	0^{a}	2^{a}	4^{b}	4^{b}	3^{b}	2^{a}	5^{b}	3^{a}
Memnoniella echinata	8^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Penicillium citrinum	8^{b}	0^{a}	7^{b}	0^{a}	0^{a}	2^{a}	0^{a}	0^{a}	2^{a}
Rhizopus oryzae	7^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Bacterium Gram (-)	9^{b}	2^{a}	5^{b}	7^{b}	4^{b}	2^{a}	8^{b}	6^{b}	10^{b}

*Mean values subscribed by the same letter(s) do not differ significantly at p = 0.05 (Row-wise comparison)

4B.4. Seed storage and its influence on microflora, seed germination and seedling development

4B.4.1. Incidence of micro-organisms

Incidence of microflora of treated seeds of *L. microcarpa* stored for a period of 1-year is given in Table 26. In control seeds most of the storage micro-organisms observed initially remained till the end of observations except *F. solani* which was not detected after 180 days of storage. The incidence of a species of *Pestalotiopsis* was observed on a few seeds stored for 90 days under dehumidified conditions at room temperature. In general the number of micro-organisms recorded on seeds under dehumidified condition was less as compared with "control" seeds. However, common storage micro-organisms continued their presence even up to 1-year of storage.

4A.4.2. Seed germination and seedling development

Generally, % germination gradually declined over increased period of storage in all the treatments including control. In most of the treated seeds, the initial germination % ranging between 8 to 13% came down to 2 to 5 % in a period of 1 year. However, the seeds stored at 4°C - dehumidified conditions recorded a germination of 7% at the end of 1-year (Table 27). Prolonged storage decreased the vigour index.

Table 26. Micro-organisms recorded on seeds of *L. microcarpa* stored for different periods under various treatments

Treatments	Day-1	Day-90	Day-180	Day-365
Captafol	5,7,10*	5,7,10	5,7,10	2,7,8,9
Captan	10	10	10	10
Carbendazim	2,3,4,5,10	2,4,5,10	2,4,5,10	2,3,7,10
Carboxin	2,3,5,10	2,3,5,9	2,3,5,9	2,3,7,10
Mancozeb	2,5,10	2,5,10	3,5,10	2,3,7,10
MEMC	2,3,5,7,10	2,3,5,10	2,3,5,10	2,3,7,10
PCNB	2,3,5,10	1,2,3,5,10	2,3,4,5,10	2,3,7,10
Thiram	2,5,7,10	2,5,10	2,5,10	2,3,10
Dehumidified cond. Room temp.	2,3,4,5,6, 7,8,10,11	1,2,3,5, 8,9,12	2,3,5,7,9	2,3,6,7
Dehumidified cond. 4° C	2,3,4,5,6 7,8,10,11	4,5,9	4,5,7,9	3,6,7
Control (Plastic container)	2,3,4,5,6, 7,8,9,10,11	2,3,4,5 6,7,9	1,2,3,4,5, 6,7,9,10	2,3,4,6 7,8,9,10
Control (cloth bags)	2,3,4,5, 6,7,8,9,10	2,3,4,5, 6,7,9,10	1,2,3,4,5 6,7,8,10	2,3,4,6,7 8,9,10,11

* 1. *Alternaria alternata*, 2. *Aspergillus flavus*, 3. *A. niger*, 4. *Curvularia lunata*, 5. *Fusarium solani*, 6. *Memnoniella echinata*, 7. *Penicillium citrinum*, 8. *Rhizopus oryzae*, 9. sterile hyphae, 10. Bacterium, 11. *Chaetomium globosum*, 12. *Pestalotiopsis* sp.

Table 27. Effect of various seed dressers and storage conditions on seed germination and vigour index of *L. microcarpa*

Treatment	Germination (%)				Vigour Index (VI)			
	Day-1	Day-90	Day-180	Day-365	Day-1	Day-90	Day-180	Day-365
Captafol	8^{a*}	8^{a}	9^{ab}	2^{a}	326.3^{a}	422.7^{a}	218.1^{ab}	188.0^{a}
Captan	13^{c}	10^{ab}	10^{b}	3^{ab}	517.8^{ab}	420.8^{a}	416.2^{abc}	261.6^{a}
Carboxin	11^{ab}	8^{a}	8^{ab}	5^{ab}	577.2^{b}	403.5^{a}	408.8^{abc}	192.4^{a}
Carbendazim	11^{ab}	6^{a}	5^{a}	3^{ab}	518.5^{ab}	293.0^{a}	322.2^{abc}	234.0^{a}
Mancozeb	11^{ab}	11^{a}	11^{b}	5^{ab}	581.0^{b}	537.0^{a}	550.3^{c}	197.8^{a}
MEMC	11^{ab}	8^{a}	8^{ab}	4^{ab}	531.9^{ab}	393.0^{a}	392.3^{abc}	216.5^{a}
PCNB	11^{ab}	7^{a}	8^{ab}	2^{a}	406.8^{ab}	278.7^{a}	261.7^{ab}	189.9^{a}
Thiram	11^{ab}	6^{a}	7^{ab}	5^{ab}	487.5^{ab}	304.8^{a}	303.4^{abc}	266.0^{a}
Dehumidified cond. Room temp.	11^{ab}	8^{a}	6^{a}	5^{ab}	508.8^{ab}	303.9^{a}	373.6^{abc}	220.6^{a}
Dehumidified cond. 4°C	10^{ab}	10^{ab}	10^{b}	7^{b}	387.7^{ab}	439.9^{a}	467.6^{bc}	238.6^{a}
Control (Plastic container)	10^{ab}	6^{a}	5^{a}	4^{ab}	441.5^{ab}	295.3^{a}	195.2^{a}	217.8^{a}
Control (Cloth bags)	10^{ab}	6^{a}	5^{a}	4^{ab}	437.1^{ab}	294.3^{a}	195.2^{a}	217.8^{a}

*Mean values in a column superscribed by the same letter(s) do not differ significantly at p = 0.05

Analysis of variance of data on % seed germination and vigour index related to days of storage, treatment was observed non significant (Table 28).

Table 28. Analysis of variance of germination and vigour index of seeds of *L. microcarpa* stored for 1 year

Sources	Vigour Index			Germination		
	DF	MSS	F	DF	MSS	F
Day	3	943.5	31.2**	3	472.3	34.7**
Treatment	11	88.4	2.9*	11	35.4	2.6*
Day x Treatment	33	20.3	0.7ns	33	8.0	0.6ns
Residual	144	30.2	-	144	13.6	-

** significant at p= 0.01
* significant at p= 0.05
ns non-significant

4B.5. Seedling diseases and their management

4B.5.1.Damping-off

4B.5.1.1. Occurrence

Post emergence damping-off of seedlings of *L. microcarpa* was recorded in all the beds raised at Peechi during 1989 season. There were an average of 3-5 active damping-off patches/standard bed (Plate 9A).The disease was also recorded

in a few beds at Kurigadda of Haliyal Forest Division, Karnataka. In Nilambur, seedlings raised in wooden trays, suffered a heavy loss of ca. 33% of the seedlings, due to damping-off (Plate 9B).

4B.5.1.2. Symptomatology and causal organism

The disease appeared within 2 weeks after germination of seeds and was seen in the form of irregular patches. A water-soaked constricted area appeared at the soil level causing the seedlings to fall over. The causal organism was identified as *Rhizoctonia solani* Kuhn state of *Thanatephorus cucumaris* (Frank.) Donk. IMI NO. 326295).

4B.5.1.3. Pathogenicity

Pathogenicity of the isolate was confirmed on 20 young seedlings (2 to 4-week-old) raised in sterile soil, which were transplanted in aluminium trays with infested soil. Fungal growth was observed within 24 h on the soil and damping-off was observed on the 4th day and all the seedlings died within a week.

4B.5.1.4. *In vitro* evaluation of fungicides

Evaluation of fungicides in poisoned food method (PFM) indicated that carbendazim and MEMC were the only fungicides which gave ED_{100} at all the concentrations tested; carboxin,

A

B

PLATE 9. A, View of the nursery bed of *L. microcarpa* showing damped-off seedlings; B, a close view showing the toppled seedlings.

PCNB and thiram which gave > ED_{70} in all concentrations were also included for evaluation under soil fungicide screening method (SFSM). ED_{100} was achieved by carbendazim and MEMC, but at the highest concentration of 0.2 % and 0.0250% a.i respectively. Carboxin was also effective in all the 3 concentrations but with an $\geq ED_{70}$. In thiram 70% inhibition was achieved only at the highest concentration i.e., 0.2% a.i. (Table 29). In analysis of variance of data on % inhibition related to fungicides and concentration for both the methods separately indicates high significance (Table 30).

4B.5.1.5. Control measures

Small- scale field trials conducted at Peechi indicated that pre sowing soil drench of seed beds with MEMC (0.006% a.i.) was the best treatment in controlling the damping-off. In the "Control" beds the mean number of active damping-off patches was 4.33/bed, while the MEMC drenched beds did not record any disease patches at all. The seed treatment with captan and mancozeb was not effective as the seed bed treated with them recorded 2.5 and 3.5 active patches/bed. In comparison beds drenched with carboxin, carbendazim, fytolan and thiram recorded low disease incidence as the mean number of active damping-off patches was only 0.33/bed.

Table 29. Evaluation of fungicides against *R. solani* causing damping-off in *L. microcarpa* using various methods

Fungicide and concentration	% a.i.	% inhibition over control	
		PFM*	SFSM+
Captafol (Difoltan)	0.05	66.7	
	0.1	66.7	Not tested
	0.2	72.2	
Captan (Deltan)	0.05	77.8	
	0.1	77.8	Not tested
	0.2	79.3	
Carbendazim (Bavistin)	0.05	100	23.3
	0.1	100	44.4
	0.2	100	100
Carboxin (Vitavax)	0.05	86.7	72.0
	0.1	88.9	76.8
	0.2	88.9	80.7
Copper oxychloride (Fytolan)	0.05	50	
	0.1	77.8	Not tested
	0.2	77.8	
Mancozeb (Dithane M-45)	0.05	75.6	
	0.1	78.9	Not tested
	0.2	80.0	
MEMC (Emisan)	0.006	100	24.1
	0.0125	100	30.0
	0.0250	100	100
PCNB (Brassicol)	0.05	77.8	9.6
	0.1	81.1	21.1
	0.2	83.3	27.8
Thiram (Thiride)	0.05	72.2	45.9
	0.1	76.3	54.8
	0.2	83.3	74.1
Ziram (Ziride)	0.05	61.1	
	0.1	66.7	Not tested
	0.2	66.7	

*Poisoned food method; +Soil fungicide screening method

Table 30. Analysis of variance of data on % inhibition of *R. solani* causing damping-off in *L. microcarpa*

Source	Poisoned food method			Soil fungicide method		
	DF	MSS	F	DF	MSS	F
Treatment	9	1373.3	12483.3**	4	3852.6	535.5**
Concentration	2	325.2	2956.0**	2	6997.6	972.7**
Treatment x Concentration	18	655.6	72.1**	8	997.4	138.6**
Residual	60	0.11	-	30	7.2	-

** significant at p = 0.01

4B.5.2. Root rot

4B.5.2.1. Occurrence

Root rot disease was recorded in container seedlings (ca. 3-5 months old) at Nilambur during 1989. This disease was observed in a very less proportion (< 1%) at Nilambur and was not recorded in any of the seed bed/container beds surveyed.

4B.5.2.2. Symptomatology and causal organism

Root rot caused slow wilting of seedlings. The initial symptom was the change of pigmentation in top leaves from normal green to light yellow Within a week the lower leaves were also affected. In some cases even the root collar zone was affected. Usually 3 to 4 month old seedlings were

affected. *Pythium middletonii* Sparrow (IMI No. 326291) was consistently isolated from the affected parts.

4B.5.2.3. Pathogenicity

Pathogenicity of the isolate was confirmed on 2- to 3-month-old seedlings. Fungal growth was observed the next day on the soil surface and wilting was recorded on the 5th day. Mortality of seedlings (ca. 80%) was recorded on the eighth day.

4B.5.2.3. *In vitro* evaluation of fungicides

In-vitro evaluation of fungicides employing PFM indicated that MEMC and thiram were the best fungicides inhibiting the radial growth of mycelium in all the 3 concentrations tested. Captan gave ED_{100} only at two concentration of 0.1 and 0.2% a.i. while captafol, PCNB, ziram and copper-oxychloride inhibited 75-87% of the radial growth of the mycelium (Table 31). Analysis of variance of the data on % inhibition related to fungicides, concentration and their interaction were highly significant (Table 32).

Since this disease is not economically important in the nurseries, no small scale field trial was attempted.

Table 31. Evaluation of fungicides against *P. middletonii* causing root rot of *L. microcarpa* using poisoned food method

Fungicides and concentration	% a.i.	% inhibition over control
Captafol (Difoltan)	0.05	83.9
	0.1	84.8
	0.2	85.6
Captan (Deltan)	0.05	86.7
	0.1	100
	0.2	100
Carbendazim (Bavistin)	0.05	22.2
	0.1	55.6
	0.2	59.4
Carboxin (Vitavax)	0.05	2.6
	0.1	22.6
	0.2	55.6
Copper oxy chloride (Fytolan)	0.05	87.2
	0.1	87.8
	0.2	87.8
Mancozeb (Dithane M-45)	0.05	20.6
	0.1	26.9
	0.2	55.6
MEMC (Emisan)	0.006	100
	0.0125	100
	0.0250	100
PCNB (Brassicol)	0.05	78.1
	0.1	80.0
	0.2	85.9
Thiram (Thiride)	0.05	100
	0.1	100
	0.2	100
Ziram (Ziride)	0.05	75.9
	0.1	83.3
	0.2	86.7

Table 32. Analysis of variance of data on % inhibition of *P. middletonii* causing root rot in *L. microcarpa*

Source	DF	MSS	F
Treatment	9	6500.3	1558.6**
Concentration	2	2224.9	533.5**
Treatment X Concentration	18	392.7	94.2**
Residual	60	4.2	-

** significant at p = 0.01

4C. *PTEROCARPUS MARSUPIUM*

4C.1. Seed health testing methods

Numerous micro-organisms made their appearance in certain testing methods, while they were absent in others. In SB and PDA methods 15 micro-organisms were recorded on non-surface sterilised seeds with varying incidence, followed by MEA method with 10 micro-organisms, 2,4-D and DF methods respectively with 9 and 8 micro-organisms (Table 33). Except MEA method, actinomycetes were recorded in all other methods. The incidence of *Alternaria infectoria* was significantly higher in SB method, while it did not occur at all in 2,4-D DF and PDA methods. *Aspergillus ochraceus* was recorded very frequently in all the methods, and its incidence did not differ significantly . *Botryodiplodia theobromae* occurred in PDA, MEA and SB methods, wherein it was not recorded in 2,4-D and DF methods *Chaetomium globosum* grew abundantly in 2,4-D, DF and PDA methods. *Fusarium moniliforme* var. *intermedium* was observed only in PDA and MEA methods. A *Marasmius* sp. was recorded only in SB method. The incidence of *Myrothecium roridum* was the highest in DF method followed by SB, 2,4-D and PDA methods while it was absent in MEA method. (Plate 10 & 11).

In the case of surface sterilised seeds, the number of micro-organisms recorded was reduced to nine and the percent incidence was also less as compared with non-surface

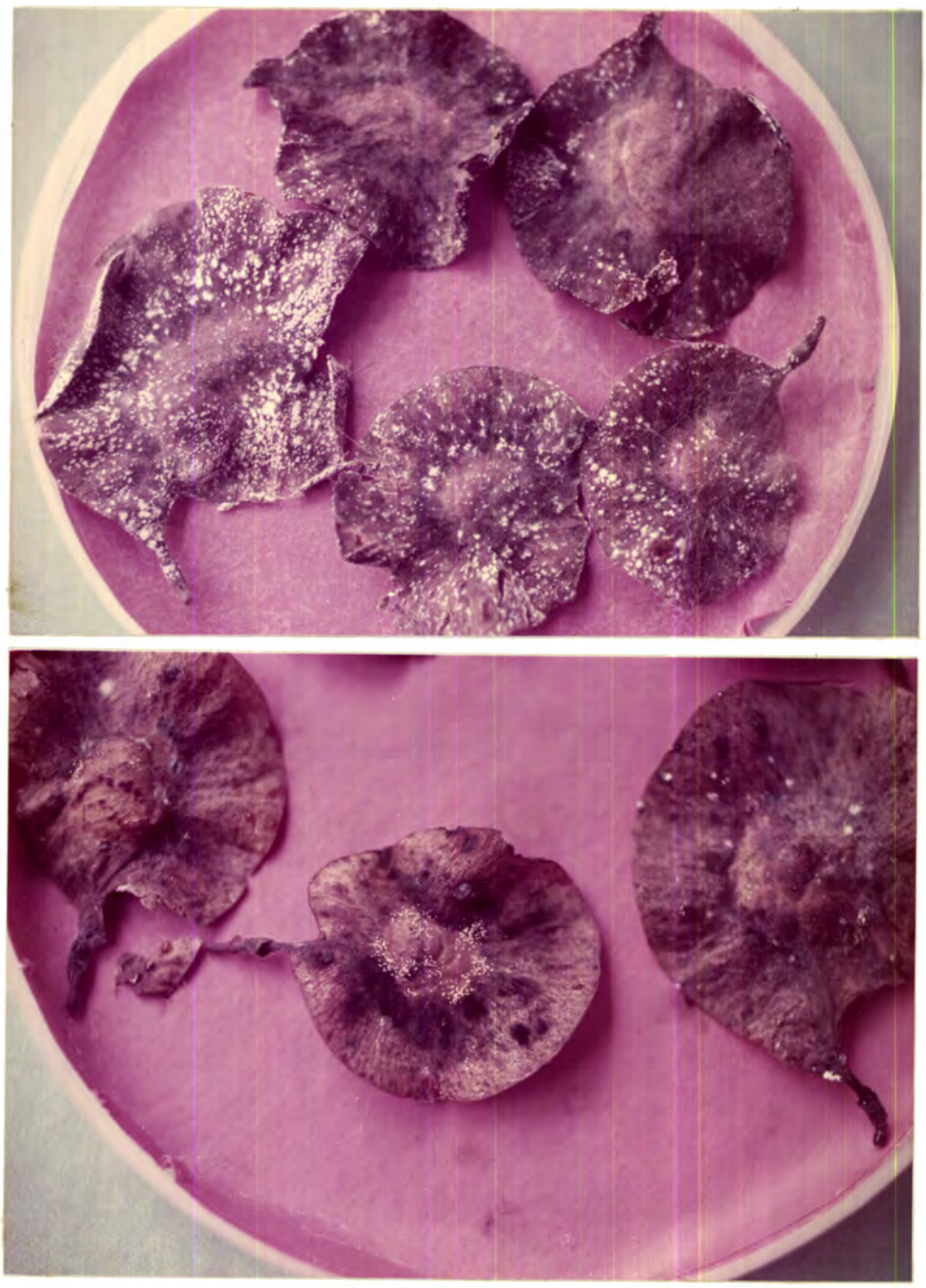


PLATE 10. *Pterocarpus marsupium*. A, Growth of actinomycetes and other micro-organisms; B, Growth of *A. ochraceus* and *Alternaria infectoria*

A

B

PLATE 11. A, *Trichurus spiralis*; B, *Marasmius* sp. growing on the seeds of *Pterocarpus marsupium*.

sterilised seeds. Even after surface sterilisation field fungi like *F. moniliforme* var. *intermedium*, *M. roridum* and *A. infectoria* were recorded. The incidence of storage micro-organisms like Actinomycetes, various species of *Aspergillus*, *Chaetomium globosum*, *Cladosporium herbarum*, *Memnoniella echinata*, *Penicillium citrinum*, *Rhizopus oryzae* and *Trichurus spiralis* was less (Table 34) as compared with non-surface sterilised seeds. Among all the methods, PDA method appeared to be the best in the expression of micro-organisms, followed by DF, SB, 2,4-D and MEA methods, but high incidence of certain micro-organisms also occurred in DF, SB and 2,4-D methods (Table 34).

4C.2. Seed microflora and their significance

4C.2.1. Dry seed examination

The seeds of *P. marsupium* could be graded into three categories by dry seed examination (Plate 12). The percentage occurrence of round and apparently healthy seeds was the highest (55.5%) followed by discoloured seeds (27 %) and small and deformed seeds (17.5%). The weight of 100 seeds was the highest in round seeds (77. 3 g), followed by discoloured category (73.5 g) and small and deformed seeds (33.0 g). A pooled sample of 100 seeds weighed 67.4 g.

Table 33. Percent incidence of spermoplane micro-organisms in in different seed health testing methods on non-surface sterilised seeds of *P. marsupium*

Micro-organism	Methods				
	SB	2,4-D	DF	PDA	MEA
Actinomycetes	54^{c*}	66^{c}	96^{d}	18^{b}	0^{a}
Alternaria infectoria E.Simmons	40^{c}	0^{a}	0^{a}	0^{a}	20^{b}
Aspergillus candidus Link.	4^{b}	0^{a}	0^{a}	6^{b}	0^{a}
A. flavus Link.	8^{b}	4^{b}	0^{a}	22^{c}	6^{b}
A. niger van.Tieghem	4^{a}	0^{a}	6^{ab}	22^{c}	18^{bc}
A. ochraceus Wilhelm.	36^{a}	44^{a}	62^{a}	44^{a}	34^{a}
A. versicolor (Vuill.) Tiraboschi	0^{a}	0^{a}	0^{a}	2^{a}	0^{a}
Botryodiplodia theobromae Pat.	14^{b}	0^{a}	0^{a}	10^{b}	40^{c}
Cladosporium herbarum (Pers.) Link. ex Gray	48^{c}	12^{b}	0^{a}	0^{a}	18^{bc}
Chaetomium globosum Kunze.	0^{a}	52^{b}	40^{b}	28^{b}	0^{a}
Fusarium moniliforme Sheldon var. *intermedium* Neish & Leggett	0^{a}	0^{a}	0^{a}	8^{b}	18^{b}
Memnoniella echinata (Riv.) Galloway	8^{b}	10^{b}	0^{a}	2^{a}	16^{b}
Marasmius sp.	10^{b}	0^{a}	0^{a}	0^{a}	0^{a}
Myrothecium roridum Tode: Fr.	38^{c}	12^{b}	42^{c}	8^{b}	0^{a}
Penicillium citrinum Thom.	0^{a}	0^{a}	4^{a}	2^{a}	4^{a}
Trichothecium roseum (Pers.) Link.ex Gray	4^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Trichurus spiralis Hasselbr.	14^{b}	12^{b}	18^{b}	0^{a}	0^{a}
Rhizopus oryzae Went & Prinsen Geerligs	50^{b}	50^{b}	12^{a}	32^{b}	42^{b}
sterile hyphae (black)	4^{b}	0^{a}	0^{a}	2^{a}	0^{a}

*Mean values superscribed by the same letter(s) do not differ significantly at p = 0.05 (Row-wise comparison)

Table 34. Percent incidence of spermoplane micro-organisms in different seed health testing methods on surface sterilised seeds of *P. marsupium*

Micro-organisms	Methods				
	SB	2,4-D	DF	PDA	MEA
Actinomycetes	20^{bc*}	32^{c}	20^{bc}	6^{b}	0^{a}
Alternaria infectoria	20^{b}	0^{a}	0^{a}	0^{a}	8^{b}
Aspergillus candidus	0^{a}	0^{a}	4^{a}	2^{a}	0^{a}
A. flavus	2^{a}	6^{a}	22^{b}	10^{a}	2^{a}
A. niger	2^{a}	0^{a}	18^{b}	10^{b}	10^{b}
A. ochraceus	22^{ab}	32^{c}	42^{c}	16^{ab}	14^{a}
Botryodiplodia theobromae	4^{a}	0^{a}	0^{a}	10^{b}	18^{b}
Chaetomium globosum	0^{a}	18^{b}	26^{b}	10^{ab}	0^{a}
Cladosporium herbarum	24^{b}	2^{a}	0^{a}	0^{a}	14^{ab}
Fusarium moniliforme var. *intermedium*	0^{a}	0^{a}	0^{a}	6^{b}	8^{b}
Memnoniella echinata	0^{a}	6^{b}	0^{a}	2^{a}	16^{c}
Myrothecium roridum	14^{b}	5^{b}	6^{b}	0^{a}	0^{a}
Penicillium citrinum	0^{a}	0^{a}	0^{a}	2^{a}	0^{a}
Rhizopus oryzae	38^{c}	20^{b}	0^{a}	14^{b}	14^{b}
Trichurus spiralis	4^{b}	0^{a}	6^{b}	0^{a}	0^{a}
sterile hyphae (black)	0^{a}	0^{a}	6^{b}	2^{a}	0^{a}

*Mean values superscribed by the same letter(s) do not differ significantly at p = 0.05 (Row-wise comparison)

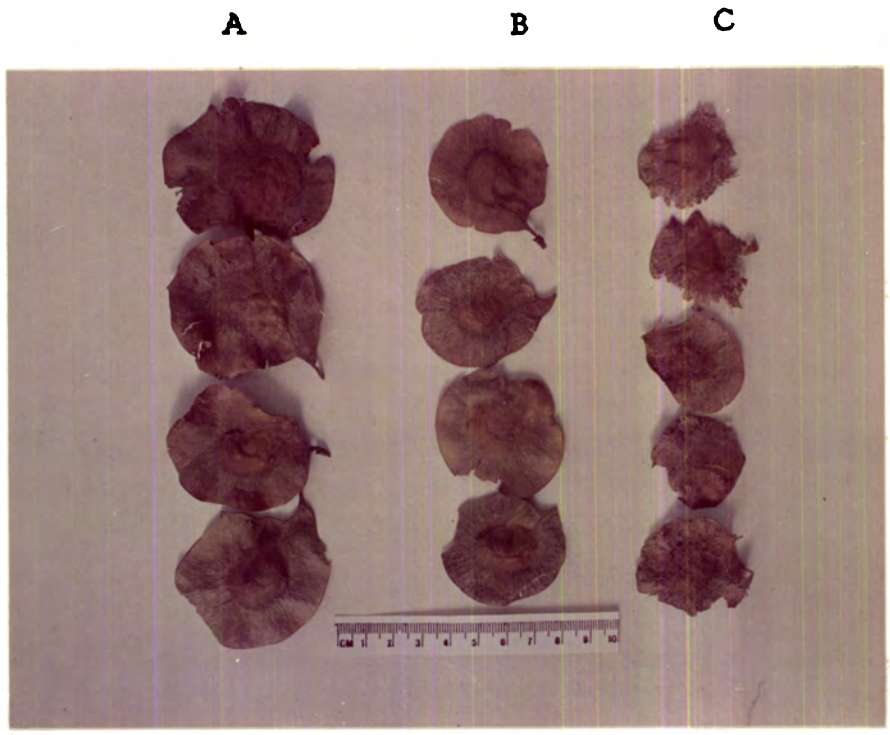


PLATE 12. Seeds of *P. marsupium* showing apparently healthy (A), discolored (B) and small and discolored (C) categories.

4C.2.2. Incidence of micro-organisms in different categories of seeds

In round and apparently healthy category of seeds, 13 micro-organisms were detected in non-surface sterilised seeds, while only 10 occurred in surface sterilised seeds. Generally the incidence of various micro-organisms was higher in non-surface sterilised seeds as compared to surface sterilised seeds. A total of 14 micro-organisms were recorded on non-surface sterilised discoloured seeds while only 11 micro-organisms were detected on surface-sterilised seeds. The incidence of Actinomycetes, *A. ochraceus, C. herbarum, M. roridum* and *R. oryzae* was higher in this category as compared with apparently healthy seeds. Surface sterilisation greatly reduced the incidence of most of the micro-organisms Although a number of micro-organisms detected in small seeds did not differ much as compared with other categories, the incidence of various micro-organisms was higher. Micro-organisms which showed higher incidence were Actinomycetes, *A. infectoria, Aspergillus* spp., *B. theobromae, C. herbarum, M. roridum, T. spiralis* and *R. oryzae* (Figs.13 & 14).

4C.2.3. Pathogenicity studies

In general, the viability of seeds of *P. marsupium* collected from the Peechi Range was poor with only 24% of seeds germinated in control. Seeds inoculated with

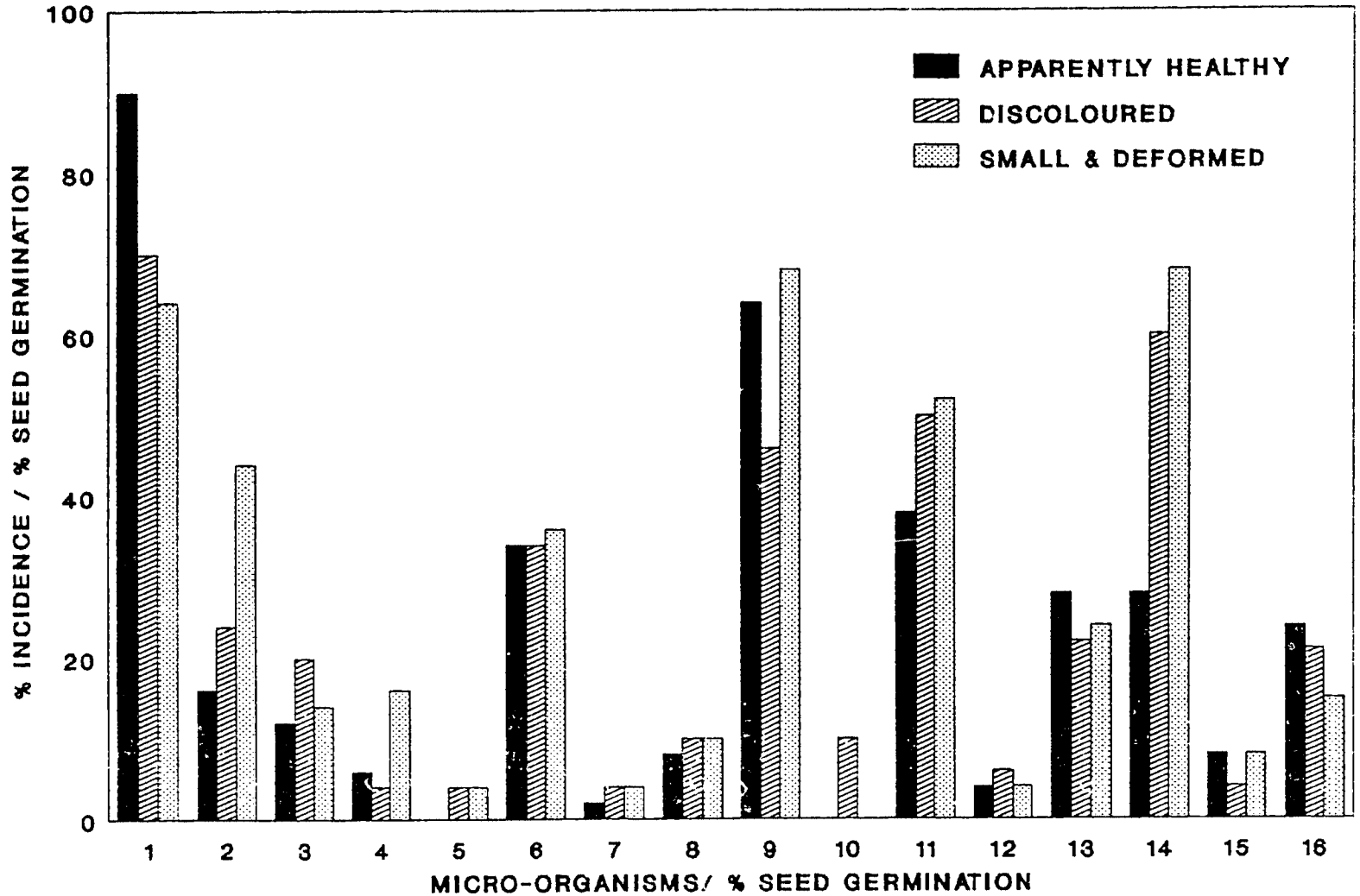


Fig.13. Percent incidence of spermoplane micro-organisms on non-surface sterilised seeds and % seed germination in different categories of seeds of *P. marsupium*.

1. Actinomycetes, 2. *Alternaria infectoria*,3. *Aspergillus candidus*,4. *A. flavus*,5. *A. niger*,6. *A. ochraceus*,7. *A. versicolor*, 8. *Botryodiplodia theobromae*, 9. *Cladosporium herbarum*, 10. *Marasmius* sp., 11. *Myrothecium roridum*, 12. *Trichothecium roseum* 13. *Trichurus spiralis*, 14. *Rhizopus oryzae*, 15. sterile hyphae (black), 16. % seed germination

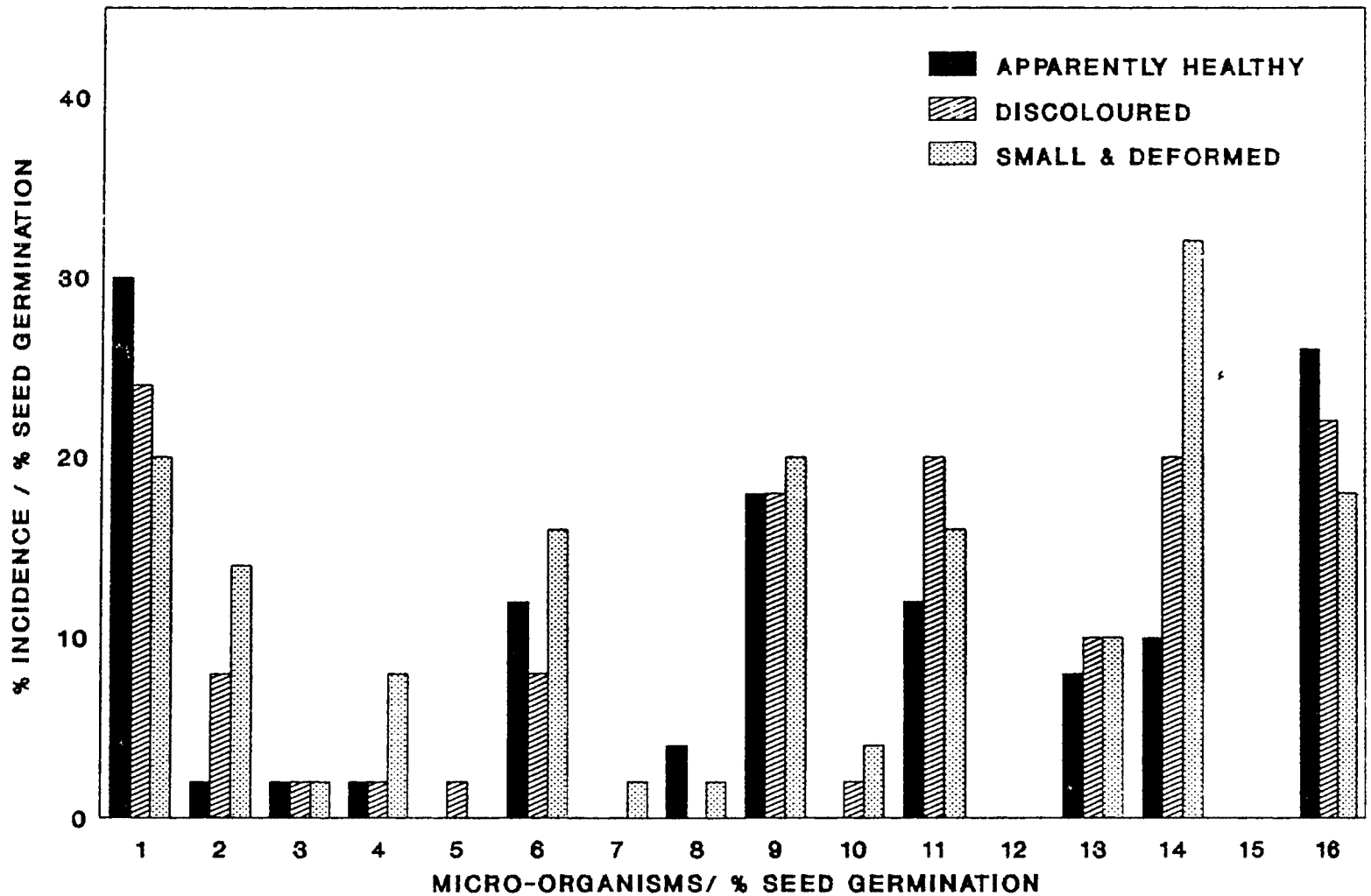


Fig.14. Percent incidence of spermoplane micro-organisms on surface sterilised seeds and % seed germination in different categories of seeds of *P. marsupium*.

1. Actinomycetes, 2. *Alternaria infectoria*,3. *Aspergillus candidus*,4. *A. flavus*,5. *A. niger*,6. *A. ochraceus*,7. *A. versicolor*, 8. *Botryodiplodia theobromae*, 9. *Cladosporium herbarum*, 10. *Marasmius* sp., 11. *Myrothecium roridum*, 12. *Trichothecium roseum*, 13. *Trichurus spiralis*, 14. *Rhizopus oryzae*, 15. sterile hyphae (black), 16. % seed germination

A. flavus, B. theobromae and *F. moniliforme* var. *intermedium* showed reduced seed germination in the range of 4 to 8 %. Delayed germination was observed in seeds treated with *Aspergillus niger, A. ochraceus, C. globosum* and *Trichurus spiralis* (Fig. 15) and decay of seeds (2%) was recorded when treated with *Fusarium moniliforme* var. *intermedium*. Shoot length was considerably reduced in the seeds treated with *A. flavus* and *A. ochraceus*, while it was enhanced in the case of *T. spiralis* (Fig.16 A). In other treatments the shoot length did not differ appreciably as compared with control seedlings. *B. theobromae* and *A. niger* reduced the root length considerably in comparison with control seedlings (Fig.16 A), while it was slightly higher in treatments of *A. infectoria* and *M. roridum*. The vigour index of control seedlings and seedlings under treatments of *Alternaria infectoria* and *C. globosum* did not differ appreciably, while the vigour index of the seedlings in treatments of *A. flavus, B. theobromae* and *F. moniliforme* var. *intermedium* was very low (Fig. 16 B). However, the vigour index of other treatments which was lower as compared with control did not differ among each other.

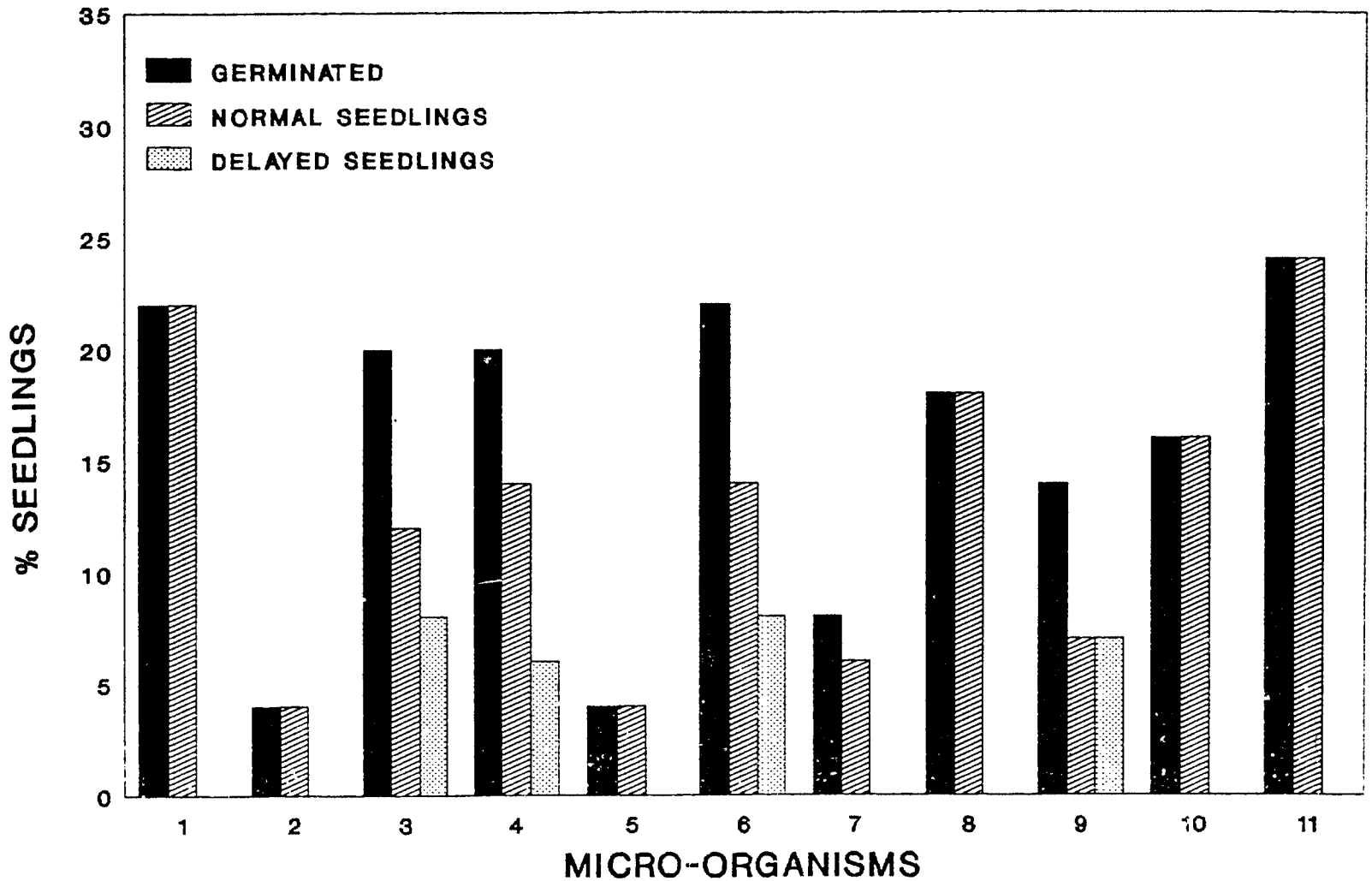
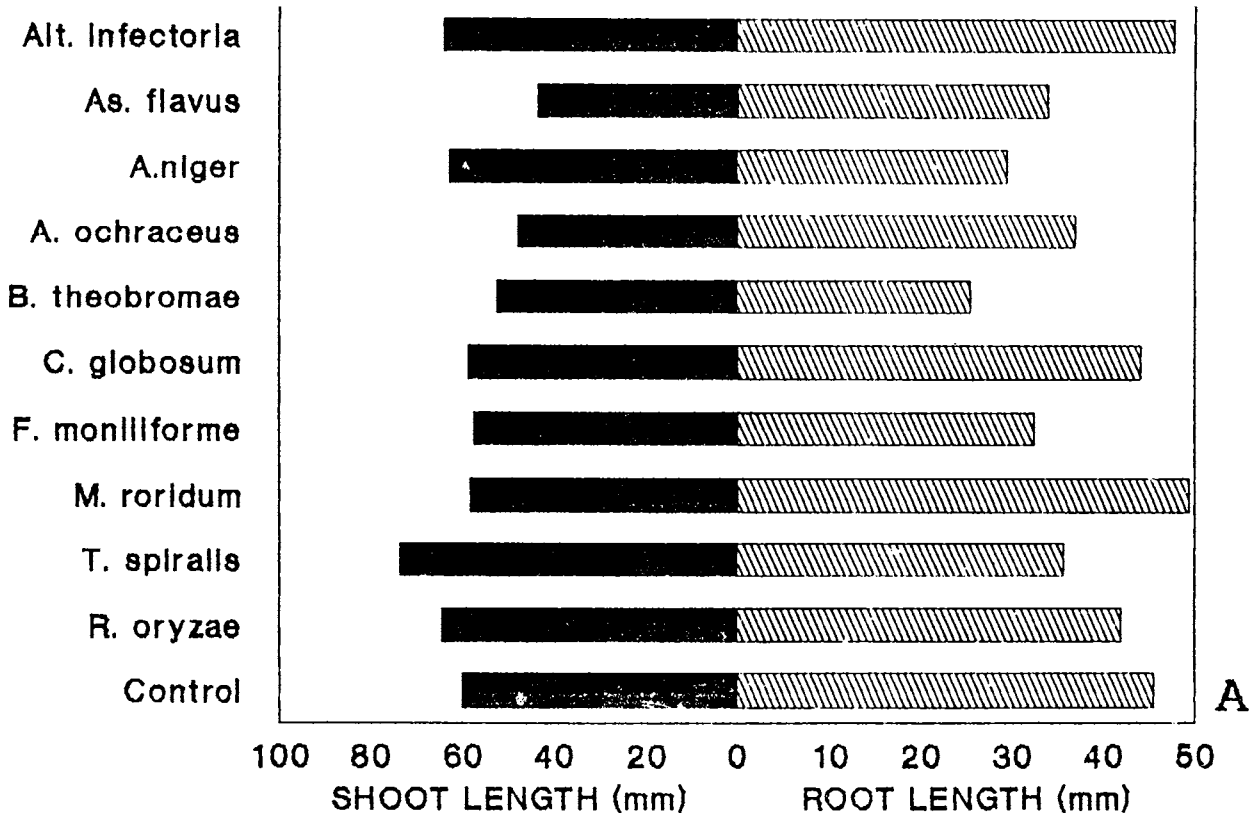


Fig.15. Effect of various micro-organisms on seed germination and seedling emergence of *P. marsupium*.

1. *Alternaria infectoria*, 2. *Aspergillus flavus*, 3. *A. niger*, 4. *A. ochraceus*, 5. *Botryodiplodia theobromae*, 6. *Chaetomium globosum*, 7. *Fusarium moniliforme* var. *intermedium*, 8. *Myrothecium roridum*, 9. *Trichurus spiralis* 10. *Rhizopus oryzae*, 11. Control.



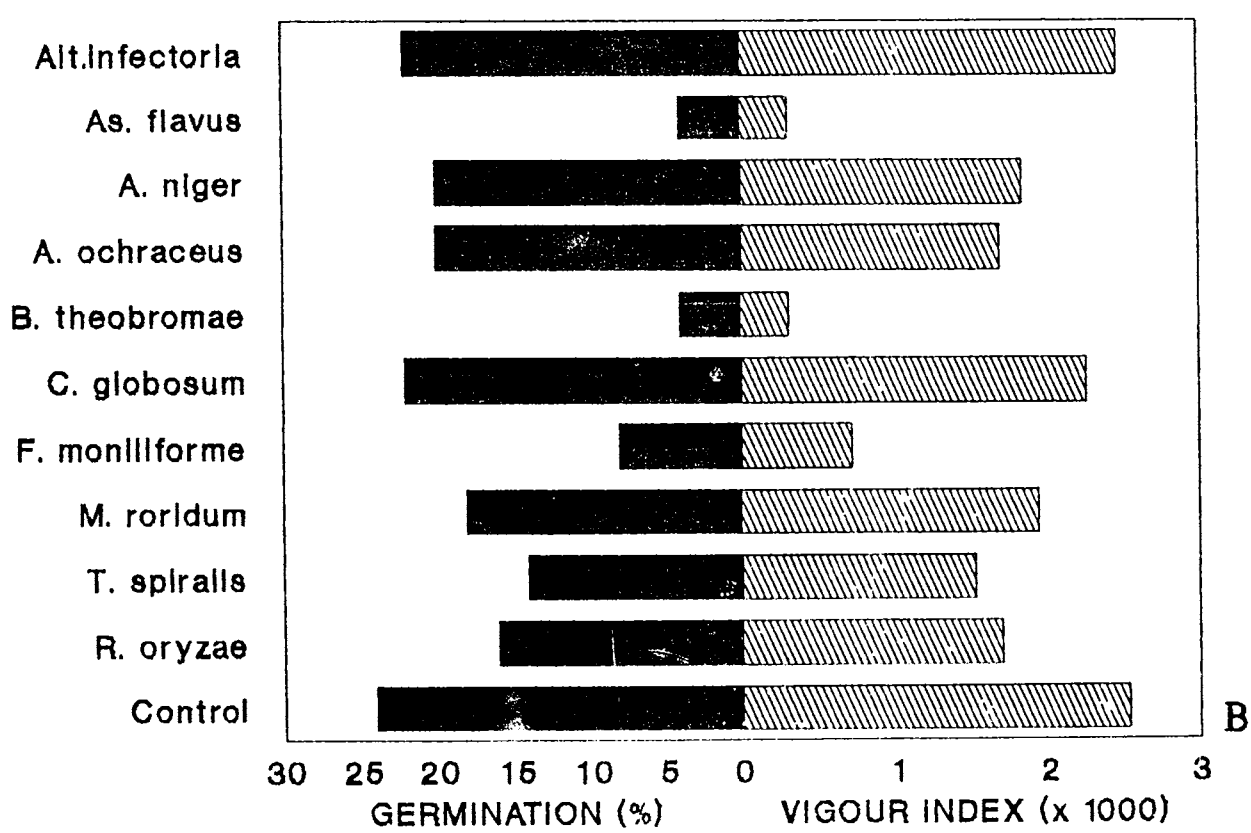


Fig.16. Effect of various micro-organisms on shoot and root length (A) seed germination and vigour index (B) of *P. marsupium*.

4C.3. Management of seed microflora

4C.3.1. Hot water treatment

The germination was significantly reduced in the treatments of 50°C and 60°C for 30 min., as compared with other treatments and control (Table 35). The shoot length did not differ significantly in any of the treatments, while the root length was significantly higher in treatments of 60°C-30 min. followed by treatment of 50°C-30 min.; The vigour index was significantly reduced in treatments of 50° and 60°C for 30 min.

Table 35. Effect of hot water treatment on seed germination and growth of seedlings of *P. marsupium*

Observations	50°C		60°C		Control
	15 min.	30 min.	15 min.	30 min.	Control
Germination (%)	22^{bc*}	10^{a}	26^{c}	12^{ab}	26^{c}
Shoot length (mm)	51.5^{a}	49.7^{a}	57.0^{a}	57.3^{a}	52.7^{a}
Root length (mm)	29.0^{ab}	38.6^{b}	31.3^{ab}	52.6^{c}	25.1^{a}
Vigour index (VI)	1710.0^{b}	836.9^{a}	2226.6^{b}	1269.3^{a}	2012.7^{b}
No. of micro-organism recorded	5	5	9	10	13

* Mean values with the same superscript(s) do not differ at p = 0.05 (Row-wise comparison)

The number of micro-organisms developed on hot water treated seeds were less as compared to control (Table 36).

Table 36. Effect of hot water treatment on the % incidence of spermoplane micro-organisms of *P. marsupium*

Micro-organism	50°C		60°C		Control
	15 min.	30 min.	15 min.	30 min.	
Actinomycetes	48^{a}	56^{b}	68^{b}	28^{a}	100^{c}
Alternaria infectoria	0^{a}	0^{a}	0^{a}	0^{a}	16^{b}
Aspergillus candidus	0^{a}	0^{a}	8^{a}	4^{a}	72^{b}
A. flavus	48^{c}	44^{c}	0^{a}	5^{a}	24^{b}
A. niger	4^{a}	20^{b}	? 8^{a}	0^{a}	12^{b}
A. ochraceus	0^{a}	4^{a}	24^{b}	8^{a}	72^{c}
Botryodiplodia theobromae	0^{a}	0^{a}	0^{a}	0^{a}	12^{b}
Chaetomium globosum	0^{a}	0^{a}	64^{b}	66^{b}	0^{a}
Cladosporium herbarum	0^{a}	0^{a}	32^{b}	85^{c}	88^{c}
Fusarium moniliforme var. *intermedium*	12^{b}	0^{a}	0^{a}	4^{a}	12^{b}
Memnoniella echinata	0^{a}	0^{a}	48^{b}	80^{b}	0^{a}
Myrothecium roridum	0^{a}	0^{a}	0^{a}	0^{a}	12^{b}
Penicillium citrinum	0^{a}	0^{a}	0^{a}	0^{a}	12^{b}
Rhizopus oryzae	16^{a}	40^{a}	16^{a}	10^{a}	88^{b}
Trichurus spiralis	0^{a}	0^{a}	8^{a}	52^{b}	36^{b}

* Mean values with the same superscript(s) do not differ at p = 0.05 (Row-wise comparison)

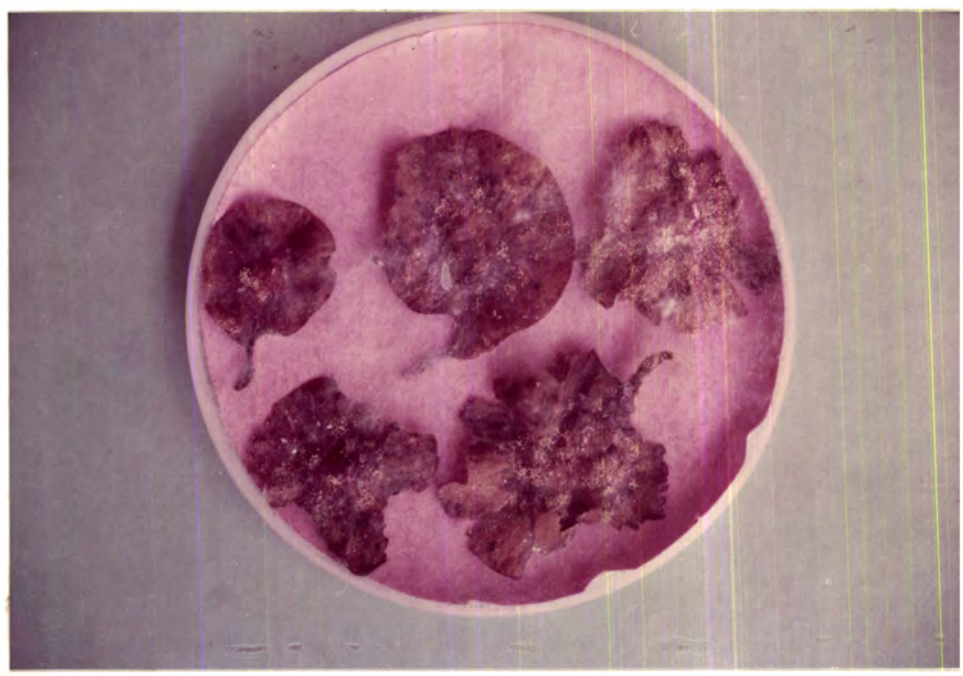
The incidence of field fungi like *A. infectoria*, *B. theobromae* and *M. roridum* was completely inhibited in all the treatments. Interestingly high incidence of storage fungi like *C. globosum* and *M. echinata* which were not recorded on control seeds was observed on seeds treated at 60°C for 15 and 30 min.

4C.3.2. Chemical Treatment

Captan was the most effective fungicide having the highest vigour index of the seedlings followed by thiram, captafol, MEMC and carboxin(Table 37). The % seed germination and shoot length in the above treatments were significantly higher when compared with control, while this was not the case with the root length. The shoot length in other treatments was not significantly different from control (Table 37) but the root length in the treatments, viz., carbendazim, mancozeb, PCNB was significantly lower as compared with control.

All the fungicides were effective in reducing the number of micro-organisms from 13 in control to 0 to 4 in various treatments (Plate 13). *Alternaria infectoria* and *B. theobromae* were inhibited completely in all the treatments. Though *M. roridum* was detected only on seeds treated with PCNB its incidence was significantly lower than in control. *Fusarium moniliforme* var. *intermedium* was recorded in seeds treated with captan, mancozeb and carboxin; its incidence was significantly higher in carboxin treated seeds.

A



B

PLATE 13. A, Incidence of micro-organisms on control seeds B, seeds treated with MEMC in *P. marsupium*.

Table 37. Effect of various seed dressers on % seed germination and growth of seedlings of *P. marsupium*

Treatment	Germination (%)	Mean shoot length(mm)	Mean root length(mm)	Vigour index	No. of micro-organisms recorded
Captafol	38^{b*}	70.6^{de}	38.8^{d}	4133.5^{cd}	3
Captan	42^{c}	77.8^{e}	39.0^{d}	4814.0^{d}	2
Carbendazim	25^{a}	42.8^{a}	23.6^{ab}	1659.8^{a}	2
Carboxin	35^{b}	54.6^{bc}	37.6^{d}	3213.1^{c}	3
Mancozeb	25^{a}	46.6^{ab}	21.7^{a}	1694.5^{a}	3
MEMC	38^{b}	60.4^{c}	34.0^{cd}	3539.2^{cd}	0
PCNB	34^{ab}	57.9^{c}	28.1^{bc}	2908.7^{abc}	2
Thiram	40^{b}	64.1^{cd}	39.8^{d}	4145.9^{cd}	4
Control	24^{a}	43.8^{a}	37.2^{d}	1923.9^{ab}	13

*Mean values in a column superscribed by the same letter(s) do not differ significantly at p = 0.05.

Actinomycetes were not controlled by captafol, carbendazim, carboxin and thiram treatments but their incidence was much lower than in control seeds. Most of the other storage fungi were more or less completely arrested except for *A. flavus, A. candidus, C. herbarum* and *R. oryzae* which made their erratic appearance on some treated seeds (Table 38).

Table 35. Effect of various seed dressers on % incidence of spermoplane micro-organisms of *P. marsupium*

Micro-organism	Control	Captan	Captafol	Carbendazim	Carboxin	Mancozeb	MEMC	PCNB	Thiram
Actinomycetes	72^{c*}	0^{a}	4^{a}	20^{b}	12^{b}	0^{a}	0^{a}	0^{a}	16^{b}
Alternaria infectoria	48^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Aspergillus candidus	16^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	4^{a}
A. flavus	4^{a}	0^{a}	0^{a}	0^{a}	0^{a}	4^{a}	0^{a}	0^{a}	0^{a}
A. niger	4^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
A. ochraceus	100^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Botryodiplodia theobromae	10^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Cladosporium herbarum	92^{c}	0^{a}	8^{b}	0^{a}	0^{a}	0^{a}	0^{a}	20^{b}	8^{b}
Fusarium moniliforme var. *intermedium*	12^{bc}	4^{ab}	0^{a}	0^{a}	24^{c}	8^{b}	0^{a}	0^{a}	0^{a}
Memnoniella echinata	16^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Myrothecium roridum	42^{c}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	12^{b}	0^{a}
Rhizopus oryzae	100^{c}	5^{a}	4^{a}	52^{b}	4^{a}	4^{a}	0^{a}	0^{a}	5^{a}
Trichurus spiralis	24^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}

*Mean values with the same superscript(s) do not differ significantly at p = 0.05 (Row-wise comparison).

4C.4. Seed storage and its influence on microflora, seed germination and seedling development

4C.4.1. Incidence of micro-organisms

The incidence of micro-organisms on treated seeds of *P. marsupium* stored for various period is given in Table 39. It was observed that most field fungi like *M. roridum, F. moniliforme* were either reduced or eliminated as the period of storage increased. In most of the seeds treated with fungicides, only storage fungi like *Aspergillus* spp., *Rhizopus oryzae, Penicillium citrinum* and *Cladosporium herbarum* survived. On seeds stored under dehumidified conditions, there were fewer micro-organisms than in control seeds. *Penicillium citrinum* and *Chaetomium globosum* appeared on seeds in non-fungicidal treatment

4C.4.2. Germination and seedling development

As the storage period increased the germination of seeds gradually decreased and reached a minimum of 3-10% by the end of 1 year in all the treatments except in PCNB and dehumidified conditions at 4°C where the % seed germination was ca. 13-16%. Interestingly the seeds stored under dehumidified condition at room temperature also gradually lost the viability, and the % germination was not significantly different from control. The % germination of seeds stored in cloth bags

and plastic containers did not differ significantly. The vigour index of seeds treated with carbendazim, MEMC, thiram and seeds kept under laboratory conditions declined significantly at the end of 1 year of storage (Table 40).

Table 39. Micro-organisms recorded on seeds of *P. marsupium* stored for different periods under various treatments

Treatments	Day-1	Day-90	Day-180	Day-365
Captafol	1,13,15*	1,3,4,5,6,13	1,2,4,5,6,13	1,4,5,13
Captan	13,15	2,8,11	2,8,11,13	4,5,13
Carbendazim	1,13	1,2,8,13	1,2,8,13	2,4,5,13
Carboxin	1,15	1,2,3,4,5	1,2,4,5,8,13	1,4,5,13
Mancozeb	4,13,15	5,6,11,14	1,3,4,6,13,15	1,4,6,13
MEMC	Nil	2,13	2,13	4,5,13
PCNB	8,11	1,2,4,5,8,15	1,3,4,6,8,15	1,4,5,6
Thiram	1,3,8,13	1,3,4,5,6	1,3,4,6,	4,5,6,8,13
Dehumidified cond. Room temp.	1,2,3,4,5,6, 7,8,9,11,12, 13,14,15	1,2,4,5,13 16,17	3,4,5,6,8, 13,15	1,3,4,5,6 8,11,13
Dehumidified cond. 4°C.	1,2,3,4,5,6 7,8,9,11,12, 13,14,15	1,2,6,8,	1,2,3,5,6 15,17	1,2,4,5,8, 17
Control (Plastic-containers	1,2,3,4,5,6 7,8,9,10,11, 12,13,14,15	1,2,3,4,5 6,8,11,13	2,3,4,5,6 8,11,13,15	4,5,6,12, 17
Control (Cloth bags) (Cloth bags)	1,2,3,4,5,6 7,8,9,11,12 13,14	1,2,3,4,5 6,8,9,11,13	1,2,3,4,5,6 7,9,10,13,17	1,3,4,5,6 10,11,13,17

*1. Actinomycetes, 2. *Alternaria infectoria*, 3. *Aspergillus candidus*, 4. *A. flavus* 5. *A. niger*, 6. *A. ochraceus*, 7. *Botryodiplodia theobromae*, 8. *Cladosporium herbarum*, 9. *Memnoniella echinata*, 10. *Marasmius* sp., 11. *Myrothecium roridum* 12. *Trichurus spiralis*, 13. *Rhizopus oryzae*, 14. sterile hyphae, 15. *Fusarium moniliforme* var.*intermedium* 16. *Chaetomium globosum*, 17. *Penicillium citrinum*

Table 40. Effect of various seed dressers and storage conditions on seed germination and vigour index of *P. marsupium*

Treatment	Germination (%)				Vigour Index (VI)			
	Day-1	Day-90	Day-180	Day-365	Day-1	Day-90	Day-180	Day-365
Captafol	38^{c*}	18^{ab}	18^{bc}	10^{bc}	4133.5^{cd}	1338.5^{bc}	1367.1^{bcd}	600.6^{ab}
Captan	42^{c}	33^{d}	18^{bc}	10^{bc}	4814.0^{d}	2164.9^{cd}	1279.4^{bcd}	801.6^{ab}
Carbendazim	25^{ab}	14^{a}	14^{b}	3^{a}	1659.8^{a}	1377.2^{bc}	1015.6^{b}	392.0^{a}
Carboxin	35^{bc}	32^{cd}	16^{b}	10^{bc}	3213.1^{bc}	3083.8^{de}	1150.3^{bc}	1185.7^{bc}
Mancozeb	25^{ab}	16^{ab}	6^{a}	4^{ab}	1694.5^{a}	1695.7^{ab}	410.5^{a}	721.9^{ab}
MEMC	38^{C}	17^{ab}	5^{a}	4^{ab}	3539.2^{bcd}	1425.1^{ab}	306.3^{a}	403.7^{a}
PCNB	34^{abc}	24^{bc}	22^{cd}	16^{c}	2908.7^{b}	1992.4^{abc}	1602.1^{de}	1453.6^{c}
Thiram	40^{c}	32^{cd}	18^{bc}	4^{ab}	4145.9^{cd}	3298.6^{e}	1461.9^{cde}	303.2^{a}
Dehumidified cond. Room temp.	25^{ab}	21^{abc}	17^{bc}	8^{ab}	1936.4^{a}	1890.4^{ab}	1390.4^{cde}	504.6^{ab}
Dehumidified cond. 4°C	23^{a}	28^{bcd}	26^{d}	14^{c}	1801.2^{a}	2869.4^{cde}	1707.1^{e}	1267.3^{bc}
Control (plastic containers)	24^{a}	16^{ab}	7^{a}	4^{ab}	1923.9^{a}	1200.7^{a}	484.1^{a}	364.8^{a}
Control (cloth bags)	24^{a}	18^{ab}	6^{a}	4^{ab}	1914.5^{a}	1266.3^{a}	483.1^{a}	309.2^{a}

*Mean values in a column with the same superscript(s) do not differ significantly at p = 0.5

Analysis of variance of data on germination % and vigour index related to days of storage and different treatments indicated that a significant interaction was present (Table 41).

Table 41. Analysis of variance of germination and vigour index of seeds of *P. marsupium* stored for 1 year

Sources	Germination			Vigour index		
	DF	MSS	F	DF	MSS	F
Day	3	3165.5	199.4**	3	6199.5	175.0**
Treatment	11	251.5	15.8**	11	597.2	16.9**
Day x treatment	33	55.5	3.5**	33	191.1	5.4**
Residual	144	15.9	-	135	35.4	-

** significant at p = 0.01

4C.5. Seedling diseases and their management

4C.5.1. Collar rot

4C.5.1.1. Occurrence

A few seedlings (< 1%) of less than 2-month-old were found to be affected with a collar rot disease during May 1988 in a nursery maintained at Peechi The disease was also recorded in a few container seedlings (less than 2-month-old) during June 1988 kept for transplanting at Kurigadda of Haliyal Forest Range and Division, in Karnataka State .

4C.5.1.2. Symptomatology and causal organism

Water soaked lesions appeared in the collar region which develop into necrotic area Subsequently the affected tissue decayed, causing a constriction at the collar region and the seedlings toppled. Young seedlings of < 2- month- old were usually found infected with collar rot disease. *Rhizoctonia solani* Kuhn state of *Thanatephorus cucumeris* (Frank) Donk (IMI 328621) was consistently isolated from the affected seedlings.

4C.5.1.3. Pathogenicity

Pathogenicity of the isolate was confirmed using 2-month-old healthy seedlings of *P. marsupium*. On the 3rd day mycelium was observed growing on the root collar zone which caused water soaked lesions. Typical symptoms of collar rot appeared from 5th day onwards and ca. 80% of the test seedlings were toppled.

4C.5.1.4. *In vitro* evaluation of fungicides

In poisoned food method, carboxin, carbendazim, and MEMC were found effective at all the concentrations tested, while ED_{100} for PCNB and thiram was obtained at concentrations of 0.1% and 0.2% a.i. (Table 42).The above mentioned five fungicides were further evaluated using soil fungicide screening method. However, in this method it was found that complete inhibition over control was achieved by MEMC and carboxin in two higher concentrations whereas carbendazim gave ED_{100} only at 0.2%(a.i.).

Table 42. Evaluation of fungicides against *R. solani* causing collar rot off in *P. marsupium* using various methods

Fungicides and concentration	% a.i.	% Inhibition over control PFM*	SFSM+
Captafol (Difoltan)	0.05	69.8	
	0.1	78.1	Not tested
	0.2	77.0	
Captan (Deltan)	0.05	76.3	
	0.1	76.3	Not tested
	0.2	76.3	
Carbendazim (Bavistin)	0.05	100	58.1
	0.1	100	79.6
	0.2	100	100
Carboxin (Vitavax)	0.05	100	59.3
	0.1	100	100
	0.2	100	100
Copper oxychloride (Fytolan)	0.05	50	
	0.1	58.1	Not tested
	0.2	80.4	
Mancozeb (Dithane M-45)	0.05	75.9	
	0.1	78.1	Not tested
	0.2	79.3	
MEMC (Emisan)	0.006	100	69.3
	0.0125	100	100
	0.025	100	100
PCNB (Brassicol)	0.05	91.3	24.1
	0.1	100	29.6
	0.2	100	30.8
Thiram (Thiride)	0.05	84.1	3.7
	0.1	100	13.7
	0.2	100	51.9
Ziram (Ziride)	0.05	58.1	
	0.1	60.6	Not tested
	0.2	68.5	

*Poisoned food method; +Soil fungicide screening method

In analysis of variance of data on % inhibition related different fungicides and concentration for both the methods separately indicates highly significant interaction (Table 43).

Table 43. Analysis of variance of data on % inhibition of *R. solani* causing collar rot in *P. marsupium*

Sources	Poisoned food method			Soil fungicide method		
	DF	MSS	F	DF	MSS	F
Treatment	9	2178.3	881.9**	4	9726.2	989.9**
Concentration	2	433.3	175.4**	2	4361.3	443.9**
Treatment x Concentration	18	86.0	34.8**	8	383.4	39.0**
Residual	60	2.5	-	30	9.8	-

** significant at p = 0.01

Since this disease was neither serious nor a common one, no chemical control field trials were conducted

4C.5.2. Seedling blight

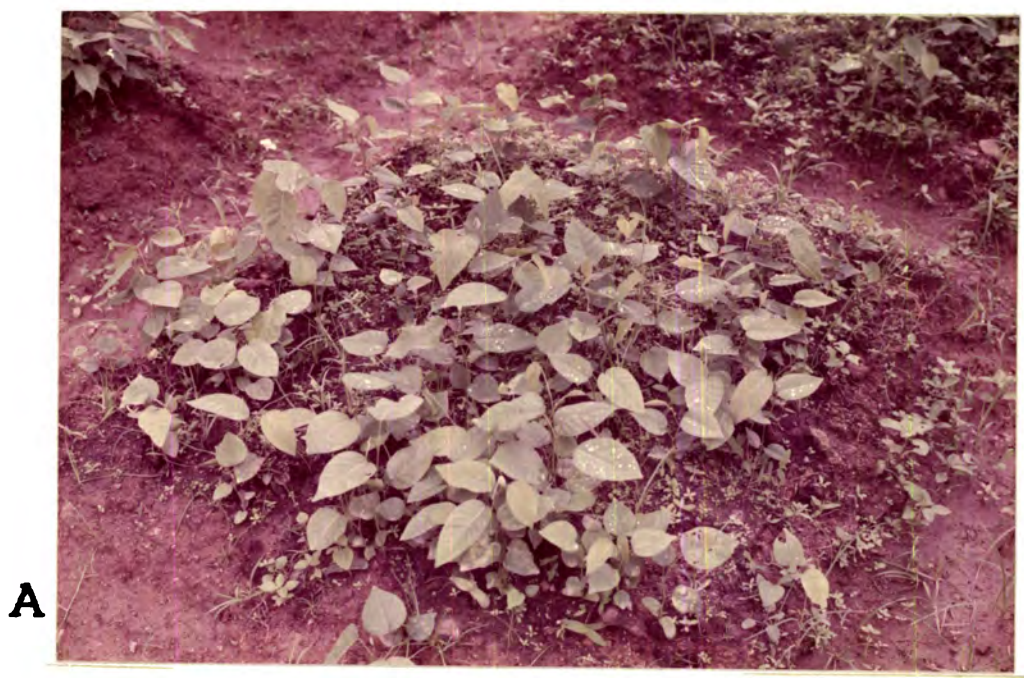
4C.5.2.1. Occurrence

Seedling blight disease was recorded in ca. 2 to 3-month-old seedlings in all the nursery beds raised at Peechi

during 1989. The diseases was noticed during late June and it continued till October end. Thereafter the incidence of the disease gradually declined , as the plants grew old and sturdy. Initially the incidence of the disease was 15.4%, which increased to 32.3% in July, 32.4% in August and then decreased to 3.8% and 3.3% respectively in September and October. The seedling blight disease was not observed in any of the nurseries surveyed in Karnataka during June 1988.

4C.5.2.2. Symptomatology and causal organism

Initially, small brownish-yellow spots appeared in concentric rings on the lamina of young leaves. These spots later enlarged in size and coalesced to form large necrotic areas (Plate 14) which sometime covered nearly 2/3 of the lamina. The infection spread rapidly through contact and rain splash. The diseased leaves were shed causing premature defoliation. Occasionally stem infection was also observed in some cases and such seedlings dried up soon. On the affected leaves and stem small off-white sclerotia developed. *Sclerotium rolfsii* Sacc. (IMI No. 3536504) was consistently isolated from the affected parts.



A

B

C

PLATE 14. *Pterocarpus marsupium*. A, View of the nursery bed; B, Initial leaf spots of leaf blight disease; and C, blighted seedlings caused by *Sclerotium rolfsii*.

4C.5.2.3. Pathogenicity

Pathogenicity of the isolate was confirmed by inoculating ten 6 to 8- week-old seedlings of *P. marsupium*. Young and mature leaves were inoculated by placing a sclerotium in a drop of sterile water. Ten seedlings were also inoculated with 5-10 sclerotia around the root collar zone in soil and incubated as mentioned under 3.7.7. In the latter case none of the seedlings developed infection . However, sclerotia placed on the young and mature leaves germinated and caused leaf blight within 5 to 7 days and seedling blight after 15 days.

4C.5.2.4.*In vitro* evaluation of fungicides

All concentrations of carboxin and thiram were found inhibitory while MEMC, PCNB and captan were effective only at higher concentrations. The efficacy of these fungicides (Table 44) was further tested using soil method, in which thiram and carboxin were found to be very effective While MEMC was effective with an ED_{100} at the concentration of 0.0125 % and 0.025% a.i. PCNB and captan were not effective (Table 44). In analysis of variance of data on % inhibition related to fungicides and concentration for both the methods separately indicates highly significant interaction (Table 45).

Table 44. Evaluation of fungicides against *S. rolfsii* causing seedling blight in *P. marsupium* using various methods

Fungicides and concentration	% a.i.	% inhibition over control	
		PFM*	SFSM+
Captafol (Difoltan)	0.05	48.5	
	0.1	74.5	Not tested
	0.2	77.0	
Captan (Deltan)	0.05	75.2	0
	0.1	87.2	40.7
	0.2	100	50.0
Carbendazim (Bavistin)	0.05	0	
	0.1	47.8	Not tested
	0.2	81.3	
Carboxin (Vitavax)	0.05	100	100
	0.1	100	100
	0.2	100	100
Copper oxychloride (Fytolan)	0.05	0	
	0.1	0	Not tested
	0.2	18.5	
Mancozeb (Dithane M-45)	0.05	34.8	
	0.1	38.5	Not tested
	0.2	61.1	
MEMC (Emisan)	0.006	82.4	81.5
	0.0125	100	100
	0.0250	100	100
PCNB (Brassicol)	0.05	84.6	13.0
	0.1	100	16.7
	0.2	100	22.2
Thiram (Thiride)	0.05	100	100
	0.1	100	100
	0.2	100	100
Ziram (Ziride)	0.05	27.8	
	0.1	60.0	Not tested
	0.2	70.0	

*Poisoned food method; +Soil fungicide screening method

Table 45. Analysis of variance of data on % inhibition of *S. rolfsii* causing seedling blight in *P. marsupium*

Source	Poisoned food method			Soil fungicide method		
	DF	MSS	F	DF	MSS	F
Treatment	9	9046.5	264.1**	4	15103.1	3669.7**
Concentration	2	4937.3	144.1**	2	1023.4	248.7**
Treatment x Concentration	18	470.6	13.7**	8	376.9	91.6**
Residual	60	34.4	-	30	4.1	-

** significant at p= 0.01

4C.5.2.5. Control measures

Pilot scale field trials conducted during 1990 at Peechi indicated that pre-sowing soil drenching of carboxin (0.2% a.i.) or thiram (0.2% a.i.)or MEMC (0.0125% a.i.) was most effective in controlling this disease. Control seed beds had an initial disease incidence of 10% in June which increased to 16.4 % during July-August. In another treatment where the seeds were dipped in various fungicides the disease appeared late in July only in the treatments of captan (0.3% a.i.) and mancozeb (0.3% a.i.) where the disease incidence was 9.7 and 6.9 % respectively. However, drenching of seed beds with carbendazim (0.2% a.i.) and PCNB (0.2% a.i.) was not effective and disease incidence of 7.7% and 8.2% was observed during June itself.

4D. *XYLIA XYLOCARPA*

4D.1. Seed Health testing methods

Among the five different seed health testing methods, the standard blotter method was found superior to others, in the expression of most of the spermoplane micro-organisms in both surface sterilised and non-surface sterilised seeds of *Xylia xylocarpa* (Table 46 & 47). Actinomycetes expressed on non-surface sterilised seeds in SB, 2,4-D and DF methods whereas on sterilised seeds only in DF method. *Fusarium pallidoroseum* was detected on non-surface sterilised seeds in all methods except MEA method while on surface sterilised seeds also, it was not detected in MEA method. Except DF method, *Aspergillus flavus* was recorded in higher percentage in all the methods on non-surface sterilised seeds; its occurrence on surface sterilised seeds in all the methods was not significantly different from each other. In SB and MEA methods the incidence of *Rhizopus oryzae* and *Penicillium citrinum* was significantly different from other methods while the expression of *R. oryzae* in SB method was significantly different involving surface sterilised seeds. *Chaetomium globosum* which was detected in SB and PDA methods on non-surface sterilised seeds could not be detected by any of the other methods on surface sterilised seeds. The incidence of *Cladosporium herbarum* on non-surface sterilised seeds by SB method was significantly higher than that of other methods.

Table 46. Percent incidence of spermoplane micro-organisms in different seed health testing methods on non-surface sterilised seeds of *X. xylocarpa*

Micro-organism	Methods				
	SB	2,4-D	DF	PDA	MEA
Actinomycetes	2^{a*}	2^{a}	7^{b}	0^{a}	0^{a}
Aspergillus flavus Link.	10^{bc}	18^{bc}	2^{a}	5^{ab}	21^{c}
A. niger van Tieghem	15^{b}	15^{b}	1^{a}	6^{a}	19^{b}
A. ochraceus Wilhelm	0^{a}	1^{a}	1^{a}	0^{a}	0^{a}
A. versicolor (vuill.) Tiraboschi	0^{a}	0^{a}	1^{a}	0^{a}	1^{a}
Chaetomium globosum Kunze.	2^{a}	0^{a}	0^{a}	3^{a}	0^{a}
Cladosporium herbarum (Pers.) Link. ex Gray	18^{b}	7^{a}	5^{a}	2^{a}	5^{a}
Fusarium pallidoroseum (Cooke.) Sacc.	2^{a}	9^{b}	2^{a}	6^{b}	0^{a}
Penicillium citrinum Thom.	15^{c}	6^{ab}	3^{ab}	5^{ab}	12^{c}
Rhizopus oryzae Went & Prinsen Geerligs	40^{b}	7^{a}	6^{a}	11^{a}	29^{b}
Trichoderma sp.	0^{a}	2^{a}	1^{a}	0^{a}	0^{a}
Trichothecium roseum (Pers.) Link. ex Gray	1^{a}	0^{a}	0^{a}	0^{a}	0^{a}
sterile hyphae (white)	2^{a}	0^{a}	0^{a}	1^{a}	1^{a}
Bacterium Gram (-)	5^{b}	0^{a}	1^{a}	1^{a}	1^{a}

*Mean values superscribed by the same letter(s) do not differ significantly at $p = 0.05$ (Row-wise comparison).

In general, surface sterilisation of seeds reduced the incidence of surface-borne saprophytic organisms like Actinomycetes, *A. flavus, A. niger, C. herbarum, P. citrinum,* and *R. oryzae*. Low incidence of *Phomopsis* sp., which was not detected on non-surface sterilised seeds in any of the methods, was recorded only on sterilised seeds by PDA method (Table 47).

Table 47. Percent incidence of spermoplane micro-organisms in different seed health testing methods on surface sterilised seeds of *X. xylocarpa*

Micro-organism	Methods				
	SB	2,4-D	DF	PDA	MEA
Actinomycetes	0^{a}	0^{a}	5^{b}	0^{a}	0^{a}
Aspergillus flavus	5^{a}	3^{a}	1^{a}	3^{a}	4^{a}
A. niger	7^{b}	3^{ab}	1^{a}	1^{a}	1^{a}
Cladosporium herbarum	5^{b}	0^{a}	2^{b}	2^{b}	2^{b}
Fusarium pallidoroseum	2^{a}	4^{b}	3^{b}	3^{b}	0^{a}
Penicillium citrinum	4^{a}	0^{a}	3^{a}	3^{a}	2^{a}
Phomopsis sp.	0^{a}	0^{a}	0^{a}	3^{a}	0^{a}
Rhizopus oryzae	10^{b}	4^{a}	4^{a}	3^{a}	2^{a}
Bacterium Gram (-)	7^{b}	3^{ab}	1^{a}	1^{a}	1^{a}

* Mean values with the same superscript(s) do not differ significantly at p = 0.05 (Row-wise comparison)

4D.2. Seed microflora and their significance

4D.2.1. Dry seed examination

The dry seed examination revealed the presence of apparently healthy, discoloured, and discoloured and shrivelled seeds (Plate 15). The percent occurrence of apparently healthy seeds was the highest (50.6%) followed by discoloured (28.6%) and shrivelled (20.8%) seeds. The weight of 100 seeds was also higher in apparently healthy seeds (40. 1 g), followed by discoloured seeds (27.2 g) and shrivelled seeds (20.5g), while the 100 seed weight of pooled sample was 31.8 g.

4D.2.2. Incidence of micro-organisms in different categories of seeds

In apparently healthy seeds the incidence of various micro-organisms varied from 1-30% incidence of *R. oryzae* was the highest. From non-surface sterilised seeds only nine micro-organisms were recorded, while only five occurred on surface sterilised seeds. The percent germination of apparently healthy seeds was higher as compared to other categories of seeds in SB method. Discoloured seeds harboured twelve species of micro-organisms on non-surface sterilised seeds, whereas only nine species were detected on surface sterilised seeds. The highest incidence (38%) was recorded by *R. oryzae*. A total of fourteen species of micro-organisms

A B C

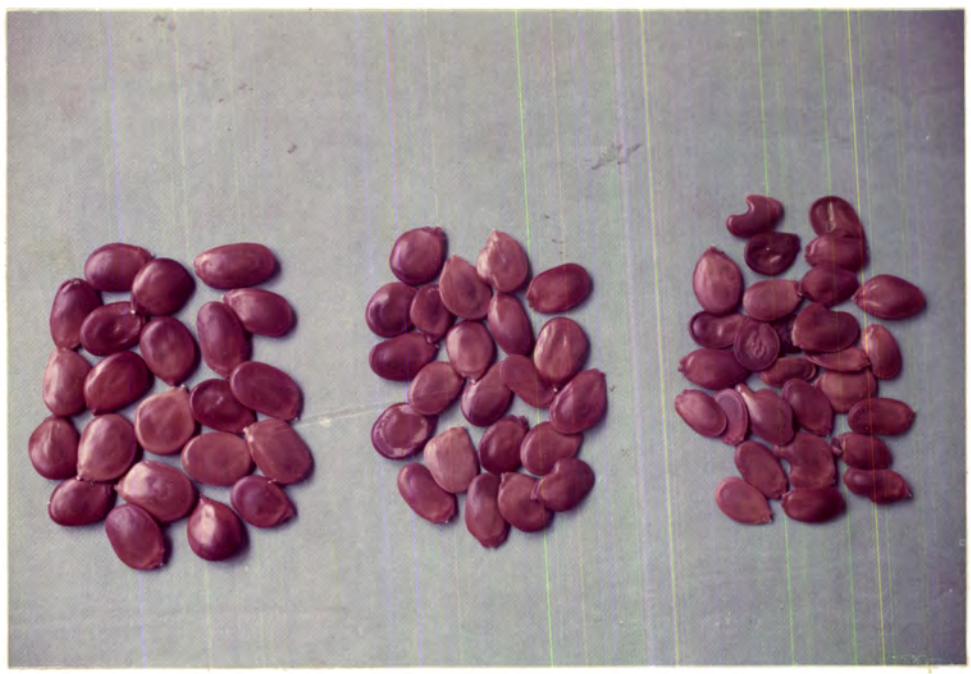


PLATE 15. Seeds of *Xylia xylocarpa* showing apparently healthy (A), discolored (B) and shrivelled (C) categories.

were recorded on shrivelled category of seeds varying in incidence from 2% -40% *Botryodiplodia theobromae*, not detected in any of the other categories, was recorded on non-surface sterilised seeds of this category (Figs.17 & 18).

4D.2.3. Pathogenicity studies

The effect of various micro-organisms on seed germination, seedling development, shoot and root lengths, and vigour index is given in Fig. 19 & 20. The percent seed germination was reduced from 59% in untreated control to 18%, when the seeds were inoculated with spores of *A. flavus* which caused suspected radicle decay symptoms (Plate 16). However, the percent reduction in germination by other micro-organisms ranged between 17 to 34%. Bacterium Gram(-), *C. herbarum* and *F. pallidoroseum* caused the maximum distortion of seedlings. Delay in seed germination was noted in seeds treated with *P. citrinum, A. flavus, A. niger* and *R. oryzae* (Fig.19). *Rhizopus oryzae* grew on treated seeds as well as on soil and caused seed decay. Profuse growth actinomycetes was also noticed on the seed coat (Plate 17). However, none of the micro-organisms tested caused any lesions on cotyledons and blight of seedlings. Shoot and root lengths were considerably reduced in seeds treated with *A. flavus*. However, root length was slightly higher in seeds treated with *R. oryzae*, while the

PLATE 16. *Xylia xylocarpa*. A, Profuse growth of *A. flavus* on a seed; B, An emerging seedling showing radicle decay

A

B

PLATE 17. A, Profuse growth of *Rhizopus oryzae* and B, Actinomycetes growing on the seed coat of *X. xylocarpa* in a nursery.

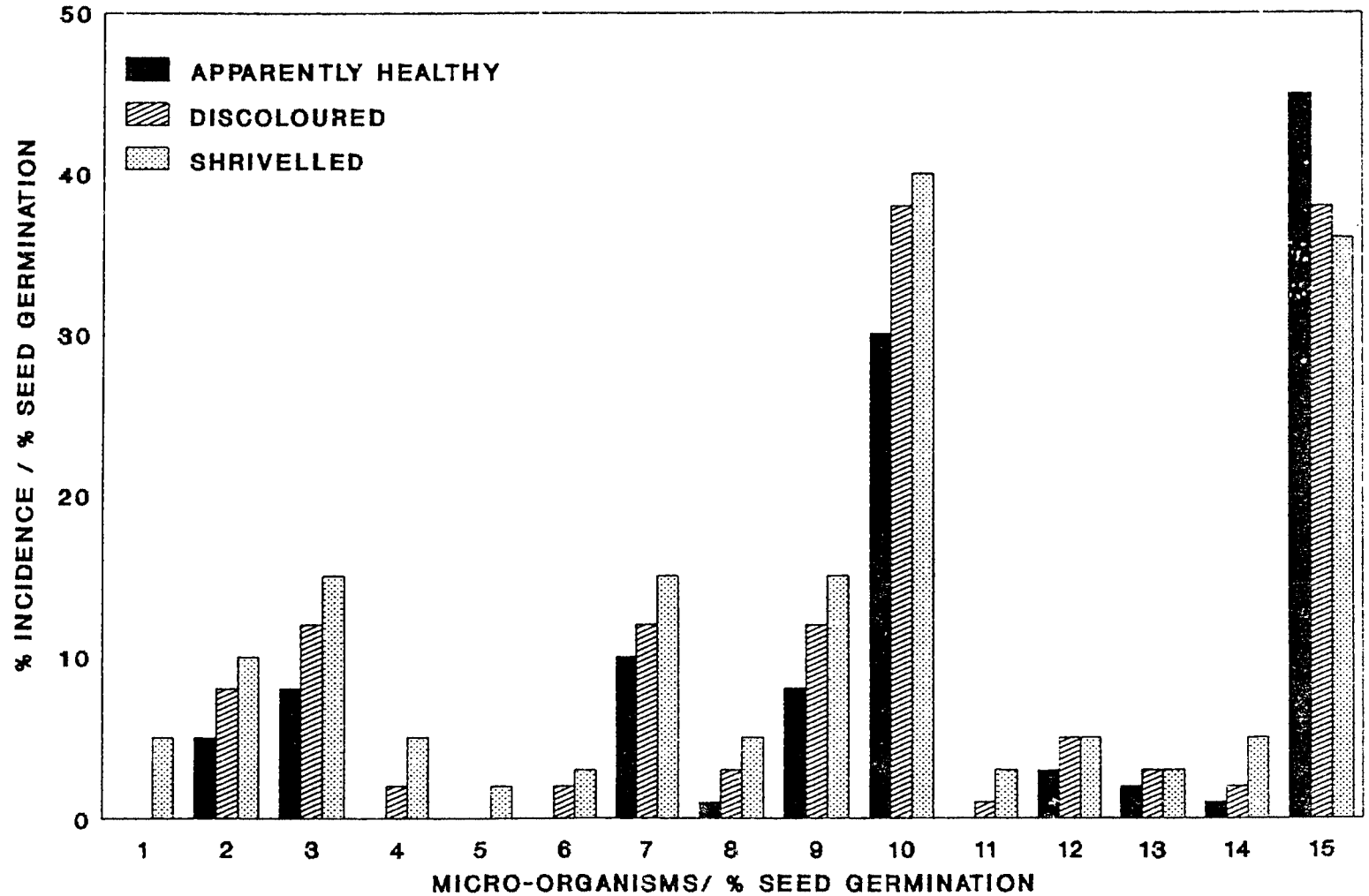


Fig.17. Percent incidence of spermoplane micro-organisms on non-surface sterilised seeds and % seed germination in different categories of seeds of *X. xylocarpa*.

1. Actinomycetes,2. *Aspergillus flavus*, 3. *A. niger*, 4. *A. versicolor*, 5. *Botryodiplodia theobromae*, 6. *Chaetomium globosum*, 7. *Cladosporium herbarum*, 8. *Fusarium pallidoroseum*, 9. *Penicillium citrinum*, 10. *Rhizopus oryzae*, 11. *Trichothecium roseum*, 12. sterile hyphae(white), 13. sterile hyphae (black), 14. Bacterium Gram (-), 15. % seed germination.

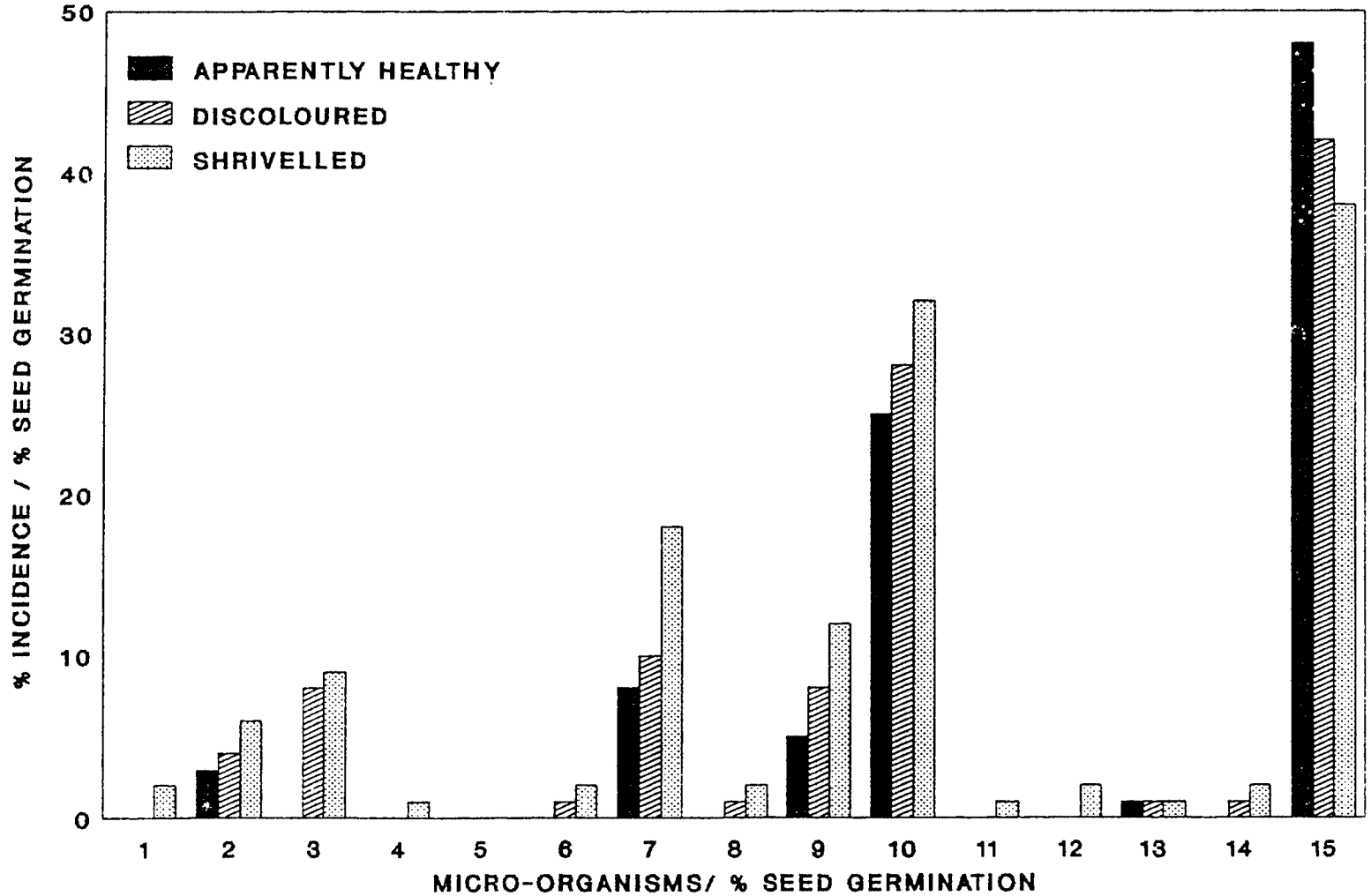


Fig.18. Percent incidence of spermoplane micro-organisms on surface sterilised seeds and % seed germination in different categories of seeds of *X. xylocarpa*.

1. Actinomycetes,2. *Aspergillus flavus*, 3. *A. niger*, 4. *A. versicolor*, 5. *Botryodiplodia theobromae*, 6. *Chaetomium globosum*, 7. *Cladosporium herbarum*, 8. *Fusarium pallidoroseum*, 9. *Penicillium citrinum*, 10. *Rhizopus oryzae*, 11. *Trichothecium roseum*, 12. sterile hyphae(white), 13. sterile hyphae (black), 14. Bacterium Gram (-), 15. % seed germination.

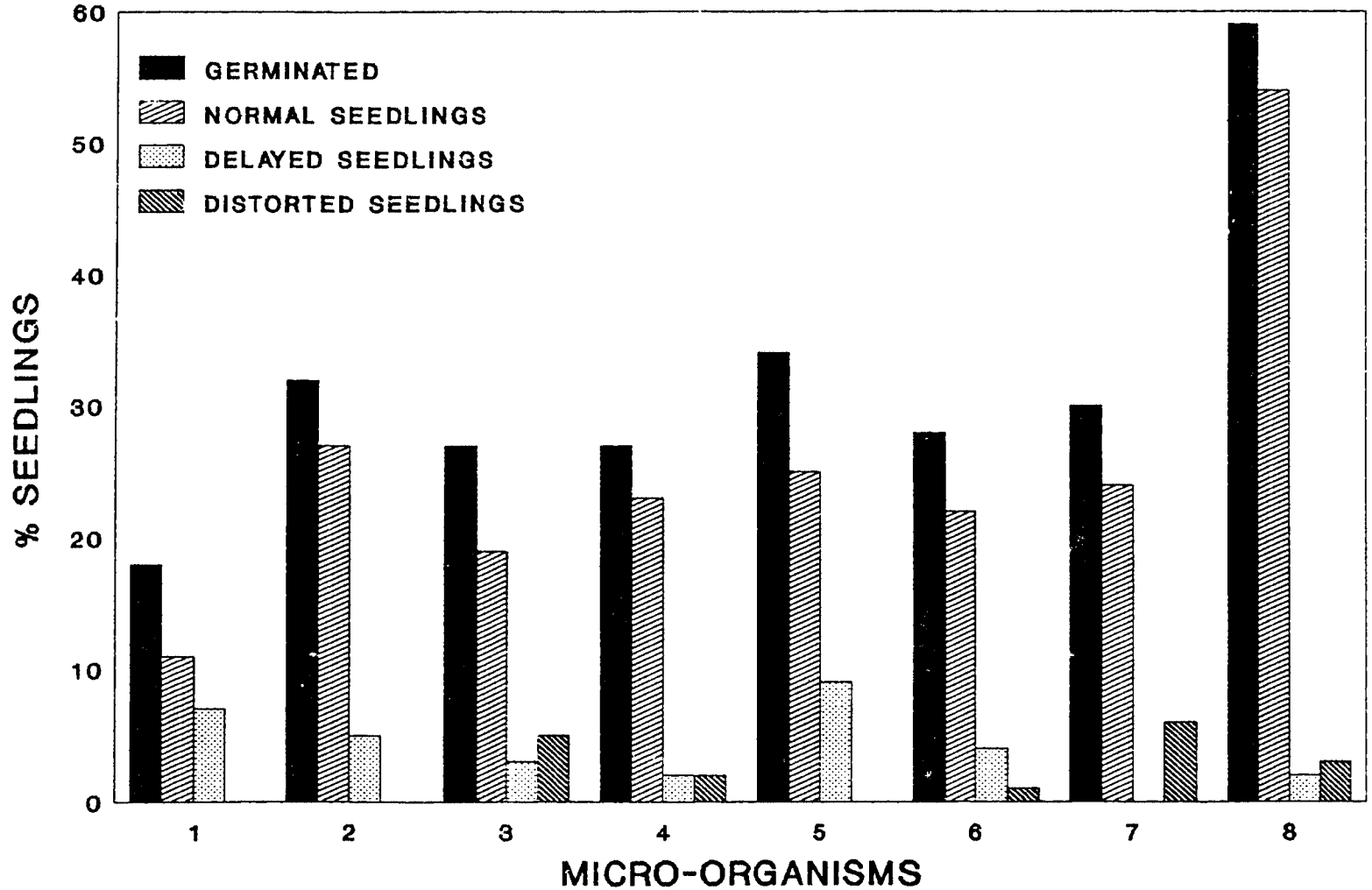


Fig.19. Effect of various micro-organisms on seed germination and seedling emergence of *X. xylocarpa*.

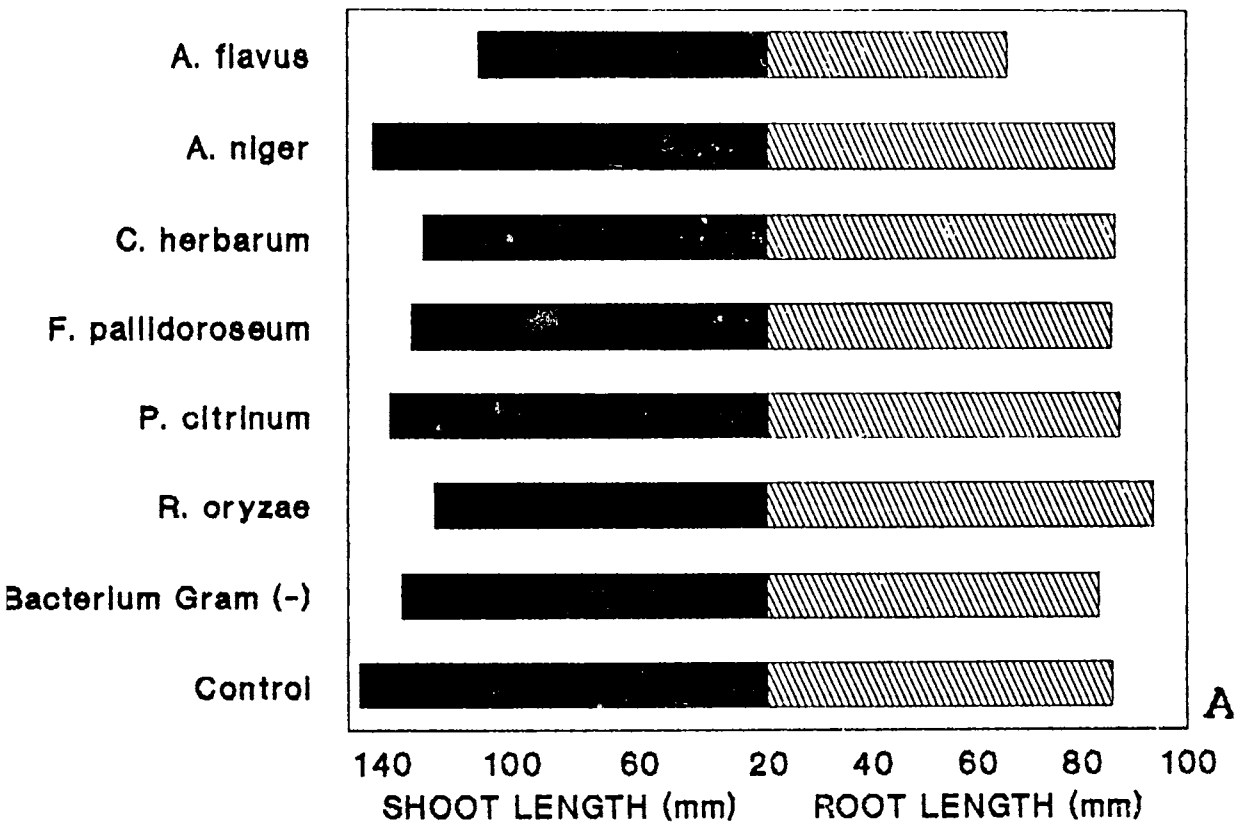
1. *Aspergillus flavus*, 2. *A. niger*, 3. *Cladosporium herbarum*, 4. *Fusarium pallidoroseum*, 5. *Penicillium citrinum*, 6. *Rhizopus oryzae*, 7. Bacterium Gram (-), 8. Control.

shoot length was less as compared with control. In other cases root length did not show much difference, although the shoot length did change. Vigour index was the lowest in seeds treated with *A. flavus*, followed by *C. herbarum*, *F. pallidoroseum*, *R. oryzae*, bacteria, *A. niger* and *P. citrinum* (Fig. 20).

4D.3. Management of seed microflora

4D.3.1. Hot water treatment

The germination percentage of the seeds subjected to hot water treatment at 50°C for 30 min. and 60°C for 15 and 30 min., was significantly lower from untreated control and treatment of 50°C- 15 min. The shoot length increased considerably as compared with untreated control in treatments involving 50°C-15 min., 60°C-30 min., while the root length was observed significantly higher in treatment at 60°C-30 min. and 60°C-15 min. The vigour index in various treatments did not show significant difference except the treatment of 50°C-30 min. (Table 48). Hot water treatment at different temperature did induce sloughing-off the seed coat.



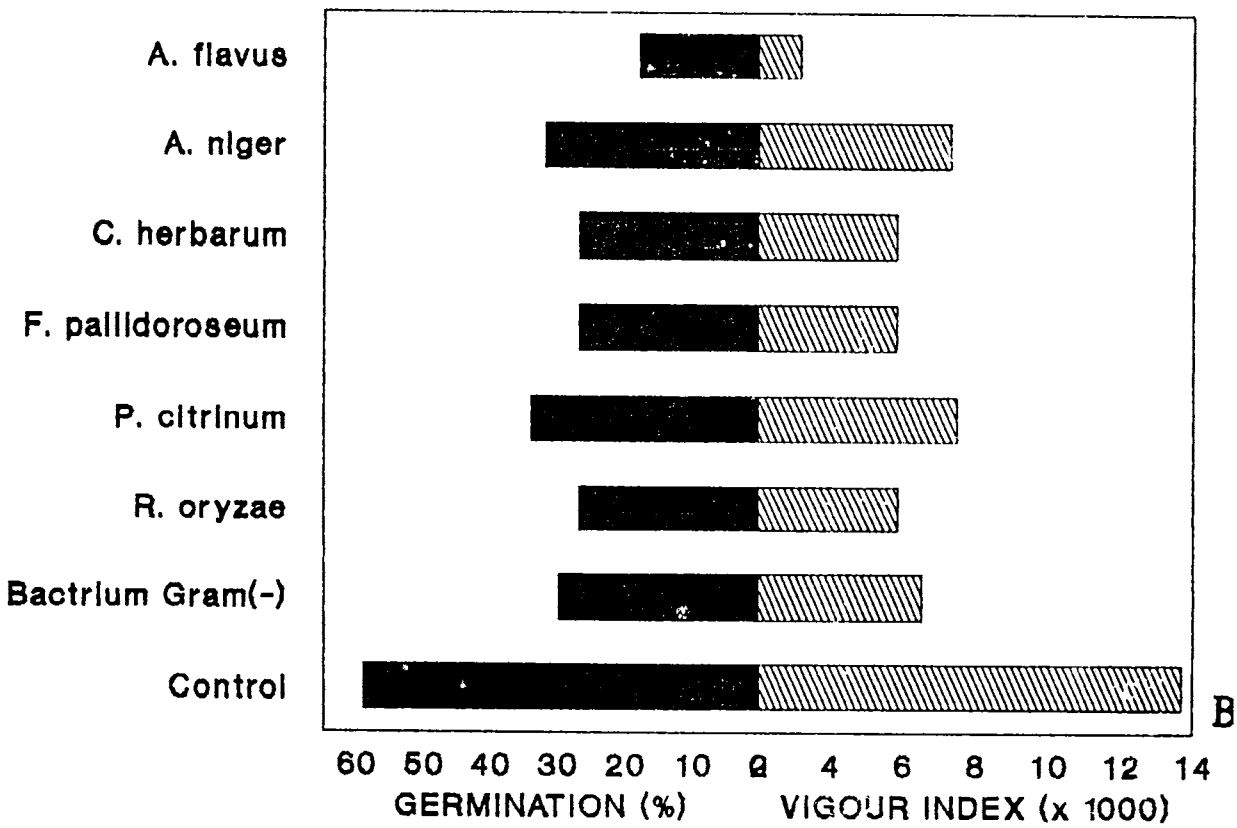


Fig.20. Effect of various micro-organisms on shoot and root lengths (A); seed germination and vigour index (B) of *X. xylocarpa*.

Table 48. Effect of hot water treatment on seed germination and growth of seedlings in *X. xylocarpa*

Observations	50°C		60°C		Control
	15 min.	30 min.	15 min.	30 min.	
Germination (%)	52[b*]	38[a]	36[a]	38[a]	58[b]
Shoot length(mm)	125.5[ab]	77.2[a]	117.9[ab]	145.5[b]	117[ab]
Root length (mm)	44.4[a]	57.3[a]	89.8[b]	93.9[b]	49.9[a]
Vigour index(VI)	8782.7[b]	5174.5[a]	7433.5[b]	9007.2[b]	9306.1[b]
No. of micro-organism recorded	4	2	4	2	9

* Mean values superscribed by the same letter (s) do not differ significantly at p = 0.05 (Row-wise comparison)

The number of micro-organisms developed also reduced from nine in control to two to four in different treatments (Table 49). However, *R. oryzae* and *A. flavus* maintained their high incidence in most of the treatments. Incidence of *Rhizopus oryzae* was not significantly different except in treatments of 60°C - 30 min. and control. Surprisingly *F. pallidoroseum* was not suppressed both in control and 50° - 15 min., while it was completely inhibited in other treatments. The incidence of *A. niger* was inhibited only in treatments of 50°C and 60°C-30 min., while *A. versicolor, C. herbarum, C. globosum* and sterile hyphae were completely inhibited in all the treatments.

Table 49. Effect of hot water treatment on the incidence of spermoplane micro-organisms of *X. xylocarpa*

Micro-organism	50°C		60°C		Control
	15 min.	30 min.	15 min.	30 min.	
Aspergillus flavus	12^{ab*}	10^{a}	38^{bc}	16^{b}	22^{bc}
A. niger	2^{a}	0^{a}	2^{a}	0^{a}	4^{b}
A. versicolor	0^{a}	0^{a}	0^{a}	0^{a}	2^{a}
Cladosporium herbarum	0^{a}	0^{a}	0^{a}	0^{a}	6^{b}
Chaetomium globosum	0^{a}	0^{a}	0^{a}	0^{a}	4^{b}
Fusarium pallidoroseum	4^{a}	0^{a}	0^{a}	0^{a}	6^{b}
Penicillium citrinum	0^{a}	0^{a}	2^{a}	0^{a}	14^{b}
Rhizopus oryzae	48^{a}	40^{a}	44^{a}	62^{b}	72^{b}
sterile hyphae	0^{a}	0^{a}	0^{a}	0^{a}	2^{a}

*Mean values with the same superscript(s) do not differ significantly at p = 0.05 (Row-wise comparison)

4D.3.2.. Chemical Treatment

Thiram was the most effective fungicide in giving the highest vigour index of seedlings followed by mancozeb and carbendazim. Germination percentage and vigour index of seeds showed a significant increase in all the treatments as compared with control (Table 50). While the shoot length of various treatments did not differ significantly from the control,

the root length in in captafol was significantly lower than the control.

Table 50. Effect of various seed dressers on seed germination and growth of seedlings of *X. xylocarpa*

Treatment	Germination (%)	Mean shoot length (mm)	Mean root length (mm)	Vigour index	No.of micro-organisms recorded
Captafol	60^{bc*}	105.7^{a}	41.0^{a}	8737.6^{bc}	2
Captan	57^{b}	104.2^{a}	57.4^{d}	9167.8^{bc}	-
Carbendazim	62^{bc}	108.9^{a}	44.9^{ab}	9267.8^{bc}	2
Carboxin	61^{bc}	102.4^{a}	48.1^{bc}	9110.9^{bc}	-
Mancozeb	65^{bc}	106.1^{a}	55.0^{cd}	10173.9^{bc}	-
MEMC	61^{bc}	105.5^{a}	47.8^{bc}	9265.4^{bc}	-
PCNB	57^{b}	100.5^{a}	51.1^{bc}	8561.5^{b}	2
Thiram	74^{c}	102.4^{a}	53.5^{cd}	11463.5^{d}	2
Control	51^{a}	101.5^{a}	48.7^{bc}	7660.2^{a}	11

*Mean values with the same superscript(s) in a column do not differ significantly at p = 0.05

All the eight fungicides were effective in inhibiting the occurrence of a number of micro-organisms. While carboxin, MEMC, captan and mancozeb inhibited completely the development of all micro-organisms; only 2 micro-organisms

were recorded in treatments with PCNB, thiram, carbendazim and captafol. The incidence of the micro-organisms was significantly lower than in untreated control (Table 51), except in the case of *Memnoniella echinata* and a bacterium. Interestingly , *M. echinata* was only recorded on seeds treated with captafol and PCNB.

4D.4. Seed storage and its influence on microflora, seed germination and seedling development

4D.4.1. Incidence of micro-organisms

The incidence of seed micro-organisms on treated seeds of *X. xylocarpa* showed that most fungi were either eliminated or their incidence decreased gradually with the increasing storage period. In treated seeds, the occurrence of storage fungi viz., *Aspergillus* spp., *Rhizopus oryzae, Cladosporium herbarum, Penicillium citrinum* etc., was low. However, a profuse growth of *R. oryzae* was recorded on seeds treated with carbendazim. In the case of control seeds, the occurrence of micro-organisms did not change appreciably, except for the appearance of *M. echinata* in a few seed samples stored for 180 days and more. In seeds stored under dehumidified conditions at room temperature and at 4°C, the number of micro-organisms were less as compared to seeds stored under laboratory conditions (Table 52).

Table 51. Effect of various seed dressers on the incidence of spermoplane micro-organisms of *X. xylocarpa*

Micro-organism	Control	Capta-fol	Captan	Carben-dazim	Carb-oxin	Manco-zeb	MEMC	PCNB	Thira
Actinomycetes	4^{b*}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Aspergillus flavus	10^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	2^{a}	2^{a}
A. niger	16^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Cladosporium herbarum	22^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Chaetomium globosum	2^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Fusarium pallidoroseum	4^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Memnoniella echinata	0^{a}	2^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	4^{a}	0^{a}
Penicillium citrinum	18^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Rhizopus oryzae	42^{c}	0^{a}	0^{a}	16^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Trichoderma sp.	2^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
sterile hyphae	2^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Bacterium Gram(-)	4^{b}	6^{b}	0^{a}	14^{b}	0^{a}	0^{a}	0^{a}	0^{a}	4^{b}

*Mean values with the same superscript(s) do not differ significantly at p 0.05 (Row-wise comparison)

Table 52. Micro-organisms recorded on seeds of *X. xylocarpa* stored for different periods under various treatments

Treatments	Day-1	Day-90	Day-180	Day-365
Captafol	4,11*	2,3,4	2,3	2,3
Captan	Nil	Nil	Nil	Nil
Carbendazim	4,9	9	9	9
Carboxin	Nil	Nil	Nil	2,3,9
Mancozeb	Nil	Nil	3	3,9
MEMC	Nil	Nil	Nil	2,9
PCNB	2,11	2	5	2,3
Thiram	2,4	2	2	2,3
Dehumidified cond. Room temp.	1,2,3,4,5,6,7, 8,9,10,12	2,3,7,9	2,3,8,9	2,3,5,8
Dehumidified cond. 4°C	1,2,3,4,5,6,7, 8,9,10,12	2,3,7,9	3,5,7,9	2,3,5,8
Control(Plastic-containers)	1,2,3,4,5,6,7, 9,10,12	2,3,5, 7,8,9	2,3,5,7,8, 9,11,12,	2,3,5,7, 8,9,11
Control (cloth bags)	1,2,3,4,5,6,7, 8,9,10,12	2,3,5, 7,8,9	2,3,5,7,8, 11,12	2,3,5,7, 8,9

* 1. Actinomycetes, 2. *Aspergillus flavus*, 3. *A. niger*, 4. Bacterium, 5. *Cladosporium herbarum*, 6. *Chaetomium globosum*, 7. *Fusarium pallidoroseum*, 8. *Penicillium citrinum*, 9. *Rhizopus oryzae*, 10. *Trichoderma* sp., 11. *Memnoniella echinata*, 12. sterile hyphae.

4D.4.2.. Germination and seedling development

As the storage period increased the percent seed germination in various treatments decreased gradually (Table 53). The control seeds which were stored either in cloth bags or in closed plastic containers, where the initial germination of about 52%, reduced to about 30% after 90 days of storage, and ca. 15% after 180 days of storage; there was complete loss of viability at the end of one year. This was the case in most of the fungicide treated seeds. However, the seeds stored under low temperature and dehumidified condition, the germination percent reduced from the initial 55% to 26% after 365 days of storage. Even the seeds stored at room temperature under dehumidified conditions, did not lose germinability completely and ca. 17 % seeds germinated even after 1 year. The vigour index of seeds treated with thiram, captan, captafol, mancozeb came down considerably at the end of 1 year of storage. The vigour index of seeds stored under dehumidified condition at 4°C and room temperature did not differ significantly. Analysis of variance of data pertaining to seed germination and vigour index of storage of seeds of *Xylia xylocarpa* for 1 year showed that the interaction between them was highly significant (Table 54).

Table 53. Effect of various seed dressers and storage conditions on seed germination and vigour index of *X. xylocarpa*

Treatment	Germination (%)				Vigour index			
	Day 1	Day-90	Day-180	Day-365	Day-1	Day-90	Day-180	Day-365
Captafol	60^{bcd*}	35^{ab}	18^{bcd}	12^{c}	8737.8^{bc}	4282.0^{ab}	1609.7^{abc}	350.4^{b}
Captan	57^{abc}	44^{c}	14^{bcd}	4^{ab}	9167.8^{c}	6348.4^{d}	2118.3^{bcd}	690.5^{b}
Carbendazim	62^{bcd}	33^{ab}	6^{a}	0^{a}	9267.8^{c}	5374.6^{bc}	848.1^{a}	0^{a}
Carboxin	61^{bcd}	38^{bc}	14^{bcd}	0^{a}	9110.9^{c}	6160.3^{bc}	2298.8^{bcd}	0^{a}
Mancozeb	65^{cd}	43^{bc}	14^{bcd}	4^{ab}	10173.9^{d}	6241.3^{bc}	1560.8^{abc}	769.8^{b}
MEMC	61^{bcd}	39^{bc}	10^{ab}	0^{a}	9265.4^{c}	5971.4^{bc}	1324.2^{ab}	0^{a}
PCNB	57^{ab}	36^{bc}	12^{bc}	0^{a}	8561.5^{bc}	5062.5^{abc}	1480.2^{abc}	0^{a}
Thiram	74^{d}	41^{c}	16^{bcd}	4^{ab}	11463.5^{c}	5633.0^{bc}	1912.4^{bcd}	415.4^{b}
Dehumidified cond. Room temp.	53^{a}	37^{bc}	21^{de}	17^{d}	7975.4^{a}	5416.5^{bc}	3186.6^{de}	2620.7^{c}
Dehumidified cond. 4°C	55^{ab}	40^{abc}	26^{e}	26^{d}	7963.1^{a}	6068.8^{cd}	4494.1^{e}	3940.9^{c}
Control (plastic containers)	51^{a}	29^{a}	15^{bcd}	0^{a}	7660.2^{a}	3821.7^{a}	2323.3^{bcd}	0^{a}
Control (cloth bag)	52^{a}	32^{ab}	17^{cd}	0^{a}	7886.3^{a}	4629.1^{abc}	2607.1^{cd}	0^{a}

*Mean values in a column with the same superscript(s) do not differ significantly at p = 0.05.

Table 54. Analysis of variance of germination and vigour index of seeds *X. xylocarpa* stored for 1 year

Source	Germination			Vigour index		
	DF	MSS	F	DF	MSS	F
Day	3	12382.4	390.8**	3	23667.9	581.9**
Treatment	11	181.0	5.7*	11	334.4	5.0*
Day x Treatment	33	122.4	3.4*	27	459.0	6.8*
Residual	144	31.7	-	122	67.3	-

** significant at p = 0.01
* significant at p = 0.05

4D.5. Seedling diseases and their control

4D.5.1. Seedling blight

4D.5.1.1. Occurrence

There were no diseases recorded in the experimental nurseries raised at Peechi and Nilambur during 1988, and 1989 season, and in the various nurseries surveyed in Haliyal Forest Division of Karnataka. However, a seedling blight disease was recorded (<5%) in a few container seedlings (ca. 6 months old) kept for transplanting at Nilambur during September 1989.

4D.5.1.2. Symptomatology and causal organism

Initially the lower leaves turned light yellow and within a week all of them were affected and the apical portion of seedlings showed wilting. In severe cases complete defoliation occurred and the whole aerial portion dried up; however, no root infection was observed (Plate 18). *Rhizoctonia solani*. Kuhn anamorph of *Thanatephorus cucumeris* (Frank) Donk. (IMI NO. 326296) was consistently isolated from the affected portions.

4D.5.1.3. Pathogenicity

The pathogenicity of the isolate was confirmed in an artificial inoculation experiment. The typical disease symptoms were observed after 5- 7 days of incubation in infested soil.

4D.5.1.4. *In vitro* evaluation of fungicides

Laboratory screening using poisoned food method indicated that carbendazim and MEMC were the best fungicides in inhibiting the radial growth of *R. solani* completely, followed by PCNB, carboxin and thiram. However,these fungicides gave promising results in the soil fungicide screening method only at the highest concentration tested (Table 55). Carbendazim (0.2% a.i.), carboxin (0.2% a.i.) and MEMC (0.025% a.i.) were the best fungicides in inhibiting the growth of the pathogen completely.

A

B

PLATE 18. *Xylia xylocarpa* .A, View of a healthy nursery at Peechi; B, seedling blight symptoms leading to complete defoliation.

Table 55. Evaluation of fungicides against *R. solani* causing seedling blight in *X. xylocarpa* using various methods

Fungicides and concentration	% a.i.	% inhibition over control PFM*	SFSM+
Captafol (Difoltan)	0.05	72.2	
	0.1	76.7	Not tested
	0.2	79.3	
Captan (Deltan)	0.05	72.6	
	0.1	75.2	Not tested
	0.2	77.8	
Carbendazim (Bavistin)	0.05	100	21.1
	0.1	100	43.3
	0.2	100	100
Carboxin (Vitavax)	0.05	84.4	76.7
	0.1	85.6	83.3
	0.2	87.0	87.0
Copper oxychloride (Fytolan)	0.05	64.6	
	0.1	75.4	Not tested
	0.2	80.7	
Mancozeb (Dithane-M-45)	0.05	72.8	
	0.1	73.9	Not tested
	0.2	75.8	
MEMC (Emisan)	0.006	100	57.4
	0.0125	100	75.9
	0.0250	100	100
PCNB (Brassicol)	0.05	83.3	20.0
	0.1	83.3	32.2
	0.2	88.9	33.0
Thiram (Thiride)	0.05	66.7	41.9
	0.1	70.4	43.0
	0.2	73.7	43.3
Ziram (ziride)	0.05	50.4	
	0.1	61.1	Not tested
	0.2	70.0	

*Poisoned food method; +Soil fungicide screening method.

In analysis of variance of data on % inhibition in both the methods showed significant interaction between fungicides and concentrations (Table 56).

Table 56. Analysis of variance of data on % inhibition of *R. solani* causing seedling blight in *X. xylocarpa*

Source	Poisoned food method			Soil fungicide metho		
	DF	MSS	F	DF	MSS	F
Treatment	9	1449.4	176.6**	4	5249.4	2015.1*
Concentration	2	328.0	40.0**	2	3876.4	1488.0*
Treatment x Concentration	18	33.5	4.1*	8	762.7	292.8*
Residual	60	8.2	-	30	2.6	-

** significant at p = 0.01
* significant at p = 0.05

As this disease was neither a serious nor a common one and occurred only in a few container seedlings, no pilot scale field trials attempted.

5. DISCUSSION

5. DISCUSSION

In India, over 90 million hectares of land has been classified as wastelands and the deficit of fuel wood and fodder has been estimated to the tune of over 133 and 150 million ton respectively. To fulfill the twin objective of afforesting the waste lands and to solve fuel wood and fodder crisis, it was estimated that ca. 3 billion seedlings would be required to plant every million hectare of waste land (Bachketi, 1986). For meeting this demand of large scale forestry activities, in spite of genetical advantage of clonal propagation, seed is still the main source of planting material. Forestry is becoming increasingly dependent on a constant supply of good quality seeds. Quality seeds are essential for the production of healthy crops. Till now studies on cause for poor germination was directed at seed dormancy, seed size and maturity and very little attention was given to the seed deterioration due to micro-organisms. Seed health testing is primarily concerned with the evaluation of the presence or absence of disease causing organisms viz., fungi, bacteria, virus and nematodes, with fungi, the most important group of micro-organisms causing loss of seed viability. Micro-organisms affect the developing fruit, invade the seed and thus make the seed unhealthy. Further, when the fruit falls on the ground, the seed is subjected to further invasion by

forest floor decay micro-organisms. Spermoplane micro-organisms can cause decay and death of the seed or indirectly weaken the seed thereby predisposing attack by soil fungi. Seeds of several conifers and other soft wood and hardwood tree species are prone to attack by fungi (Mittal *et al.*, 1990). However, pathogenicity of many of these fungi has been much debated, because most of the spermoplane micro-organisms are usually moulds, and are saprophytic in nature. But now they are also being studied as causal agents of pre- and post-emergence loses in forestry crops (Gibson,1957; Mittal, 1979 Vijayan,1988)

Current information on seed pathology of forest tree species in India is very meagre and is mainly concerned with listing of fungi associated with a particular seed lot (Mittal *et al.*, 1990). No work has been carried out on i. the evaluation of seed health testing methods to come out with the best method to record the maximum number of micro-organisms, ii. tree seed storage and development of microflora and iii. tree seed disorders and their management and hence the present study was undertaken on the four major indigenous tree species of Kerala.

5.1. Seed pathological studies

5.1.1.Seed health testing methods

The selection of a particular method for seed health testing generally depends upon the type of micro-organisms associated with the seed and possibly handling convenience. Among the various seed health testing procedures developed to test the health of the seed and identify the pathogens carried with them, standard blotter (SB) and agar plate methods are the most widely used (ISTA, 1966,1976,1985). Seed health testing methods mainly developed for agricultural crops, have never been evaluated for forestry seeds in India or else where, and this is the first time such an attempt has been made. This is considered essential in view of certain inherent characteristics and requirement of forestry seeds. Forest tree seed varies greatly in size and shape; for example seeds of *Haldina cordifolia* are very minute, light in weight (10 million/kg) with viability extending up to an year (Troupe, 1985) while many dipterocarp seeds are winged, large and whose viability last only for a few days (Mohanan and Sharma, 1991). Considering the enormous variability in size, availability of tree seeds and other factors, the prescribed number of seeds for seed testing i.e., 400 (ISTA, 1976, 1985) is highly impractical for forestry seeds. For example, in the present

investigation, the size of the seeds of *Pterocarpus marsupium* is the largest (49.5x42.5 mm), followed by *Xylia xylocarpa* (14 x 9.7 mm), *Lagerstroemia microcarpa* (8.8 x 3.9 mm) and *Albizia odoratissima* (6.6 x 4.7 mm) and thereby the number of seed available per kilogram is also less. In addition, highly variable flowering and fruit setting is observed in indigenous trees and generally a good seed setting is recorded only in alternate years. Hence, a compromise was made in the selection of number of seeds to over come practical problems for seed testing and appropriately 400 seeds each of *L. microcarpa* and *A. odoratissima* with small seed size and 50 and 100 seeds each of *P. marsupium* and *X. xylocarpa* with large seed size were respectively used. Based on the results obtained for large sized seeds, it may be advisable to reduce the number of seeds, as this is not only convenient for handling but also there is no loss in the realistic picture of the spermoplane microflora.

Although, in the present investigation, SB method is found highly suitable than other methods for routine seed health testing of forest trees, other methods have their own significance on the development of micro-organisms. Comparison of various seed health testing methods (Table 57) indicates that for all the four species used in the investigation SB

method is found the best. SB method not only provides quantitative and qualitative assessment of micro-organisms, but ease in handling, less material requirements makes it very economical.

In *Albizia odoratissima*, actinomycetes and *Colletotrichum gloeosporioides* and in *L. microcarpa*, *Fusarium solani* are observed in higher incidence in 2,4-D method on non- surface sterilised seeds, while a species of *Phomopsis* expressed well in PDA and MEA methods on surface sterilised seeds. Similar result is obtained in the case of *Pterocarpus marsupium* where *F. moniliforme* var. *intermedium* grew well in PDA and MEA methods, while higher percentage of *M. roridum* is detected on non- surface sterilised seeds in SB and DF method. In *Xylia xylocarpa* also, SB method appears to be superior to other methods; however, *F. pallidoroseum* is detected better in 2,4-D and SB method on non-surface sterilised seeds than others. In the present investigation also high incidence of *F. moniliforme* and *F. solani* in blotter and PDA methods confirm the earlier observations of Neergaard (1973) and Bilgrami *et al.* (1979) who found that SB and agar methods are best for *F. moniliforme* and *F. solani* for the seeds of urad, mung and masour. High incidence of *Fusarium moniliforme* var. *intermedium* recorded from *P. marsupium* in

Table 57. Comparison of various seed health testing methods

Tree species	Seed health testing methods									
	SB		2,4-D		DF		PDA		MEA	
	NSS	SS	NSS	SS	NSS	SS	NSS	SS	NSS	SS
A.odoratissima										
TN	14	8	8	6	8	8	9	7	7	7
TN1	3	2	3	1	3	1	2	-	2	1
MHI	Ch	Af	Pc	Pc	Pc	Pc	Ch	Af	Pc	Pc
L. microcarpa										
TN	9	9	8	5	10	6	10	6	9	7
TN1	6	2	3	3	3	-	1	-	-	-
MHI	Pc	B	Fs	Fs	Ro	Ro	Ph	Ph	Af	Ph
P. marsupium										
TN	15	10	9	8	9	9	14	13	10	9
TN1	11	6	8	7	7	8	10	12	9	8
MHI	Act	Ro	Act	Ao	Act	Ao	Ao	Ao	Ro	Bt
X. xylocarpa										
TN	11	7	9	5	11	8	9	8	8	6
TN1	6	5	6	-	3	1	5	-	5	-
MHI	Ro	Ro	Af	Ro	Act	Act	Ro	Ro	Ro	Af

NSS, Non-surface sterilised; SS, surface sterilised; TN, Total number of micro-organisms recorded; TN1, Total number with > 5% incidence; MHI, Micro-organisms showing highest incidence; Act, Actinomycetes; Af, *Aspergillus flavus*; Ao, *A. ochraceus*; Bt, *Botryodiplodia theobromae*; B, Bacterium; Ch, *Cladosporium herbarum*; Fs, *Fusarium solani*; Me, *Memnoniella echinata*; Ph, *Phomopsis* sp.; Pc, *Penicillium citrinum*; Ro, *Rhizopus oryzae*

pus oryzae in all the seeds except the seeds of *P. marsupium*, and *Myrothecium roridum* in *P. marsupium* was encountered. On the contrary, a few fungi are detected in high incidence in 2,4-D method as compared with other methods. They are actinomycetes, *A. flavus* on the seeds of *A. odoratissima; Rhizopus oryzae* on *P. marsupium* and *Fusarium pallidoroseum* on *X. xylocarpa*. Earlier, Neergaard (1977) reported that 2,4-D method was effective in detecting *Phoma* spp. on crucifers and soybean seeds (Prasad *et al.*, 1985) while Shivanna (1989) observed that *A. flavus* and *C. dematium* grew well in 2,4-D method as compared with other methods.

agar plate method than SB method is in conformity with the the observations of Nath *et al.* (1970) who recorded 2 - 5% increase in the incidence of *F. moniliforme* on mung been seeds. Similar results were also obtained by Vijayan (1988) on non-surface sterilised seeds of *Acacia catechu, Cassia glauca, Dalbergia sissoo* and *Leucaena leucocephala* and Mittal (1983b) on *Cedrus deodora*. Higher incidence of *F. solani* on seeds of *L. microcarpa* in SB method confirms the earlier observations of Neergaard (1973) Bilgrami *et al.* (1979) and Vijayan (1988) in many crops.

The deep freeze (DF) method does not appear to be suitable in routine seed health testing as in most of the cases only saprophytic fungi grew. However, certain fungi such as *Chaetomium globosum* and *Myrothecium roridum* expressed well in DF than other methods in the non-surface sterilised seeds of *P. marsupium*. Earlier, the DF method was reported to favour the growth of various species of *Fusarium* and *Septoria* in cereals (Neergaard, 1973) and *Alternaria porri* in onion (Limonard, 1966). However, various species of *Fusarium* and *Alternaria* encountered in the study did not grow well in DF method.

In 2,4-D method poor growth of certain micro-organisms such as *Alternaria alternata* on seeds of *L. microcarpa, Rhizo-*

pus oryzae in all the seeds except the seeds of *P. marsupium*, and *Myrothecium roridum* in *P. marsupium* was encountered. On the contrary, a few fungi are detected in high incidence in 2,4-D method as compared with other methods. They are actinomycetes, *A. flavus* on the seeds of *A. odoratissima; Rhizopus oryzae* on *P. marsupium* and *Fusarium pallidoroseum* on *X. xylocarpa*. Earlier, Neergaard (1977) reported that 2,4-D method was effective in detecting *Phoma* spp. on crucifers and soybean seeds (Prasad *et al.*, 1985) while Shivanna (1989) observed that *A. flavus* and *C. dematium* grew well in 2,4-D method as compared with other methods.

Pre-treatment of seeds with chlorine is advocated in seed health testing (ISTA, 1966) and counts of seed-borne pathogens are generally reduced due to pre-treatment (Lin, 1948; Lo, 1973; De Tempe,1962,1963; Sutherland *et al.*,1978), which indicates that these fungi are superficially located on the seed surface. During the present investigation, however, it was found that pre-treatment with sodium hypochlorite (5%) does not always reduces the fast growing saprophytic fungi and hence, 0.1% solution of mercuric chloride was used. Pre-treatment of seeds of *A. odoratissima, L. microcarpa, P. marsupium* and *X. xylocarpa* reduce the incidence of most saprophytic fungi. A few fungi which occurs in low percentage

on non-surface sterilised seeds are eliminated completely. However, the incidence of a Gram (-) bacterium shows only a slight decline in *A. odoratissima* and *L. microcarpa*. Pre-treatment of seeds is advisable as it not only reduces the counts of fast growing micro-organisms but also facilitates the growth of certain slow growing fungi in blotter tests.

The present investigation has thus showed that among all the methods tried SB method is the best for observing the highest incidence /maximum number of most micro-organisms on the seeds of four tree species tried. Furthermore, this method is found to be very reliable for the detection of *A. flavus, A. niger, C. herbarum, Fusarium* spp., *Alternaria infectoria* and *R. oryzae*. Although agar method also supported high incidence of the above fungi, 2,4-D and DF methods were less effective as compared with other methods.

5.1.2. Seed microflora and its significance

Dry seed examination of seeds is very useful as it distinguishes different categories of seeds like apparently healthy, discoloured, shrivelled, and discoloured and small seeds. In all the four tree species, less weight observed for most of the discoloured, shrivelled and small seeds as compared with apparently healthy looking seeds, is possibly due

to microbial deterioration of seeds during seed maturation stage, which may have got further aggravated due to high humidity as also has been reported by Neergaard (1977). Adlakha and Joshi (1974) have also observed that wheat seeds (*Triticum aestivum* L.) infected with *Drechslera sorokiniana* were weighed light than the healthy seeds and were small and discoloured. Black discolouration of wheat seeds was found to be due to *Alternaria alternata* and various species of *Curvularia* and *Phoma* (Agarwal, 1970). Johnson and Jones (1962) and Shivanna (1989) reported that purple discolouration of cluster been is due to *Cercospora kikuchii* and various other fungi respectively.

All the category of seeds harboured both saprophytic and parasitic fungi, the former being in abundance in discoloured, shrivelled and small seeds. Removal of such discoloured and shrivelled seeds before sowing will help in minimising the carry over of fungal inoculum to seedlings and subsequently to the field. The apparently healthy seeds of all the four species always have low incidence of spermoplane micro-organisms compared with the deformed discoloured and small seeds. This observation conform to earlier report by Shivanna (1989) on cluster beans that the fungi such as *Alternaria alternata,*

A. flavus, A. niger, C. dematium, Cladosporium sp., *F. equiseti, F. semitectum, F. solani* and *Phoma* spp. occurred in high abundance on discoloured and deformed seeds than the healthy seeds. During the course of present investigation, the storage fungi viz., *Aspergillus* spp., *Cladosporium herbarum, Penicillium citrinum* and *Rhizopus oryzae* occurred in high percentage, while colonisation by field fungi viz., *Fusarium* spp., *Botryodiplodia theobromae, Colletotrichum gloeosporioides, Myrothecium roridum* and *Alternaria infectoria* was low. The ability of saprophytic fungi to occur in higher percentage may be due to the rapid germination of spores, quick hyphal invasion high competitive nature and their ability to utilise a wide variety of substrata. In addition warm and humid climate of Kerala possibly also helps to increase the occurrence of storage fungi like *Aspergillus* spp., *Penicillium citrinum, Cladosporium herbarum* and *Rhizopus oryzae*. A similar statement has been advocated by Pongpanich (1990) in Thailand who found that many of the fungi associated with 60 forest tree seeds were saprophytic and parasitic fungi were rarely found.

Among the four tree species *L. microcarpa* records the least number of micro-organisms (11 fungi and a Gram (-) bacterium) followed by *X. xylocarpa* (13 fungi and a Gram (-)

bacterium), *A. odoratissima* (13 fungi, a Gram (-) bacterium and an actinomycete) and *P. marsupium* (18 fungi and an actinomycete). The fungal organisms belong to 16 genera viz., *Alternaria, Aspergillus, Botryodiplodia, Curvularia, Colletotrichum, Cladosporium, Chaetomium, Fusarium, Marasmius, Myrothecium, Memnoniella, Penicillium, Phomopsis, Rhizopus, Trichothecium, Trichoderma* and mycelia sterilia (black and white). Seeds of different tree species have common as well as exclusively associated micro-organisms. The common micro-organisms include, *Aspergillus flavus, A. niger, Penicillium citrinum* and *Rhizopus oryzae*. The exclusive fungi were *A. stellatus, C. gloeosporioides* on *A. odoratissima; Curvularia lunata* and *Alternaria alternata* on *L. microcarpa; Alternaria infectoria, Botryodiplodia theobromae* and a *Marasmius* sp. on *P. marsupium*, and *F. pallidoroseum* on *X. xylocarpa*. The observations are similar to those of Vijayan (1988) who recorded 22 fungi on *Acacia catechu* , 13 on *Cassia fistula*, 14 on *Cassia glauca*, 16 on *Cassia nodosa*, 16 on *Dalbergia sisso* 26 on *Leucaena leucocephala* and 19 on *Shorea robusta*. He also reported that the seeds of different tree crops have common as well as exclusively associated seed micro-organisms. The common ones include *Alternaria alternata, Aspergillus flavus, A. niger , A. tamari, Rhizopus nigricans* and mycelia sterilia.

Surprisingly, in artificial inoculation trials, a large number of micro-organisms, hitherto known to be saprophytes, show their pathogenic behaviour to germinating seeds of four tree species. Of the 11 micro-organisms tested, *Aspergillus flavus, A. stellatus, C. herbarum, F. moniliforme, F. solani, T. spiralis* and *R. oryzae* proved to be harmful to *Albizia odoratissima*. In *Lagerstroemia microcarpa*, out of 9 micro-organisms tested, *A. niger, F. solani* and *M. echinata* are found to be pathogenic, while *A. flavus, B. theobromae, F. moniliforme* var. *intermedium* and *R. oryzae* are pathogenic to *Pterocarpus marsupium*. In *Xylia xylocarpa, A. flavus, C. herbarum, F. pallidoroseum* and *R. oryzae* are pathogenic. This may be inferred from the results that high incidence of such saprophytes should not be ignored as they are capable of affecting the seed health considerably.

Occurrence of various species of *Aspergillus* on forest tree seeds has been reported by earlier workers (Urosevic, 1961; 1979; Mittal, 1979; Sharma and Mohanan, 1980; Archana and Mehrotra, 1982). Majority of the *Aspergillus* spp. detected on seeds of various forest tree species (Vijayan 1988) were seed inhabiting and caused seed rot and reduced germination. In all the four tree species tested *A. flavus* cause considerable damage to seed health by bringing down the germinability

and seedling vigour, being maximum in *P. marsupium* and *X. xylocarpa*. It also produces suspected 'afla' root like symptoms in *X. xylocarpa* as reported in other crops (Chohan and Gupta, 1968). *Aspergillus niger* causes symptoms similar to that of *A. flavus* and is highly pathogenic to the seeds of *L. microcarpa* and to a lesser extent to *X. xylocarpa* but it does not cause any post-emergence mortality as reported in other crops by Gibson,1957; Kumari and Karan, 1981; Mittal, 1983a, 1986; Munjal and Sharma, 1976.

Myrothecium roridum which has a wide host range (Munjal, 1960; Shivanna and Shetty, 1986), reduces the germination of the seeds of *P. marsupium*. There is no report on *M. roridum* affecting seeds of forestry tree species. However, this fungus has been reported to be seed-borne pathogen causing heavy seedling mortality in tomato (Srivastava and Tandon, 1966), Mung bean (Nath *et al.*, 1970), cotton (Srinivasan and Kannan, 1974). Dake (1980) and Shivanna (1989) have also reported *M. roridum* causing reduced germination in cotton and cluster bean respectively. Recently *M. roridum* has been reported to cause leaf spot in *Bombax ceiba* (Sharma *et al.*, 1985). However, the chances of *M. roridum* becoming internally seed-borne in *P. marsupium* are very less, as fruits of *P. marsupium* are very hard.

Various species of *Alternaria* and *Curvularia* have been reported to be seed-borne pathogens (Neergaard, 1977). However, in the present investigation, *A. infectoria* occurring on the seeds of *P. microcarpa* does not appear to be pathogenic. *Curvularia lunata* causes germination loss and reduces the seedling growth in *L. microcarpa*. This fungus has been reported to be pathogenic in agricultural crops such as cluster bean (Chand and Verma, 1961; Shivanna, 1989) *Eucalyptus* hybrid (Mittal, 1986) *Picea abies* and *Pinus sylvestris* (Urosevic, 1961) and some conifers (Munjal and Sharma, 1976).

Fusarium moniliforme, a wilt causing pathogen (Booth, 1971) isolated from *Albizia odoratissima* and *Pterocarpus marsupium* brings about reduction in seed germination and distortion of seedings. Nath *et al.*, (1970) have also observed reduction in seed germination in mung bean due to *F. moniliforme*. This fungus is also known to cause lesion on seedlings and mortality in cowpea (*Vigna chinensis* (L.) Kumari and Karan, 1981) and in soybean (Lee, 1984) and cluster bean (Shivanna, 1989).

Fusarium pallidoroseum, isolated from the seeds of *Xylia xylocarpa* also causes reduction in seed germination and distorted seedlings. Recently, Mohanan and Leise (1991) reported foliar infection of various species of rattans caused by

F. pallidoroseum. *Fusarium solani* which has a wide host range is known to affect the seed germinability in mung bean (Nath *et al.*,1970), broad bean (*Vicia faba* L.), bean (*Phaseolus vulgaris* L) and pea (*Pisum sativum* L) (Neergaard, 1977). *Fusarium solani* recorded from *Albizia odoratissima* and *Lagerstroemia microcarpa* causes decay of seeds, reduction in seed germination and distortion of seedlings which is in agreement with earlier studies (Shivanna, 1989; Vijayan, 1988).

Rhizopus oryzae, a common saprophyte, caused severe germination loss in *X. xylocarpa* and grows profusely on the germinating seeds affecting the vigour. However, it causes only moderate germination loss in *P. marsupium* and its high incidence should be given due consideration during seed health testing for this fungus hitherto known to be pathogenic. In an another study , artificial inoculations by *R. oryzae* reduced seed germination and affected the growth and development of seedlings of *Eucalyptus* hybrid (Mittal, 1986). Urosevic (1961) reported decrease in germination in *Picea abies* and *Pinus sylvestris* by species of *Rhizopus*. Pathogenic behaviour of various species of *Rhizopus* to some conifers (Munjal and Sharma, 1976); *Shorea robusta, Pinus roxburghii* and *Pinus wallichiana* (Mittal and Sharma,1982 abcd) conform with the present observations.

The results clearly indicate that the dry seed examination yields very important information in respect of spermoplane micro-organisms and it should be recommended for the forestry seeds as also has been practiced for seed health tests in maize (Kumar and Shetty, 1983). Removal of such 'abnormal' and unhealthy seeds and appropriate seed treatment procedures can help in minimising the carry over of fungal inoculum to seedlings and subsequently to the field. From the above results it is evident that in addition to known pathogens, occurrence of various saprophytic fungi, which act as facultative parasites is harmful to the seeds of four tree species tested. In order to reduce/minimise the loss in viability/germinability of seeds due to these micro-organisms appropriate means should be adopted right from the seed collection to seed storage.

5.1.3. Management of seed microflora

It is quite apparent from the results that seed microflora harbouring the four forest tree species not only deteriorates the quality of seeds but also affects subsequently the seed health. To prevent the bio deterioration of seeds, it is essential to manage the seed microflora to an acceptable level. Generally scarification of seeds of some

forest tree species like *Acacia nilotica*, *A. catechu*, *A. auriculiformis*, *A. campylicantha*, *A. albida*, *Albizia lebbcek*, *Cassia fistula*, *C. siamea*, *Leucaena leucocephala*, *Delonix regia*, *Bauhinia variegata*, *B. racemosa* and *Strychnos nuxvomica* to increase the germination percentage (Ram Prasad and Kandya, 1992). But no work has been done on the effect of hot water treatment on the suppression of seed microflora and hence the present study.

Among the various methods tried, hot water treatment at 50° and 60° for 15 and 30 min. duration gives inconsistent results for seeds of various tree species. In *A. odoratissima* 60°C-30 min. is highly effective as it gives the best vigour index (VI). Interestingly, hot water treatment does not control *F. moniliforme* in all the treatments, and significantly higher incidence is recorded, particularly at 50°C and 60°C-15 min. exposures. Persistence of this fungus may be due to deep seated infection and its resistance to high temperatures (Neergaard, 1977).

Since hot water treatment is inhibitory to the germination of seeds of *L. microcarpa* and *P. marsupium*, especially at longer duration, it cannot be practiced even though, various micro-organisms are either eliminated or their incidence reduced. In *X. xylocarpa*, though the hot water treatment does not

affect the seed germination significantly and reduces the microflora at 50°C-15 min. as compared with control, other parameters do not have any significant increase and hence, it cannot be practiced. The results of this study gets support from the following literature available on hot water treatment of seeds of various plant species. Shivanna (1989) reported that hot water treatment of cluster bean seeds at 50°C- 30 min. and 60°C- 30 min. decreased the incidence of many fungi. He further observed that the seed treatment at 50°C and 55°C for 15 and 30 min. did not affect the seed germinability, while at 60° C, seed germination was considerably reduced. He also observed that the root length was reduced only at 55° C and 60° C for 15 - 30 min., while the shoot length was inhibited in all the treatments. These observations are similar to present findings where, excepting *A. odoratissima*, in all other cases, hot water treatment was inhibitory to seed germination and other parameters. On the contrary Venkatasubbaiah *et al.* (1984) reported that hot water treatment of seeds of *Leucaena leucocephala* at 85°C for 5 min. gave a better germination of 63 % as compared with control seeds (44%). They also observed that reduction in fungal contamination might be due to the sensitive nature of mycoflora to heat therapy or their superficial presence. However, fungal species like *Fusarium*

solani, and *F. moniliforme*, *Trichoderma harzianum*, *Verticilliun* sp. and *Cladosporium* sp. etc. were prevalent on hot water treated seeds. These observations are similar to this study as fungi such as *F. moniliforme*, *Chaetomium globosum*, Gram (-) bacterium, *C. herbarum* on *A. odoratissima*; *F. solani*, *C. globosum*, *P. citrinum* on *L. microcarpa*, *A. flavus*, *F. moniliforme* var. *intermedium*, *Memnoniella echinata*, *Chaetomium globosum* and *Cladosporium herbarum* on *P. marsupium* and *A. flavus* and *F. pallidoroseum* on *X. xylocarpa* are not completely eliminated and continue to be prevalent on seeds treated with hot water. This may be attributed to their deep seated nature as well as resistance to high temperature (Neergaard, 1977). Recently, Donald and Lundquist (1984) also reported that hot water treatment of *Eucalyptus* seeds at 50 ° for 5, 10 and 20 min. not only restricted fungal development but also enhanced seed germination. Zazzerini *et al.*(1985) used hot water treatment as a means of controlling various species of *Alternaria* on safflower seeds. In the present investigation also *A. infectoria* recorded on the seeds of *P. marsupium* is completely eliminated by hot water treatment.

It may be concluded from the results that although the hot water treatment shows some degree of protection against

micro-organisms, it has deleterious effects on seed health except in *A. odoratissima*. In view of this, hot water treatment cannot be used widely for forestry tree species without proper investigation.

In recent times, seed treatment with fungicides has become an integral part of routine seed storage in agricultural crops due to their protective as well as therapeutic values. Seed dressing with fungicides not only reduces the incidence of microflora, but also gives protection from soil-borne pathogens. In the present investigation, eight commonly available seed dressers are evaluated against seed-borne infection, seed germination and growth of seedlings of *A. odoratissima, L. microcarpa, P. marsupium* and *X. xylocarpa*. It is evident from the results that different fungicides behaved differently to various seeds and micro-organisms. No single seed dresser is the "most effective" for all the four types of seeds tested. It is possibly due to differences in seed micro-organisms, their incidence and the physiology of the mature seed. A comparison of results indicates the two best fungicidal treatments in influencing various parameters like shoot and root lengths, seed germination and vigour index of various species (Table 58). Captan emerges as the best fungicide for improving the seed health parameters in all the

Table 58. Two best fungicidal seed treatments in improving various parameters

Species	Seed germination	Shoot length	Root length	Vigour index	Suppression of microflora
A. odoratissima	Captan	Captan	Carboxin	Captan	Captafol
	Carboxin	Carbendazim	MEMC	Carboxin	Mancozeb
L. microcarpa	Captan	Mancozeb	Carboxin	Mancozeb	Captan
	Mancozeb	Carboxin	MEMC	Carboxin	Mancozeb
P. marsupium	Captan	Captan	Thiram	Captan	MEMC
	Thiram	Captafol	Captafol	Thiram	Captan
X. xylocarpa	Thiram	Carbendazim	Captan	Thiram	Captan
	Mancozeb	Mancozeb	Mancozeb	Mancozeb	Mancozeb

four species tested. In *A. odoratissima*, captan is the best fungicide as far as the seed germination, shoot length and vigour index is concerned. In *L. microcarpa* it is superior to others in improving the seed germination and suppression of seed microflora. In the case of *P. marsupium*, seed germination shoot length and seedling vigour are influenced by captan. Root length and suppression of seed microflora are influenced by the same fungicide in *X. xylocarpa*. This observations is in agreement with the studies by Vijayan (1988) where captan brought about higher seed germination, root elongation and inhibition of micro-organisms in seeds of *Cassia glauca*. Unlike

other treatments, *F. solani* was suppressed on the captan treated seeds. Captan has also been reported to be effective against *F. moniliforme* and *F. oxysporum* and *F. equiseti* (Sinha and Khare, 1977). The incidence of *F. solani, F. moniliforme* and *F. equiseti* were reported to be significantly reduced due to thiram, followed by mancozeb and captan in cluster beans (Shivanna, 1989). In the present investigation, captan inhibits the growth of many micro-organisms, except *P. citrinum* on *A. odoratissima* and *F. moniliforme* var.*intermedium* and *R. oryzae* on *P. marsupium*. Captafol, mancozeb, captan and MEMC are the best fungicides in reducing the incidence of microflora on treated seeds. However, a Gram (-) bacterium could not be controlled by any of the seed treatments and in fact its incidence shows significant increase in MEMC, carboxin and mancozeb treatments. Curiously the incidence of *Fusarium moniliforme* is significantly higher on the PCNB treated seeds than the control. PCNB is also found less effective to *F. moniliforme, F. solani* and *F. equiseti* affecting cluster bean seeds (Shivanna, 1989).

Mancozeb (Dithane-M-45) appears to be the second best fungicide in giving better seed health parameters (Table 58) than others. Seeds of *L. microcarpa* treated with mancozeb show the best vigour. In *X. xylocarpa* mancozeb rank second best for

all the seed health parameters. In suppressing the seed microflora of *A. odoratissima*, mancozeb is the best fungicide. Mancozeb is also reported to be second best fungicide in reducing the incidence of *Fusarium solani, F. moniliforme* and *F. equiseti* in cluster bean seeds (Shivanna, 1989). The influence of mancozeb appears to be superior in *L. microcarpa* and *Xylia xylocarpa* in giving better vigour index than control. In seeds of *Cedrus deodora*, Mittal (1983b) also observed that Dithane-M-45 treatment resulted in increased growth and development of seedlings and reduction of fungal infection. Vijayan (1988) also reported complete control of microflora of *Cassia fistula* by seed dusting with Dithane-M-45 or Bavistin or captan @ 0.25% seed weight.

In the present study, thiram shows promise only in *X. xylocarpa* in giving the best seed germination and vigour index. In *P. marsupium*, it ranks second for seed germination and vigour index, while its effect is not significantly different from control in *L. microcarpa* Thiram is found inhibitory to seed germination and vigour in *A. odoratissima*. The fungal infection of many vegetable crops is known to be reduced by thiram. Thiram was found to be as effective as captafol, mancozeb and captan on elongation of root of *Acacia catechu* but it could not suppress the growth of some of the

micro-organisms. In *Dalbergia sissoo* thiram was the second best in root elongation and the best in the suppression of micro-organisms (Vijayan, 1988). Thiram has been also reported to accelerate the seed germination by reducing the fungal infection considerably in *Pinus roxburghii* (Mittal and Sharma, 1982c). They also found that thiram and mancozeb are the best fungicides for *P. wallichiana* as only one fungal species could develop on the seeds (Mittal, 1982d). On the contrary thiram was not effective as a seed dresser in *Shorea robusta* (Mittal and Sharma, 1982b).

Certain fungicidal treatments affect the seed germination and growth of seedlings adversely. Captafol, MEMC and thiram not only reduce the germination but also decreases the vigour index in *A. odoratissima* Captafol treated seeds of *L. microcarpa* show reduced vigour index and carbendazim and mancozeb retard the vigour index of *P. marsupium* . The reduction in germination and seedling vigour is possibly due to the toxicants or non-compatibility of seed dressers. Mittal (1986) while working on microflora of *Eucalyptus* hybrid and its control has also reported adverse effects of all the nine fungicides viz., captan, Agrosan GN, Brassicol, Ceresan, thiram, Dithane M-45, Panoctine, RH-2161 and Bavistin on the germination and seedling development.

In addition fungus such as *Rhizopus oryzae* shows occasional growth in other treatments grows profusely on treated seeds of carbendazim. Mittal and Sharma (1981c) while working on the effect of some fungicides to control some tree seed-borne fungi also found that Bavistin SD (carbendazim) was not effective against *P. canadense* and *R. oryzae*. The inhibition of *Curvularia lunata* on the seeds of *L. microcarpa* was controlled by all the treatments except carbendazim, which is in agreement with similar studies (Karwasra *et. al.*, 1979). Shivanna (1989) reported that the increase in the germinability of fungicide treated seeds of cluster bean may be due to the reduction or elimination of fungal inoculum associated with the seed surface and the decrease in vigour may be due to fungi still remained inside the seeds or due to the retardation of growth of seedlings by the phytotoxicity of the fungicides. The same explanation may also be true for the trend of results obtained for seed dressers in the present study

The present investigation has thus confirmed the use of seed dressers for improving the seed germination and seedling vigour, and for the control of seed-borne microflora of forestry seeds. But there is a need for caution in the indiscriminate use of fungicides as they may affect the seed health adversely as observed in the present investigation.

5.1.4. Seed storage and its influence on microflora, seed germination and seedling growth

Various factors such as moisture content, temperature and relative humidity affect the germinability of seeds during storage (Harington, 1972; Christensen,1973; Roberts,1983). In addition, the seed-borne micro-organisms, especially fungi play a dominant role in determining the quality and longevity of seeds (Christensen, 1957,1973; Christensen and Lopez, 1963). According to Neergaard (1977) many fungi belonging to species of *Alternaria, Colletotrichum, Drechslera* and *Phoma* possibly survive on the seeds for many years. Storage fungi are generally classified as saprophytes like species of *Aspergillus, Cladosporium, Curvularia Penicillium* and *Rhizopus* and field fungi are parasitic fungi which include species of *Alternaria, Botryodiplodia, Colletotrichum, Fusarium, Graphium, Macrophomina, Phomopsis* and *Stemphylium* as reported in numerous forestry tree species (Pongapanich, 1990). The longevity of some seed-borne parasitic or field fungi is only for a few months in storage, while many other saprophytic fungi may survive on tree seeds for many years (Pongapanich, 1990). Species of *Aspergillus* and *Penicillium* have been reported to be principal fungi responsible for deterioration of stored seeds (Justice and Bass, 1978). The continued presence

of storage fungi on seeds of forestry importance as observed in the present study is a matter of concern.

Generally, field fungi dominate in the first phase of storage and storage fungi or saprophytes become dominant later. This may be due to the gradual change in the nutritive condition of the seeds. The gradual reduction in the incidence of field fungi during prolonged storage may be attributed to the lowering of moisture content of seeds before storage. The initial low moisture content of the seeds do not favour the colonisation by storage fungi. However, the increased activity of other fungi during the subsequent period of storage makes the seed vulnerable to the attack by storage fungi as possibly happens with the seeds of the four species in the present study. This is in agreement with the observations made by Shivanna (1989) who reported that the storage period of 18 - 21 months can bring down the incidence of some seed-borne fungi like *M. roridum* and *F. oxysporum* on the seeds of cluster beans. After the advent of storage fungi, the field fungi decline slowly and gradually. He had also reported the increase in the incidence of storage fungi like *Aspergillus flavus, A. versicolor* and *Penicillium* sp. which he attributed to the decline of field fungal population and increase in the moisture content of seeds of cluster bean.

During the course of the present investigation, it was found that the seeds stored up to 12 months show gradual reduction in the number of some seed-borne micro-organisms in all the four tree species; the number of micro-organisms recorded on seeds stored under dehumidified condition are less as compared with the seeds stored under laboratory conditions. In *A. odoratissima*, most of the storage fungi recorded initially are observed till the end of storage period. However, in seeds stored under de-humidified conditions at 4°C and room temperature most field fungal population is eliminated. However seeds stored at room temperature, harboured low incidence of *Fusarium solani*. In the case of *L. microcarpa*, *Fusarium solani* occurs up to 180 days of storage. *Myrothecium roridum* and *Fusarium moniliforme* var. *intermedium* recorded on the seeds of *Pterocarpus marsupium* are either eliminated or reduced over 1- year period of storage. In *X. xylocarpa*, *Fusarium pallidoroseum* is recorded till the end of storage but under de-humidified conditions it is eliminated after 180 days of storage. Generally, species of *Fusarium* except *Fusarium moniliforme* are known to survive for 2 to 3 years on seeds (Neergaard, 1977) and seeds stored under dehumidified conditions at low and room temperature harboured less number of micro-organisms as compared with control seeds. These findings are in agreement with studies conducted by Shivanna

(1989). All the *Fusarium* spp. encountered on the seeds of four tree species are pathogenic, and storage of seeds under dehumidified conditions may be useful in eliminating these pathogens.

In the present investigation, the seed germination of all the four indigenous tree species gradually declines, as the period of storage increases. Generally viability of forestry seed vary from species to species and for most tree species seldom exceeds three years under natural conditions (Crocker and Barton, 1953) which could be increased to many folds under regulated storage conditions (Barton, 1961). Reduction in germinability due to increase in storage fungi has also been reported in maize (Lopez and Christensen, 1967) and in cluster bean (Shivanna, 1989). Abdullah (1970) has attributed the fall in seed germination during storage period due to the toxic metabolites excreted by fungi, which could be either parasites or saprophytes.

In recent times seed dressing with fungicides has become an integral part of routine seed storage of Agricultural crops. Seed treatment with fungicides not only control seed microflora but also known to improve seed germination in some forestry tree species (Jamaluddin *et al.*, 1985; Mittal, 1979; Mittal and Sharma, 1981abc; Vijayan 1988). Literature

pertaining to reduction of seed germination due to fungicide treatment and storage in forestry seeds are lacking. Recently Moreno and Vidal (1981) and Moreno *et al.*, (1985) reported improvement in the viability of maize seeds by reducing the moisture content of seeds and storing them with fungicides at 85 % r.h. They found that the seeds treated with benomyl, captan, captafol, carbendazim, chlorothalanil, and thiabendazole with 9.8 % initial moisture content showed 82-93% germination after 150 days of storage, in comparison with 14% in untreated control. In the present study, seeds stored with fungicides under laboratory conditions show gradual decline in germination and seedling vigour as compared with untreated control seeds,indicating that storing seeds after seed treatment with various fungicides is not found effective.

The present study clearly shows that seeds can be stored under dehumidified conditions at low or room temperature without much loss in germinability and seedling vigour as compared with seeds stored under normal laboratory condition and treated with fungicides. Earlier Gupta and Sood (1978) reported that in *Dendrocalamus strictus*, storage of seeds over calcium chloride prolong the viability for more than 34 months, while the untreated seeds lost the viability in eight months. Later, Soman and Seethalakshmi (1989) reported a rapid loss in the viability of seeds of *Bambusa arundinacea* stored

in a plastic container at laboratory conditions within two months; the seeds stored at low (4° C) and room temperature over calcium chloride in partially evacuated/non-evacuated desiccators, germination deterioration was gradual, reaching 10% or less after 413 days. The results of the present investigation also show a similar trend. Seeds kept in non-evacuated desiccators over anhydrous calcium chloride lose the germinability slowly as compared with seeds stored in plastic containers in the laboratory. The possible explanation given for this is storage over anhydrous calcium chloride brings about reduction in weight of the seeds and partial evacuation helps to provide low levels of oxygen which helps to minimise the rate of respiration. Further the seeds stored under low temperature have reduced rate of metabolic activities and inactivation of enzymes helps to retain the seed viability. Some studies have also provided indirect evidences of micro-organisms in affecting the seed viability. Soman and Seethalakshmi(1989) reported that filter paper cultures revealed the presence of fungi like *Aspergillus, Cephalosporium, Cladosporium, Fusarium, Penicillium* and *Trichoderma* which also would have contributed towards the decline in germinability of *B. arundinacea*. Recently Mohanan (1990) while working on the seed microflora of stored bamboo seeds observed a comparatively low incidence of seed micro-organisms on seeds stored

under low temperature(15°C) and higher germination (82%) as compared with the seeds stored at 28±2°C (63%).

Thus, the present investigation revealed that the seed microflora is decreased gradually during the period of storage; but some of the important pathogens like *F. moniliforme*, *F. solani*, *F. pallidoroseum*, *M. roridum*, *C. gloeosporioides*, and *B. theobromae* do not completely disappear during storage and continue to harbour the seeds till 180 days of storage. However, none of the above mentioned pathogens cause any seedling diseases in the tree species studied. Such pathogens having greater viability during long storage can be eliminated by appropriate seed dressing chemicals. But storing seeds treated with fungicide cannot be practiced for the forestry seeds as they lose the viability rapidly as observed in the case of four tree species.

5.2. Seedling diseases and their management

In Kerala the tropical moist deciduous forests occupy 4010 sq. km of the total forest area of 9400 sq.km (KSLUB, 1989) which mainly consists of valuable indigenous trees. However, information on seedling diseases and their management of many indigenous trees is completely lacking. Sharma *et al.*(1985) made a valuable and exhaustive survey of diseases in

nurseries and plantations of seven forest plantation species in Kerala and reported a number of diseases, majority of them of minor significance. They also found that in nurseries facultative parasites such as *Rhizoctonia solani* and *Sclerotium rolfsii* have emerged as serious pathogens. *Rhizoctonia solani* has already gained the reputation of being a worldwide, destructive and most serious plant pathogen attacking a very wide range of plant parts (Parmeter, 1970).. *R. solani* has already become a serious pathogen in *Albizia falcataria* causing web blight (Sharma and Sankaran, 1987), in teak causing collar rot (Mohamed Ali and Florence, 1992), in *Bombax ceiba* and in *Ailanthus triphysa* causing collar rot (Sharma *et al*, 1985). In addition, it has been recorded to cause web blight of *Azadirachta indica* (Sankaran *et al.*, 1986), damping-off of *Causarina equisetifolia* (Mohanan and Sharma, 1989) collar rot of *Acacia auriculiformis* (Mohanan and Sharma, 1988), and damping-off of *Eucalyptus* sp. (Sharma *et al.*, 1985).

Sclerotium rolfsii is a common soil-borne pathogen and is known to parasitise seedlings of various tree species (Browne, 1968). *Sclerotium rolfsii*, another opportunistic pathogen has also been found to cause many diseases of seedlings such as collar rot of *Gmelina arborea* (Maria Florence and Sankaran, 1987), leaf blight of *Azadirachta indica* (Sankaran *et al*, 1986), collar rot of *Swietenia macrophylla*

(Sankaran *et al.*, 1984), leaf blight of *Pterocarpus santalinus* (Sankaran *et al.*, 1988) and seedling wilt of *Eucalyptus* sp. (Sharma *et al.*, 1985) and other forest seedlings (Maria Florence *et al.*, 1985). These findings are in agreement with the observation of the present investigation where many of the nursery diseases reported for the first time from the indigenous species also belong to facultative parasites such as *Rhizoctonia solani* and *Sclerotium rolfsii*. *Rhizoctonia solani* causes post emergence damping-off of *L. microcarpa*, collar rot of *P. marsupium* and seedling blight of *X. xylocarpa*, while a seedling blight caused by *S. rolfsii* is recorded only from *P. marsupium*. It is of paramount importance to observe such "ubiquitous and opportunistic" pathogens infecting the native species. It clearly shows that *R. solani* and *S. rolfsii* infect the native species too and we may have to assess, how serious they may become, when these species are raised in large scale.

In vitro evaluation of fungicides using two methods gives interesting results. Fungicides which give promising results in the poisoned food method (PFM), failed to give the same when screened in soil fungicide screening method (SFSM). For example, *R. solani* causing damping-off of *L. microcarpa* is inhibited by MEMC and carbendazim at all the concentrations

in poisoned food method while in soil fungicide screening method 100% inhibition is obtained only in the highest concentration of 0.0125% and 0.2%(a.i.) respectively. In the case of collar rot isolate of *R. solani* from *P. marsupium* complete inhibition is obtained by carbendazim, carboxin, MEMC and thiram in poisoned food method while complete inhibition was observed in MEMC (0.0125 and 0.0250% a.i.) and carboxin (0.1 and 0.2% a.i.) and in carbendazim only at the highest concentration (0.2% a.i.) in soil fungicide screening method. *Rhizoctonia solani* causing seedling blight of *X. xylocarpa* was inhibited completely in poisoned food method by carbendazim and MEMC in all concentrations, while in soil method it is achieved in the highest concentration only. In the case of *S. rolfsii* causing seedling blight of *P. marsupium* also, complete inhibition is obtained by carboxin, PCNB, thiram in poisoned food method, while complete inhibition is recorded in thiram and carboxin; PCNB is not effective in soil method. This clearly indicated that for sclerotial fungi like *R. solani* and *S. rolfsii* soil fungicide screening method is more reliable than the poisoned food methods (Sharma and Sankaran, 1987). This could be one of the reasons for obtaining erroneous results in field screening, using the most effective fungicide obtained through poisoned food method for sclerotial fungi (Martin *et al.*, 1984).

Rhizoctonia solani and *S. rolfsii* affecting different plant parts such as collar, seedling, etc., of a species possibly suggest the existence of strains/biotypes in these pathogens. The fungicidal screening experiments also give a clear indication to this effect since no single chemical proved to be the most effective one for controlling various diseases (Table 59). This finding is in agreement with studies carried out with *R. solani* isolates from different crops such as mung bean (Kataria and Grover,1978) and *Albizia falcataria* (Sharma and Sankaran, 1987).

Table 59. Nursery diseases of four tree species and their pathogens and the most effective fungicide for controlling them

Tree species	No. of diseases and pathogen associated	Most serious disease and pathogen	Most effective fungicide for the serious disease
A. odoratissima	Nil	Nil	Nil
L. microcarpa	2; *R. solani*, *P. middletonii*	Damping off; *R. solani*	MEMC (0.006% a.i.)
P. marsupium	2; *R. solani*, *S. rolfsii*	Seedling blight *S. rolfsii*	carboxin (0.2%a.i.)
X. xylocarpa	1; *R. solani*.	Nil	carbendazim (0.2% a.i.)

Differential behaviour of various isolates of *R. solani* to various fungicides have been earlier reported by Sharma *et al.*(1985); Sankaran, (1987); Mohamed Ali and Florence,(1992). A few fungicides found effective against *R. solani* and *S. rolfsii* in earlier studies did not show much promise against these pathogens in this study. For example pentachloronitro-benzene (PCNB) was found to be very effective against *R. solani* and had been widely used to control *Rhizoctonia* diseases (Galindo *et al*; 1982; Bains and Jhotty 1983; Gurkin and Jenkins, 1985). Another fungicide found effective against *Rhizoctonia* diseases was carbendazim (Shehata *et al.*, 1982. Grover and Kataria, 1985). Carboxin was also reported to be effective against *R. solani* (Martin *et al.*, 1984). However, in the present investigations, only MEMC is found consistently effective in bringing about complete control while other fungicides behave differentially. These are in agreement with earlier observations that in spite of the fact that quite a large number of fungicides have been tried and found effective against *R. solani* there is a lack of agreement between different reports on the efficacy of a particular fungicide (Grover and Kataria, 1985; Sharma *et al*, 1985; Sharma and Sankaran, 1987).

Even though MEMC has come out as the best fungicide in controlling most of the seedling diseases reported in the present study, a word of caution has to be put forward as the injurious properties of MEMC is well known (Torgeson, 1969) and therefore is banned from use in most of the developed countries. However, its continued availability in most of the developing countries, due to the less cost and its high effectiveness against such pathogens cannot stop its usage, but one should be restrained to use it indiscriminately especially near the habitats.

In vivo studies confirm the promise of the soil method in identifying the most effective fungicide as *R. solani* is controlled only by pre-sowing soil drenching of MEMC (0.006% a.i.) while the other fungicides found effective in other methods are not effective. In the case of *P. marsupium*, *Sclerotium rolfsii* causing seedling blight is effectively controlled by pre-sowing soil drenching of one of these fungicides (carboxin (0.2%) or thiram (0.2%) or MEMC (0.0125% a.i.) found promising in the soil method. Of the various methods of fungicidal application attempted the results clearly suggest that for affording effective protection against *R. solani* and *S. rolfsii*, the soil of the nursery beds should be treated with fungicides, especially MEMC before sowing. Seed dressing

or soaking was not very effective. These observations get support from the earlier results of Sharma and Sankaran (1987) who reported effective protection against *Rhizoctonia* web blight of seedlings of *Albizia falcataria* was achieved by treating the nursery soil of the nursery with carbendazim before raising the seedlings.

The present investigation reveals that the four indigenous tree species viz., *Albizia odoratissima, Lagerstroemia microcarpa, Pterocarpus marsupium* and *Xylia xylocarpa* have very few seedling diseases and that none can be treated as of serious nature spreading into epidemic proportions. This confirms the generally held view that trees in their natural habitat seldom suffer from serious disease problems In accordance with the concept put forward by Elton (1958) on the susceptibility of monoculture stand, literature also suggests that exotics are inherently more prone to diseases than native or indigenous species (Heather and Griffin, 1978; Vaschko, 1983).

Based on the above discussion it may be concluded that *A. odoratissima* and *X. xylocarpa* emerge as the best indigenous tree species for afforestation programme, considering that they have (i) less seed pathological problems, (ii) none or very few seedling diseases of uneconomic importance and

(iii) higher seed germinability. However, to make the planting programme a success, before a species is finally decided for a given geoclimatic area, other factors such as pest problems and silvicultural aspects of these species also have to be taken into account. It is hoped that these findings will go a long way in the planting of indigenous tree species in its natural habitat, as it will not only improve the ecosystem, but also help to exploit the plantation potential of useful indigenous tree species, especially when the wood resources in the country are depleting rapidly due to deforestation.

SUMMARY

SUMMARY

In forestry, availability of healthy seeds is an important factor in raising planting stock. Initial seed health and storage conditions are the major factors governing the germinability of seeds. Like seeds of agricultural and horticultural crops, forest tree seeds are also liable to be affected by micro-organisms during storage, which affects the germination, and reduces the viability. Further introduction of seed-borne diseases into newly sown crops/areas on account of using unhealthy seeds is also not ruled out. Availability of healthy stock of seedlings is intrinsic for raising plantations and to meet this requirement elimination of nursery diseases by appropriate chemicals is of prime importance. As exotic tree species may become susceptible to various native pathogens, it is generally considered better to select indigenous tree species for large scale plantations as they are well adapted to local environment. However, before taking up large scale afforestation programme involving any indigenous tree species, it is essential to have knowledge about seed disorders and seedling diseases and their management. With a view to select appropriate tree species with fewer seed disorders and seedling disease problems for use in further plantation programme, four indigenous tree species such as *Albizia odoratissima* (L.f) Benth., *Lagerstroemia microcarpa* Wt.,

Pterocarpus marsupium Roxb. and *Xylia xylocarpa* (Roxb.) Taub. were evaluated to meet the above parameters.

The results of the study are presented in two parts. The seed pathology constitutes the first part, while the seedling diseases and their management form the second part. Seed health testing methods, seed microflora and their significance, management of seed microflora, seed storage and its influence on seed germination and seedling growth were carried out under seed pathological studies. The occurrence of various seedling diseases, their symptomatology, causal organisms and pathogenicity tests, *in vitro* evaluation of fungicides and disease control measures in the nursery are included in the second part.

1. SEED PATHOLOGICAL STUDIES

1.1. Seed health testing methods

The main objective of this study was to ascertain the most suitable seed health testing method for forestry tree species. Five seed health testing methods viz., standard blotter (SB), 2,4-D, deep freeze (DF), potato-dextrose agar (PDA) and malt extract agar (MEA) using both surface sterilised and non-surface sterilised seeds were evaluated to obtain maximum information on seed microflora.

In *Albizia odoratissima*, of the fifteen micro-organisms recorded on non-surface sterilised seeds, except actinomycetes, all were detected in SB method; actinomycetes were detected only in 2,4-D and DF methods. Low incidence of *Fusarium solani* (Mart.) Sacc. was observed only in SB method. Surface sterilization with 0.1% $HgCl_2$ reduced the incidence of many micro-organisms except a Gram (-) bacterium.

In *Lagerstroemia microcarpa* PDA, DF and SB methods were equally effective as most of the micro-organisms grew well. *Alternaria alternata* (Fr.) Keissler was detected only in PDA and DF methods and a Gram (-) bacterium had significantly higher incidence in SB method as compared with others. Surface sterilisation reduced the incidence of certain micro-organisms and eliminated a few others.

In *Pterocarpus marsupium*, 15 micro-organisms were recorded with varying intensities in SB method followed by other methods. Actinomycetes did not appear in MEA method. *Alternaria infectoria* E. Simmons *Botryodiplodia theobromae* Pat. and *Myrothecium roridum* Tode: Fr. grew well in SB, MEA and DF methods respectively. Surface sterilisation brought down the percent incidence, as well as the number of micro-organisms in all the methods.

Xylia xylocarpa recorded a total of 11 micro-organisms in SB method. *Fusarium pallidoroseum* (Cooke.) Sacc. and *Cladosporium herbarum* (Pers.) Link ex Gray were detected mainly on non-surface sterilised seeds. Surface sterilisation of seeds reduced the incidence of a number of storage fungi and to a lesser extent the field fungi.

1.2. Seed microflora and their significance

The studies were mainly taken up with a view to generate data on micro-organisms associated with the seeds of four tree species and ascertain how do they affect the quality of seeds, which consequently may affect / influence the seedling vigour.

Macroscopic examination revealed the occurrence of apparently healthy, discoloured and discoloured and deformed seeds in *A. odoratissima*. The incidence of seed microflora was higher in deformed seeds as compared to other categories. The germination percentage was lower viz., 6% in deformed seeds whereas it was 21% in apparently healthy seeds, indicating the superiority of selecting apparently healthy seeds for sowing. Of the 15 micro-organisms detected by different methods in *A. odoratissima* viz. Actinomycetes[1], *Aspergillus flavus* Link.[2], *A. niger* van Tieghem[3], *A. stellatus* Curzi.[4], *A. versicolor* (Vuill.) Tiraboschi[5] *Cladosporium herbarum* (Pers.) Link ex

Gray[6], *Colletotrichum gloeosporioides* (Penz.) Penz & Sacc.[7] *Fusarium moniliforme* Sheld.[8], *F. solani* (Mart.) Sacc.[9], *Myrothecium roridum* Tode:Fr[10] *Penicillium citrinum* Thom[11], *Rhizopus oryzae* Went & Prinsen Geerligs[12], *Trichurus spiralis* Hasselbr.[13], sterile hyphae[14] and a bacterium Gram (-) [15], except 1,5 10 and 14, all were tested for their pathogenicity. *Fusarium moniliforme, F. solani, C. herbarum, A. stellatus, T. spiralis* and *R. oryzae* affected the seed germination and growth of seedlings. Vigour index was the lowest in treatments involving both the species of *Fusarium* followed by *Cldosporium herbarum, A. flavus, A. stellatus, T. spiralis* and *R. oryzae*.

In *Lagerstroemia microcarpa* also apparently healthy, discoloured and discoloured and broken seeds were encountered. The incidence of various micro-organisms was higher in the latter two categories as compared with apparently healthy seeds. Twelve micro-organisms viz.,*Alternaria alternata*[1], *Aspergillus flavus*[2], *A. niger* [3], *Curvularia lunata* (Wakker) Boedijn [4], *Fusarium solani*[5], *Memnoniella echinata*(Riv.) Galloway[6], *Phomopsis* sp.[7] *Penicillium citrinum* [8], *Rhizopus oryzae* [9] sterile hyphae (black)[10], sterile hyphae (white)[11], and a bacterium Gram (-) [12] were recorded by different methods on the seeds of *L. microcarpa*. Except 10 and 11, all were

checked for their pathogenicity. *F. solani* was found to be highly pathogenic since it reduced the germination (5%) as compared with control (13%). The vigour index was the lowest in *F. solani* (226) followed by *A. niger* (292), while the untreated control recorded a vigour index of 595.

Dry examination of seeds in *Pterocarpus marsupium* also revealed three categories of seeds. The number of micro-organisms was lower in apparently healthy seeds as compared with other categories. Surface sterilisation greatly reduced the incidence of micro-organisms and improved germination percentage. The micro-organisms recorded by different methods were Actinomycetes[1], *Alternaria infectoria* [2], *Aspergillus candidus*[3] Link ex Link, *A. flavus* [4], *A. niger* [5], *A. ochraceus* Wilhelm.[6], *A. versicolor*[7] *Botryodiplodia theobromae* [8], *Cladosporium herbarum*[9], *Chaetomium globosum* Kunze.[10], *Fusarium moniliforme* Sheldon var. *intermedium* Neish & Leggett.[11], *Memnoniella echinata* [12], *Marasmius* sp.[13], *Myrothecium roridum* [14], *Penicillium citrinum* [15], *Trichothecium roseum* (Pers.) Link ex Gray[16], *Trichurus spiralis* [17] *Rhizopus oryzae* [18] and sterile hyphae (black)[19] of which 2,4,5,6,8,9,11,14,12,17 and 18 were tried for pathogenicity. *A. flavus, B. theobromae* and *F. moniliforme* var. *intermedium* caused reduced germination; in addition the latter also caused decay of seeds (2%). The

vigour index was the lowest in the case of seeds treated with *B. theobromae, A. flavus* and *F. moniliforme* var. *intermedium* indicating that they are pathogenic to seeds of *P. marsupium*.

In *Xylia xylocarpa* also apparently healthy, discolored and discolored and shrivelled seeds were encountered. The incidence of various micro-organisms was higher in the latter two categories as compared with apparently healthy seeds. Actinomycetes[1], *Aspergillus flavus*[2], *A. niger*[3], *A. ochraceus*[4], *A. versicolor*[5], *Chaetomium globosum*[6] *Cladosporium herbarum*[7], *Fusarium pallidoroseum*[8], *Penicillium citrinum*[9] *Rhizopus oryzae*[10], *Trichoderma* sp.[11], *Trichothecium roseum*[12], sterile hyphae (white)[13] and a bacterium Gram (-)[14] were recorded. Pathogenicity of only 2,3,7,8,9,10 and 14 were tested *A. flavus* reduced the seed germination considerably. Bacterium *C. herbarum* and *F. pallidoroseum* caused distortion of seedlings. Vigour index was the lowest in seeds treated with *A. flavus, C. herbarum, F. pallidoroseum* and *R. oryzae*.

1. 3. Management of seed microflora

Various methods such as hot water treatment and fungicidal seed dressing were evaluated for their efficacy in reducing the seed-borne micro-organisms and consequently improve the seedling vigour. The efficacy of hot water treatment was evaluated at 50°C and 60°C for 15 and 30 min. In the chemical

control experiment, commonly available seed dressers viz., captafol, captan, carbendazim, carboxin, mancozeb, MEMC (Methoxy ethyl mercuric chloride), PCNB (Penta chloro nitro benzene), and thiram were tested

In general, hot water treatment was effective in *Albizia odoratissima* as higher vigour index was achieved over control in all the treatments. The number of micro-organisms also reduced from 12 in control to 6 - 9 in other treatments; *M. echinata* and *C. globosum* were the new fungi recorded after hot water treatment. Captan was the best fungicide as far as the seed germination and shoot length were concerned followed by carboxin, mancozeb, and carbendazim On the contrary, MEMC, captafol and thiram appeared to be harmful as there was reduction in the germination percentage.

In the case *L. microcarpa*, though the number of micro-organisms was reduced, the hot water treatment was not at all effective as the seed germination was completely inhibited in 60° and 50°C-30 min. treatments. In treated seeds, *Curvularia lunata* and *C. herbarum* were completely eliminated and incidence of *F. solani* was significantly reduced. Curiously, high incidence of *P. citrinum* was recorded on the treated seeds. Chemical treatment with mancozeb was most

effective followed by carboxin, MEMC, carbendazim and captan. Captafol and PCNB were not effective.

The hot water treatment was not effective in the case of *P. marsupium* also. Although germination of seeds of *P. marsupium* was greatly reduced in 50° and 60°C-30 min. treatments, at 15 min. exposure it remained unaffected. Root length was greatly enhanced at 50°C and 60°C - 30 min. while shoot length did not change appreciably. However, the number of micro-organisms reduced from 13 in control to 5-10 after hot water treatment. In chemical control studies, captan was the most effective fungicide in respect of seedling vigour, followed by thiram, captafol, MEMC and carboxin. In none of the treatments germination was affected All the fungicides were effective in reducing the number of micro-organisms; MEMC completely inhibited the growth of all the micro-organisms

In *Xylia xylocarpa* hot water treatment was not effective as it reduced the seed germination except for treatment at 50°C-15 min. The number of micro-organisms also reduced from 9 in control to 2-4 in various treatments. Interestingly, *A. flavus* and *R. oryzae* recorded higher incidence in most of the treatments as compared with control. Thiram was most effective as a seed dresser followed by mancozeb and carbendazim. Though, the germination was enhanced in these

treatments the shoot length did not change appreciably. Seeds treated with captan, carboxin, MEMC and mancozeb were completely free from micro-organisms

1.4. Storage and its influence on microflora, seed germination and seedling growth

Seeds of all the four tree species were stored separately in plastic containers and cloth bags for one year in the laboratory. Other treatments included fungicidal seed dressing storage of seeds in a desiccator over calcium chloride at room temperature and at 4°C The number of micro-organisms were enumerated at Day-1, Day-90, Day- 180 and Day-365 following SB method. At each sampling the germination percent and vigour index were also worked out for all the treatments.

In *A. odoratissima*, most storage fungi recorded initially continued their presence till the end of the storage period. However, seeds stored under dehumidified conditions recorded less number of micro-organisms as compared with laboratory storage. Field fungi like *F. moniliforme* and *F. solani* were not observed after 90 days of storage under dehumidified conditions. The germination and vigour index gradually decreased as the period of storage increased. In seeds stored under dehumidified conditions at 4°C, the germination percentage reduced from 24% to 11% after 1 year

Similar results were obtained for the seeds of *L. micr-ocarpa*. Most storage fungi continued to appear till the end of storage period. The number of micro-organisms was less on seeds stored under dehumidified conditions. The seed germination showed a reduction from 10% to 4% in control untreated seeds while it varied from 10% to 7% in seeds stored at 4°C under dehumidified conditions.

In *P. marsupium*, the incidence of field fungi like *Myrothecium roridum* and *F. moniliforme* var.*intermedium* were either reduced or they were completely eliminated during the period of storage. Seeds treated with fungicides recorded only storage fungi. The number of micro-organisms was less in seeds stored under dehumidified conditions as compared with other treatments. The vigour index gradually decreased as the storage period increased. Storage of seeds at 4°C under dehumidified condition was effective as the reduction in germination was only from 23% to 14% over 1-year of storage as compared with 24 % to 4% in control.

X. xylocarpa also yielded similar results. The number of micro-organisms was less on seeds stored under dehumidified condition at room temperature and 4°C as compared with control. Storage of seeds treated with fungicides was not effective as the germination was completely lost over 1 year period

of storage. In dehumidified conditions at room temperature and 4°C, germination percentage showed a reduction from 55 at Day-1 to 17-26 at Day- 365, respectively.

2. SEEDLING DISEASES AND THEIR MANAGEMENT

In nurseries no seedling diseases were recorded from *Albizia odoratissima* indicating that it is virtually free from seedling diseases.

From *L. microcarpa*, two seedling diseases viz., damping-off and root rot were recorded of which the former was a serious disease, caused by *Rhizoctonia solani* Kuhn. Evaluation of fungicides against *R. solani* using poisoned food method (PFM) indicated that only carbendazim, MEMC, carboxin, PCNB and thiram were the most effective ones. However, in soil fungicide screening method (SFSM), carbendazim and MEMC only gave 100% inhibition over control at the highest concentration of 0.2% and 0.0125% (a.i.) respectively. Small scale nursery trials indicated that pre-sowing soil drenching with MEMC (0.006% a.i.) gave adequate control of damping-off. Root rot disease was not found to be a serious one as only < 1% container seedlings were affected. *In vitro* evaluation of fungicides against *Pythium middletonii* Sparrow. using PFM indicated MEMC

and thiram as the most effective fungicides inhibiting the pathogen at all the three concentrations tested.

Pterocarpus marsupium recorded two seedling diseases viz., collar rot caused by *R. solani* and seedling blight caused by *Sclerotium rolfsii* Sacc. Collar rot did not appear to be a serious disease as it occurred in low incidence. High incidence (ca. 32%) of seedling blight was observed during monsoon period (June - September). *In vitro* evaluation indicated the superiority of MEMC against *R. solani* followed by carboxin, and to a lesser extent carbendazim. Other fungicides were not effective. Against *S. rolfsii* carboxin and thiram were found effective in all concentrations tested in PFM, while MEMC and captan brought about inhibition only at higher concentrations. A pilot scale nursery trial indicated that pre-sowing soil drenching of carboxin or thiram (0.2% a.i.) or MEMC (0.0125% a.i.) was most effective in controlling seedling blight completely.

X. xylocarpa recorded no seedling disease in nurseries except an economically unimportant seedling blight disease caused by *R. solani* in a few container seedlings. *In vitro* evaluation of fungicides using PFM indicated that carbendazim

and MEMC were the most effective ones followed by PCNB, carboxin and thiram while these gave promising results only at the highest concentration tested in SFSM.

From the study, it may be concluded that the seeds of four indigenous tree species harboured rich seed microflora as in the case of agricultural crops with storage or saprophytic fungi as the predominant ones. Although a few field fungi recorded in the study did not cause any seed-borne diseases in nurseries. Besides, the tree species tested have a few common seed microbes as well as some microbes exclusively associated showing substrate preference. In general, SB method was superior to others as more micro-organisms were recorded and surface sterilisation of seeds reduced the micro-organisms both qualitatively and quantitatively. Dry examination of seeds revealed the presence of apparently healthy, discolored and shrivelled and deformed seeds and the incidence of micro-organisms in the former category was less as compared with the others. Hot water treatment was not at all effective for the four forestry species tested. However, fungicidal seed dressing was effective as it reduced the incidence of many micro-organisms as well improved the seedling vigour. Storage of seeds treated with fungicides was not found effective in maintaining the viability of seeds; however, storage of seeds

under dehumidified conditions can be tried in special circumstances. Very few seedling diseases were found to be associated with the four indigenous tree species and this support the generally held view that indigenous tree species rarely suffer from serious disease problems.

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