1. Introduction

Cylindrocladium leaf blight (CLB) caused by *Cylindrocladium quinqueseptatum* Boedijn and Reitsma was the first serious disease recorded in eucalypt nurseries and plantations in South India (Sehgal *et al.*, 1969). Subsequently, another serious disease namely pink disease caused by *Phanerochaete salmonicolor* (Berk. and Broome) Julich (= *Corticium salmonicolor*), came to the forefront (Seth *et al.*, 1978). By the end of 1970s, both the diseases had spread in epiphytotic proportions throughout Kerala state affecting eucalypts significantly; the disease incidence was especially high in high rainfall areas. The pink disease caused stem cankers in two- to four-year-old eucalypt trees resulting in significant loss in yield. Cylindrocladium leaf blight (CLB) affected the seedlings in eucalypt nurseries and coppice shoots and foliage in young plantations. When large-scale planting of eucalypts began during the early 1960s, apparently there was not much problem posed by CLB. However, within a few years CLB became a serious problem in raising healthy nurseries, accounting for up to almost 100 per cent seedling mortality in seedbeds and containers in high rainfall areas during the monsoon (June-September). Thus, CLB emerged as one of the most serious diseases of eucalypt in Kerala and elsewhere which required immediate attention as it affected the eucalypt at all the growth stages (Mohanan, 1995). *C. quinqueseptatum* causing foliar diseases of *Eugenia caryophyllata, Anacardium occidentale, Acacia auriculiformis, Hevea brasiliensis* and *Terminalia paniculata* (Sarma and Nambiar, 1978; Sharma and Mohanan, 1982; Mohanan and Sharma, 1982, 1984, 1985b, 1986; Nair and Jaysree, 1986), adopted the susceptible eucalypts in Kerala and within a few years caused epiphytotic of CLB after the initial inoculum build up. This way, *C. quinqueseptatum*, a pathogen of minor importance in eucalypts in Australia has become the major pathogen posing serious threat to eucalypt plantation programmes in Kerala (Mohanan and Yesodharan, 2005). Considering the magnitude of CLB, its control is necessary to provide healthy seedlings for the afforestation programmes in the state. But there is a large gap in information on various aspects
of CLB. For adopting strategies for the control of CLB in nurseries and plantations, a clear understanding of the epidemiology of the disease is a prerequisite. Except for some preliminary epidemiological studies on CLB of *E. microcorys*, in Australia no detailed information was available on this aspect. Varied types of symptoms of CLB observed in seedlings, saplings and mature trees of different *Eucalyptus* species in various parts of Kerala possibly indicate the association of more than one species of *Cylindrocladium*. In this situation, knowledge is necessary not only on various species of *Cylindrocladium* associated but also on their geographic distribution.

The most common method of controlling fungal diseases like CLB in forest nurseries is by chemicals. There are numerous examples to show that fungal diseases can be effectively and economically managed by fungicides. For a chemical management strategy to be successful, especially in forest nursery, where the seedlings are intensively managed, behaviour of pathogen on host – the infection process, the factors responsible for infection and subsequently its spread and variation in virulence – should be clearly understood. This helps in applying the suitable chemicals at appropriate time to gain the maximum benefit from the treatments. To be more effective, the strategy of chemical management should form a part of the nursery management practices. In view of the fact that nursery practices, especially the seed rate, watering schedule, shade, etc. for raising eucalypt seedlings are found to vary greatly and large-scale mortality of seedlings has been recorded, there was a need to standardize the nursery practices to suit different climatic zones (high and low rainfall regions) in Kerala.

In plantations, where CLB causes extensive defoliation and die-back of shoots during the initial two to five years of establishment, the most appropriate disease management strategy has to be of introducing disease resistance by way of planting eucalypt provenances/species resistant to CLB rather than chemical management which will be not only impractical but also cost prohibitive. This approach requires the knowledge of degree of resistance available in various eucalypt provenances/species, which can be exploited against CLB through selection or breeding. For this, a large number of eucalypt provenances/species need to be screened against the existing population of *Cylindrocladium* spp. which may possess genetical variability as being composed of even physiological races. Beside the host resistance, appropriate cultural practices to be followed during the establishment of a plantation need to be investigated to provide significant protection against CLB. Extensive field survey, laboratory experiments, and nursery trials were carried out to generate information on epidemiology and control of CLB. Only very brief data of results of various experiments and field trials are provided here. The details on materials and methods, statistical analyses of data, results, etc. of various experiments are described by Sharma and Mohanan (1991a).
2. Symptoms
CLB is the most serious disease prevalent in eucalypt nurseries and young plantations affecting growth of plants. The disease caused extensive to complete premature defoliation accompanied by die-back of tender shoots during peak period of monsoon (July-August). Defoliated twigs generally developed new shoots within one month. The initial symptom was appearance of minute grayish-black water-soaked lesions on the leaves of any maturity. Later, these lesions coalesced to form larger necrotic areas, which, on drying, turned brown giving typical blighted appearance. In high humid areas, the initial symptoms observed on leaves of *E. grandis* (Fig. 1) and *E. tereticornis* (Fig. 2) were large grayish-black irregular spots, sometimes covering the entire leaf. Such severe foliage infection caused premature defoliation.

3. Cylindrocladium Species Associated with CLB
A preliminary survey conducted during 1979 indicated that CLB was responsible for serious damage in eucalypt nurseries (Fig. 3) and plantations in Kerala (Sharma and Mohanan, 1982). Since, *Cylindrocladium* was found to be associated with a variety of diseases affecting different plant parts in eucalypt of varying maturity, occurrence of more than one species was suspected. To ascertain this, extensive disease survey in eucalypt nurseries (>70 nurseries), plantations of *E. grandis*, *E. globulus*, *E. tereticornis* (>30 plantations) and trial plots of *E. alba*, *E. camaldulensis*, *E. citriodora*, *E. torelliana* situated in different geographical and climatic zones of the Kerala state was carried out during 1979-1982. A total of 10 species of *Cylindrocladium*, viz., 1. *C. quinqueseptatum* Boedijn and Reitsma, 2. *Calonectria pyrochroa* (Desm.) Sacc. and its anamorph *Cylindrocladium ilicicola* (Hawley) Boedijn and Reitsma, 3. *Calonectria floridana* Sobers and its anamorph *Cylindrocladium floridanum* Sobers and Seymore, 4. *Calonectria induciata* (Seaver) Crous and its anamorph *Cylindrocladium theae* (Petch) Alf. and Sobers, 5. *Cylindrocladium clavatum* Hodges and May, 6. *Calonectria camelliae* Venkataramani and Venkata Ram and its anamorph *Cylindrocladiella camelliae* (Venkataram and C.S.V. Ram) Boesw., 7. *C. parvum* Anderson, 8. *Calonectria curvata* (Boed. and Reists.) L. Lombard, M.J. Wingfield and Crous and its anamorph *Cylindrocladium curvatum* Boedijn and Reitsma, 9. *Calonectria morganii* Crous, Alfenas and M.J. wingfield and its anamorph *Cylindrocladium scoparium* Morgan, 10. *C. colhaunii*, were found associated with various diseases of eucalypts (Sharma and Mohanan, 1982; Mohanan and Sharma, 1984, 1985a). In a number of instances, more than one species was recorded from the same *Eucalyptus* species. *C. quinqueseptatum* was found distributed throughout the state, irrespective of host species of *Eucalyptus* or geographical location. However, other species had discernible spatial distribution with narrow host range. *C. ilicicola* and *C. theae* were localized only in high ranges. The occurrence of many *Cylindrocladium* species,
some localized in a particular geographical area and their causing various diseases of eucalypt at all growth stages is suggestive of complex problems associated with control measures.

For planning disease management strategy of CLB, a clear understanding of its epidemiology is essential. Hence, detailed investigations were undertaken to study the conidial germination (Fig. 4), severity of CLB in eucalypt plantations in relation to intercropping with taungya and rainfall, relative susceptibility of eucalypt provenances to CLB, cultural variation of *Cylindrocladium* isolates, pathogenic variation in *C. quinquepartitum*, *in vitro* evaluation of fungicides against *Cylindrocladium* species, nursery trials for managing seedling diseases of eucalypts, etc. were carried out.

4. *In vitro* and *in vivo* Conidial Germination of *Cylindrocladium*

For obtaining optimum conidial germination, two techniques, viz., hanging drop and cavity slide were compared. Two-month-old *E. grandis* seedlings were used for *in vivo* conidial germination studies (Sharma and Mohanan, 1991a, b). Seedlings were inoculated with the conidial suspension on adaxial and abaxial surfaces till runoff. Leaves obtained from four to six hours of incubation period were processed for scanning electron microscopy by freeze drying and gold coating under vacuum. These were observed using Hitachi S-540 SEM for the formation of infection structures and penetration.

Results of *in vitro* conidial germination revealed that conidia usually produce a single germ tube from each end cell but development of germ tubes from intercalary cells was also not uncommon and up to five germ tubes were recorded from a single conidium. However, the growth of the terminal germ tube was more rapid than that produced from the intercalary cells. Within 30-45 minutes of germination, a thick walled septum was formed adjacent to conidial wall. Elongation of the germ tube was rapid after six hours and branching occurred within an hour. Statistical analyses of data on conidial germination showed that both the techniques (GT) gave similar results. Interaction T x GT was found to be highly significant (P<0.01). Among the three conidial concentrations tried, germination differed significantly being the highest at lowest concentration of 1.5 x 10\(^3\) m\(^{-1}\). Cluster analysis indicated that hanging drop method using the lowest conidial concentration at 25°C gave maximum conidial germination. Conversely, in the cavity slide technique, maximum conidial germination was obtained at 20°C and not at 25°C. Temperature also influenced the germination greatly as the conidia germinated only at 20°, 25° and 30°C and was not at 10°, 15° and 35°C.

*In vivo* conidial germination details were similar to that for *in vitro* studies except that *in vivo* germination of conidia began after three hours of incubation and branching of germ tubes occurred during four to five hours of incubation (Sharma and Mohanan, 1990, 1991a). Occasionally, fusion of germ tubes was observed only
Fig. 1. CLB affected *E. grandis* plantation.

Fig. 2. CLB in *E. tereticornis*.

Fig. 3. *Cylindrocladium* seedling blight in nursery.

Fig. 4. Conidia of *Cylindrocladium*.
the leaf surface. Growth of the germ tube was faster on younger leaves than on mature. Also, the germ tube length was significantly greater on adaxial surface than on abaxial surface of young leaves. Appressorium formation occurred after four hours of incubation. Stomatal penetration rarely occurred. In the case of stomatal penetration, the appressorium was formed over the stomata covering the entire stomatal opening, while for direct penetration, appressoria were formed at any place over the epidermal cell. The efficiency of *Cylindrocladium* is evident from the results where each conidium produced two to four germ tubes which, subsequently, branch further, thus, bringing about multiple infections through one conidium.

5. Severity of CLB in Relation to Cultural Practices and Rainfall
Initially, CLB begins to appear on leaves of lower branches near the ground and spreads upwards to higher branches. However, in seedlings and young trees the infection may initiate at any part. Since no information was available on the influence of climatic conditions and cultural practices followed during the stand establishment phase such as cultivation of tapioca (*Manihot utilissima*) as taungya crop on the severity of CLB, these studies were undertaken.

High CLB severity coincided with rainfall months whereas high relative humidity alone during dry period did not seem to favour infection considerably. During January-March of 1982 though CLB severity remained very low, it showed an upward trend especially after showers during March-May. After heavy rainfall during June, the severity increased rapidly and it was high during July and August. Subsequently, as the rainfall declined, the CLB severity also declined gradually to low level by December 1982. The cultivation of tapioca as a taungya crop in the eucalypt plantation contributed to severity of the disease. The overall high CLB severity during 1983 was possibly due to the congenial conditions such as high rainfall and greenhouse conditions by tapioca which helped to build up high inoculum potential for disease development. Positive correlation of CLB severity with the high rainfall pattern appears to have some management implications. *E. tereticornis*, which is highly susceptible to CLB may not be suitable for the high rainfall areas of Kerala and it is advisable to manipulate the present cultural practices so as to minimize the disease hazards (Sharma and Mohanan, 1992b).

6. Relative Susceptibility of Eucalypt Provenances to CLB
The long-term solution for managing CLB is possibly to raise resistant provenances/species of *Eucalyptus*. To find out potential of introducing resistance as a strategy of CLB management, artificial inoculation tests were carried out to assess the relative susceptibility of different eucalypt provenances to three predominant species of *Cylindrocladium*: i.e., *C. clavatum*, *C. ilicicola* and *C. quinqueseptatum*. 
Seeds of 46 provenances belonging to 16 species of *Eucalyptus* were obtained from the Commonwealth Scientific and Industrial Research Organization (CSIRO), Canberra, Australia, whereas seeds of *E. grandis* and *E. tereticornis* were obtained from Tamil Nadu Forest Department for raising seedlings, and nine-month-old seedlings were used for inoculation studies.

Quantitative assessment of relative susceptibility of eucalypt provenances to three *Cylindrocladium* spp., causing CLB under identical experimental conditions revealed a great deal of variation. Among the three *Cylindrocladium* species, the variance for *C. ilicicola* (CI) was the least followed by that of *C. quinqueseptatum* (CQ) and *C. clavatum* (CC). This indicated a closer relationship between the susceptibility level of the provenances to *C. ilicicola* than the other two species. The percentage of provenances giving resistant reaction was highest (60) to CI, lowest to CC (19.14) and intermediate to CQ (35.41). A reverse trend was observed for the provenances giving highly susceptible (HS) and susceptible (S) reactions. It possibly implies that CC is the most virulent species and CI, the least.

In general, there appeared to be no correlation between level of susceptibility and subgenus/section of the genus *Eucalyptus* as the response of different provenances varied significantly from resistant to susceptible within a subgenus/section. The relative susceptibility of different provenances of eucalypt species also varied considerably to three *Cylindrocladium* species. This is clearly evident from the responses of provenances of *E. grandis* and *E. tereticornis*. Of the eight provenances of *E. grandis*, including Local TN, three gave resistant reaction (R), two susceptible (S) and three highly susceptible (HS) to CQ. Similar varying responses were observed for CC and CI, the respective figures for R, MS and S reactions being 1, 3, 2 and 2, 1, 1. The susceptibility reactions of provenances of *E. tereticornis* also varied greatly depending on the *Cylindrocladium* spp. However, there were three provenances of *E. tereticornis* (12944, 13277, 13319) which gave identical resistant reactions to three *Cylindrocladium* spp. Besides, *E. tessellaris* 12967, *E. cloeziana* 13278, *E. urophylla* 12896 and *E. camaldulensis* 12964 also gave identical reactions to three *Cylindrocladium* spp. However, there were 15 provenances which gave similar (either resistant or susceptible) reactions to at least two *Cylindrocladium* spp. (Sharma and Mohanan, 1992c). The study revealed a total of 17 provenances with fewer restricted necrotic lesions per unit area as compared to those with fewer but large lesions. These are *E. tessellaris* 12967, *E. cloeziana* 12945, *E. deglupta* 12322, *E. grandis* 13022, 13025, 12970, and *E. camaldulensis* 12181 to CI; *E. citriodora* 12379, *E. grandis* 12409 and *E. urophylla* 13357 to CC; *E. propinqua* 12800 to CQ; *E. tereticornis* 13277 and *E. brassiana* 13412 to CQ and CI; *E. tereticornis* 13319 and *E. brassiana* 13397 to CC and CI; and *E. tereticornis* 12944 and *E. brassiana* 13415 to CQ, CC and CI. These provenances may prove to be superior in the field to other resistant provenances. Control of CLB
of eucalypts in future will be based on raising provenances/species with durable field resistance. As a first step in this direction, this study has identified provenances with relatively resistant reaction which might be a good indicator of field tolerance to CLB.

7. Cultural Variation in *C. quinqueseptatum*

During routine isolation of *C. quinqueseptatum* (CQ) from diseased *Eucalyptus* materials, collected from different parts of Kerala, a great deal of cultural variation was observed in the isolates. This together with differences recorded in leaf blight reactions on various *Eucalyptus* species to field isolates gave an indication to the existence of physiological strains in CQ. Since, sources of resistance in eucalypts to CLB are not clearly understood, for an effective and viable tree selection programme it is essential to assess the variation in pathogenicity and virulence of the CQ population.

Cultural characteristics and diameter growth of 10 isolates of CQ were studied on nine different media, viz., Czapek dox agar (CDA), glucose asparagine agar (GAA), glucose tyrosine agar (GTA), glucose yeast extract agar (GYEA), glucose limabean agar (GLBA), malt extract agar (MEA), potato dextrose agar (PDA), vegetable agar (V8) and yeast malt agar (YMA). Five CQ isolates, viz., 897, 947, 961, 963 and 1080 having very distinct cultural and morphological characters were selected and effect of 11 carbon (C) and 13 nitrogen (N) sources on growth and MS production were compared (Sharma and Mohanan, 1991a, 1992a).

The results revealed that cultural characters such as radial growth, colony characters, sporulation and microsclerotia (MS) production form an important criterion for ascertaining differences among CQ isolates as they differ considerably from medium to medium (Sharma and Mohanan, 1992a). Though PDA and YMA were the best media for growth, sporulation and MS production, MEA was the best medium as it could discern a maximum number of six isolates as distinct from the others. This means that not all the media are equally suitable in discerning the differences among the isolates. This could be due to differences in nutritional requirements of the isolates. Growth rate (GR), which is possibly closely related to the ability of an isolate to utilize nutrients in a particular growth medium appears to be of limited use in discerning the isolate differences. This is due to the fact that GR of CQ isolates was statistically significant only on four media (GYEA, MEA, PDA, GLBA). However, on GLBA all the 10 isolates were separated into three distinct groups, indicating that this medium is better for discerning isolates on the basis of their GR. Overall growth of five CQ isolates was found to be better on C sources than N sources, but both appear to be dependable characters in differentiating the isolates into strains. Though, statistically significant differences are found in the utilization of C and N sources, the latter is better as it helped in distinguishing four
isolates (897, 947, 961, 1080) as compared to three (947, 963 and 1080) in the former. Better growth of most of the isolates was recorded in organic N sources, especially the peptide and protein than inorganic sources. Monosaccharide and disaccharide sugars seem to be preferred by most of the isolates. For the production of MS, casein hydrolysate is found to be the best N source for all the isolates. The study revealed that eucalypt isolates of \textit{C. quinqueseptatum} show remarkable differences in their cultural characters on a given medium and in their capacity to utilize C and N sources. This indicates that they could be different strains which may also vary in virulence (Sharma and Mohanan, 1991a, 1992a).

8. Pathogenic Variation in \textit{C. quinqueseptatum}

As sources of resistance in eucalypts to CLB are not understood, for an effective and viable tree selection for disease resistance it is essential to assess the variation in pathogenicity of population of CQ and also to know whether different CQ isolates possess general or specific variance. To achieve the above objectives five monoconidial isolates of CQ (755, 897, 947, 968, 1080) were tested on a set of 11 differential provenances of \textit{Eucalyptus} selected on the basis of their susceptibility to CLB, viz., \textit{E. tessellaris} 12967, \textit{E. brassiana} 13412, \textit{E. tereticornis} 13398, \textit{E. urophylla} 12895, \textit{E. saligna} 13027, \textit{E. brassiana} 13415, \textit{E. grandis} TN Local and \textit{E. propinqua} 12800. A cluster analysis (Calinski and Corsten, 1985) was done for the mean leaf lesions (cm$^{-2}$) for various eucalypt differential provenance (P) and isolate (I) combinations to distinguish and separate resistant (R), susceptible (S) and highly susceptible reactions (HS) (Sharma and Mohanan, 1991a, b).

\textit{Eucalyptus} differential provenances showed significant differences in CLB susceptibility ranking of different provenances isolates. Susceptibility ranking of different provenances to five isolates of CQ also differed significantly indicating differential interaction which is clearly evident by the two-way ANOVA (Sharma and Mohanan, 1991b). The isolates and provenances differed significantly at $p=0.001$ in virulence and susceptibility, respectively and the interaction between them was also significant at $p=0.001$. This showed that relative CLB susceptibility between provenances depended on the CQ isolates. Similarly, the relative virulence between isolates depended upon the provenances. Since the mean square of isolates was greater than that of provenances, it possibly indicated that disease severity is mainly governed by the genetically different isolates and also that the provenances have a closer genetic relationship.

Cluster analysis of mean leaf lesions of 55 combinations of isolates and differential provenances showed three statistically significant clusters with mean lesions of 7.66, 24.25 and 56.11 cm$^{-2}$ representing, respectively R, S and HS combinations. In R cluster, there were as many as 37 combinations involving all 11 provenances and five isolates while S cluster had only 10 combinations, all excepting one provenance; i.e.,
E. propinqua 12800 and only three isolates (755, 897, and 1080). In HS cluster, there were only four combinations involving isolate 13412, 13397, E. grandis 13020 and E. propinqua 12800. The latter clearly indicated that isolate 947 is the most virulent of five. It is evident from the results that the dynamics of virulence in the population of CQ is much more complex than expected. High statistically significant isolate x provenance (I x P) interaction clearly shows the specificity in horizontal resistance (HR) in various eucalypt provenances. It could result in positive selection pressure by the chosen provenances on the population of the CQ strains.

9. In Vitro Evaluation of Fungicides against Cylindrocladium

Despite the economic importance of eucalypt seedling blight and leaf blight no proper management measures have been worked out in a systematic manner. In laboratory studies, Anahosur et al. (1977) found Bavistin and Thiram as highly effective (ED<sub>100</sub>) in inhibiting the growth of C. quinquepartitum in poisoned-food technique. Since there are more than one species of Cylindrocladium associated with various diseases of eucalypts, and fungicides were not evaluated using soil-fungicide technique to confirm the inhibition of microsclerotia (MS) production by the pathogen, these observations have little importance in controlling Cylindrocladium diseases in Kerala. With the objective of affording chemical management of Cylindrocladium diseases in nurseries and plantations, various fungicides were evaluated in vitro for their efficacy against two major species; i.e., C. quinquepartitum and C. ilicicola. C. camelliae, C. floridanum and C. parvum were also included in some of the screening methods to find out fungicides, if any, equally effective against all the five species of Cylindrocladium.

A total of 22 fungicides were evaluated against various Cylindrocladium spp. following conidial germination technique (CGT), poisoned-food technique (PFT) and soil fungicide screening technique (SFT). The purpose of employing three techniques was to ascertain the efficacy of fungicides in inhibiting conidial germination and mycelial growth and rendering microsclerotia non-viable.

9.1. Comparison of Efficacy of Fungicides

It is evident from the results that there is a differential effect of many fungicides depending upon the species of Cylindrocladium. Similarly, the Ed<sub>100</sub> of fungicide also varied depending upon the species. However, there was a few fungicides which were more or less equally effective against all the Cylindrocladium spp. tested using a particular screening technique. In conidial germination technique (CGT), of the 22 fungicides tested against C. quinquepartitum (CQ), 12 showed cent per cent conidial inhibition at 0.1 per cent a.i. For C. floridanum (CF), 10 out of 15 fungicides were highly effective. But for C. ilicicola (CI) which appears to be more tolerant than other species, only 6 out of 18 fungicides were found to be
effective. In PFT, carbendazim, benomyl, busan-30 and sodium azide were equally effective against all the four species of Cylindrocladium (CQ, CI, CF, CP). However, copper oxychloride, kitazin, guazatine, terrazole are highly effective against CI but not against CQ, CF and CP. In SFT, carbendazim stood out as the only fungicide effective against all the three species of Cylindrocladium (CQ, CF, CI); none of the others caused even 50 per cent inhibition in growth. On comparing the effective fungicides for various Cylindrocladium spp., it is amply clear that carbendazim is the only fungicide consistently effective against all the five species of Cylindrocladium (Sharma and Mohanan, 1991c).

10. Nursery Trials for Controlling Seedling Diseases of Eucalypts

Under conducive microclimatic conditions, especially in high rainfall areas (>3,500 mm annual rainfall), Cylindrocladium diseases in nursery may cause even 100 per cent mortality of seedlings, thus posing practical problems to foresters in meeting the requirement of stock for raising a planned area of plantations. Chemical management of seedling diseases in nursery appears to be the only solution, because it can be easily integrated with other nursery management practices. With this in view, the efficacy of the fungicides evaluated in the laboratory was further tested in the nursery trials conducted during three consecutive years at Chandhanathode, Wayanad, Kerala. E. grandis and E. tereticornis seedlings were raised and a total of 32 treatments consisting of 14 non-systemic fungicides, five systemic fungicides, two soil fumigants and 9 combinations of fungicide/fumigants. Solar heating treatments were given in the experimental nursery (Sharma and Mohanan, 1991a).

The results of first year nursery trials indicated that even five treatments of most of the effective fungicides could not provide a total protection against Cylindrocladium infection. Though, fungicides in various treatments reduced the disease incidence, yet they varied greatly in their effectiveness in controlling the disease. The ones which survived against a heavy pathogen pressure and provided a total control were systemic fungicides: carbendazim and benomyl and a non-systemic, captan. These results are in conformity to earlier findings of Engelhard (1971) who reported the effectiveness of benomyl against Cylindrocladium rot of Azalia cuttings caused by C. scoparium. Fumigation with chemicals has been used commercially for many years to control certain plant pathogens present in the upper few centimeters of soil. However, Cylindrocladium leaf blight could not be controlled in the present trials. It means, microsclerotia of Cylindrocladium are not affected by methyl bromide or Di-trapex. Solar heat treatment, where mulching with polythene sheet increased the soil temperature from 37° to 43.5°C, resulted in reduced damping-off and seedling blight as compared to untreated control. On comparison of efficacy of different fungicidal treatments against various diseases in nursery trials conducted in three consecutive years, carbendazim stood out as the best, as it control led
besides CLB other diseases too. Its efficacy increased when used in combination with other fungicides such as MEMC, mancozeb and quintozene. During the third year trials when prophylactic treatments were given just after sowing of seeds, the best treatment where no damping-off, web blight and seedling blight appeared and other diseases were subsequently controlled effectively, is a combination of MEMC, mancozeb and carbenazim in the first application followed by second and third applications of carbenazim alone. By applying the fungicides initially at pre-emergence stage, the damping-off, web blight and seedling blight caused by *Cylindrocladium*, *Rhizoctonia* and *Pythium* were controlled. Subsequently, carbenazim treatment controlled effectively all the *Cylindrocladium* diseases (Sharma and Mohanan, 1991a).

11. Effect of Some Nursery Practices on Incidence and Severity of Diseases and Growth of Eucalypt Seedlings

Under the conventional method practised in Kerala, the eucalypt seedlings are raised in seedbed nurseries during December-January, and pricked out in polythene containers during February/March. These container nurseries are maintained till the time they are out planted during June after the onset of monsoon. During this period, seedlings are exposed to disease hazards and any lapse in management of nursery may accentuate the disease situation, resulting in large-scale mortality of seedlings (Sharma et al., 1984, 1985). Some of the important nursery practices which appear to have direct bearing on the incidence and severity of seedling diseases are shading, watering frequency and quantity of water, and seed rate. The objectives of this study was to investigate the effect of different types of shading over the nursery, moisture regimes and seed rates on the incidence and severity of nursery diseases and growth of eucalypt seedlings with a view to standardize nursery practices for raising healthy and disease-free seedlings. Experimental nursery was raised at Chandhanathodu, Wayanad, Kerala. For shade treatment, besides conventional coconut leaf thatch (CLT), coir mat (CM) of 7mm mesh was used. Two seed rates, viz., 2.6 g m\(^{-2}\) (SR1) and 7.0 g m\(^{-2}\) (SR2) equivalent of 40 g and 100 g per standard seedbed were used. Soil moisture regimes, viz., 11 l m\(^{-2}\) (MR1) and 14 l m\(^{-2}\) (MR2) per day were regulated by appropriate frequency of watering (Sharma and Mohanan, 1992b).

Microclimatic conditions under two shade treatments: Microclimatic conditions under coir mat (CM) and coconut leaf thatch (CLT) varied significantly. The average light intensity under CLT was about 15 times less as compared to CM. Average ambient and soil temperatures were higher under CM (26\(^{\circ}\) and 24.3\(^{\circ}\)C, respectively) than under CLT. Also the soil water potential (SWP) was generally higher in seedbed with low moisture regime (MR1) than in high moisture regime (MR2).
11.1. Incidence and Severity of Seedling Diseases
Incidence and severity of web blight, damping-off and seedling blight were severely affected by various nursery practices. Web blight appeared and persisted for a long duration under CLT than CM; in MR1-SR1 of both the shade treatments no disease was recorded. Average number of foci and area under disease progress curve (AUDPC) were significantly higher for CLT shading than those of CM. High moisture regime (MR2) and high seed rate (SR2) had significantly higher disease severity than low moisture regime and low seed rate as evidenced by average number of foci and AUDPC and the disease progress rate (Sharma and Mohanan, 1992b). Incidence of damping-off was also affected significantly by the type of shading as it appeared first and persisted for a long period under CLT than CM; a similar trend was also observed for MR2 and SR2 treatments. However, disease severity as expressed by AUDPC and the disease progress rate did not differ in both the shade treatments. Seedling blight was recorded first under CM and a week later in CLT but it persisted for a longer period in the latter than in former. The disease severity was significantly higher in MR2 of CM than of CLT. Though high disease severity was correlated well with high seed rate (SR2) of all the treatments of CM and CLT, significantly higher disease severity in MR2 than in MR1 was observed only in CM (Sharma and Mohanan, 1992b).

11.2. Growth of Seedlings
Since the microclimatic conditions differed considerably under CM and CLT, the growth of seedlings of *E. grandis* also showed variation under the two shade treatments. Development of leaves was much faster under CM than under CLT. The shoot growth was exponential in both the shade treatments. At 105-day of emergence, the length of root and shoot was significantly higher in seedlings of both the moisture regimes under CM than CLT (Sharma and Mohanan, 1991a, 1992b).

11.3. Introduction of Root Trainer in Nurseries
During the past few years, forest nursery practices in India have undergone tremendous modifications based on various microclimatic, edaphic and biotic factors, including host, pest and pathogen association (Mohanan, 2000a). Consequently, seedling health has been given more importance which further widened the scope of phytosanitary problems. However, introduction of root trainers in forestry sector and thereby the technological changes in seedling production has had a major impact on nursery management (Mohanan, 2000b, 2003, 2007). As soilless or soil-free potting media are used in root trainers, common soil-borne diseases damping-off, seedling blight, wilt, etc. seldom occur in root trainer nurseries. Another advantage of root trainers is that the seedlings require a maximum period of 90 days growth and hence rigorous management is possible during this comparatively short period of
maintenance than in conventional nurseries, where seedlings have to be maintained for two to four months. For example, eucalypt seedlings have to be maintained in the seedbeds for three to four months and thereafter in polythene containers for two to three months. In root trainer nurseries, even if foliage disease occurs, the affected seedlings can be easily removed from the blocks and replaced with other healthy seedlings, thereby avoiding the spread of disease in nursery. Since, the root trainer seedlings exhibit uniform growth performance, prophylactic fungicidal treatment, if required, and maintenance of seedling quality are easier than in conventional nursery system (Mohanan, 2003; Mohanan et al., 2005).

12. Discussion and Conclusion

Since the genus Cylindrocladium was originally established for a Mucedinaceae fungus, Cylindrocladium scoparium Morgan on dead pod of honey locust (Gleditschia triacanthus L.) in Indonesia, several Cylindrocladium species have frequently been reported as pathogenic. C. quinqueseptatum Boedijn and Reitsma, isolated in Indonesia in 1941 by W.C. Sloof from clove leaves and published by Reitsma and Sloof (1950) after establishing its pathogenicity, has emerged as one of the serious pathogens of eucalypts in Australia, Brazil, India, Indonesia, Malaysia and Mauritius (Bakshi et al., 1972; Peerally, 1974; Sharma et al., 1985; Ferriera, 1989; Sharma and Mohanan, 1991a). At present, 52 Cylindrocladium species and 37 Calonectria species were recognized based on sexual compatibility, morphology and phylogenetic interference from specimens collected from different plant species including Eucalyptus (Crous et al., 1998; Park et al., 2000; Crous, 2002; Old et al., 2003). Association of 10 species of Cylindrocladium with eucalypts in Kerala indicates their potential threat to susceptible eucalypts in exotic environment. Among the 10 species, C. ilicicola, C. quinqueseptatum and C. theae are the major pathogens affecting eucalypts at different growth stages in nurseries and plantations. In Brazil, which has the largest area under eucalypt plantations, 13 species of Cylindrocladium have been recorded, the prominent species being C. crotalariae, C. ilicicola, C. quinqueseptatum and C. scoparium. However, in Australia, the home of eucalypts, only C. quinqueseptatum and C. scoparium have been reported, and only the former species is known to cause severe shoot blight of E. microcorys in Queensland (Pitkethley, 1976). This variation in dominant species in different geographical area appears to be closely related to Eucalyptus species grown and climatic conditions, and to a lesser extent, the presence of hosts other than eucalypts on which different Cylindrocladium species occur. The specialized nature of Cylindrocladium species is clearly evident from their distribution pattern in the Kerala state and their causing diseases of specific plant parts. There appears to be an ecological balance between various Cylindrocladium species which governs their temporal and spatial distribution within a geographical area.
The present study reveals that *C. quinqueseptatum* has specialized into physiological strains varying greatly in virulence to adopt eucalypts. *E. tereticornis*, commonly called Mysore hybrid possibly has considerable genetic variability. This variability in the host may have exerted the selection pressure on *C. quinqueseptatum* to evolve into different physiologic strains. Origin of strains is further substantiated by the fusion of germ tubes, originating from the same conidium or different conidia observed on the leaf surface. Of the five strains of *C. quinqueseptatum* identified, four (isolate nos. 755, 897, 947 and 1080) have specific virulence or wide variability in their reactions which possibly means that eucalypt differential provenances may have...
population lines. The most virulent isolates can be tested on different eucalypt germplasm and the apparently resistant sources can be DNA fingerprinted and used in breeding programmes. Variation among C. quinqueseptatum isolates, is not known in northern parts of India.

1. Genetic variability in North Indian Isolates of C. quinqueseptatum

Pandey (2010) collected 82 isolates of C. quinqueseptatum from three north Indian states, viz., Punjab, Haryana and Uttarakhand, and subjected them to Random amplified polymorphic DNA (RAPD) analysis for quantifying polymorphism and identifying different populations. It was done with an idea to use representative isolates from each population for artificial inoculation and screening of resistant germplasm. Apart from determining taxonomic identity by microscopic examination of different isolates, 26 isolates were characterised using ITS regions of ribosomal DNA and nine for beta tubulin gene regions. The ITS region sequences were then used to design species specific primers and rest of the isolates were authenticated using these primers.

1.1. Characterization of Isolates Using Random Amplified Polymorphic DNA Analysis

Random amplified polymorphic DNA (RAPD) analysis is a fast, PCR based method of genetic typing based on genomic polymorphisms. RAPD is widely used by different workers to assess the variability among the isolates within the same geographical region or within the same host species. Different isolates of C. quinqueseptatum collected from Punjab, Haryana and Uttarakhand were subjected to RAPD-PCR for identifying different population lines (Fig. 1). The pathogen repository so created will be helpful in identifying durable resistance in eucalypt germplasm by artificial inoculation with representative isolates from each population line. These resistant sources can be propagated and utilized by the industries and forest managers for raising disease-free nurseries and plantations.

Mohanty et al. (2012) analysed 73 isolates from north India by RAPD technique and identified different population lines. The four primers used in the study produced specific patterns that could differentiate C. quinqueseptatum isolates according to their geographical origin (Fig. 2). For example, 85 per cent isolates in OPE 2 clustered in accordance with their geographical location while 15 per cent isolates clustered irrespective of their geographical location. Later, more isolates were included in the study and out of 40 decamer operon primers tested initially, only three primers, viz., OPE-2, OPE-3 and OPE-5 produced consistent and reproducible polymorphic bands with all the fungal isolates and were finally used for molecular variability study.

Fig 1. Dendrogram of 82 isolates of C. quinqueseptatum showing major population lines based on combined RAPD binary matrix.
Fig 2. Random amplified polymorphic DNA (RAPD) finger prints of isolates of C. quinquesepatatum amplified with primer OPE-2 (Mohanty et al., 2012).

Amplification and Sequencing of ITS Region of Ribosomal DNA

Internal transcribed spacers (ITS-1 and ITS-2) are non-coding region of genomic DNA found in rDNA operon of fungi. ITS-1 region is found between 18S rDNA and 5.8S rDNA and ITS-2 between 5.8S rDNA and 28S rDNA. These regions are variable and exploited by the researchers for discrimination at species level in fungi and used for establishing taxonomic relationship among species of particular taxon. For amplification of this region in fungi, several universal primer pairs were designed, however, ITS-1 (forward primer) and ITS-4 (continued on next page...
(reverse primer) are frequently used by researchers. To determine the molecular diversity among the north Indian isolates of *C. quinqueseptatum*, ITS-1 and ITS-2 along with 5.8S rDNA region of 26 isolates were amplified and sequenced. Sequencing was done directly from the amplified product using primer ITS-1 at genomic laboratory of Axygen India Pvt. Ltd., New Delhi, India (Fig. 3 and 4). All the sequences were annotated using software ORF (Open Reading Frame) finder. A mini heuristic search with all the isolates yielded 70 most parsimonious trees with a length of 129 (Fig. 5). The consistency index was 0.483871, while the retention index and composite index were 0.602564 and 0.456631, respectively. A strict consensus tree was calculated. The alignment of sequences generated 490 characters from which 32 were parsimony informative (6.53%). ITS region sequences of 26 isolates and 9 of beta tubulin genes regions were deposited in NCBI, GenBank, USA. The evolutionary tree divided into five different clades on the basis of variation in ITS-1 and ITS-2 region, thus, confirming the existence of genetically differentiated lineages in the north Indian isolates of *C. quinqueseptatum*. This knowledge might be expanded for studying the epidemiology of the disease.

3. Detection of *C. quinqueseptatum* by species specific primers

Pandey *et al.* (2010) developed PCR primers to detect *C. quinqueseptatum* which cause heavy seedling mortality in north Indian states. Primers based on sequence analysis of internal transcribed spacer region 1 and 5.8S of rDNA produced PCR product of 245 bp. The internal transcribed spacer (ITS) of the ribosomal DNA (rDNA) sub unit repeat was sequenced in 26 isolates of *C. quinqueseptatum* and sequences were aligned and compared with the ITS...
sequences of other fungi in GenBank. No amplification resulted from PCR reactions on fungal DNA from 6 common forest fungi, 10 soil contaminates and six eucalypt pathogens. For amplifications directly from infected tissues, a nested primer PCR was done using two rounds of amplification. First, the entire ITS was amplified with universal fungal primer; a second round of amplification was carried out with species specific primer that amplified a 245 bp PCR product. The method detected leaf and seedling blight in artificially and naturally infected eucalypt plants. The pathogen was also detected from the soil using species specific primer. In sampling studies, C. quinqueseptatum was detected by PCR from artificially infected seedlings after six days of inoculation, before any visible symptoms were noticed. The PCR assay and direct tissue extraction methods provide tools which may be used to detect C. quinqueseptatum from soil, plant cuttings and adjoining eucalypt plantations that may be serving as recurring source of infection. Early detection may thus, limit the transmission and spread of new aggressive strains of C. quinqueseptatum in eucalypt growing regions of India.

4. Relative Virulence of C. quinqueseptatum Isolates
For evaluating variability in the virulence, 71 C. quinqueseptatum isolates collected from different parts of Punjab, Haryana and Uttarakhand were screened. Supernatant of all the isolates showed varying degree of wilting symptoms in twigs except for five isolates within 15 hours of incubation. After 30 hours of incubation all the isolates showed wilt symptoms. Results obtained after 45 hours of incubation were evaluated to differentiate the isolates on the basis of their relative virulence. From this study, it was concluded that Punjab and Haryana had most virulent isolates, hence the planting material grown in these states may be avoided for raising nurseries and plantations of eucalypt. However, more conclusive studies are needed. There appears to be correlation between the vegetative growth of pathogen isolates with their virulence as highly virulent isolates showed lesser growth rates. For screening virulent isolates, artificial inoculation of seedlings with different isolates in mist chamber will provide conclusive evidence which could be replicated in field conditions.

5. Future Prospects
From DNA finger printing of 82 isolates by RAPD analysis, 14 population lines of pathogen were identified. Representatives of each the population line needs to be utilized for identifying resistant germplasm. The isolates from Uttar Pradesh are also being collected and fingerprinted to identify different population lines. This will further contribute to a more reliable screening of resistant host germplasm. Molecular diagnostic kit for probing C. quinqueseptatum in soil, host tissues and adjacent species will be helpful in early detection of the disease, thus, enabling the industry managers and farmers to take timely remedial measures. The in vitro testing of systemic and non-systemic fungicides (nine) and antagonists (12 from four different genera) against CLBS had encouraging results. The growth of 12 isolates of C. quinqueseptatum on 10 growth media have also helped in differentiating the isolates. These approaches will support in assessing the diversity of populations as well as evolving a management strategy for the devastating pathogen. Host specificity testing and safety parameters need to be evaluated before the field application of biological control agents.

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References


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some common genes for resistance. The fifth strain, isolate no. 968, possesses general or uniform virulence within the sampled population as it gave identical reactions to all eucalypt genotypes. This clearly shows that the dynamics of virulence in the population of C. quinqueseptatum is much more complex than expected. Different eucalypt provenances show differential susceptibility to three CLB pathogens, viz., C. ilicicola (least virulent), C. clavatum (most virulent) and C. quinqueseptatum (intermediate). Most significantly, a number of provenances possess resistance to Cylindrocladium which can be exploited for the management of disease in eucalypt plantations.

Highly pathogenic nature of C. quinqueseptatum is evident from infection studies which show production of multiple germ tubes by a conidium, and causing multiple infections of CLB within a short duration through direct penetration. As expected, due to mucilage-borne conidia which are dispersed by water drops, development and spread of CLB is rain-dependent. There is a positive correlation
of CLB severity with high rainfall. A chemical management, though justifiable in nursery, is not feasible in plantations due to prohibitive operational costs. The study shows that effective control of CLB and other seedling diseases in the nursery is possible through prophylactic chemical treatment and adopting standard nursery practices. The latter should be given due importance as they can influence significantly the availability of desired quality of plantable seedlings. Though, there were a number of effective fungicides, only carbendazim effectively controlled the disease in nursery trials conducted at Chandhanathodu. Managing the nursery disease employing biological tools is innovative and environment friendly. Mohanan (2007) demonstrated the efficacy of biocontrol agents like *Trichoderma* spp. against *Cylindrocladium* spp. and *Rhizoctonis solani* in forest nurseries. It is essential to ensure healthy nursery stock for a large-scale plantation programme of eucalypts. Raising healthy seedlings depends largely on the nursery cultural practices, besides the quality of seeds. Considering the immense pressure of *Cylindrocladium* spp. in Kerala, growing provenances with durable field resistance is the only viable alternative in combating CLB in nursery and plantations.

**References**


