INTEGRATED MANAGEMENT OF LEAF ROT OF COCONUT

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I

DECLARATION

I hereby declare that this thesis entitled "**Integrated Management of Leaf Rot of Coconut**" is a *bonafide* record of research work done by me during the course of researeh and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

Certified that this thesis entitled **"Integrated Management of Leaf Rot of Coconut"** is a record of research work done independently by Ms. Vrinda. T. S. (2000 11-52) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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INTRODUCTION

1. INTRODUCTION

Coconut (*Cocos nucifera* L.), often portrayed as "the tree of life" is cultivated in more than 92 countries globally with an area of approximately 12.78 million ha producing 54802 million nuts annually. It is grown as a traditional plantation crop in India for the last many years. India is presently the leader in the coconut cultivation in world producing approximately 10.60 billion nuts from an area of 1.84 million ha with a productivity of 6891 nuts per ha contributing Rs.7000 crores to GDP, earning about Rs.313 crores as foreign exchange and sustaining ten million people (DES, 2011).

Although Kerala still ranks top in terms of area (1.08 Lakh ha) and production (6672 million nuts) of coconut in India, the productivity (4533 nuts / palm / year) is far below the national average (45 nuts / palm / year) and that of the neighbouring state (Tamil Nadu – 84 nuts / palm / year) (Kumaran *et al.*, 2002). Among the various reasons ascribed as the causes of low productivity, occurrence of the most devastating, more than a century old root (wilt) disease (RWD) is recognized as the most critical production constraint, threatening the coconut cultivation in Kerala. The malady has spread far and wide from the original foci of infection and has already inflicted enormous damage to coconut cultivation in the eight southern districts of Kerala and is fast spreading to other districts of Kerala and the neighbouring districts of Tamil Nadu and Karnataka states.

Very often root (wilt) affected palms are predisposed to and 'super infected' with leaf rot disease (LRD), another dreaded disease of coconut, resulting in the devitalization of the already weakened palm. More than 65 per cent of the RWD affected palms are superimposed with LRD in the root (wilt) endemic areas of Kerala (Srinivasan, 1991). Root (wilt) is a slow spreading, debilitating, phytoplasma disease while leaf

rot is a slow killing, fast spreading fungal disease complex totally disfiguring the palm which were considered as the "symbol of prosperity". It has been roughly estimated that an yield loss of about 968 million nuts occur due to RWD in Kerala in addition to the loss of husk, copra and oil (CPCRI, 1985a). Precise loss due to LRD alone is not available but it is presumably estimated to be 461 million nuts annually without accounting the loss of more than 60 per cent of the damaged foliage (CPCRI, 1985a). In fact, LRD is the most important factor of concern in the management of RWD since it is leaf rot which is responsible for drastic foliage destruction resulting in significant yield reduction (Koshy, 1999).

LRD was reported to have been co-evolved with RWD (Butler, 1908). It is a fungal disease complex and association of more than one fungus had been indicated (Menon and Nair, 1951). Diverse groups of fungal pathogens such as *Helminthosporium halodes*, *Colletotrichum gloeosporioides*, *Gliocladium vermoseni* and *Fusarium* spp. were implicated on the etiological role of LRD along with secondary pathogens like *Rhizoctonia solani*, *Theilaviopsis paradoxa* and *Curvularia* sp. (Menon and Nair, 1948; Radha and Lal, 1968; Srinivasan *et al.*, 1998). The primary pathogenic role of these fungi in LRD incidence need thorough investigation involving extensive isolation from different regions, for evolving strategic management of the disease. Inspite of wide spread prevalence of LRD in RWD endemic areas of Kerala, effective and sustainable management practice is still wanting. The frequent scene of grossly disfigured crown of RWD - LRD affected palms in severely infected areas is proof of this argument. Attempts to contain the disease either by using different fungicides or by following integrated prophylatic measures fall short of success. The incidence and severity of LRD is spreading alarmingly in the prominent coconut growing areas of Kerala and urgent and drastic measures to contain the disease and reduce the loss is the need of the hour.

In view of the above facts a fresh insight was envisaged in the present study to investigate on the etiology and management of LRD of coconut in the following thrust areas :

★Isolation and identification of pathogens associated with LRD of coconut in different regions during three seasons of the year

★Purification, characterization and pathogenicity of the different pathogen isolates

★Symptomatology of LRD in naturally infected palms and on artificial inoculated spindle leaflet

★Screening, selection and development of potential fungal and bacterial antagonists effective against major pathogens of LRD

★Bioassay of fungicides against these pathogens

★Compatibility among different biocontrol agents and also on compatibility of fungicides with biocontrol agents

★Integrated management of LRD of coconut in the field using selected fungicides and promising antagonists.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Studies on LRD were initiated in the early 20th century, almost in parallel with investigations on root (wilt) disease (RWD).

2.1 ORIGIN, PREVALENCE AND DISTRIBUTION

The first authentic report on incidence of 'leaf rot like' disease on the unopened spindle of coconut was made by Butler (1908) although he did not mention any pathogen associated with it. However, the disease was presumed to be in existence in the former Indian Princely states of Travancore and Cochin as early as in 1880's (Varghese, 1934; Menon, 1935; Menon and Nair, 1948,1951,1952). It needs to be assumed that the descriptions of leaf rot (Mc Rae, 1916; Menon and Nair, 1948) and leaf disease (Menon, 1935) of coconut as RWD were actually early reports of fungal leaf rot on coconut and proves the present concept that LRD is part of RWD.

Although the disease was observed only in certain pockets initially, it was gradually spreading and was found to be widely prevalent in the central Travancore region with Kollam and Punalur in the South and Oachanthuruthu and Thodupuzha in the north as boundaries (Menon and Nair, 1948). The occurrence of leaf rot increased over the years in the root (wilt) endemic areas of southern Kerala with increase in the intensity of root (wilt). Stray cases of LRD in young palms in RWD free area (Kasaragod) was also reported (Radha and Lal, 1968). However, incidence of LRD in places other than RWD endemic areas was subsequently neither reported nor confirmed. Recently heavy incidence of RWD along with LRD was noticed in coconut plantations of different age groups in Cumbum, Chenkottai, Coimbatore, Pollachi and Kulasekaram regions of Tamil Nadu (Srinivasan and Sasikala, 2001).

Palms of all age groups (from seedlings to adult palms) are infected with LRD, but incidence was more in palms below 25 years of age

(Menon and Nair, 1948; Menon and Pandalai, 1958). LRD does not normally occur on seedlings in the nursery, but is seen to occur within ten months of field planting (Koshy, 1999, 2000).

No definite information is available on the influence of soil types and topography on the occurrence of the disease. Many studies revealed that young palms with RWD were readily attacked by LRD pathogens irrespective of soil types (Srinivasan, 1991; Srinivasan *et al.*, 1998). LRD complex has not been reported from any other coconut growing tracts of the world.

2.2 INTERRELATIONSHIP OF LRD AND RWD

Many of the early observations (Butler, 1908; Mc Rae, 1916; Menon, 1935; Menon and Nair, 1948, 1951, 1952) indicated LRD as a part of RWD although it was not then realized. However, later findings corroborated the simultaneous occurrence of both diseases and the predisposition of RWD affected palms to LRD infection.

One of the early studies by Varghese (1934) showed that leaf disease of coconut occurred along with the root disease and generally both the palms showed similar type of symptoms. Further evidence was provided by Radha and Lal (1968). They also reported close relationship between occurrence of LRD and RWD in the field as well as in inoculation trials. They indicated LRD incidence on both RWD affected as well as on apparently healthy palms, but such palms expressed RWD symptoms subsequently. However, evidence of such latent infection of RWD phytoplasma in coconut palms are at present lacking. They further observed that nearly 16 – 40 per cent of the palms in RWD affected areas developed leaf rot and its intensity varied in different soil types. In another study, it was seen that palms in early, middle and advanced stages of RWD, were infected with leaf rot in 40 per cent, 79 per cent and 98 per cent of the cases respectively (George and Radha, 1973).

Mathai (1980) also correlated incidence of LRD with RWD indicating the influence of RWD on LRD.

Srinivasan (1991) reported that LRD was generally confined to RWD affected palms and on an average 65 per cent of RWD affected palms were 'super infected' with leaf rot pathogen. Srinivasan and Gunasekaran (1996a, 2000a) provided further evidence for this by inoculating two and five year old RWD affected coconut seedlings with LRD pathogen. They could observe severe leaf rot infections on the inoculated seedlings. Severe natural incidence of LRD in RWD infected seedlings in field conditions was observed (CPCRI, 1986). It is postulated that the predisposition of RWD affected palms to LRD infection might be due the already broken down defence mechanism of palms due to phytoplasma of RWD. The occurrence of both these diseases on the same palm is a unique example of phytoplasma fungal disease complex resulting in substantial crop loss (Srinivasan *et al.*, 1998).

2.3 ECONOMIC IMPORTANCE AND CROP LOSS

Precise crop loss estimation due to LRD is not available since it is difficult to separate the damages caused by RWD and LRD (Varghese, 1934; Koshy, 1999). Menon and Nair (1948) estimated the annual loss due to LRD at Rs.5.6 million. Radha *et al.* (1962) estimated that the loss in yield due to leaf rot was 70 per cent when compared to healthy palms. Joseph and Rawther (1991) estimated loss due to LRD as 461 million nuts annually in the disease endemic areas without accounting the loss in terms of damaged leaves.

2.4 SYMPTOMATOLOGY

Earliest description of symptoms of LRD was made by Butler (1908). Further descriptions on symptomatology of disease were made by McRae (1916), Sundararaman (1925), Varghese (1934) and Menon and Nair (1948, 1951, 1952). In all these descriptions the early symptom

was found to occur on the unopened spindle leaf as minute water-soaked lesion, like brown spots at the distal ends and margins of the leaflets or intermittently scattered on the laminar area. The rotting of the affected portion extends into the interior of the spindle resulting in extensive decay of the upper portions of the spindle. Subsequent workers (Radha and Lal, 1968; Lily, 1981; Srinivasan and Gunasekaran, 1992) also observed similar symptoms. Whitening and softening of the leaflets of spindle an early indication of RWD, also predispose the palm to LRD infection (Dwiedi *et al.*, 1979). Lily (1981) considered the tender un-opened spindle leaf having thinner epidermal layer and with maximum moisture content as the ideal port for the leaf rot pathogen. Further decay and deterioration of the spindle is assisted by ants, earwigs, maggots and nematodes (Varghese, 1934; Menon and Nair, 1951; Koshy, 1999). Often the rotten leaflets of spindle cement together. When the leaflets of such spindle gradually unfurl, the rotten portions dry up, turn black and blown off by wind and the broken portions and midvein become blackish and shrivelled while the leaflets of the basal part of the spindle are not infected and open normally producing the typical 'fan leaf' like symptom (Menon and Nair, 1951; Joseph and Rawther, 1991). Successively emerging spindles are progressively infected by LRD pathogens resulting in severe foliage loss as the disease progresses (Srinivasan and Gunasekaran, 1992). The progress of rotting slows down as leaflets mature. The 'fan leaf' like appearance of all leaves in the crown indicates that all the leaves had contracted LRD in succession and rotting progressed to varying degrees resulting in linear disease progression (Menon and Nair, 1951; Srinivasan, 1991). Lesions of the disease were also seen on petiole, midrib and midveins (CPCRI, 1996; Srinivasan and Gunasekaran, 2000a). Study on the quantitative pattern of the LRD symptom in different whorls of palm indicated that the inner whorls of leaf are more vulnerable to infection (Srinivasan and Gunasekaran, 1992). In exceptional cases the leaves of middle whorl of

palm become yellow (mid-whorl yellowing symptom) and LRD lesions appear on such leaves as dark brown lesions which further coalesce resulting in severe, blighting of the leaf lamina (CPCRI, 1985b).

Radha *et al.* (1961) developed a four grade scale while Mathai (1980) used a six grade scale to quantify the disease. The indexing system was further modified by Srinivasan and Gunasekaran (1996b) and they formulated a five point grading system for satisfactory computation of disease severity.

2.5 ETIOLOGY

2.5.1 Fungal Disease Complex

The early report of involvement of microorganisms with LRD was by McRae (1916) from Cochin who indicated the presence of a salmon coloured *Penicillium* like fungus on infected portions. Sundararaman (1925) also made similar observation. Sundararaman (1929) noticed the association of *Fusarium* sp. with shoot rot of coconut while McRae (1929) observed *Gloeosporium* sp. in diseased palms. Varghese (1934) also gave the indication of fungal etiology of leaf rot. Detailed investigations on the etiology of LRD led to the isolation of diverse organisms such as *Helminthosporium halodes*, *Gloeosporium* sp., *Gliocladium* sp., *Pestalotia* sp., *Fusarium* sp., *Thielaviopsis paradoxa*, *Rhizoctonia solani*, etc. (DAF, 1938, 1940; Menon and Nair, 1948, 1951, 1952). Pathogenicity trials with these fungi indicated that *H. halodes*, *Gloeosporium* sp. *G. roseum* and *Pestalotia* sp. produced typical symptoms associated with LRD (Menon and Nair, 1948, 1951). However, it was noticed that *H. halodes* induced infection within 12 hours and the rest in 48 hours. Hence they considered *H. halodes* as the most virulent pathogen and the rest acted only as secondary parasites. Pathogenicity was established using single and mixed inocula of different fungi. *Helminthosporium* sp. alone caused 84 per cent infection while it was 93 per cent with mixed inocula (Menon and Nair, 1951).

Later studies by Radha and Lal (1968), Lily (1963, 1981) and CPCRI (1979) further confirmed the etiological role of *H. halodes* (*Bipolaris halodes*) with LRD. However culture filtrate of *H. halodes*, applied on tender leaves of coconut failed to induce any toxic effect (CPCRI, 1981). Apart from the fungi recorded above, *Curvularia* sp. and *Diplodia* sp. were also isolated from LRD infected coconut leaves (CPCRI, 1985b). Sathiarajan *et al.* (1988) isolated *H. halodes*, *Gloeosporium* sp., *Gliocladium roseum*, *Fusarium* sp, *Diplodia* sp. *Pestalotia palmarum* and *Thielaviopsis paradoxa* from disease specimen collected from southern districts of Kerala state and found that *H. halodes* was pathogenic. In addition to *H.halodes*, *Gloeosporium* sp., *Gliocladium roseum*, *Fusarium* spp. and *Macrophomina phaseolina* were isolated from leaf rot affected coconut palms in Orissa (Mishra *et al.*, 1989).

A detailed morphological study of *H. halodes* was undertaken by Shanmughom (1963) and reported that the spores of fungus produced secondary spores from primary spores and the coconut isolate of the fungus was designated as *H. halodes* var. *nuciferae* var. nov. The spore characteristics of *Helminthosporium* sp. was studied by Menon and Pandalai (1958) who observed that fungal spores were airborne and enveloped in drops of dew/rain water.

Most of these works were mainly centered around *H. halodes* as causal agent of LRD based on isolations from a few plants. The complex nature of the disease warranted elaborate studies on etiology by isolations from a large number of palms from different locations during varying seasons. This work was taken up Srinivasan and Gunasekaran (1993) and they isolated various fungi in different frequencies. Based on specific taxonomic criteria they concluded that the fungi identified previously as *H. (Bipolaris) halodes*, *Gloeosporium* sp. and *G. roseum* were actually *Exserohilum rostratum* (Drechsler) Leonard and Suggs., *Colletotrichum gloeosporioides* (Penzig and Sacc.) and *Gliocladium vermoeseni* (Biourge) Thom. respectively and attributed leaf rot as a

fungal disease complex. Detailed pathogenicity studies of these fungi further proved their etiological nature in LRD incidence (Srinivasan and Gunasekaran, 1994a, 1996a).

The fungi associated with LRD (14 different species) were grouped into three categories *viz.*, group A, B and C based on the frequency pattern, and relative association with the disease (CPCRI, 1994). In addition to this association, documentation, characterization and pathogenicity of *Thielaviopsis paradoxa* (Dade) C. Moreau, *R. solani* Kuhn., *Mortierella elongata* Linnem, *Curvularia* sp., *Acremonium* sp., *Thielavia microspora* Mouch., *T. terricola* (J. Gilman and E. V. Abbott.) Emmons., *Chaetomium brasiliense* Batista and Pont. (Srinivasan and Gunasekaran, 1994b, Srinivasan *et al.*, 1995); *Cylindrocladium scoparium* (Srinivasan and Gunasekaran, 1995a). *Fusarium solani* and *F. moniliformae* var. *intermedium* (Srinivasan and Gunasekaran, 1996a) were also recorded. Subsequent studies by Srinivasan *et al.* (1995) showed frequent association of *C. gloeosporioides*, *E. rostratum*; *G. vermoeseni*, *F. solani*, *F. moniliforme* var. *intermedium*, *T. paradoxa* and *Pestalotiopsis palmarum* with the disease. They also observed profuse mycelial growth and spore masses in between infected leaflets. The *in vitro* associative interaction of predominant fungi with LRD was studied by Srinivasan and Gunasekaran (1995b) and they opined that the behaviour of the predominant fungi of LRD was more of associative nature rather than antagonistic, having etiological significance for a disease complex. *Fusarium* spp. had competitive interaction over certain less frequent fungi. Repeated isolations and pathogenicity trials (Srinivasan and Gunasekaran, 1996c,d, 2000a) proved that *C. gloeosporioides* is the principal pathogen of LRD. The etiological role of *F. solani* and *F. moniliformae* associated with LRD was further proved by their frequent isolations from LRD infected coconut palms (Srinivasan and Gunasekaran, 1999).

E. rostratum inoculated leaves took up infection faster while *G. vermoeseni* could induce infection only when inoculated with injury. Mixed inoculum of the spores of *E. rostratum*, *C. gloeosporioides* and *G. vermoeseni* gave higher incidence compared to independent inoculation with these fungi (Srinivasan and Gunasekaran, 1994a). Behaviour of these fungi was evaluated in potted seedlings and field palms to elucidate their relative role in disease incidence and expression (Srinivasan and Gunasekaran, 1996a). Among them *C. gloeosporioides* and *E. rostratum* were aggressive and produced intense lesions on RWD palms. LRD symptoms were reproduced in field planted coconut seedlings having mild symptoms of RWD by artificial inoculation with *C. gloeosporioides* and *E. rostratum* individually and in combination (CPCRI, 1996). However, in healthy seedlings (free from RWD in pots and field planted palm) these fungi produced only restricted spots (Srinivasan and Gunasekaran, 1996a).

Menon and Nair (1951) studied growth requirement of the fungi associated with the disease. *H. halodes* grew best in oat meal agar and *Gloeosporium* sp. in Brown's agar. Optimum growth of both fungi was obtained around neutral pH while high acidity and alkalinity had a deleterious effect.

2.5.2 Other Associated Factors of LRD

Individual and collective role played by other invaders and colonizers of leaf rot affected spindle leaf such as mealy bugs, mites, nematodes etc. in aggrevating /synergising and disseminating the LRD was emphasized by Nadakkal (1965). The high proneness of LRD affected palms to red palm weevil attack was indicated by Koshy (1999). According to him nematodes *Aphelenchoides ligarhiensis*, *Panagrolaimius rigidus* and *Rhabditis* sp. are associated with rotting of spindles.

2.6 EPIDEMIOLOGY

Incidence, severity, pathogens composition and spread of LRD is directly related with season and prevailing weather conditions. Leaf rot infection was found to be more severe during the seasons when atmospheric humidity was at its maximum (Menon and Nair, 1951). They also observed highest number of spores in atmosphere during south west monsoon (June-August). Similar observation was made by Radha *et al.* (1961) and they correlated periods of high humidity (above 98 %) and low temperature (below 27^0C) prevailed during monsoon period with disease. Subsequent studies by Radha and Lal (1968) also confirmed this finding. Any atmospheric condition, which provided free water or wetness to leaf surface, such as rainfall or dew during the dry months and compactness of the foliage, also favoured fungal infections (CCRS, 1962; 1965). Dwiedi *et al.* (1979) observed that the frequency of LRD rotting/necrosis in spindles of RWD affected palms were at the same level during rainy and summer seasons, but severity was visually greater in rainy season. Results of study conducted by Mathai (1980) indicated that leaf rot disease intensity was maximum during September-October (north-east monsoon period) and minimum in April-June period.

Isolation studies conducted by Srinivasan and Gunasekaran (1996c) showed that fungal recovery from infected pieces was in the range of 42-50 per cent in August and 60-70 per cent in December among the different grades of spindle infection. The influence of weather variables in population dynamics of core pathogens were recorded by sequential monthly isolations from the spindles of diseased palms (Srinivasan and Gunasekaran, 1996d). They observed that the incidence of *C. gloeosporioides* was higher in frequency and population during monsoon with peak in June/July. *C. gloeosporioides* was implicated as the principal pathogen of LRD during monsoon. Incidence and population of *E. rostratum* was less strongly correlated with weather,

while *Fusarium* spp. were most commonly observed during the dry seasons of January-May.

2.7 DISEASE MANAGEMENT

2.7.1 *In vitro* Study

Menon and Nair (1951) tested copper sulphate, mercuric chloride and phenol against the leaf rot fungi. Mercuric chloride and phenol were found to be lethal at various concentrations tested. Prasannakumari *et al.* (1960) obtained complete inhibition of mycelial growth of *H. halodes* by 1 % Bordeaux mixture, 0.3 % Fungimar copper and 0.5 % Kirti copper. Sathiarajan *et al.* (1988) found that Foltaf, Maneb, Panolil, Captaf, Hinosan and Kitazin were effective in preventing the growth of leaf rot pathogen (*B. halodes*) under *in vitro* condition. *In vitro* assay of few contact fungicides (Indofil M-45, Fytolan, Captan and Thiram) and systemic chemicals (Contaf, Calixin and Aureofungin-Sol) against *C. gloeosporioides*, *E. rostratum* *G. vermoeseni*, *F. solani*, *F. moniliformae* and *T. paradoxa* by poisoned food technique revealed that contaf (Hexaconazole) completely inhibited the growth of pathogens while Indofil M-45 completely inhibited *E. rostratum* at different concentrations used. *C. gloeosporioides* growth was inhibited only up to 88 per cent at the 0.5 per cent of Indofil M-45. Fytolan also caused 100 per cent suppression of growth of *C. gloeosporioides* (Srinivasan and Gunasekaran, 1998).

2.7.2 Field Study

2.7.2.1 Phytosanitation

In one of the earliest field management studies, Varghese (1934) reported cutting down of the innermost infected spear and one or more of the surrounding infected leaves and protecting the cut ends with Bordeaux paste or coal tar and burning infected material as method of management of LRD. Similar management practice was also

recommended by Radha (1984). Koshy (1999, 2000) also recommended cutting and removal of rotten portions of the spindle and the adjacent two fully opened leaves for LRD management.

2.7.2.2 Chemical Control

Patel (1938) indicated the effectiveness of Bordeaux mixture for shoot rot control in coconut. Menon and Nair (1951) found that two copper oxychloride fungicides 'Bar cop' and 'Oxi-cop' at concentration of 0.2 per cent were as effective as one per cent Bordeaux mixture in ameliorating LRD in the fields. The efficacy of Bordeaux mixture was further confirmed by Menon and Nair (1952). Based on the studies at Vadayar and Malankara estate Gregory (1960) noticed that aerial spraying of oil based copper fungicides was useful in controlling LRD. However, according to Samraj *et al.* (1966) the aerial spraying of oil based copper fungicides was not effective in protecting the spindle leaf of coconut against LRD. Field trial at Kayamkulam (CCRS, 1963) indicated that Bordeaux mixture reduced the intensity of leaf rot by 74 per cent followed by Kirti copper by 65.6 per cent and Fytolan by 59.6 per cent. Subsequent studies (CCRS, 1970) at the 400 acres of coconut garden at Vypeen island using Oleocop and Fycol 8 with Orobex 964 oil suspension showed the effectiveness of aerial spray.

Spraying of Bordeaux mixture (1 %), Dithane M-45 (0.2 %) and Cuman (0.4 %) with or without manuring in alluvial, leterite and sandy loam soils did not show much variation on the effect of these fungicides (CPCRI, 1972). Spraying Bavistin (0.1 %), Bordeaux mixture (1 %), Difolatan (0.3 %, 0.5 %), Fytolan (0.3 %) and Kitazin (0.3 %) also did not provide good control in a trial involving spraying three times a year for three consecutive years (CPCRI, 1982). Prophylatic basal application of systemic fungicides (Actidione, Bavistin, Benlate) at the rate of 4 g/healthy palm twice a year also failed to prevent the incidence of leaf rot (CPCRI, 1983). Mathai *et al.* (1984) could not obtain any significant

reduction in number of leaves infected by LRD pathogens in a trial involving fungicide (Benlate), bactericide (Agrimycin-100), nematicide (Dasanit) and different plant nutrients (N, P, K, Ca, Mg and Zn). However Radha (1984) recorded that removal and burning of infected parts of leaves along with application of one per cent Bordeaux mixture or (0.5 %) copper oxychloride controlled and prevented the spread of LRD. Sequential spraying of Bordeaux mixture (1 %), Fytolan (0.5 %) and Dithane M-45 (0.3 %) four times a year on leaf rot affected palms in farmers garden controlled the disease very effectively (CPCRI, 1985b). Kitazin 1 % spray was almost as effective as Bordeaux mixture (1 %) in leaf rot control (Sathiarajan *et al.*, 1988).

Result of the field fungicidal trial by different methods of application such as pouring into leaf axil / around the spindle, spraying on the crown and root feeding, on the LRD palm involving contact (Indofil M-45, Fytolan) and systemic (Calixin) fungicides conducted by Srinivasan and Gunasekaran (1996b) revealed the usefulness of spraying of Indofil-M-45 and pouring of calixin into leaf axil of spindle since the disease intensity was reduced in newly emerged leaves. Combined application of either Contaf 2 ml or Indofil M-45 3g/palm with insecticide phorate 10 G at 20 g/palm was found effective in controlling leaf rot (Koshy, 1999, 2000; Koshy *et al.*, 2001). Effectiveness of mancozeb against leaf rot was reported by Koshy *et al.* (2002).

George and Samraj (1966) reported that coconut palm affected by leaf rot responded favourably to foliar application of boron but it was contradicted by Sathiaraj *et al.* (1988) stating that application of boron was not effective in preventing the incidence of leaf rot or in reducing the intensity of the disease in affected palm.

2.7.2.3 Disease Resistance

Butler (1908) emphasized the need for evolving resistant against the 'coconut palm disease of Travancore'. Accor

Radha (1961) and CCRS (1962) Andaman Ordinary and New Guinea were comparatively resistant to LRD. Natural incidence of LRD was found to be low in Chowghat Green Dwarf (CCRS, 1965, 1971). Mathai *et al.* (1991) found that among nine hybrids tested the lowest incidence of disease was in West Coast Tall (WCT) x Laccadive Dwarf and WCT x Nyior Gading. Mathai *et al.* (1985) observed lower levels of incidence of RWD and LRD in Andaman ordinary, SSG and Cochin China varieties.

2.7.2.4 Bio-control

Attempts to manage LRD using antagonistic microorganisms was initiated as early as in 1952 but much headway was not obtained under field condition. Lily *et al.* (1952) isolated a bacterium (*Bacillus anthracis*) having inhibitory effect on leaf rot pathogen *H. halodes*. Later, it was found that the culture filtrate of *B. subtilis* inhibited the growth of *R. bataticola*, *R. solani*, *Botryodiplodia theobromae* and *H. halodes* (Lily *et al.*, 1955). Antagonistic interaction study of *Pseudomonas fluorescens* with principal pathogens of LRD *viz.*, *C. gloeosporioides*, *E. rostratum*, *Gliocladium vermoeseni*, *F. solani*, *Thielaviopsis paradoxa* and *R. solani* clearly demonstrated the inhibitory effect of bacterium on growth of all these pathogens (CPCRI, 1998). In another study it was found that *P. fluorescens* isolated from rhizosphere of coconut palms inhibited the mycelial growth of both *C. gloeosporioides* and *E. rostratum* under *in-vitro* condition (Gupta *et al.*, 2000).

Although studies on the antagonistic effect of various fungal biocontrol agents on LRD pathogens are at present lacking, *Trichoderma* spp. were tested against leaf blight pathogen *Pestalotiopsis palmarum* of coconut (Karthikeyan *et al.*, 2002). The antagonist was found to be inhibitory to the leaf blight pathogen and they recommended spraying of

spore suspension of native isolates of *T. harzianum* along with adhesive material for disease control.

2.8 COMPATIBILITY OF ANTAGONISTIC MICROORGANISMS WITH FUNGICIDES

Compatibility of antagonistic microorganisms with commonly used pesticides is a pre-requisite for integration of biological control methods with chemical control in any integrated disease management strategy.

Henis *et al.* (1978) reported that PCNB at 4 µg/g soil added with *T. harzianum* had an additive effect on control of damping off of radish and synergistic effect on the decrease in inoculum density of *R. solani* propagules. Biotypes of *T. harzianum* tolerant to Captan, Captafol, Chlorothalonil, Iprodione and BAS 352 were developed by exposing conidia to increasing concentrations of the fungicides and these biotypes possessed an increased ability for biological control of white rot of onion (Papavizas, 1980). Indu and Mukopadhyay (1990) reported that there was no inhibition on radial growth of *T. harzianum* by metalaxyl at concentration as high as 50 ppm and in field trials *T. harzianum* and metalaxyl were less effective individually but when used in combination they had an additive effect providing 84 – 100 per cent disease control.

Somasekhara *et al.* (2000) reported that Captan had no inhibitory action on certain strains of *Trichoderma* spp. . Siltuang *et al.* (2000) reported that the population of *T. harzianum* were higher in triademefon treated plots. Nallathambi et al. (2001) tested the sensitivity of *T. viride* isolates against Bavistin, thiophanate methyl, Captan and Ridomil and found that some isolates showed resistance and some showed tolerance.

2.8.1 Compatibility of Biocontrol Agents

In recent years successful attempts are being made to deliver a consortium of compatible biocontrol agents with different modes of action to get satisfactory biocontrol of plant pathogens. Hubbard *et al.*

(1983) and Bin *et al.* (1991) identified strains of fluorescent pseudomonads which reduced the growth of *Trichoderma* sp. under *in vitro* conditions. But Duffy *et al.* (1996) reported that strains of fluorescent pseudomonads and *Trichoderma* sp. were compatible as their co inoculation was significantly more suppressive to take all disease of wheat. Varsney *et al.* (2000) reported that combined inoculation of fluorescent pseudomonades and *T. harzianum* resulted in growth inhibition of latter by the former. A combination of *Paecilomyces lilacinus*, *T. viride* and *P. fluorescens* as seed treatment resulted in less root rot incidence in *Vigna mungo* (Latha *et al.*, 2000). Seed treatment with talc based formulation of *T. viride* and *P. fluorescens* effectively reduced the pre and post emergence damping off of chillies (Manoranjitham and Prakasham, 2000).

MATERIALS AND METHODS

3.MATERIALS AND METHODS

All the laboratory studies connected with the thesis project were conducted in the Department of Plant Pathology, College of Agriculture, Vellayani while field trial was conducted at Regional Agricultural Research Station, Kumarakom, a severe root (wilt) (RWD) – leaf rot (LRD) endemic area in Kerala.

3.1 ISOLATION OF PATHOGENS ASSOCIATED WITH LEAF ROT OF COCONUT

Disease specimen of leaflets of unopened spindle leaves of coconut palm were collected from different areas in Thiruvananthapuram (Kazhakoottam and Kudappanakunnu), Kollam (Kottarakkara, Kilimanoor and Kollam), Alappuzha (Moncompu, Aroor and Kayamkulam) Kottayam (Kidangoor, Kumarakom and Changanacherry) and Pathanamthitta (Konni and Pathanamthitta) districts during three seasons *viz*., June – July, November – December and March – April. The microorganisms associated with leaf rot were isolated from the infected portions of leaves by the routine isolation method.

The infected portions of newly developed lesions were cut into small bits and washed in distilled water. The pieces were then surface sterilized in 0.1 per cent mercuric chloride solution for one minute and washed in sterilized distilled water by three consecutive serial transfers of the specimen in three petridishes. The pieces were plated on Potato Dextrose Agar (PDA) medium (4 pieces/plate) and incubated at room temperature. When the fungal growth was visible, mycelial bits were transferred to PDA slants and labelled.

3.2 PURIFICATION

All the fungal pathogens obtained from LRD infected lesions of coconut were purified by single spore techniques and pure cultures were maintained on PDA slants for further studies.

3.3 STUDY ON THE PATHOGENICITY OF ISOLATED ORGANISMS

Detached healthy leaflets of unopened spindle leaf of coconut were used as host for the study. The leaflets were kept in conical flasks containing sterile distilled water with the upper portion of the leaflets projecting outside the conical flask. Inoculation with the organisms was performed by placing the mycelial bit from seven days old culture of the pathogen on the inner surface of leaflet with injury by pinprick and without injury. The culture bits on the leaflets were covered with moist cotton wool and the whole leaflet was kept covered by a polythene tube moistened with water and incubated at room temperature. Leaflets with plain agar disc and moist cotton placed on the inner surface with and without injury served as control. Development of symptoms and spread of lesions were recorded daily. The pathogens were re-isolated from the lesions developed by artificial inoculation.

3.4 SYMPTOMATOLOGY OF THE DISEASE

Detailed studies were made on the symptomatology of leaf rot disease of coconut by observing the sequence and development of symptom expression of naturally infected plants in the field and also by artificial inoculation.

3.5 VIRULENCE OF DIFFERENT ISOLATES OF THE PATHOGEN

Detached leaflets of unopened spindle of healthy coconut palm were used for the study. The pathogens *viz.*, *Colletotrichum gloeosporioides* (8 isolates), *Fusarium* sp (12 isolates) and *Scytalidium* sp. (2 isolates) were grown on PDA for seven days by incubating at room temperature. The leaflets were inoculated with different isolates of the

pathogens using agar discs from seven day old cultures as described in para (3.3).

Observations on the size colour and time taken for development and spread of lesions were recorded regularly. Based on the size of lesion and time taken for the symptom expression the isolates were classified as highly virulent, virulent and mildly virulent.

3.6 CHARACTERIZATION OF PATHOGENS

The morphology and cultural characteristics of different pathogens isolated from leaf rot infected portions of coconut leaves were studied by growing them on appropriate media as detailed below (Appendix-1).

a. *Fusarium* sp.: Potato sucrose Agar medium

b. *Colletotrichum gloeosporioides* : Czapeck's medium

c. Other pathogens : PDA

Four to ten day old cultures were used for the study. Colony characters such as growth rate, type of growth and colour were recorded.

3.6.1 Slide Culture

The morphological characteristics of all the pathogens were studied by growing them in slide cultures as described by Riddel (1950). Sterile plain agar medium was poured in previously sterilized petridishes and after solidification, blocks of 6 mm square agar discs were cut out using a sterile needle. One square disc was placed at the centre of a sterile slide and each of the four sides of the agar block was inoculated with mycelial bits of the respective pathogens taken from seven-day old cultures on respective medium. A cover slip was placed on the top of the inoculated agar disc and the slides were placed in moist petridish chambers (petridish with wet filter paper in the bottom on which two glass rods kept as support for the slides) and were incubated at room temperature for two days. The coverslip was then lifted off gently and mounted on another slide using lactophenol stain. The square of agar was removed from the culture slide and another mount was prepared on it

without disturbing the fungal growth on the slide. The slides were then examined under low and medium power objectives of ordinary compound microscope and measurements of conidia were taken using ocular micrometer.

3.7 *IN VITRO* INTERACTION OF PATHOGENS ASSOCIATED WITH LEAF ROT

The *in vitro* interactions between various fungi isolated from LRD lesions were determined to estimate their synergistic/ antagonistic/ no effect among themselves.

One week old cultures of *C. gloeosporioides*, *Exserohilum rostratum*, *Gliocladium roseum*, *Fusarium* sp, *Scytalidium* sp, *Cephalosporium sacchari* and *Curvularia* sp. grown on PDA medium were used as test pathogens for the study. Dual combinations of all the above organisms were tested in petridishes containing solidified PDA medium by placing agar disc (5 mm) of one of the test fungus on one side and the other at the opposite side of each petriplate. Slow growing organisms were inoculated one/two days prior to prevent overgrowth by the fast growing fungus. Three replications were maintained for each combination. Plates inoculated with only one fungus served as check. The inoculated plates were incubated at room temperature and the interactions of both the fungi were studied daily by measuring the growth, extent of inhibition and extent of overgrowth.

3.8. EFFECT OF COMBINED INOCULATION OF LRD PATHOGENS ON DETACHED SPINDLE LEAF LETS

Detached leaf lets of unopened spindle leaf of coconut were used for the study. Altogether 21 combinations of seven fungi were tested. The inoculation technique followed was similar to 3.3. For each combinations three replications were maintained.

3.9 *IN VITRO* SCREENING OF ANTAGONISTIC MICROORGANISMS AGAINST MAJOR PATHOGENS OF LRD

The interaction of potential fungal and bacterial antagonistic microorganisms available in the Department of Plant Pathology and elsewhere with the major pathogens of LRD *viz.*, *C. gloeosporioides*, *E. rostratum* and *Fusarium* sp. were tested *in vitro* to determine their relative antagonistic potential against these pathogens.

3.9.1 *Trichoderma* sp.

Six *Trichoderma* cultures developed as biocontrol agents in the Department of Plant Pathology and one from Regional Agricultural Research Station, Kumarakom were tested by dual culture technique (Skidmore and Dickinson, 1976). Agar disc of 5 mm diameter cut from seven day old culture of the antagonist and the pathogen were placed on two opposite sides of pertriplates containing sterilized PDA and incubated at room temperature. Three replications were maintained for the experiment. The paired cultures were examined at regular intervals and the radial growth of the pathogen was recorded. Petriplates inoculated with pathogen alone served as control.

The per cent inhibition of mycelial growth was calculated using the formula.

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

Where I – Inhibition of mycelial growth, C – Growth of the pathogen in control plates (cm) and T – Growth of pathogen in dual cultures (cm) (Vincent, 1927)

3.9.2 *Pseudomonas fluorescens*

Isolates of *P. fluorescens* developed and maintained in the Department of Plant pathology were used for the present study. Altogether 29 isolates were tested to estimate their antagonistic potential against major pathogens of LRD. Kings Medium B (KMB) and PDA

were used for the test. After solidifying the media for one hour in sterilized petri dishes, culture bits of 5 mm size of the pathogen was placed at the centre of each dish. The respective bacterial isolate was then streaked 2 cm away from pathogen at the centre in a triangular fashion. Each treatment was replicated five times. Radial growth of pathogens was recorded daily. Plates without the bacterial antagonist served as check.

The per cent inhibition of mycelial growth was calculated using the formula as described in para 3.9.1.

3.10 SCREENING OF ANTAGONISTS BY LEAFLET BIT ASSAY

Three selected cultures of *Pseudomonas fluorescens* (P_{S1}, P_{40}, P_{41}) and *Trichoderma* sp. (T_9, T_6, T_{12}), which showed maximum inhibitory effect on pathogen growth *in vitro* were further tested by the leaflet bit assay. Unopened spindle leaflet pieces of approximately 18 cm length were cut from healthy coconut leaves and placed in sterile petriplates (20 cm dia.) lined inside with moist filter paper. The leaflet pieces were initially sprayed with bacterial/spore suspensions of antagonists (4×10^8 and 9×10^6 cfu respectively for *P. fluorescens* and *Trichoderma* sp.). The leaflet pieces were then inoculated with culture discs of respective pathogens by placing it inside the leaflets with pinprick. Observations were taken on the time taken for symptom development and its spread.

3.11 INTERACTION BETWEEN SELECTED CULTURES OF *P. fluorescens* AND *Trichoderma* sp.

The level of interaction between the most antagonistic Fluorescent pseudomonad (PSI) and *Trichoderma* sp. (T_9) was estimated to determine their compatibility for combined delivery in the integrated management of LRD in the field.

The test was performed in sterile petriplates containing PDA medium and specific medium for the bacterial antagonists (KMB). Agar disc (5mm) aseptically cut from five day old culture of selected

Trichoderma sp. (T_9) was seeded at one side on the petridish. A sterilized filter paper disc (5mm) impregnated with the selected *P. fluorescens* (PS_1) culture suspension (4×10^8 cfu ml^{-1}) was placed at the opposite side of the plate (Varshney *et al.*, 2000). Petridishes were incubated at room temperature. Petriplates containing *Trichoderma* sp. on one side and filter paper disc impregnated with sterile water on the other side served as check. Five replications were maintained. Radial growth of *Trichoderma* sp. was estimated. Percent inhibition of growth of *Trichoderma* sp. by *P. fluorescens* was determined as described in para 3.9.1.

3.12 BIOASSAY OF COMMONLY USED FUNGICIDES AGAINST MAJOR PATHOGENS OF LRD

The commonly used fungicides were evaluated *in vitro* against the major pathogens of LRD.

Fungicides	Concentrations (ppm)
Mancozeb	50, 100, 200
Copper oxychloride	50, 100, 200
Carbendazim	25, 50, 100
Propiconazole	25, 50, 100
Hexaconazole	25, 50, 100

The test was performed following the poisoned food technique (Zentmeyer, 1955). The required quantity of fungicides for 100 ml medium was added into 50 ml sterile water in a conical flask and mixed thoroughly. This fungicidal suspension was poured into another conical flask containing 50 ml of double strength melted PDA and mixed thoroughly. The medium was poured equally in five sterile petridishes. After solidification of medium each plate was inoculated with 5 mm agar disc containing seven day old culture of the respective pathogens *viz.*, *C. gloeosporioides*, *F. solani*, *E. rostratum* by placing it at the centre of

the dish. Plates containing non-poisoned media served as check. Five replications were maintained for each concentration of the chemical. The petridishes were incubated at room temperature and fungal colony diameter was measured daily until fungal growth was complete in the control plates.

The per cent inhibition of mycelial growth was calculated using the formula explained in para 3.9.1.

3.13 COMPATIBILITY OF FUNGICIDES WITH ANTAGONISTIC MICROORGANISMS

The experiment was conducted to test the compatibility of selected fungicides and biocontrol agents that are to be used for the field study on the integrated management of leaf rot. The culture of *Trichoderma* (T_9) and *P. fluorescens* (PS_1) which showed maximum biocontrol potential against the major pathogens of leaf rot *in vitro* were selected for the compatibility test.

The fungicides and their respective concentrations used for the trial are as follows

Fungicide	Concentrations (ppm)
hexaconzole	50
	100
	200
mancozeb	100
	200
	300
	400

3.13.1 Compatibility of *Trichoderma* sp. with Fungicides

The experiment was conducted by the poisoned food technique as described above (3.12). Observations on the radial growth and per cent inhibition of *Trichoderma* sp. were recorded.

3.13.2 Compatibility of *Pseudomonas fluorescens* with Fungicides

The poisoned specific medium (KMB medium) was prepared as detailed in para (3.12).

A loopfull of 24 h old *P. fluorescens* culture was taken using a sterilized inoculation needle and made a single straight line streak on the medium. The experiment was replicated five times and plates without any fungicides served as control. The growth of the antagonist was assessed after two days.

3.14 INTEGRATED MANAGEMENT OF LEAF ROT DISEASE IN THE FIELD

3.14.1 Mass culturing of selected antagonists for field application

The selected antagonists *viz.*, *P. fluorescens* (PS_1) and *Trichoderma* sp. isolate (T_9) were mass produced as detailed below for the field experiment.

3.14.1.1 Preparation of Talc Based Formulation of Bacterial Antagonist

The *P. fluorescens* culture (PS_1) was formulated as talc based formulation (Vidhyasekaran and Muthamilan, 1995).

The culture (PS_1) was grown in KMB broth in conical flasks and incubated at room temperature. The bacterial population was estimated (4×10^9 cfu ml^{-1}) after 48 hours of inoculation.

The ingredients of the talc based formulation are 100 g of talc, 4 g of calcium carbonate and 1 g of carboxymethyl cellulose (CMC) were mixed thoroughly and kept in polypropylene bags. The bags were then sealed and autoclaved at 1.5 kg cm^{-2} for one hour on two successive days. After sterilization, 40 ml of 2 days old broth culture of the bacterium was mixed with the carrier in each polypropylene bag under aseptic conditions. This formulation was used for mass delivery of the antagonist in the field.

3.14.1.2 Mass Multiplication of Fungal Antagonist

The selected antagonistic fungus (*Trichoderma* sp., T_9) was mass multiplied in sand-oats medium by mixing washed fine sand with oats meal in the proportion of 9:1. The mixture was moistened with water sufficient enough to promote fungal growth. It was then sterilized in one litre conical flasks at 1.02 kg cm^{-2} for one hour. Actively growing culture discs of the antagonistic fungus was aseptically introduced into the flasks and incubated at room temperature for two weeks to develop fungal growth.

For the field application it was further mass cultured in powdered and sterilized cowdung-neem cake mixture (9:1) by mixing 50 g of the sand-oats medium containing the fungal culture per kilogram of cowdung-neem cake mixture. This mixture was sprinkled with water and mixed thoroughly for optimum moisture requirement, covered with polythene sheet and incubated under moist warm conditions for one week. When greenish mould growth had sufficiently covered the medium, it was raked, sprinkled more water, again covered with polythene sheet and incubated to promote further fungal growth. After 12-15 days growth the mixture was ready for field application (Joseph, 1997).

3.14.2 Integrated Management of Leaf Rot Disease in the Field

A field experiment was conducted at Regional Agricultural Research Station, Kumarakom to evolve an integrated management practice by incorporating different management practices such as fungicides and biocontrol agents

Details of the experiment are as follows:

Design – Randomized Block Design (RBD)

Replication – Five

Treatment combinations

T_0 – Absolute control without any treatment and phytosanitation

T_1 – Spraying and pouring of mancozeb (0.4%)

T_2 – Spraying and pouring of hexaconazole (0.2%)

T_3 – Spraying and pouring bacterial suspension of talc based *P. fluorescens* (2%)

T_4 – Placing neem based Trichoderma culture @ 30 g/leaf axil

T_5 – Mancozeb + *P. fluorescens*

T_6 – Mancozeb + Trichoderma.

T_7 – Hexaconazole + *P. fluorescens*

T_8 – Hexaconazole + Trichoderma

T_9 – *P. fluorescens* + Trichoderma

T_{10}- Control with phytosanitation

Formulations of *P. fluroescens* and Trichoderma were prepared as detailed in section 3.14.1.1 and 3.14.1.2.

Approximately 10-15 year old, uniformly leaf rot affected coconut palms were selected for the experiment.

Phytosanitation was given to all the selected palms (except absolute control) by removing the severely leaf rot affected portions of spindle leaf and one or few inner most leaves. The palms were maintained as per the package of practice recommendations for coconut (KAU, 1996) by giving timely application of manures and fertilizers and adopting plant protection measures.

Pre treatment observations were taken during the month of October for all the leaves in the crown by assigning appropriate grade to each leaf based on disease severity. A disease indexing method based on five point grading system was adopted to quantity and assess the severity of disease (Srinivasan and Gunasekaran, 1996b).

Grade	Leaf area affected
0	No infection
1	Upto 25%
2	25 to 50%
3	50 to 75%
4	Above 75%

(for each leaf in the crown)

The disease index was calculated using the formula

$$DI = \frac{\text{Total numerical rating}}{\text{Total number of leaves x Maximum disease grade}} \times 100$$

The first set of treatments were given soon after phytosanitation and pre-treatment observation was recorded during the month of October. *Trichoderma* culture application was given only after 2 weeks of fungicidal application in combination treatments. First post-treatment observation on disease severity was taken during January and second sets of treatments were then given. The second set of post treatment observations were taken during the month of May followed by the third spraying. The third and final observation on disease severity was taken during August. Per cent reduction in disease severity in each treatment with that of corresponding pre treatment and disease index were calculated for all three post-treatment disease index. The temperature, humidity and rainfall at Regional Agricultural Research Station, Kumarakom were recorded during the entire duration of the field experiment.

3.15 STATISTICAL ANALYSIS

The data obtained from the studies conducted under laboratory and field conditions were statistically analysed by applying the technique of analysis of variance appropriate for the design after suitable transformations.

RESULTS

4.RESULTS

4.1 ISOLATION OF PATHOGENS ASSOCIATED WITH LEAF ROT OF COCONUT

The pathogens associated with leaf rot of coconut in different regions of southern Kerala were isolated during three seasons.

4.1.1 Kottayam District

The details of microorganisms isolated from infected unopened spindle of coconut from different tracts of Kottayam district are presented in Table 1. During the rainy season (June-July, 2001) the infected specimen from Kidangoor and Kumarakom harboured *Colletotrichum gloeosporioides* and *Fusarium* sp. while *Scytalidium* sp. was prevalent in specimen collected from Changanachery. A same spectrum of pathogens were also observed from specimen collected from Kidangoor and Kumarakom during post monsoon season (November-December, 2001). In addition, *Exserohilum rostratum* and *Gliocladium roseum* were also found associated on specimen collected from Kumarakom.

During the summer season (March-April, 2002) the predominant pathogen was *Fusarium* sp. in most of the samples collected from Kidangoor, Changanachery and Kumarakom. Presence of *Scytalidium* sp. was noted on specimen from Changanachery during summer also. One sample from Kumarakom showed the presence of *E. rostratum*, *C gloeosporioides* and *G. roseum* while in another sample, *Curvularia* sp was predominant.

4.1.2 Alappuzha District

The data on the pathogenic spectrum of leaf rot affected sample from Alappuzha district are presented in Table 2.

Table 1. Fungal pathogens found associated with leaf rot of coconut during different seasons in Kottayam district

Sl. No.	Location	Pathogens isolated		
		June - July	November- December	March - April
1.	Kidangoor	*Colletotrichum gloeosporioides* *Fusarium* sp.	*C. gloeosporioides* *Fusarium* sp.	*C. gloeosporioides*
2.	Changanacherry	*Scytalidium* sp.		*Scytalidium* sp. *Fusarium* sp.
3.	Kumarakom	*C. gloeosporioides* *Fusarium* sp.	*C. gloeosporioides* *Exserohilum rostratum* *Fusarium* sp. *Gliocladium roseum*	*Curvularia* sp. *Fusarium* sp. *C. gloeosporioides* *E. rostratum* *G. roseum*

C. gloeosporioides and *Fusarium* sp. were simultaneously present on infected portions in samples collected from Kayamkulam and Aroor during June-July season while *Scytalidium* sp. was mainly isolated from Moncompu sample during the season. *Fusarium* spp. was the common pathogen recorded on samples collected from Kayamkulam and Aroor during November-December months. The sample from Moncompu continuously indicated the presence of *Scytalidium* sp. during this season. During the summer months also *Fusarium* sp. and *C.gloeosporioides* were isolated from samples collected from these localities.

4.1.3 Kollam District

Leaf rot specimen were collected from Kollam and Kottarakkara regions in Kollam district and the results are presented in Table 3. *C. gloeosporioides* and *Fusarium* sp. were consistently found associated with the samples from Kottarakkara during all the seasons. The pathogens isolated from specimen from Kollam region were *Fusarium* sp. and *Cephalosporium sacchari* during June-July and March-April while only *Fusarium* sp. was detected during November-December season.

4.1.4 Thiruvananthapuram and Pathanamthitta Districts

The results of the study on the isolation of pathogens associated with leaf rot in Thiruvananthapuram and Pathanamthitta district are presented in Table 4. It was found that *C. gloeosporioides* was predominantly present during June-July and November-December seasons while *Fusarium* sp. was involved in causing the disease during November-December and March-April on specimen obtained from Kilimanoor. Irrespective of the seasons *Fusarium* sp. alone was consistently isolated from disease specimen of Kazhakoottam and Kudappanakunnu regions.

The disease sample from Konni and Pathanamthitta areas of Pathanamthitta district indicated the presence of *C. gloeosporioides*

Table 2. Fungal pathogens associated with leaf rot of coconut during different seasons in Alappuzha District

Sl. No.	Location	Pathogens isolated		
		June - July	November- December	March - April
1.	Moncompu	*Scytalidium* sp.	*Scytalidium* sp.	
2.	Kayamkulam	*C. gloeosporioides* *Fusarium* sp.	*Fusarium* sp.	*C. gloeosporioides* *Fusarium* sp.
3.	Aroor	*C. gloeosporioides* *Fusarium* sp.	*Fusarium* sp.	*Fusarium* sp.

Table 3. Fungal pathogens associated with leaf rot of coconut during different seasons in Kollam district

Sl. No.	Location	Pathogens isolated		
		June - July	November-December	March - April
1.	Kottarakkara	*C. gloeosporioides Fusarium* sp.	*C. gloeosporioides* *Fusarium* sp.	*C. gloeosporioides* *Fusarium* sp.
2.	Kollam	*Fusarium* sp. *Cephalosporium sacchari*	*Fusarium* sp.	*Fusarium* sp. *C. sacchari*

Table 4. Fungal pathogens associated with leaf rot of coconut during different seasons in Thiruvananthapuram and Pathanamthitta districts

Sl. No.	Location	Pathogens isolated		
		June - July	Nov-Dec	Mar - Apr
1.	Kilimanoor	*C. gloeosporioides*	*C. gloeosporioides* *Fusarium* sp.	*Fusarium* sp.
2.	Kazhakuttam	*Fusarium* sp.	--	*Fusarium* sp.
3.	Kudappanakunnu	*Fusarium* sp.	--	*Fusarium* sp.
4.	Konni	*C. gloeosporioides*	*Fusarium* sp.	*C. gloeosporioides* *Fusarium* sp.
5.	Pathanamthitta	*C. gloeosporioides*	*Fusarium* sp.	*C. gloeosporioides* *Fusarium* sp.

alone during June-July, *Fusarium* sp alone during November-December and both the species during March-April.

4.2 PURIFICATION

Cultures of *C. gloeosporioides*, *F. solani*, *F. moniliformae*, *F. oxysporum*, *E. rostratum*, *C. sacchari*, *G. roseum*, *Scytalidium* sp. and *Curvularia* sp. were purified by single spore method and maintained on PDA slants for further studies.

4.3 PATHOGENICITY STUDIES

Altogether seven different fungal species were found to be pathogenic in causing LRD on healthy spindle leaflets of coconut in different regions of the study (Table 5 and 6).

The average time taken for initiation of symptoms by the different pathogens varied from three to seven days when inoculated by pin-pricking the leaflet *Scytalidium* sp. took the least time of three days for the expression of symptom while *Fusarium solani* took a maximum time of seven days. The average time taken for initiation of symptom by *C. gloeosporioides*, *E. rostratum*, *G. roseum*, *C. sacchari*, and *Curvularia* sp. ranged from 4 to 6 days.

Study on the initiation of symptoms on uninjured leaflets (without pin-prick) indicated that all pathogens developed symptoms on uninjured leaflets except *Curvularia* sp. The average time taken for the development of initial symptom varied from a minimum of 7 days (*C. gloeosporioides* and *Scytalidium* sp.) to maximum of 10 days (*Cephalosporium sacchari*.).

Study on the progression of lesion size (Table 6) revealed that the maximum lesion size of 9.5 x 2.0 cm (10 DAI) and 14.5 x 3.5 cm (15 DAI) was produced by *Scytalidium* sp. followed by *E. rostratum* (6.8 x 2.0 cm and 12.7 x 3.5 cm) and *G. roseum* (5.0 x 2.0 cm and 10.5 x 3.5 cm). The minimum lesion size of 3.0 x 2.0 cm (10 DAI) and 8.5 x 3.3 cm (15 DAI) was noticed in leaflets inoculated with *Curvularia*

Table 5. Time taken for symptom expression by the different pathogens

Pathogen	On injured leaf lets, days	On uninjured leaf lets, days
Colletotrichum gloeosporioides	4	7
Fusarium solani	7	9
Exserohilum rostratum	4	8
Gliocladium roseum	5	8
Cephalosporium sacchari	5	10
Curvularia sp.	6	No infection
Scytalidium sp.	3	7

Table 6. Development of lesion on coconut leaflets after inoculation with the different pathogens

Pathogens	Lesion size			
	10 DAI		15 DAI	
	Length, cm	Breadth, cm	Length, cm	Breadth, cm
Colletotrichum gloeosporioides	4.00	1.50	9.50	3.50
Fusarium solani	3.40	1.50	7.60	3.40
Exserohilum rostratum	6.80	2.00	12.70	3.50
Gliocladium roseum	5.00	2.00	10.50	3.50
Cephalosporium sacchari	4.50	1.70	10.50	3.40
Curvularia sp.	3.00	2.00	8.50	3.30
Scytalidium sp.	9.50	2.00	14.50	3.50

sp. All the seven fungi were reisolated and the characters studied, thus proved Koch's postulates.

4.4 SYMPTOMATOLOGY

4.4.1 Based on Natural Infection

The initial symptom of LRD of coconut usually appeared at the distal portions of distal leaflets of the unopened spindle. It developed as tiny water-soaked reddish brown to dark brown spots/lesions on laminar area, tips or margins of leaflets, enlarged in size and coalesced freely leading to extensive rotting. The rotting gradually advanced into the interior of the spindle and tan coloured mould growth could be noticed on the surface of the affected areas. Many insects like ants, earwigs, flies were attracted towards the rotten portions of the spindle and emanated a fowl smell. Often the tips of the rotten leaflets of the spindle were cemented together while the base of the leaflets gradually unfurled. Later rotten portions dried, turned black and blown off by wind protruding out blackened and shrivelled ends of mid vein of the leaflets As these infected leaves matured, the leaflets at the basal portion normally unfurled without any damage while those at the distal half are almost completely rotten and broken off producing the typical fan leaf like symptom. In severe instances of infection, all the successively emerging spindles were progressively infected by the enormous inoculum of the pathogen present in the crown. As a result all the leaves of the crown showed fan leaf like symptom in palms at advanced stage of infection. When very acute rotting of the spindle occurs, the infected portions appear whip like exhibiting severe distortions (Plate 1).

Rarely pathogen of leaf rot infect mature leaflets also particularly on palms which exhibited mid-whorl yellowing symptom. Under such condition reddish brown lesions developed on the margins and laminar area which enlarged and coalesced together resulting in extensive blighting of the leaf.

(A) Leaf rot affected crown of coconut palm

(B) Spindle leaf showing leaf rot symptom

(C) Leaflet of leaf rot affected spindle

Plate 1. Leaf rot symptoms of coconut palm

4.4.2 Based on Artificial Inoculation

On artificial inoculation on detached leaflets the symptom appeared as tiny water soaked light brown lesions. Later these lesions enlarged and spread both length and breadth wise and covered large area and the lesion colour gradually changed into dark brown. Similar type of symptoms were produced by all the pathogens except for the size and time taken for symptom development (Plate 2).

4.5 VIRULENCE OF DIFFERENT ISOLATES OF PATHOGENS

4.5.1 Isolates of *Fusarium* sp.

Altogether 12 isolates of *Fusarium* spp. were obtained from infected leaflets of spindle of coconut collected from different regions (Table 1 to 4). The comparative virulence of these isolates in causing leaf rot infection was assessed by inoculating the isolates on healthy leaflets of spindle and measuring both the time taken for symptom expression and the progressive lesion size. The data are presented in Tables 7 and 8. It indicated that F_1 and F_{11} isolates obtained from Kidangoor and Konni produced the symptom both on injured leaflets (6 days) and uninjured leaflets (9 days) with the minimum time. Maximum time for the development of symptom on injured leaflets was noticed with F_6, F_7, F_9 and F_{12} isolates (9 days) while isolates F_7 and F_9 took 12 days for symptom development on uninjured leaflets.

Data on the lesion size (Table 8) indicated that there was variation in the size of lesion noticed among different isolates. Maximum lesion size of 3.7 x 1.5 cm (10 DAI) by F_1 isolate followed by F_{11} (3.5 x 2.0 cm) while F_{12} produced the smallest lesion (1.5 x 1 cm). All the other isolates produced lesions having an intermediate size. Similar trend was noticed at 15 DAI (9.4 x 2.5 cm by F_1 isolate and 8.5 x 2.5 cm by F_{11} isolate as against 6.8 x 1.9 cm by F_{12} isolate).

Based on size of the lesions and time taken for initiation of symptom, isolates were classified into three categories.

(A) *Colletotrichum gloeosporioides*

(B) *Exserohilum rostratum*

(C) *Fusarium moniliformae*

(D) *Scytalidium* sp.

(E) *Cephalosporium sacchari*

(F) *Curvularia* sp.

(G) *Gliocladium roseum*

Plate 2. Leaf rot symptoms produced on leaflet by artificial inoculation of different pathogens

Table 7. Time taken for symptom development by *Fusarium* spp.

Isolates	On injured leaflets, days	On uninjured leaflets, days
F_1 (Kidangoor)	6	9
F_2 (Kumarakom)	6	11
F_3 (Changanacherry)	8	10
F_4 (Kayamkulam)	7	10
F_5 (Aroor)	7	10
F_6 (Kottarakkara)	9	11
F_7 (Kollam)	9	12
F_8 (Kilimanoor)	8	11
F_9 (Kazhakuttam)	9	12
F_{10} (Kudapanakunnu)	8	11
F_{11} (Konni)	6	9
F_{12} (Pathanamthitta)	9	11

Table 8. Development of lesion produced by different isolates of *Fusarium* spp. 10 and 15 days after inoculation

Isolates	Lesion size			
	10 DAI		15 DAI	
	Length cm	Breadth cm	Length cm	Breadth cm
F_1	3.70	1.50	9.40	2.50
F_2	3.40	1.40	7.60	2.40
F_3	2.80	1.50	7.40	2.80
F_4	3.40	1.50	8.50	2.50
F_5	2.50	1.30	8.50	2.50
F_6	2.10	1.60	8.50	2.50
F_7	3.00	1.40	8.40	2.40
F_8	2.50	1.10	8.00	2.40
F_9	2.10	1.00	7.10	2.00
F_{10}	2.40	1.20	8.50	2.70
F_{11}	3.50	2.00	8.50	2.50
F_{12}	1.50	1.00	6.80	1.90

1. Highly virulent (lesion size > 3.4 x 1.4 cm and time taken for symptom expression < 7 days) – F_1, F_2, F_4 and F_{11}.
2. Virulent (lesion length ranged between 2.4 - 3.4 x 1.0 cm and time taken for symptom expression 7-8 days)- F_3, F_5, F_8, F_{10}
3. Mildly virulent (All the rest of isolates) F_6, F_7, F_9, F_{12}

Isolate F_3 (*F. solani*) was chosen as the test pathogen of *Fusarium* sp. for further studies.

4.5.2 Isolates of *Colletotrichum gloeosporioides*

The data on the time taken for symptom expression indicated that isolates of *C. gloeosporioides* obtained from Kidangoor and Pathanamthitta (C_1 and C_8) produced the symptoms after four days of inoculation (shortest period) while the rest of the isolates took 5-6 days (Table 9). The time taken for symptom expression on uninjured leaflet varied from 8-9 days. Isolates of C_1, C_3 and C_8 produced symptom after 8 days of inoculation while the rest took nine days.

The virulence of the isolates was further tested by measuring the lesion size 10 and 15 days after inoculation and the data indicated that maximum lesion size (length) after 10 DAI was produced by isolate C_1 (4.5 cm) followed by C_4 (4.2 cm), while the lesion size was minimum with C_5 isolate (3 cm) (Table 10). A similar trend was noticed 15 DAI also (maximum lesion size of 11.5 by C_1 isolate followed C_4 (11 cm). Based on the time taken for symptom expression and lesion size *C. gloeosporioides* isolates were categorized into three *viz.*, highly virulent, virulent and mildly virulent.

Highly virulent isolates - lesion size ≥ 4 cm (10 DAI) and ≤ 5 days for symptom expression – C_1, C_2, C_4 and C_8.

Virulent isolates - lesion size ranged from 3.5 – 3.95 cm (10 DAI) and symptom expression from 5-6 days – C_3, C_6, C_7.

Mildly virulent isolates - lesion size ≤ 3.5 cm (10 DAI) and 6 days or more for symptom expressions - C_5.

Table 9. Time taken for symptom development by *Colletotrichum gloeosporioides*

Isolate	On injured leaf lets, days	On uninjured leaf lets, days
C1 (Kidangoor)	4	8
C2 (Kumarakom)	5	9
C3 (Kayankulam)	5	8
C4 (Aroor)	5	9
C5 (Kottarakkara)	6	9
C6 (Kilimanoor)	5	9
C7 (Konni)	6	9
C8 (Pathanamthitta)	4	8

Table 10. Development of lesions produced by different isolates of *Colletotrichum gloeosporioides*, 10 and 15 days after inoculation

Isolates	Lesion size			
	10 DAI		15 DAI	
	Length, cm	Breadth, cm	Length, cm	Breadth, cm
C_1	4.50	1.10	11.50	2.50
C_2	4.00	2.00	10.00	2.00
C_3	3.50	1.40	10.00	2.50
C_4	4.20	1.70	11.00	2.50
C_5	3.00	1.20	9.50	2.00
C_6	3.80	1.50	10.50	2.00
C_7	3.50	1.40	9.80	2.50
C_8	4.00	1.50	10.50	1.90

Isolate C_8 was chosen as the test pathogen of *C. gloeosporioides* for further study.

4.5.3 Isolates of *Scytalidium* sp.

The two isolates of *Scytalidium* sp. also varied in their pathogenicity. Isolate S_1 (Moncompu) was found to be more virulent as it took only 3 days for symptom expression and produced larger size (9.5 x 3 cm) lesions at 10 DAI, while isolate S_2 (Changanacherry) took 5 days for lesion development and the size of lesions was comparatively less (7.2 x 2 cm).

4.6 CHARACTERISATION OF LRD PATHOGENS

The LRD associated pathogens were isolated, purified, and identified based on morphological and cultural characteristics.

4.6.1 *Colletotrichum gloesoporioides* (Penzig) Penzig and Sacc.

On Czapeck's medium, colour of the colony was found varied from light grey to dark grey or dirty white depending upon the isolates (Plate 3A). The acervuli were varied from spherical to saucer shaped. They were setose or non-setose. Conidia were mostly cylindrical with rounded ends, aseptate and the size were varied from 10.23 -14.03 x 3.3 - 4.01 µm. They were hyaline individually but orange coloured in mass (Plate 4A).

4.6.2 *Fusarium* spp.

Fusarium spp. isolated from leaf rot affected coconut palms were identified as *F. solani* Martius (Sacc.), *F. moniliformae* Sheldon and *F. oxysporum* Schl. Ex Fries (Plate 3G, 4B and 4C). Characters of these species are given in Table 11. Based on these characteristics isolates F_1, F_2, F_4, F_5 and F8 were identified as *F. solani*, isolates F_3, F_6, F_{10}, F_{11}, F_{12} were identified as *F. moniliformae* and isolates F_7 and F_9 were identified as *F. oxysporum*.

Table 11. Cultural and morphological characters of *Fusarium* spp.

Characters	*Fusarium solani*	*Fusarium moniliformae*	*Fusarium oxysporum*
Colony diameter after 10 days	9 cm	9 cm	9 cm
Aerial mycellium	Present	Present	Present
Average length of macroconidia	25.50μ	20.17 μ	18.30 μ
Average width of macroconidia	3.3 μ	3.3 μ	3.3 μ
Microconidia	Present, rod shaped	Present, abundant in chains, clavate shaped	Present, rod shaped
Chalmydospores	Present, chained	Absent	Present
Conidial mass on PSA	Cream coloured	Pinkish, dark blue later complete blue	Light pink
Reverse on PSA	Cream	Concentric, radiating and dark blue zones	Peach
Apical cell shape	Conical	Conical	Conical
Apical cell length	Less than penultimate cell	Equal to penultimate cell	Less than penultimate cell
Location	Aroor, Kidangoor, kumarakom, Kayamkulam, Kilimanoor	Changanacherry, Kudapanakunnu, Konni, Pathanamthitta	Kollam, Kazhakootam

4.6.3 *Exserohilum rostratum* (Drechsler) Leonard and Suggs.

On PDA medium, the colony was black in colour and appeared as felty growth (Plate 3B). It took six days to complete the growth in 9 cm petridish. Conidiophores were single, brown and measured 26.4 – 33.0 x 6.6 µm size. The conidia were straight, cylindrical, 4 – 9 septate, end cell cut off by a thick dark septa and measured. 33 - 69.3 x 13.2 - 19.8 µm (Plate 4D).

4.6.4 *Cephalosporium sacchari* Butler

On PDA medium colony was white initially and later turned light pinkish in colour (Plate 3E). Deep pink colour formation was noticed on the media. The fungus took ten days for attaining complete growth in the petridish (9 cm). Hyaline, oblong and one celled conidia (6.6 9.9 x 3.3 µm) were produced from the tip of the ultimate branches and many aggregated to from 'heads' which were easily shed (Plate 4E).

4.6.5 *Gliocladium roseum* Bainier

On PDA medium, the colour of the colony was salmon above while light yellow below. Texture of the colony was granular (Plate 3D). Within five days of growth the fungus completely covered the 9 cm petridish. Conidiophores were with divergent verticillium like branches measured 9.9 - 16.5 µm size, phialides in whorls of three to four and 9.9 x 3.3 µm in size. Conidia were in columns with slightly asymmetrical apex and obliquely round base (Plate 4F).

4.6.6 *Scytalidium* sp.

On PDA medium the colonies were effuse and black in colour (Plate 3C). Some of the hyphae were smooth, narrow, cylindrical and colourless while others were thick, pale to mid-brown with occasional darker swollen cells and often with thick dark septa. The hyphae often lie parallel to one another and closely adpressed forming bundles. Setae and hyphopodia absent. Conidiophores micronematous, mononematous

or sometimes synnematous, branched or unbranched, straight or flexuous, colourless or brown and smooth. Conidiogenous cells were fragmented and formed arthroconidia. Arthroconidia were integrated, intercalary, determinate and cylindrical, dolliform or ellipsoidal. Conidia catenate, separating, simple smooth and one celled. Two kinds of conidia were noticed, *viz.*, colourless thin walled cylindrical with 9.9 – 13.2 x 3.3 – 6.6 µm size and broader, dark brown, thick walled, oblong with 9.9 x 5 – 6.6 µm size. The fungus took three days to cover completely 9 cm petridish (Plate 4G).

4.6.7 *Curvularia* sp.

Colonies were black velvety, conidia four celled and measured 19.8 – 29.01 x 9.9 – 13.2 µm, middle cell large, broad and dark. It took seven days for the completion of growth in 9 cm petridish (Plate 3F).

4.7 INTERACTION OF PATHOGENS ASSOCIATED WITH LEAF ROT

The results indicated that the growth of *C. gloeosporioides* along with *F. solani*, *E. rostratum* and *Scytalidium* sp. freely merged with each other without development of inhibition zone and without affecting their sporulation (Table 12) while *C. gloeosporiodes* mildly inhibited the growth of *Curvularia* sp., *Cephalosporium sacchari* and *G. roseum*. When *Fusarium* sp. was allowed to interact with *Scytalidium* and *Curvularia* sp. free merging of colonies was found without growth inhibition. When it was grown along with *C. sacchari*, *G. roseum* and *E. rostratum* it showed moderate overgrowth and slight growth inhibition of these fungi

When *E. rostratum* was grown with *G. roseum* and *C. sacchari* growth of both these fungi ceased at the point of contact while growth suppression and mild inhibition of *Curvularia* sp. and *Scytalidium* sp. were observed in the presence of *E. rostratum*.

(A) *C. gloeosporioides*

(B) *E. rostratum*

(C) *Scytalidium* sp.

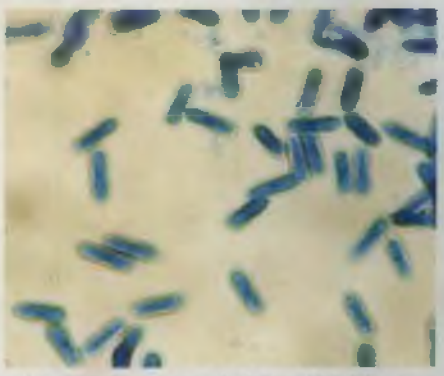
(D) *G.roseum*

(E) *Cephalosporium sacchari*

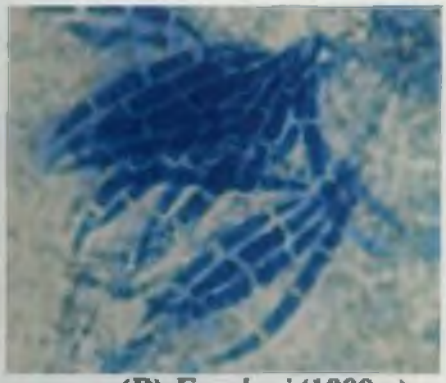
(F) *Curvularia* sp

(G) *Fusarium* spp.

Plate. 3. Growth of different pathogens on culture media



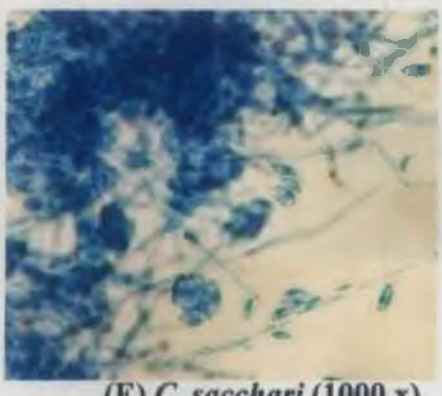
(A) *C.gloeosporioides* (1000 x)



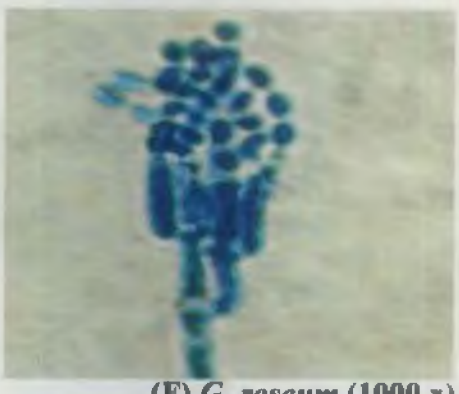
(B) *F. solani* (1000 x)

(C) *F.moniliformae* (1000 x)

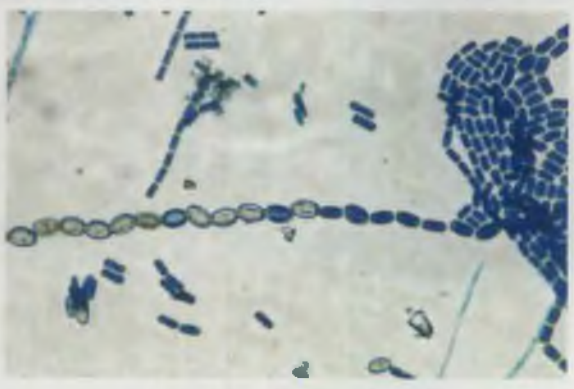
(D) *E. rostratum* (200 x)



(E) *C. sacchari* (1000 x)



(F) *G. roseum* (1000 x)



(G) *Scytalidium sp.* (200 x)

Plate 4. Photomicrographs of conidia of different pathogens

Table 12. Nature of interactions shown by different pathogens *in vitro*

Sl. No.	Pathogen combinations	Nature of interactions
1	*Colletotrichum gloeosporioides x Fusarium solani*	No inhibition, free merger of growth by both fungi, normal sporulation
2	*C. gloeosporioides x Exserohilum rostratum*	No inhibition, slight overgrowth by *E. rostratum*, sporulation normal in both fungi
3	*C. gloeosporioides x Cephalosporium sacchari.*	Slight inhibition of growth and sporulation of *C. sacchari* by *C. gloeosporioides*, growth ceases at the point of contact
4	*C. gloesporioides x Curvularia sp.*	Perfect stoppage of growth at point of contact by both fungi slight inhibition of growth of *Curvularia* sp. by *C. gloeosporioides* noticed
5	*C. gleosporioides x Scytalidium sp.*	*C. gloeosporioides* overgrew *Scytalidium* sp. mild inhibition of *Scytalidium* sp. by C. *gloeosporioides*
6	*C. goleosporioides x Gliocladium x roseum*	Inhibition of growth of *G. roseum* at the point of contact of *C. gloeosporioides* mild inhibition zone developed
7	*F. solani x Exserchilum rostratum*	Mild inhibition of *Exserohilum rostratum* by *F. solani*

Table continued

8	*F. solani x Curvularia sp.*	No inhibition, both fungi freely merges each other, normal sporulation
9	*F. solani. x Cephalosporium sacchari.*	Moderate overgrowth of *F. solani* sp. on *Cephalosporium sacchari*, growth and sporulation of *Cephalosporium sacchari* were mildly suppressed
10	*F. solani x G. roseum*	*F. solani* overgrows *G. roseum.*
11	*Fusarium sp x Scytalidium sp*	Both fungi overgrow each other without any inhibition over growth is more intense by *F. solani*, partial inhibition of *Scytalidium* sp. by *F. solani.*
12	*E. rostratum x C. sacchari.*	Growth gradually ceases at point of contact. No suppression of growth and sporulation
13	*E. rostratum x Curvularia sp.*	Mild inhibition of *Curvularia* sp. by *E. rostratum*
14	*E. rostratum x Scytalidium sp.*	Intense overgrowth of *E. rostratum* along with slight inhibition of growth of *Scytalidium* sp.

Table continued

15	*E. rostratum x G. roseum*	No inhibition, mycelial growth and sporulation of both cultures normal. Growth ceases at the point of contact
16	*Curvularia sp. x C. sacchari*	Growth ceases at point of contact, no inhibition of growth and sporulation
17	*Curvularia sp. x Scytalidium sp.*	*Scytalidium* overgrows over *Curvularia* sp. suppression of growth of *Curvularia* sp.- sporulation normal
18	*Curvularia sp. x G. roseum*	No suppression of growth of both fungi
19	*C. sacchari x Scytalidium sp.*	*Scytalidium* sp. overgrows *C. sacchari* suppression of growth of *C. sacchari* sporulation normal
20	*C. sacchari. x G. roseum*	Slight overgrowth by *C. saachari*
21	*G. roseum x Scytalidium sp.*	Moderate inhibition of growth and sporulation of *G. roseum* by *Scytalidium* sp. initially, slight overgrowth and sporulation by *G. roseum* at later stage

When *Curvularia* sp. was allowed to grow with *G. roseum* the colonies freely merged without inhibition of growth or sporulation. The growth stopped at the point of contact when it was challenged with *C. sacchari* while *Scytalidium* sp. had overgrown *Curvularia* sp. with growth suppression.

Scytalidium sp. had overgrown *C. sacchari* with growth suppression and inhibition while moderate inhibition of growth and sporulation was observed in dual cultures with *Scytalidium* sp. and *G. roseum*. *C. sacchari* also inhibited the growth of *G. roseum*.

4.8 EFFECT OF COMBINED INOCULATION OF LRD PATHOGENS ON DETACHED SPINDLE LEAFLETS

The time taken for symptom expression by different combinations of fungal pathogens of LRD varied from 3 – 6 days (Table 13). When *E. rostratum* x *Scytalidium* sp., *E. rostratum* x *C. sacchari*, *E. rostratum* x *G. roseum*, *E. rostratum* x *Curvularia* sp., *Scytalidium* sp. x *C. sacchari*, *Scytalidium* sp. x *G. roseum*, *Scytalidium* sp. x *Curvularia* sp. *F. solani* x *E. rostratum* and *F. solani*. x. *Scytalidium* sp. were inoculated simultaneously symptoms were expressed within three days. The combination of *F. solani* and *Curvularia* sp. took the maximum period of six days.

Lesion size ranged between 4.5 x 2.5 cm to 15 x 2.5 cm (10 DAI) (Table 13). Maximum lesion size of 15 x 2.5 cm was shown by *E. rostratum* x *Scytalidium* sp. followed by *Scytalidium* sp. x *C. sacchari*, (14.3 x 2.5 cm), *E. rostratum* x *C. sacchari* (14 x 2.6 cm). The minimum lesion size (4.5 x 2.5 cm) was noticed by *G. roseum* x *Curvularia* sp. and *C. gloeosporioides* x *Curvularia* sp. combinations

Table 13. Details of symptom expressed by combined inoculation of pathogens

Sl. No.	Pathogen combination	Time taken for initiation of symptom days	Lesion size, cm
1.	*Colletotrichum gloeosporioides* x *Fusarium solani*	4	7.00x 2.40
2.	*C. gloeosporioides* x *Exserohilum. rostratum*	5	6.00 x 2.50
3.	*C. gloeosporioides* x *Scytalidium* sp.	4	9.50 x 2.50
4.	*C. gloeosporioides* x *Cephalosporium sacchari*	5	4.80 x 2.50
5.	*C. gloeosporioides* x *Gliocladium roseum*	5	4.50x 2.50
6.	*C. gloeosporioides* x *Curvularia* sp.	5	5.70x 2.30
7.	*F. solani x E. rostratum*	3	8.00 x 2.50
8.	*F. solani x Scytalidium sp.*	3	8.00 x 2.40
9.	*F. solani x C. sacchari*	5	6.00 x 2.50
10	*F. solani x G. roseum*	5	8.50 x 2.50
11.	*F. solani x Curvularia* sp.	6	3.50 x 2.10
12.	*E. rostratum x Scytalidium*	3	15.00 x 2.50
13.	*E. rostratum x C. sacchari*	3	14.00 x 2.60
14.	*E. rostratum x G. roseum*	3	10.30 x 2.50
15.	*E. rostratum x Curvularia* sp.	3	10.50 x 2.50
16.	*Scytalidium sp. x C. sacchari*	3	14.30 x 2.50
17.	*Scytalidium sp. x G. roseum*	3	7.50 x 2.60
18.	*Scytalidium sp. x Curvularia* sp.	3	6.50 x 2.50
19.	*C. sacchari x G. roseum*	4	12.30 x 2.40
20.	*C. sacchari x Curvularia* sp.	5	5.50 x 2.50
21.	*G. roseum x Curvularia* sp.	5	4.50 x 2.50

4.9 *IN VITRO* SCREENING OF ANTAGONISTIC MICROORGANISMS AGAINST MAJOR PATHOGENS OF LRD

4.9.1 Screening of Fungal Antagonists

The results on the evaluation of different cultures of *Trichoderma* sp. for their antagonistic potential against *C. gloeosporioides*, *F. solani* and *E. rostratum* are presented in Table 14.

4.9.1.1 C. gloeosporioides

The inhibitory ability of the seven cultures of Trichoderma ranged from 17.25 per cent (T_2) to 25.56 per cent (T_9). Culture T_9 (25.56 %), T_6 (25.56 %), T_{12} (24.17 %) and T_{10} (24.03 %) were on par in their antagonistic effect on *C. gloeosporioides*. The minimum antagonistic ability was noted with T_2 (17.25 %) (Plate 5A).

4.9.1.2 F. solani

Antagonistic ability of T_9 (31.99 %), T_6 (30.86 %), T_k (27.74 %) and T_{10} (23.91 %) against *F. solani* were on par and their effects were significantly higher than that of T_{11} (15.74 %) and T_2 (14.09 %) (Plate 5B).

4.9.1.3 E. rostratum

The antagonistic effect of Trichoderma culture on the growth of *E. rostratum* was significantly higher with T_9 (48.00 %) and T_6 (46.77 %) while the minimum antagonistic effect was shown by T_k (29.67 %) and T_{10} (30.70 %) (Plate C). Cultures T_{11} (40.77 %), T_2 (40.27 %) and T_{12} (39.00 %) also exerted significantly higher antagonistic property on E. rostratum as compared to T_k and T_{10}.

In all trials T_9 and T_6 showed consistently higher per cent inhibition on growth of pathogens. But overgrowing ability of T_9 was found to be more as it had completely overgrown different pathogens in the present study within nine days. Hence T_9 culture was selected for further study in the present investigation.

Table 14. *In vitro* evaluation of antagonistic potential of Trichoderma cultures against leaf rot pathogens

Sl. No.	*Trichoderma* culture	Per cent inhibition growth, %		
		Colletotrichum gloeosporioides	*Fusarium solani*	*Exserohilum rostratum*
1.	T_2	17.25 (4.15)	14.09 (3.75)	40.27 (39.37)
2.	T_6	25.56 (5.06)	30.86 (5.55)	46.77 (43.13)
3.	T_9	25.56 (5.06)	31.99 (5.57)	48.00 (43.83)
4.	T_{10}	24.03 (4.90)	23.91 (4.89)	30.70 (33.63)
5.	T_{11}	19.30 (4.39)	15.74 (3.97)	40.77 (39.66)
6.	T_{12}	24.17 (4.92)	23.05 (4.80)	39.00 (38.63)
7.	T_k	22.70 (4.75)	27.74 (5.27)	29.67 (32.98)
	CD	0.67	0.72	3.15
		Figures in parenthesis are transformed values($\sqrt{x}$)		Figures in parenthesis are transformed in angles

4.9.2 Screening of Bacterial Antagonists

The antagonistic potential of 29 isolates/culture of *P. fluorescens* was tested *in vitro* against the three major pathogens of LRD and the results of their effect on *C. gloeosporioides*, *F. solani* and *E. rostratum* are presented in the Table 15.

4.9.2.1 C. gloeosporioides

The data indicated that Ps1 culture of *P. fluorescens* exerted significantly higher antagonistic ability (74.86 %) on *C. gloeosporioides* (Plate 6A) compared to other cultures in KMB. The cultures P_{35} (0 %), P_{22} (0.35 %), P_{25} (1.50 %) and P_{33} (1.53 %) did not significantly inhibit the growth of *C. gloeosporioides*.

Similar trend was also noticed when *P. flourescens* tested in PDA.

4.9.2.2 F. solani.

The data on the interaction of *P. fluorescens* isolates with *F. solani* revealed that Ps_1 culture possessed significantly higher antagonistic potential against *F. solani.* (77.01 %) in KMB (Plate 6B). The inhibitory effect of culture P_{10} (0.59 %) and P_{20} (1.30 %) was the minimum.

A similar trend was noticed when the antagonism was tested in PDA.

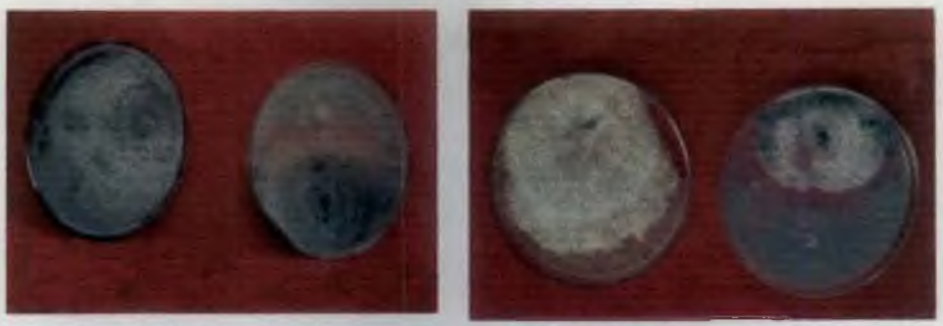
4.9.2.3 E. rostratum

The results of screening test with *E. rostratum* showed that Ps_1 culture exerted the significantly higher antagonistic effect (76.09 %) in KMB medium while the lowest effect was observed with P_{36} culture (2.39 %). Isolates P_{30}, P_{40} and P_{41} also showed more than 40 per cent inhibition on growth of *E. rostratum*. In PDA medium also Ps_1 showed maximum inhibition capacity against *E. rostratum* (75.73 %) and the minimum per cent inhibition was shown by P_{36} (3.05 %). Moderately

Table 15. *In vitro* evaluation of antagonistic potential of *Pseudomonas fluorescens* against core pathogens of leaf rot

Sl. No.	*Pseudomonas fluorescens* isolates	Per cent inhibition *of C. gloeosporioides*		Per cent inhibition of *Fusarium solani*		Per cent inhibition *of E. rostratum*	
		On KMB	On PDA	On KMB	On PDA	On KMB	On PDA
1.	P_1	26.42 (30.92)	17.73(24.90)	27.77 (31.79)	27.60(31.69)	20.52(26.93)	16.40(23.89)
2.	P_2	23.44 (28.94)	13.58(21.62)	26.18(30.76)	21.48(27.61)	18.94(25.79)	19.19(25.98)
3.	P_3	11.53 (19.83)	5.27(13.27)	12.85(21.00)	14.45(22.34)	11.25(19.59)	7.64(16.05)
4.	P_4	23.71 (29.13)	21.16(27.39)	41.34(40.00)	31.03(33.85)	22.03(27.98)	21.00(27.28)
5.	P_{13}	11.52 (19.83)	9.10(17.56)	0.59(4.40)	8.49(16.94)	8.31(16.75)	9.96(18.40)
6.	P_{14}	20.25 (26.73)	11.70(20.01)	13.85(21.84)	17.20(24.51)	14.85(22.66)	11.76(20.06)
7.	P_{15}	11.15 (19.50)	8.21(16.65)	35.68(36.66)	21.29(27.48)	11.61(19.91)	11.51(19.83)
8.	P_{16}	9.23 (17.68)	10.84(19.22)	7.36(15.74)	15.45(23.14)	8.37(16.81)	8.68(17.13)
9.	P_{17}	9.85 (18.29)	12.38(20.60)	8.57(17.02)	10.71(19.10)	13.34(21.41)	11.26(19.61)
10	P_{18}	9.37 (17.81)	6.87(15.19)	11.03(19.39)	10.17(18.60)	12.09(20.44)	12.27(20.50)
11	P_{19}	6.61 (14.89)	5.17(13.15)	9.30(17.75)	8.72(17.18)	8.31(16.75)	8.17(16.61)
12	P_{20}	5.08 (13.03)	3.19(10.29)	1.30(6.55)	3.38(10.59)	4.47(12.21)	6.10(14.30)
13	P_{21}	11.96 (20.22)	9.10(17.56)	10.06(18.48)	9.20(17.66)	12.52(20.71)	11.26(19.61)
14	P_{22}	0.35 (3.41)	2.29(8.71)	10.44(18.84)	12.16(20.41)	4.76(12.59)	4.28(11.93)
15	P_{23}	16.18 (23.71)	9.81(18.26)	21.44(27.57)	15.92(23.51)	16.29(23.80)	15.37(23.08)
16	P_{24}	30.99 (33.82)	29.6(32.96)	28.19(32.06)	22.66(28.42)	26.29(30.83)	22.31(28.19)
17	P_{25}	1.50(7.04)	2.60(9.29)	24.69(29.78)	19.18(25.94)	11.35(19.68)	9.29(17.74)
18	P_{26}	40.63(39.59)	33.85(35.58)	37.00(37.45)	31.79(34.32)	29.74(33.03)	22.30(28.18)
19	P_{27}	5.38(13.40)	9.47(17.92)	9.21(17.66)	8.73(17.18)	12.97(21.10)	11.01(19.38)
20	P_{28}	6.08(14.27)	4.96(12.86)	7.04(15.38)	6.96(15.30)	8.17(16.61)	6.62(14.91)
21	P_{30}	26.06(30.69)	19.70(26.35)	44.99(42.11)	44.51(41.85)	41.74(40.23)	41.26(39.97)
22	P_{32}	40.73(39.64)	34.10(35.73)	28.31(32.13)	22.23(28.13)	25.58(30.37)	21.79(27.83)
23	P_{33}	1.53(7.10)	1.80(7.71)	8.99(17.44)	8.11(16.54)	11.23(19.57)	11.26(19.61)
24	P_{34}	23.68(29.10)	18.93(25.79)	9.62(18.06)	11.90(20.18)	11.80(20.09)	10.94(19.32)
25	P_{35}	0(0)	1.80(7.71)	4.20(11.82)	6.62(14.91)	6.75(15.05)	6.90(15.23)
26	P_{36}	8.62(17.07)	7.92(16.35)	1.41(6.81)	4.31(11.98)	2.39(8.89)	3.05(10.06)
27	P_{40}	46.82(43.16)	40.24(39.37)	41.02(39.80)	40.06(39.27)	41.70(40.20)	38.97(38.63)
28	P_{41}	42.65(40.75)	40.26(39.38)	48.80(44.29)	40.73(39.66)	52.86(46.62)	48.19(43.96)
29	P_{51}	74.86(59.89)	69.60(56.54)	77.01(61.32)	80.35(63.69)	76.09(60.70)	75.73(60.49)
CD		3.64	1.91	2.300	2.47	1.36	1.47

Figures in parenthesis are values after angular transformation



(A). *C.gloeosporioides* x T_9 (B). *F. solani* x T_9

(C) *E. halodes* x T_9

Plate 5. Inhibition of LRD pathogens causing leaf rot by *Trichoderma* sp.

(A) .*C.gloeosporioides* x Ps_1

(B). *F. solani* x Ps_1

Plate 6. Inhibition of LRD pathogens causing leaf rot by *P.fluorescens*

higher percentage of inhibition was also exerted by P_{41} (48.19 %) and P_{30} (41.26 %).

4.10 SCREENING OF ANTAGONISTS BY LEAFLET BIT ASSAY

4.10. 1 Trichoderma Cultures

Irrespective of the cultures it took four, six and four days respectively in *C. gloeosporioides*, *F. solani* and *E. rostratum* inoculated leaflet bits for symptom development (Table 16). Variation was noticed on the length of lesion produced by different pathogens in the presence of different Trichoderma cultures. The lesion size produced by *C. gloeosporioides* was found to be minimum (3.8 cm) in presence of T_9 culture while it was 4.5 cm in presence of T_6 and T_{12} cultures. Similarly the lesion size produced by *F. solani*. (3.2 cm) and *E. rostratum* (4.0 cm) were also minimum in presence of T_9 culture as against 3.9 cm and 4.5 cm in presence of T_6 and 4.5 and 6.0 cm in presence of T_{12} isolate.

4.10.2 *Pseudomonas fluorescens*

The data indicated that the development of symptoms produced by *F. solani*. *C. gloeosporioides*. *E. rostratum* were delayed in presence of different *P. fluorescens* isolates. Maximum time for symptom development was taken by *F. solani* (10 days) followed by *E. rostratum* (8 days) and *C. gloeosporioides* (6 days) in the presence of Ps_1 culture as against six, four and four days in the respective controls (Table 17). Data on the lesion length also indicated that maximum reduction in lesion length occurred in the presence of Ps_1 culture. Among the pathogens, minimum lesion length was produced by *F. solani* (1.5 cm) in the presence of Ps1 culture followed by *E. rostratum* (2.5 cm) and *C. gloeosporioides* (3.1 cm) as against 4.7 cm, 6.2 cm and 5.4 cm each in their respective controls. Lesion length produced by these pathogens in the presence of P_{40} and P_{41} isolates were also reduced as compared with the control.

Table 16. Evaluation of antagonistic ability of Trichoderma cultures by leaflet bit assay

Pathogen	Time taken for symptom expression, days				Length of lesion after 10, days			
	T_9	T_6	T_{12}	Control	T_9	T_6	T_{12}	Control
Colletotrichum gloeosporioides	4.00	4.00	4.00	4.00	3.80	4.50	4.50	5.80
Fusarium solani	6.00	6.00	6.00	6.00	3.20	3.90	4.50	5.00
Exserohilum rostratum	4.00	4.00	4.00	4.00	4.00	4.50	6.00	6.50

Table 17. Evaluation of antagonistic ability of *P. fluorescens* by leaflet bit assay

Pathogen	Time taken for symptom expression, days				Length of lesion after 10 DAI, cm			
	Ps_1	P_{40}	P_{41}	Control	Ps_1	P_{40}	P_{41}	Control
Colletotrichum gloeosporioides	6.00	5.00	5.00	4.00	3.10	4.50	4.00	5.40
Fusarium sp.	10.00	8.00	8.00	6.00	1.50	3.40	3.60	4.70
Exserohilum rostratum	8.00	6.00	6.00	4.00	2.50	4.30	4.50	6.20

Based on the *in vitro* interaction and leaflet bit assay of various isolates / cultures of *Trichoderma* sp. and *P. fluorescens* tested against the three major pathogens of LRD, T_9 culture of Trichoderma and Ps_1 culture of *P. fluorescens* were selected for further evaluation in the present study.

4.11 INTERACTION BETWEEN SELECTED FLUORESCENT PSEUDOMONAD AND TRICHODERMA

P. fluorescens (Ps_1) inhibited the mycelial growth (T_9). A clear zone of inhibition was noticed in dual culture with 38.5 per cent inhibition of mycelial growth of T_9 by Ps_1 (Plate 7).

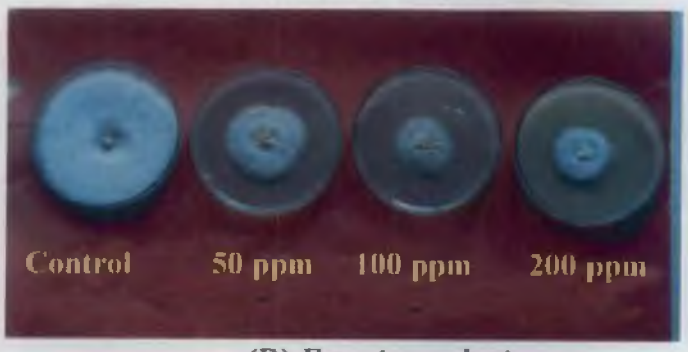
4.12 BIOASSAY OF COMMONLY USED FUNGICIDES AGAINST MAJOR PATHOGENS OF LRD

All the fungicides tried *viz.*, copper oxychloride, mancozeb, carbendazim, hexaconazole and propiconazole inhibited the growth of all the pathogens at all concentrations tested (Table 18). All concentrations of carbendazim (Plate 10A), hexaconazole (Plate 12A) and propiconazole (Plate 11A) (25, 50 and 100 ppm) resulted in 100 per cent inhibition on the *C. gloeosporioides* growth while only highest concentration of Copper oxychloride (200 ppm) and mancozeb (200 ppm) produced complete inhibition of its growth (Plate 8A and 9A). A similar trend was exhibited by these fungicides on the growth of *F. solani*. The fungicides carbendazim, hexaconazole (Plate 12B) and propiconazole resulted in complete inhibition of fungal growth at all the concentrations tested. Copper oxychloride and mancozeb gave higher inhibition of fungal growth (87.61 and 64.48 %) at higher concentration (200 ppm). However, it was noted that the minimum effect on growth suppression was by mancozeb (Plate 8B).

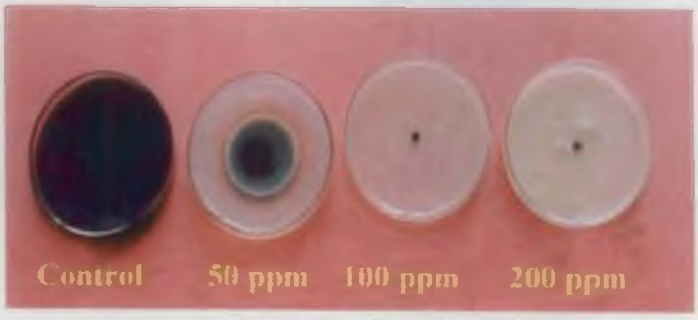
All concentrations of hexaconazole and propiconazole resulted in complete inhibition of growth of *E. rostratum* (Plate 11B and 12C). Mancozeb also produced complete inhibition at (100 and 200 ppm)

Plate. 7. Inhibition of *Trichoderma* sp. by *P.fluorescens*

(A) *Colletotrichum gloeosporioides*

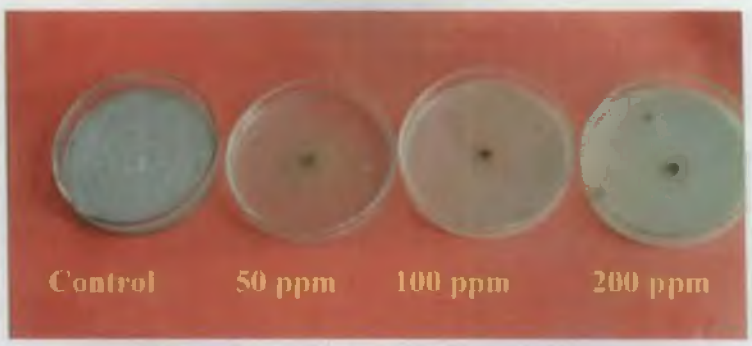


(B) *Fusarium solani*

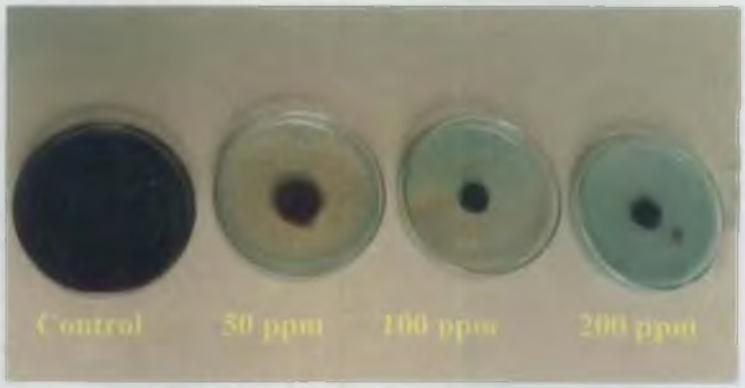


(C) *Exserohilum rostratum*

Plate. 8. Effect of mancozeb on growth of LRD pathogens

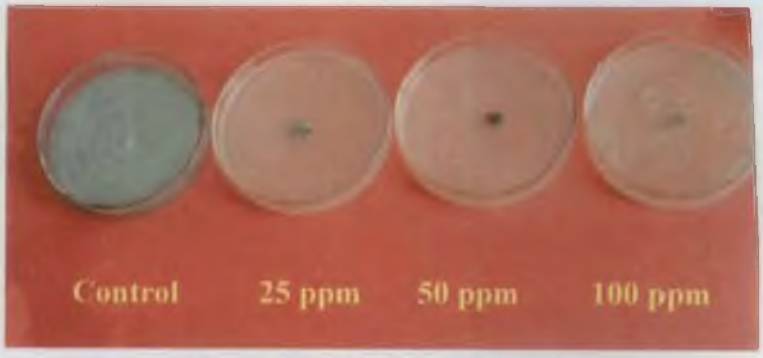


(A) *Colletotrichum gloeosporioides*

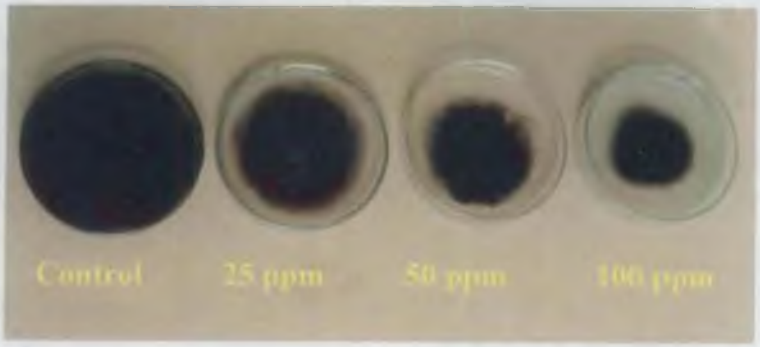


(B) *Exserohilum rostratum*

Plate 9. Effect of copper oxychloride on growth of LRD pathogens

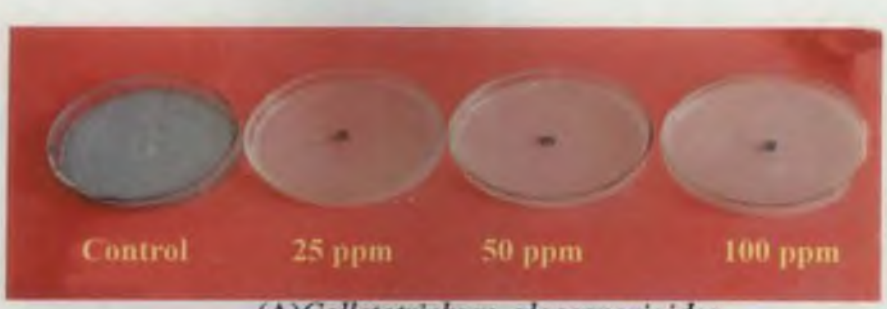


(A) *Colletotrichum gloeosporioides*

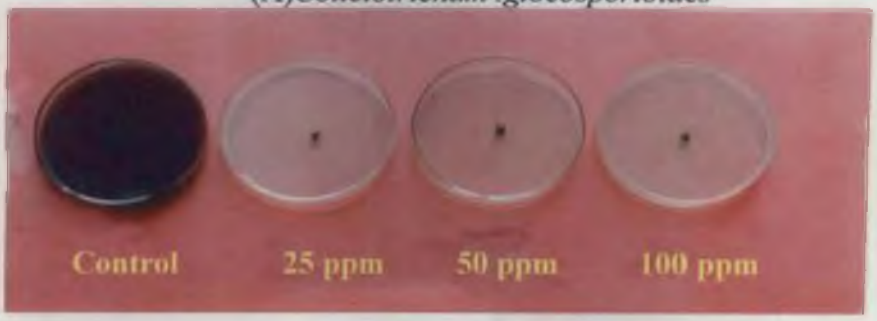


(B) *Exserohilum rostratum*

Plate 10. Effect of carbendazim on growth of LRD pathogens

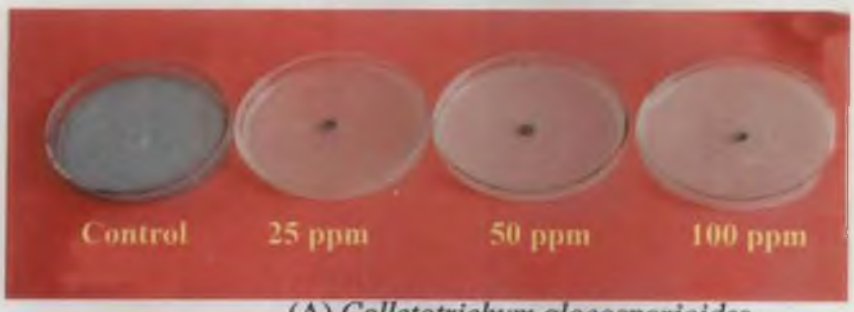


(A)*Colletotrichum .gloeosporioides*



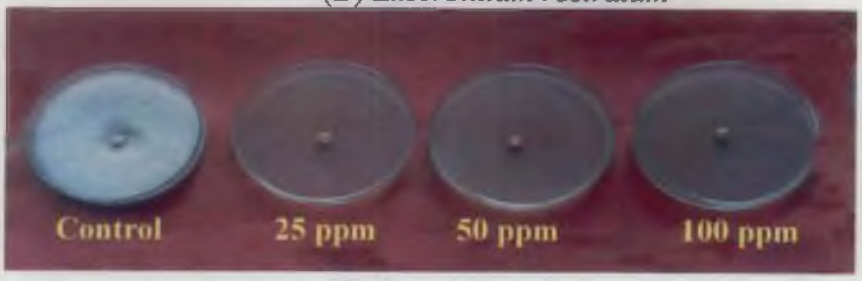
(B) *Exserohilum rostratum*

Plate 11. Effect of propiconazole on growth of LRD pathogens



(A) *Colletotrichum gloeosporioides*

(B) *Exserohilum rostratum*



(C) *Fusarium solani*

Plate 12. Effect of hexaconazole on growth of LRD pathogens

Table 18. Effect of fungicides on radial growth of major pathogens of LRD in solid media

Fungicide	Concentration, ppm	Per cent Inhibition growth, %		
		Colletotrichum gloeosporioides	*Exserohilum rostratum*	*Fusarium solani*
Copper oxychloride	50	50.02 (44.99)	57.81 (49.47)	72.74 (58.50)
	100	81.10 (64.13)	75.61 (60.37)	78.28 (62.19)
	200	100.00 (90.00)	82.69 (65.39)	87.61 (69.36)
Mancozeb	50	68.24 (55.68)	68.25 (55.68)	40.57 (39.55)
	100	74.71 (59.78)	100 (90.00)	58.46 (49.85)
	200	100.00 (90.00)	100 (90.00)	64.48 (53.40)
carbendazim	25	100 (90.00)	10.63 (19.02)	100 (90.00)
	50	100 (90.00)	20.35 (26.81)	100 (90.00)
	100	100 (90.00)	37.10(37.51)	100 (90.00)
Hexaconazole	25	100 (90.00)	100 (90.00)	100 (90.00)
	50	100 (90.00)	100 (90.00)	100 (90.00)
	100	100 (90.00)	100 (90.00)	100 (90.00)
Propiconazole	25	100 (90.00)	100 (90.00)	100 (90.00)
	50	100 (90.00)	100 (90.00)	100 (90.00)
	100	100 (90.00)	100 (90.00)	100 (90.00)
CD		1.15	1.34	1.70

Number in parenthesis are transformed means in angles

concentrations (Plate 8C). The inhibitory effect of carbendazim was only 37.10 per cent even at the recommended dose of 100 ppm (Plate 10B).

4.13 COMPATIBILITY OF FUNGICIDES WITH ANTAGONISTIC MICROORGANISMS

4.13.1 With Trichoderma Culture

Hexaconazole completely inhibited the mycelial growth of *Trichoderma* sp. at all concentrations tested (Table 19). The rate of inhibition of fungal growth in the presence of mancozeb was comparatively low at different concentrations tested. However, a delay in the sporulation of Trichoderma was noticed in presence of mancozeb (Plate 13).

4.13. 2. With *Pseudomonas fluorescens*

The growth of *P. fluorescens* was not inhibited even by the highest concentration of mancozeb tried while in hexaconazole treated media growth of *P. fluorescens* was inhibited drastically during the first 24 hours. Sparse growth was noticed 48 h after inoculation and the growth became normal from 72 h onwards.

4.14 INTEGRATED MANAGEMENT OF LEAF ROT OF COCONUT IN THE FIELD

The severity of leaf rot in the experimental palms was estimated initially before the application of treatments as per cent disease index and is given as pre-treatment disease index in Table 20. The pre-treatment per cent disease index varied from 21.80 to 41.80. Observations taken two months after the first set of treatments showed that there was a 23.29 per cent increase in disease severity in T_6 (absolute control) while a reduction in disease severity was noticed in all other treatments with a maximum of 41.53 per cent in T_2 (hexaconazole) and least (0.90 %) in T_6 (Mancozeb + Trichoderma). More than 30 per

Table 19. Evaluation of compatibility of fungicides with *Trichoderma* sp.

Fungicide	Concentration, ppm	Inhibition on radial growth of *Trichoderma* sp., %
Mancozeb	100	1.33
	200	6.67
	300	8.67
	400	10.67
Hexaconazole	100	100
	200	100

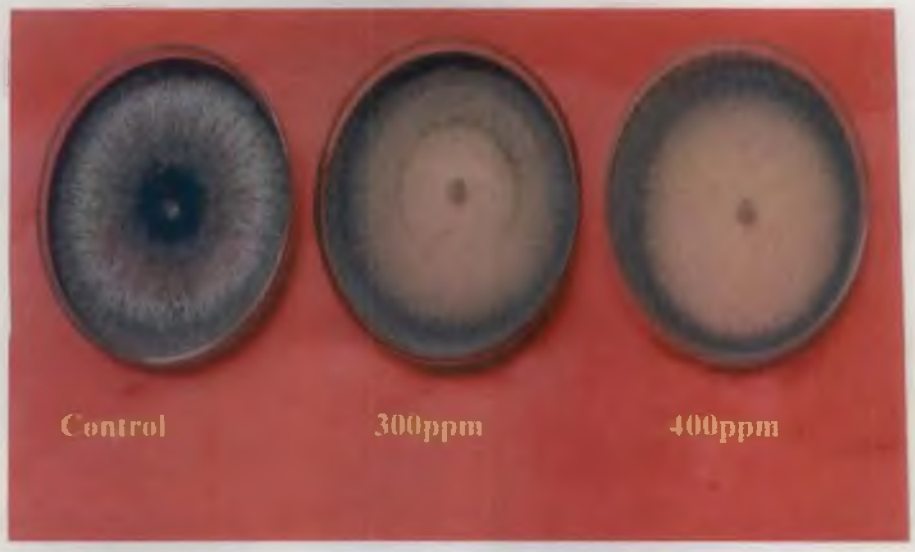


Plate 13. Effect of mancozeb on growth of *Trichoderma* sp.

Table 20. Field evaluation of fungicides and biocontrol agents against leaf rot pathogens

Treatment	Reduction in disease severity, %			
	Pre-treatment disease index October 2001	After 1st spraying January 2002	After 2nd spraying May 2002	After 3rd spraying August 2002
Absolute control (T_0)	40.20	-23.29 (13.29)	-59.02 (11.87)	-56.25 (11.99)
Mancozeb (T_1)	29.64	35.22 (15.34)	33.83 (15.29)	37.61 (15.41)
Hexaconazole (T_2)	41.80	41.53 (15.54)	59.05 (16.10)	64.74 (16.27)
Pseudomonas fluorescens (T_3)	32.12	33.86 (15.29)	54.91 (15.97)	54.51 (15.95)
Trichoderma (T_4)	21.80	20.43 (14.85)	21.33 (14.88)	13.97 (14.63)
Mancozeb + *P. fluorescens* (T_5)	22.00	29.79 (15.16)	33.45 (15.28)	19.87 (14.83)
Mancozeb + Trichoderma (T_6)	33.46	0.90 (14.17)	12.75 (14.59)	11.60 (14.55)
Hexaconazole + *P. fluorescens* (T_7)	28.94	24.70 (14.99)	30.66 (15.19)	24.58 (14.99)
Hexaconazole + Trichoderma (T_8)	27.18	17.78 (14.75)	14.06 (14.63)	21.52 (14.88)
P. fluorescens + Trichoderma (T_9)	27.98	13.58 (14.61)	15.34 (14.67)	12.65 (14.58)
Control (T_{10})	33.22	4.51 (14.30)	8.97 (14.46)	5.44 (14.33)
				CD – 2.09

Data in parenthesis indicate value after $\sqrt{x + 200}$ transformation

cent reduction in disease severity was observed in T_1 (Mancozeb, 35.22 %) and T_3 (*P. fluorescens*, 33.86 %) and more than 20 per cent reduction in T_4 (Trichoderma, 20.43 %), T_5 (Mancozeb + *P. fluorescens*, 29.79 %) and T_7 (hexaconazole + *P. fluorescens*, 24.70 %) while the reduction was less than 20 per cent in T_8 (hexaconazole + Trichoderma, 17.78 %), T_9 (*P. fluorescens* + Trichoderma, 13.58 %) and T_{10} (Control, 4.51 %).

When the observations were taken three months after second round of treatment a similar trend was noticed. The LRD increased by 59.02 per cent in T_0 (absolute control) while in other treatments there was a reduction in disease severity. Above 50 per cent reduction was achieved in T_2 (hexaconazole, 59.05 %) and T_3 (*P. fluorescens*, 54.91 %); above 30 per cent reduction in T_1 (Mancozeb, 33.83 %), T_5 (Mancozeb + *P. fluorescens*, 33.45 %) and T_7 (Hexaconazole + *P. fluorescens*, 30.66 %) and above 20 per cent reduction in T_4 (Trichoderma, 21.33 %) while the disease reduction was less than 20 per cent in T_6 (Mancozeb + Trichoderma, 12.75 %), T_8 (Hexaconazole + Trichoderma, 14.06 %), T_9 (*P. fluorescens* + Trichoderma, 15.34 %) and T_{10} (Control, 8.97 %).

The data after the last set of treatments indicated that there was 56.25 per cent increase in disease severity in T_0 (absolute control) while in all other treatments disease was reduced to various degrees with the maximum reduction in T_2 (hexaconazole, 64.74 %) and lowest reduction in T_{10} (control, 5.44 %). The reduction was more than 30 per cent in T_3 (*P. fluorescens*, 54.51 %) and T1 (Mancozeb, 37.61 %) and more than 20 per cent in T_7 (hexaconazole + *P. fluorescens*, 24.58 %) and T_8 (hexaconazole and Trichoderma, 21.52 %) while the reduction was less than 20 per cent in T_4 (Trichoderma, 13.97 %), T_5 (Mancozeb + *P. fluorescens*, 19.87 %), T_6 (Mancozeb + Trichoderma, 11.60 %) and T_9 (*P. fluorescens* + Trichoderma, 12.65 %).

DISCUSSION

5. DISCUSSION

Combined leaf rot – root (wilt) disease incidence in coconut is a unique example of fungal – phytoplasma disease complex threatening coconut cultivation, the backbone of agriculture in Kerala. A fresh insight is envisaged in the present study to analyse the etiological nature of LRD and to evolve a sustainable management practice to tackle the disease.

The pathogens associated with LRD were isolated from coconut palms from different parts of five districts of southern Kerala, *viz*., Kottayam, Allapuzha, Pathanamthitta, Kollam and Thiruvananthapuram during three seasons of the year (June – July, November – December and March – April, 2001 - 2002). A spectrum of seven different fungi were isolated and found to be pathogenic in initiating LRD on spindle of coconut in these areas. The results of isolations from Kottayam district showed that both *Colletrichum gloeosporioides* and *Fusarium* spp. were involved in causing the disease during June – July and November – December periods, while *Fusarium* sp. was more frequently isolated from LRD lesions during March – April (Table 1). Many of the earlier works (Srinivasan and Gunasekaran, 1993, 1996c, 1996a, 2000a) also indicated that *C. gloeosporioides* is the major pathogen of LRD responsible for initiating the infection during the rainy season in the presence / absence of other pathogens while *Fusarium* spp. predominates during summer season resulting in aggravation and perpetuation of the disease. *Exserohilum rostratum* was isolated along with *C. glocosporioides* and *Fusarium* sp. only once during November – December. According to Srinivasan and Gunasekaran (1996d) also, association of *E. rostratum* with LRD was less frequent compared to *C. gloeosporioides* and *Fusarium* sp. *Gliocladium roseum* was isolated from a palm at Kumarakom during November–December and

March – April and thus its etiological role also in the causation of LRD was proved. *Gliocladium roseum / G. vermoseni*, had been documented as a major pathogen of LRD by different workers (Menon and Nair, 1948; Mishra *et al.*, 1989; Srinivasan and Gunasekaran, 1993; Sathiarajan *et al.*, 1988). The repeated isolations of *Scytalidium* sp. from Changanacherry showed the possible etiological role of this hitherto unreported fungus in the leaf rot disease complex. *Curvularia* sp. was isolated during March – April from one location and its pathogenicity was proved. However, its etiological role is doubtful since the organism had not been isolated frequently and it failed to produce any symptoms on uninjured leaflets during artificial inoculation studies. Further, its pathogenicity and etiology had not been proved although, it was previously isolated from LRD affected leaflets of coconut (CPCRI, 1985b., Srinivasan and Gunasekaran, 1994b, 1996a)

The data from Alapuzha district (Table 2) also reiterated the combined etiological role of *C. gloeosporioides* and *Fusarium* spp. in the initiation of LRD during rainy season while infection by *C. gloeosporioides* subsides during post monsoon and *Fusarium* spp. predominated subsequently. Further confirmation on the etiological role of *Scytalidium* sp. was obtained from this district based on its repeated isolations from Moncompu during rainy season and later.

The repeated isolations of *C. gloeosporioides* and *Fusarium* sp. from one location and *Cephalosporium sacchari* and *Fusarium* sp. from another location in Kollam district during all the seasons of the year are further proof on the major role played by *C. gloeosporioides* and *Fusarium* sp. in the etiology of the disease (Table 3).

The results in Thiruvananthapuram and Pathanamthitta districts are also not different from that of other districts (Table 4). The consistent isolation of *C. gloeosporioides* and *Fusarium* spp. either alone or in combination reconfirmed the earlier reports of their primary role in

LRD incidence (Srinivasan and Gunasekaran, 1993, 1994, 1996a,d and 1999).

The most frequently isolated pathogens from LRD infected coconut palms during the rainy season were *C. gloeosporioides* and *Fusarium* spp. (Fig. 1). The frequency of isolation of *C. gloeosporioides* decreased during November-December and March-April, while that of *Fusarium* sp. progressively increased with summer season. This is in conformity with the earlier observations of the primary etiological role of *C. gloeosporioides* during rainy season and its subsequent loss of prominance when monsoon subsides (Srinivasan and Gunasekaran, 1996d). The present study further points to the fact that *Fusarium* spp. are as intimately associated with the disease with the same magnitude and frequency as that of *C. gloeosporioides* during the rainy season also. Its pathogenic role was further strengthened as the rainy season ends. *Fusarium* sp. then became the principal pathogen during the summer, while *C. gloeosporioides* probably undergoes quiescent phase (Srinivasan and Gunasekaran, 1996d). During the subsequent rainy season, as LRD initiated by *C. gloeosporioides* is intensified, infection by *Fusarium* spp. probably take a supporting role. However, it was *Fusarim* spp., which were associated with LRD lesions to the maximum extent followed by *C. gloeosporioides* and hence based on the present study, *Fusarium* spp. and *C. gloeosporioides* are suggested as the major pathogens of LRD.

Further, the study also elucidated a hitherto unreported fungus *Scytalidium* sp. as a possible etiological agent of LRD in certain locations. All other fungi were associative in nature and aggravated the severity of LRD.

Data on the pathogenicity studies based on the time taken for symptom expression and development of lesion size (Table 5 and 6) indicated the etiological role of six out seven different pathogens tested, in the initiation of LRD symptoms. It can be observed that the minimum

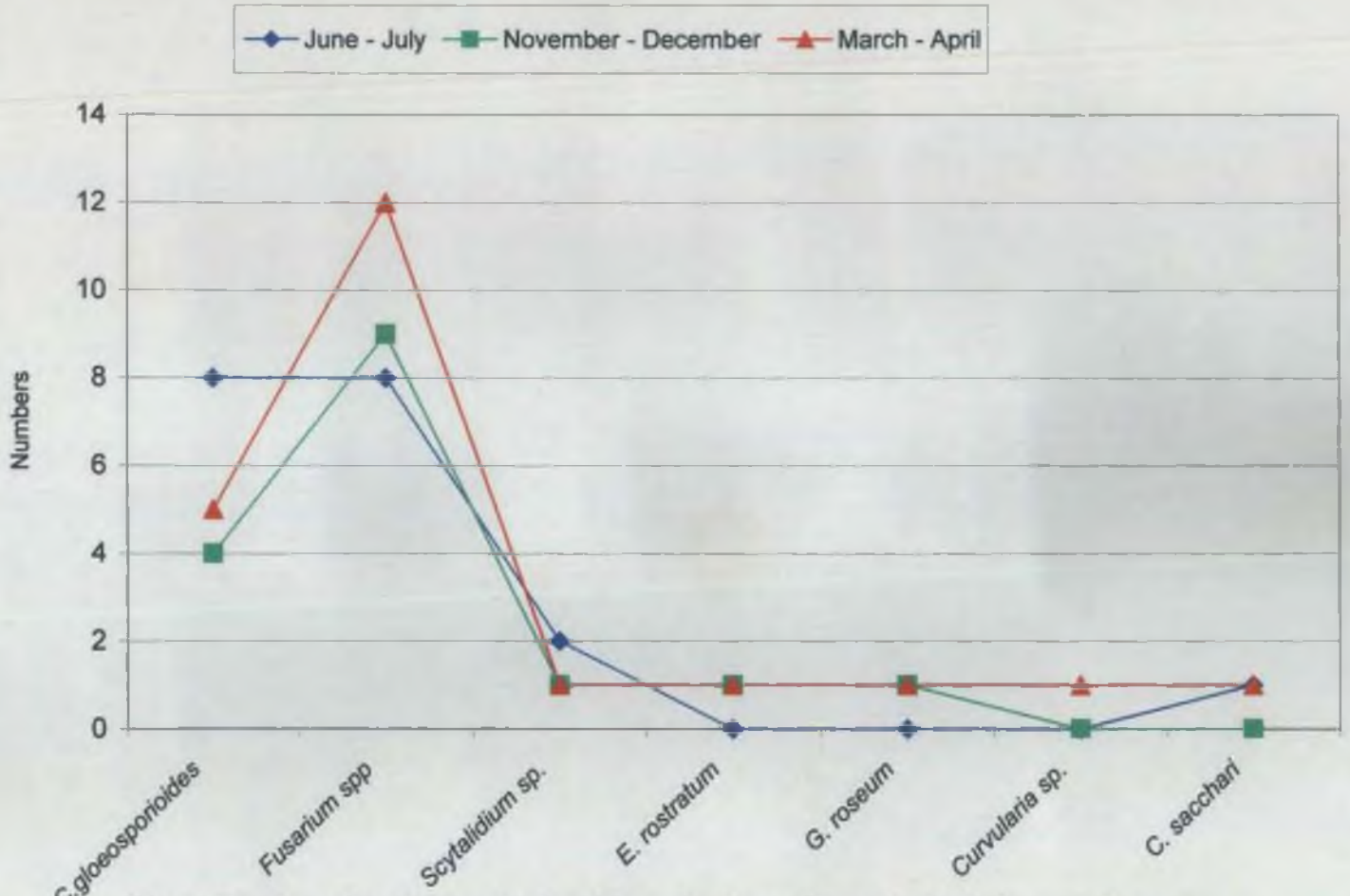


Fig. 1. Number of pathogens isolated from leaf rot affected spindle leaves of coconut during different seasons

time taken for symptom expression after inoculation with or without injury was by *Scytalidium* sp. (3 and 7 days) followed by *C. gloeosporioides* (4 and 7 days). *E. rostratum* also took less time for lesion development when compared to the other organisms (4 and 8 days). Srinivasan and Gunasekaran (1994a) also observed that *E. rostratum* developed leaf rot symptoms relatively faster as compared to *C. gloeosporioides* and *Gliocladium vermoesent*. This is indicative of the primary pathogenic role played by these fungi in the LRD incidence. The comparatively larger lesion size produced by *Scytalidium* sp. and *E. rostratum* further confirms the above finding.

The detailed isolations and the subsequent pathogenicity trials pointed out that different fungi were involved in the initiation and further aggravation of LRD in coconut in different locations. The primary pathogenic role played either singly or in combination with other fungi also varied in different localities. This is an example where more than one fungi are involved in the incidence of a disease and different groups of fungi cause the same disease in different regions seasons. It could be probable that the highly susceptible spindle of root (wilt) affected palms are predisposed to infection by LRD pathogens. Similar postulations were also indicated by Srinivasan *et al.* (1998). They opined that the palms weakened by phytoplasma with accompanied physio-chemical changes in the host, resulted in breakdown of defense mechanism, of the host predisposing it to LRD infection. The fact that more than 65 per cent of root (wilt) affected palms are super imposed with LRD incidence further confirms the above argument. Eventhough scattered reports of the occurrence of the LRD on non RWD palms are there it is not seen in large scale on non root (wilt) affected palms. Hence LRD incidence could be initiated by different, comparatively weaker fungi in different regions of RWD-LRD endemic areas. However, the inference require further proof as the palms in the present investigations were not subjected to RWD-LRD evaluation.

Detailed symptomatology of LRD recorded in the field during the present study is similar to the earlier reports (Menon and Nair, 1951., Srinivasan, 1991 and Srinivasan and Gunasekaran, 1992. Srinivasan *et al.*, 1998, Koshy, 1999). The green house inoculation trials with detached healthy leaflets of unopened spindle of coconut exhibited difference in the sequence of development of symptoms. In the field condition LRD incidence is initiated and aggravated by diverse etiological agents under the influence of varying environmental factors over a period of time unlike inoculation experiments under controlled green house condition where such variations in symptom expression can be expected.

The comparative virulence of eight isolates of *C. gloeosporioides* and 12 isolates of *Fusarium* spp. were estimated based on the lesion size and time taken for the lesion development. The results indicated that there were great differences among the different isolates developed from different regions on the time taken for lesion development and lesion size. Thus isolates C_1, C_2, C_4 and C_8 of *C. gloeosporioides*, F_1, F_2, F_4 and F_{11} of *Fusarium* spp. and S_1 of *Scytalidium* sp. were categorized as highly virulent isolates based on the time taken for symptom expression and larger lesions developed by them. The study also showed subsequently that F_1, F_2 and F_4 isolates were identified as *F. solani* and F_{11} isolate as *F. moniliformae* which proved that *F. solani* is the most prevalent form of *Fusarium* spp. associated with LRD. It is clearly documented that fungi exhibit greater variability in different regions with regard to their pathogenicity and other characteristics, which determines the predominance of isolates in such areas (Pande *et al.*, 1991. Mathur. *et al.*, 2001). The existence of such variability results in the evolution of highly virulent isolates capable of causing epiphytotic outbreaks of LRD and greater economic loss to the farmers. Further, characterization studies on the variability and grouping of these isolates

using modern molecular techniques is needed for better understanding of these pathogens.

The seven pathogens, which were isolated and pathogenicity proved, were further characterized based on morphological and cultural characteristics. The characteristics of *C. gloeosporioides* observed during the present study were similar to those given by Sutton (1992) and descriptions of *Scytalidium* sp. was suited with that of Ellis (1971). The cultural and morphological characters of *C.sacchari*, *G. roseum* and *Curvularia* sp. were matched and found to be similar to those reported by Subramanian (1961). The various isolates of *Fusarium* spp. isolated and characterized in the present study were identified as *F. solani*, *F. moniliformae* and *F. oxysporum*, based on their morphological and cultural characters and on comparison with descriptions of these species by Seifert (1996).

In vitro interaction study of different fungi associated with leaf rot showed that there was no strong inhibition or antagonism among the major pathogens of leaf rot. Thus *C. gloeosporioides* had grown and freely merged with that *Fusarium solani*. *E. rostratum* and *Scytalidium* sp. Similar was the case of *F. solani* along with *Scytalidium* sp. However slight growth inhibition was noticed when *E. rostratum* was grown in presence of *F. solani* and *Scytalidium* in presence of *E. rostratum*. Hence it can be persumed that the major pathogens such as *C. gloeosporioides*, *Fusarium solani*, *E. rostratum*, and *Scytalidium* sp. are more associative and synergistic in nature rather than antagonistic at the site of infection. Many of these fungi which were isolated from leaf rot infected spindles in combination underlines the veracity of this fact. Similar type of interactions were also noticed by Srinivasan and Gunasekaran (1995b) between these pathogens. The growth suppression or growth inhibition of fungi such as *Cephalosporium sacchari*, *G. roseum* and *Curvularia* sp. by major pathogens indicate that their role in LRD development may be secondary in nature.

The results of the combined inoculation of various pathogens of LRD in detached healthy leaflets of spindle (Table 11) showed that quicker and larger lesions were produced by combination of *E. rostratum* with other fungi such as *Scytalidium* sp. *Curvularia* sp. *C. sacchari* and *G. roseum;* combination of *Fusarium solani* with *E. rostratum* and *Scytalidium* sp. with *C. sacchari* and *Scytalidium* sp. with *C. sacchari*, *G. roseum* and *Curvularia* sp.. *Scytalidium* sp. produced symptom expression with the same speed when inoculated alone, but *E. rostratum and Fusarium solani* took more time to produce the symptoms when inoculated alone. Hence it can be inferred that the early symptom expression by combined inoculation of the above pathogens proved the involvement of more than one pathogen in initiating the infection and severity of LRD.

The results of the study on the biocontrol potential of seven cultures of *Trichoderma* sp. against *C. gloeosporioides*, *Fusarium* sp. and *E. rostratum in vitro* indicated that all the cultures varied greatly in exerting their antagonistic effect on the pathogens (Fig. 2). Most significant effect on the suppression of pathogen was exerted by T_9 culture of *Trichoderma* sp. against *C. gloeosporioides*, *Fusarium* sp. and *E. rostratum*. Evaluation of these cultures by leaf bit assay (Table 12) further reiterated the higher inhibitory effect of T_9 culture against these three pathogens, particularly in reducing the lesion size. The biocontrol potential of different species of *Trichoderma* against important plant pathogens is amply highlighted (Harman and Taylor, 1980; Haran *et al.*, 1995; Harman, 2000). It is also well documented that different isolates and species of *Trichoderma* differ greatly in exerting the antagonistic effect against various pathogens under diverse environmental conditions. Screening and selection of the most efficient isolate is crucial in the success of any biocontrol programmes. In the present study, although culture T_9 and T_6 were found to possess similar antagonistic ability, since T_9 grew better in *in vitro* condition it was selected for further study.

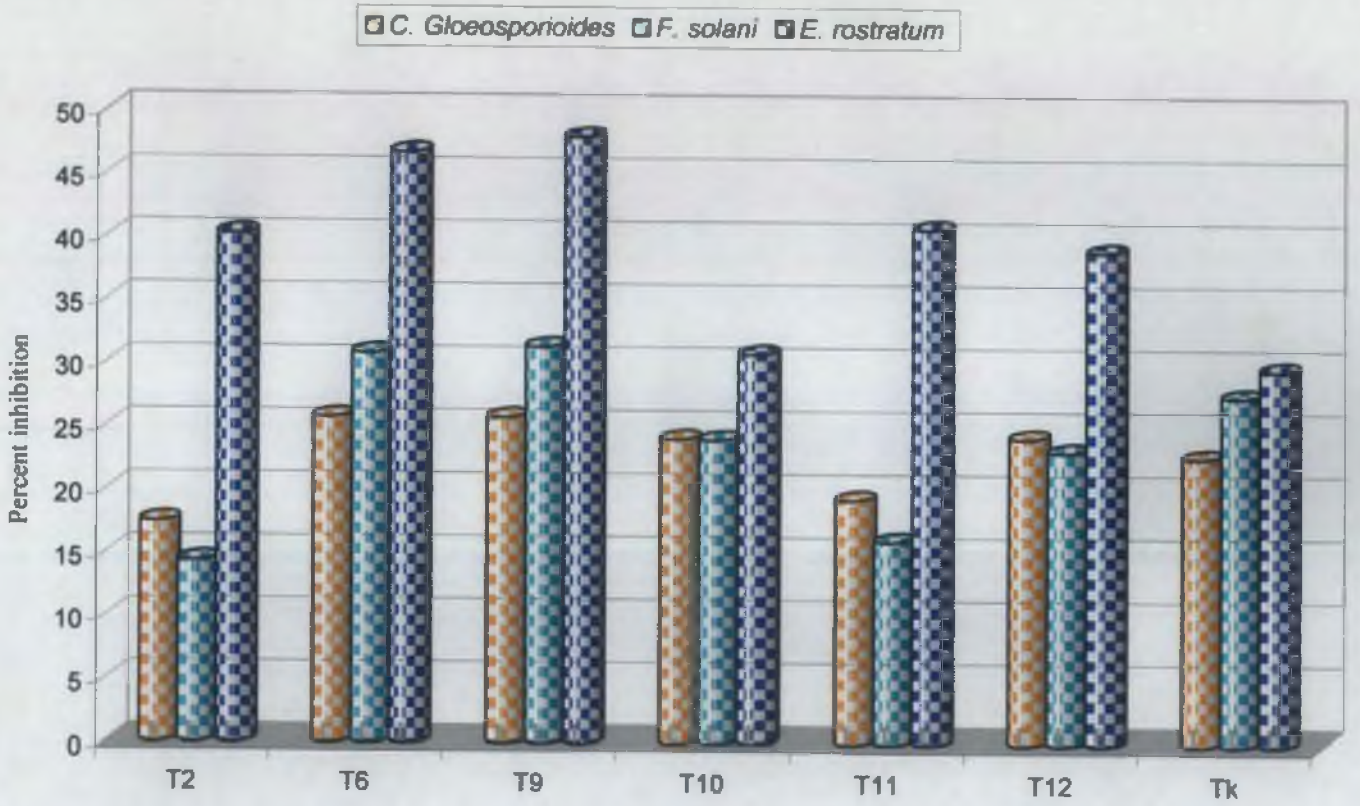


Fig. 2. Antagonistic potential of different *Trichoderma* isolates against 3 major leaf rot pathogens

Among the 29 isolates/cultures of *P. fluorescens* tested against *C. gloeosporioides*, *Fusarium* sp. and *E. rostratum*. Ps_1 culture showed the most significant inhibitory effect although a few other isolates were also found to possess excellent antagonistic property against these pathogens (Fig. 3). The results of the leaflet bit assay with selected isolates of *P. fluorescens* on the development of LRD symptoms by the major pathogens also indicated that there was delay in the development of symptoms. Maximum delay was noticed with Ps_1 culture. It further reiterated the biocontrol potential of selected isolates of *P. fluorescens* in delaying the development and severity of symptoms. The biocontrol potential of fluorescent pseudomonads has been highlighted by several workers in the past (Howell and Stipanovic, 1979; Kloepper, 1983; Mew and Rosales, 1986; Sakthivel *et al.*, 1986, Hammer, *et al.*, 1997). Fluorescent pseudomonads have the ability to establish fast and produce a variety of secondary metabolites which play an important role in plant disease suppression. The exploitation of fluorescent pseudomonads in the integrated management of LRD of coconut was investigated by Gupta *et al.* (2000). Their *in vitro* study indicated the inhibitory effect of selected isolates of *P.fluorescens* against *C. gloeosporioides* and *E. rostratum*.. The unique antagonistic ability of Ps_1 culture exhibited against *C. gloeosporioides*, *E. rostratum* and *Fusarium solani* in present study both *in vitro* and leaflet bit assay is indicative of its inherent capacity as a potential biocontrol agent against LRD. Hence Ps_1 culture was selected for further evaluation in the field along with other components of integrated management against pathogens of LRD.

The compatibility of *Trichoderma* sp. (T_9) and *P. fluorescens* (Ps_1) was tested *in vitro* to evaluate the suitability of combined delivery of these two biocontrol agents in the field. The results indicated that the fungal antagonist (T_9) was inhibited by the presence of *P. fluorescens*. Similar observation of antagonism between biocontrol agents have been detected by other workers as well (Hubbard *et al.*, 1983; Bin *et al.*, 1991

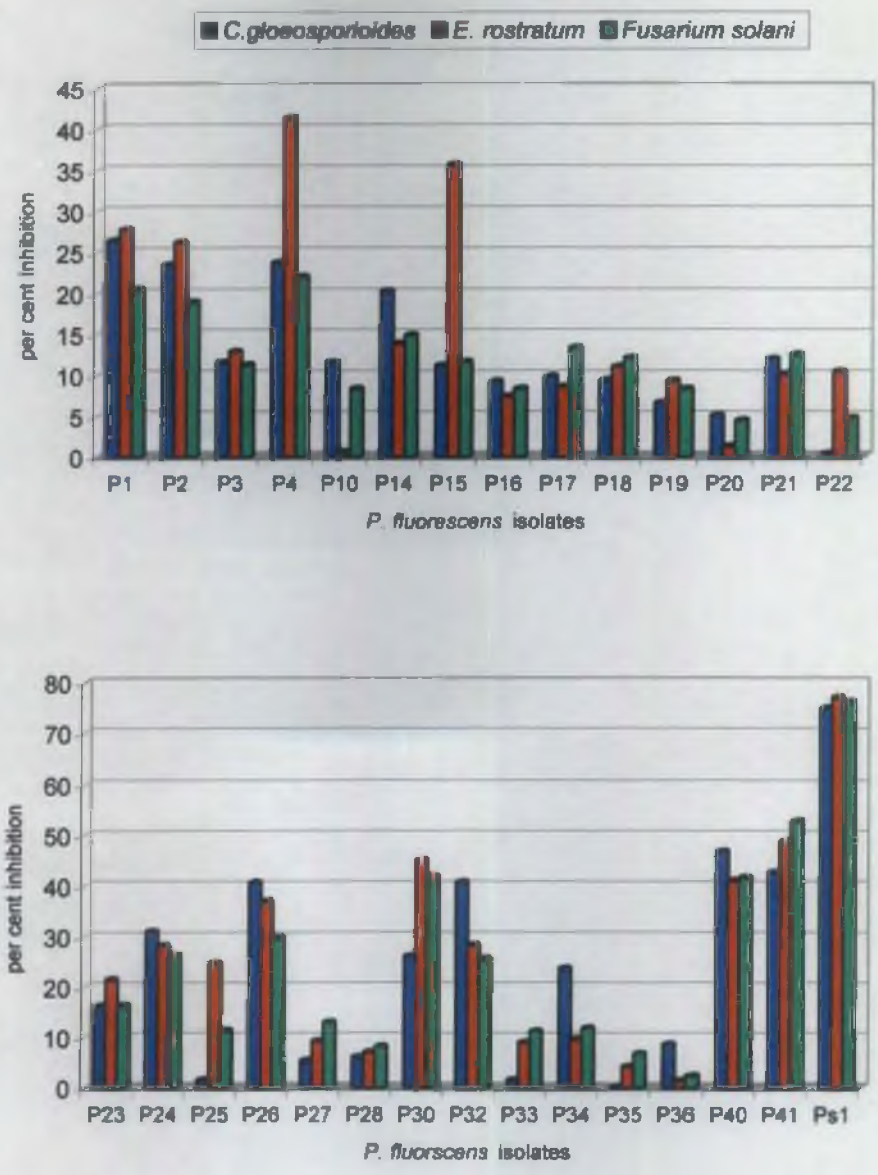


Fig. 3. ***In vitro*** **evaluation of antagonistic potential of** ***P. fluorescens*** **against major pathogens of leaf rot**

and Varshney *et al*., 2000). Compatible interactions were also not uncommon (Duffy *et al*., 1996). Hence it can be inferred that the type of interaction is governed by inherent quality of individual biocontrol agents and evaluation of the interaction is a prerequisite before a recommendation based on a consortium of biocontrol agents is made.

The bioassay of commonly used fungicides with *C. gloeosporioides*, *Fusarium solani* and *E. rostratum* at different concentrations revealed that hexaconazole and propiconazole were highly effective in inhibiting the mycelial growth of these three pathogens at all concentrations (Fig. 4). Although Carbendazim was also on par with hexaconazole and propiconazole with regard to its inhibiting response to *C. gloeosporioides* and *F. solani*, it was comparatively less effective against *E. rostratum*. The fungicidal efficacy of hexaconazole against major pathogens of LRD was evaluated *in vitro* by Srinivasan and Gunasekaran (1998) and they showed that hexaconazole exerted excellent fungicidal properties against *C. gloeosporioides*, *E. rostratum*, *Glioeladium vermoesen*, *F. solani* and *Thielaviopsis paradoxa*. Koshy *et al*. (2001) also highlighted the efficacy of hexaconazole in field control of LRD when applied along with phorate. Higher fungicidal efficacy of mancozeb against *C. gloeosporioides* and *E. rostratum* was also noted in the present study. Mancozeb has been recommended as an important and effective fungicide in sequential spraying against LRD of coconut (Srinivasan and Gunasekaran, 1996b; KAU, 1996). It was also recommended for LRD management alternating with hexaconazole (Koshy, 1999). The comparative tolerance *E. rostratum* against carbendazim has been noticed earlier (Sathiarajan, 1988). Based on the results of bioassay of the various fungicides tested and recognizing the recommendations of Srinivasan and Gunasekaran (2000b) for LRD management, hexaconazole and mancozeb were chosen for evaluation in the field trial.

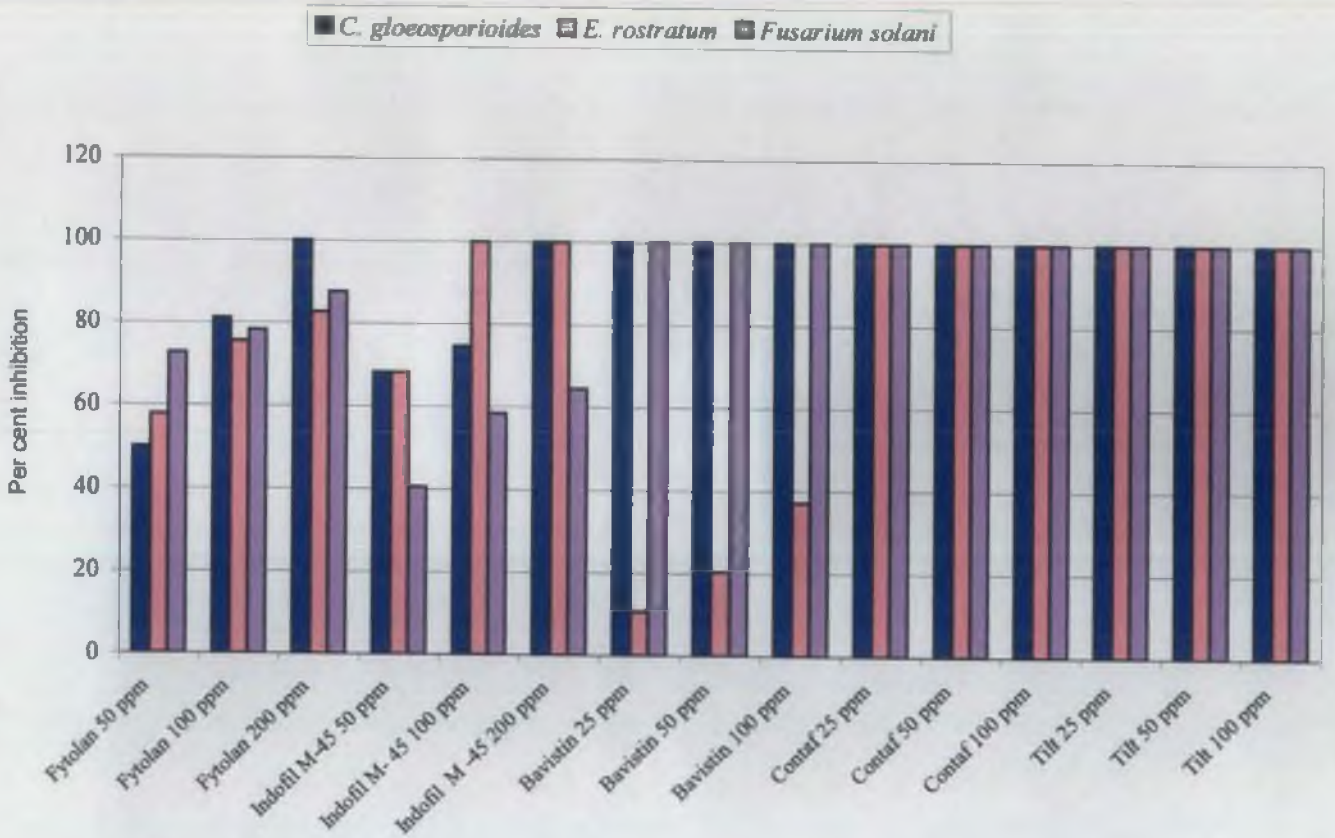


Fig. 4. Efficacy of common fungicides against major pathogens of leaf rot

Results of compatibility study of selected fungicide with selected antagonists used for the present study showed different degrees of interactions. There was no inhibition of growth of *Trichoderma* sp. by mancozeb while hexaconazole completely inhibited the radial growth of this fungus. Hence it can be concluded that Trichoderma is compatible with certain types of fungicides and incompatible with others. Henis *et al.* (1978) noticed an additive effect on the control of damping of radish when PCNB was added along with *T. harzianum*. Similarly Indu and Mukopadhyay (1990) reported that metalaxyl had no inhibitory action on radial growth of *T. harzianum*. At the same time Sharma et al. (2001) and Bhatt and Sabalpura (2001) reported that carbendazim inhibited 90 per cent growth of *T. harzianum*. Based on the compatibility test it was concluded that mancozeb could be delivered along with Trichoderma for integrated management of LRD while a waiting period of a minimum of 15 days was required for the delivery of Trichoderma after hexaconazole application.

Study on the compatibility of *P. fluorescens* and fungicides showed no inhibitory effect. Since *P. fluorescens* and the fungicides were compatible it was decided to apply the biocontrol agent and the fungicide by combined delivery.

During the start of the experiment the disease severity in the experimental plot ranged from 21.8 to 41.8 per cent. When the disease estimation was taken for a continuous period of one year the disease severity in the untreated control palms increased gradually. This clearly proves that if management practices are not followed the disease intensity will increase resulting in subsequent yield loss.

All the palms which were treated with different fungicides and biocontrol agents showed in substantial reduction in the disease intensity. However, the extent of disease reduction varied from treatment to treatment. The highest reduction of 41.53, 59.05, 64.74 per cent in disease intensity over the pretreated data was noticed after

the 1st, 2nd and 3rd application with hexaconazole (Fig. 5). This suggest that hexaconazole is a very effective fungicide in the management of LRD. Effectiveness of hexaconazole in the management of LRD has been amply demonstrated (Srinivasan and Gunasekaran, 1998, 2000b; Koshy, 1999; Koshy *et al*., 2000).

Mancozeb, the other fungicide tried, also reduced the disease intensity eventhough to a lesser extent compared to hexaconazole. The reduction in disease after the 1st (35.22), 2nd (33.83) and 3rd (37.61) spraying did not show a progressive suppression as observed with hexaconazole. Hexaconazole being a systemic fungicide could protect the emerging leaflet better than the contact fungicide mancozeb. The efficacy of mancozeb was also substantiated by previous findings (Srinivasan and Gunasekaran, 1996b; Koshy *et al* , 2002; KAU, 1996).

The two biocontrol agents tried also decreased the disease severity. The disease suppressing ability of *P. fluorescens* was more pronounced with time, two months after application disease reduction was only 33.86 compared to 54.91 and 54.51 after 2nd and 3rd application. During the initial period the reduction was less probably because the organism need more time to get established and build up the population. The inhibitory role or *P. fluorescens* against LRD pathogens eventhough was proved and found effective under *in vitro* condition, this is the first report where it has been used successfully under field condition. The LRD inhibiting ability of Trichoderma was less than that of the fungicides and *P. fluorescens*. Further, after 2nd (21.33) and 3rd (13.97) application the disease reducing ability of Trichoderma slowly decreased. Under *in vitro* conditions also Trichoderma could inhibit the LRD pathogens only to a lesser extent compared to *P. fluorescens*. It is probable that the pathogen could outcompete the biocontrol agent and multiplication of Trichoderma might have been reduced due to other micro environmental condition.

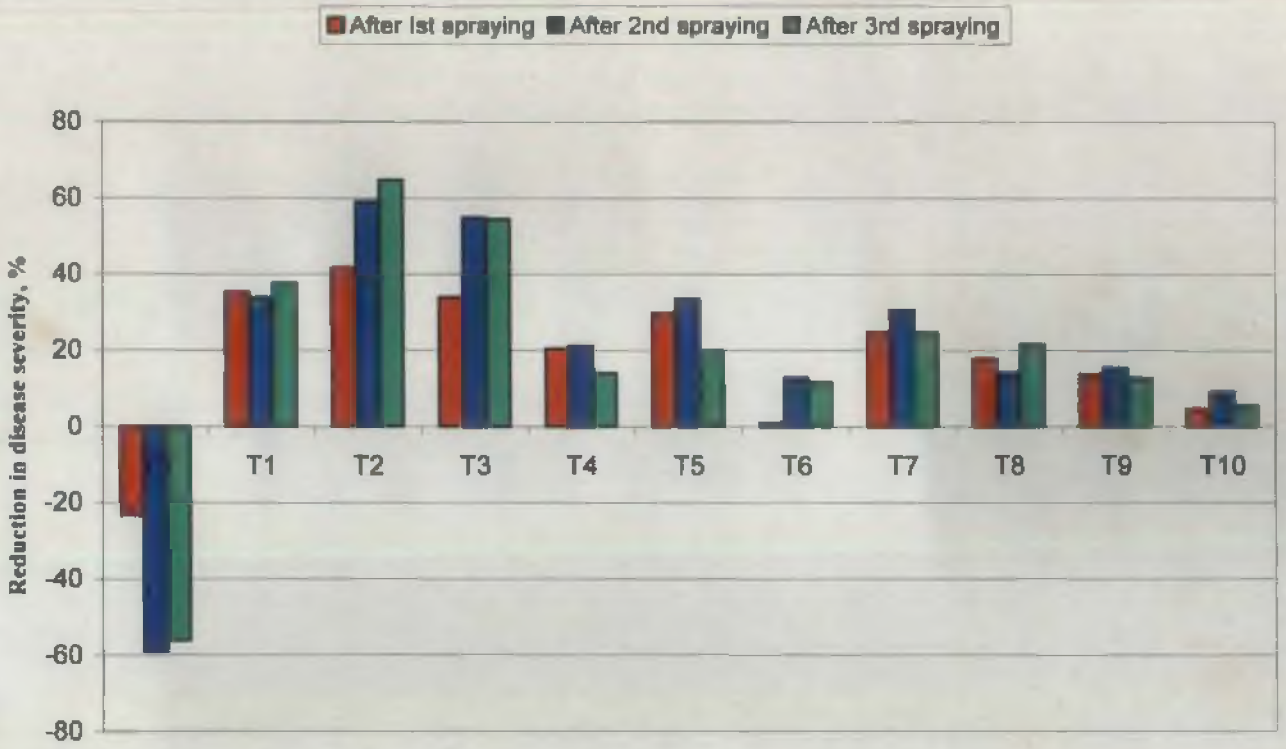


Fig. 5. Efficacy of fungicides and biocontrol agents against leaf rot of coconut in the field

The combined application of biocontrol agent with fungicide was not as effective as that of their individual application. Inhibitory nature of fungicide on *Trichoderma* and *P. fluorescens* to varying degree has been proved under *in vitro*.

Phytosanitation alone, without fungicide or biological control agent application, marginally reduced the disease. By phytosanitation it is possible to reduce primary inoculum of the pathogen available for fresh infection of newly emerging leaves. Thus, the study clearly showed that it is possible to reduce the severity of LRD to more than 50 per cent in the field by repeated application of hexaconazole or *P. fluorescens* combined with phytosanitary measures.

SUMMARY

6. SUMMARY

A detailed study was undertaken to investigate on the etiological role of different fungi associated with leaf rot of coconut in Kerala and to evolve a suitable integrated management strategy for managing the disease.

Extensive isolations of pathogens associated with LRD were carried out from five districts of Kerala *viz.*, Kottayam, Alapuzha, Kollam, Pathanamthitta and Thiruvananthapuram districts during three seasons of the year *viz.*, June – July, November – December 2001 and March – April 2002. Pathogenicity trials showed that seven different fungi *viz.*, *Colletotrichum gloeosporioides*, *Fusarium* spp. *Cephalosporium* sp., *Exserohilum rostratum*, *Gliocladium roseum*, *Scytalidium* sp. and *Curvularia* sp. were associated with LRD. *C. gloeosporioides* and *Fusarium* spp. were common during rainy seasons in most of the locations. Infection by *C. gloeosporioides* subsided after monsoon season and *Fusarium* spp. predominated subsequently. Association of *E.rostratum*, *Cephalosporium* sp., *G. roseum*, *Scytalidium* sp. and *Curvularia* sp. with LRD were also observed in certain locations. Based on the frequency of isolations and the pathogenicity tests, *Fusarium* spp. and *C. gloeosporioides* were confirmed to be the two major pathogens of LRD. The study also identified, for the first time, the involvement of fungus, *Scytalidium* sp. on the etiological role of LRD.

The results of inoculation studies of these fungi on detached healthy spindle leaflets showed that injury (pin pricking) favoured early development of symptoms. Among the various pathogens, *Scytalidium* sp. and *C. gloeosporioides* produced faster and larger symptoms.

The various pathogens isolated from LRD lesions were further studied and identified based on morphological and cultural characteristics. Thus the three *Fusarium* spp. isolated were identified as

F. solani, *F. moniliformae* and *F. oxysprorum* and *Cephalosporium* sp. as *C. sacchari*.

Detailed symptomatology of the disease was studied both in natural condition as well as by artificial inoculation on detached leaflets of healthy spindle.

The different isolates of *C. gloeosporioides*, *Fusarium* spp. and *Scytalidium* sp. obtained from various locations exhibited variation in virulence and based on the time taken for lesion development and lesion size, these isolates were grouped as highly virulent, virulent and mildly virulent. *F. solani* was identified as the most virulent form of *Fusarium* spp. associated with LRD.

The *in vitro* interaction study of the seven fungal pathogens associated with LRD showed that there was no strong inhibitory or antagonistic activity among these organisms and they were more of associative in nature. Combined inoculation of different LRD pathogens on detached spindle leaflets showed that quick and larger lesions were produced by *E. rostratum* and *Scytalidium* sp. in presence of other pathogens signifying the initiation and aggravation of LRD by more than one pathogen.

Among the various isolates of *Trichoderma* sp. and *P. fluorescens* tested *in vitro* for their antagonistic potential against *C. gloeosporioides*, *F. solani* and *E. rostratum*, culture T_9 of *Trichoderma* sp. and Ps_1 culture of *P. fluorescens* exhibited excellent antagonistic properties against all these pathogens uniformly. Hence T_9 of *Trichoderma* sp. and Ps_1 of *P. fluorescens* were chosen as biocontrol components in the integrated management of LRD in the field.

Study on the interaction between selected fungal antagonist (T_9) and bacterial antagonist (Ps_1) up on dual culture showed that Ps1 culture was strongly antagonistic to T_9 culture of *Trichoderma* sp. Hence both biocontrol agents are not suitable for combined delivery in the integrated management of LRD.

Bioassay of commonly used fungicides against the major pathogens of LRD (*C. gloeosporioides*, *E. rostratum* and *F. solani*) had shown that hexaconazole and propiconazole completely inhibited growth of all the above pathogens at all concentrations tested. Higher concentration of mancozeb and copper oxychloride also had similar effect. However, carbendazim did not show marked fungicidal activity against *E. rostratum*. Considering the overall performance of these fungicides *in vitro* and the package of practice recommendations, hexaconazole and mancozeb were selected for the integrated field management of LRD.

Study on the compatibility of selected antagonists and selected fungicides revealed that Ta culture of Trichoderma was compatible with mancozeb and Ps, culture of *P. fluorescens* was compatible with mancozeb and hexaconazole and hence their combinations are safe for combined delivery. However *Trichoderma* sp. was not compatible with hexaconazole as the fungicide completely inhibited the fungal growth.

The results of the field experiment on the integrated management of leaf rot of coconut highlighted the significance of phytosanitation in achieving satisfactory level of control. Among the various treatments employed application of hexaconazole resulted in greater reduction of disease severity. Based on the field experiment, it is recommended that phytosanitation followed with application of hexaconazole can be adopted for substantial reduction in the severity of LRD.

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*Originals not seen

INTEGRATED MANAGEMENT OF LEAF ROT OF COCONUT

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8. ABSTRACT

The study entitled as "Integrated management of leaf rot of coconut" was conducted at the Department of Plant Pathology, College of Agriculture, Vellayani and field trial was conducted at RARS, Kumarakom. Extensive isolations, pathogenicity and characterization studies of the pathogens associated with leaf rot infected spindle leaf of coconut from different regions of five southern districts of Kerala revealed that *Colletotrichum gloeosporioides*, *Fusarium solani*, *Fusarium moniliformae*, *Fusarium oxysporum*, *Cephalosporium sacchari*, *Exserohilum rostratum*, *Gliocladium roseum*, *Nigrospora* sp., *Neotaldium* sp. and *Curvularia* sp. played significant role in the etiology of LRD. Based on frequency of isolations, *Fusarium* spp. and *C. gloeosporioides* were identified as major pathogens of LRD in these areas. The present study constitutes the first report of the etiological role of *Neotaldium* sp. in leaf rot incidence.

Symptomatology of the disease based on natural incidence in the field and upon artificial inoculation were clearly described. The *in vitro* interactions of different dual combinations of LRD pathogens were found to be predominantly associative rather than inhibitory in nature. The rapid lesion development of certain dual combinations of the pathogens upon artificial inoculation further signified the associative nature of these pathogens.

Extensive *in vitro* screening and leaf bit assay of different antagonistic microorganism identified T_9 culture of *Trichoderma* sp. Ps_1 culture of *Pseudomonas fluorescens* as two potential agents against the major pathogens of LRD. The *in vitro* interactions study of the selected fungal and bacterial antagonist showed that both are incompatible and hence unsuitable for combined delivery. Bioassay results showed that fungicides hexaconazole and

propiconazole completely inhibited the growth of *C. gloeosporioides*, *E. rostratum* and *F. solani* while mancozeb was more effective at higher concentrations. None of the fungicides was inhibitory to *P. fluorescens* while *Trichoderma* sp. was inhibited by hexaconazole and hence the fungal antagonist was unsuitable for combined delivery with the fungicides. Evaluation of different components of disease management indicated that phytosanitation coupled with hexaconazole application was effective in reducing disease severity of leaf rot in the field.

APPENDIX

APPENDIX – I

1. Czapek's agar

Sucrose	- 30g
$NaNO_3$	- 2g
K_2HPO_4	- 1g
$MgSO_4\ 7H_2O$	- 0.5g
KCl	- 0.5g
$FeSO_4$	- 0.01g
Distilled water	- 1l
Agar	- 18g

2. Potato Dextrose Agar (PDA)

Potato	- 200g
Dextrose	- 20g
Agar	- 20g
Distilled water	- 1l

3. Potato Sucrose Agar (PSA)

Potato water	- 500 ml
Sucrose	- 20g
Agar	- 20g
Distilled water	- 500 ml

4. King's medium B (KMB)

Peptone	- 20g
K_2HPO_4	- 2.5g
Glycerol	- 15 ml
$MgSO_4\ 7H_2O$	- 6g
Agar	- 15g
Water	- 1l