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LEAF ROT DISEASE

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Coconut leaf rot disease (LRD) is important as it is widely prevalent in association with root (wilt) disease (RWD) in Southern districts of Kerala state. The extensive destruction of leaf in LRD infected so palm is so conspicuous that the RWD palms draw the farmers' easy attention after the onset of LRD. The disease is over a century old and investigations had begun almost in parallel with the investigations on RWD. The results on different aspects of LRD investigations are summarised in this chapter.

Inter-relationship of LRD with RWD

Since the beginning of the century LRD seemed to have been considered as a part of RWD. Varghese (1934) observed that leaf disease of coconut occurred along with the root disease and generally the same palm showed symptoms of both diseases. Radha and Lal (1968) reported close relationship between occurrence of LRD and RWD in the field as well as in inoculation trials. They observed that nearly 16 to 40% of the palms in the root (wilt) affected areas developed leaf rot and its intensity varied in different types of soils. Srinivasan (1991) reported that the LRD was generally confined to RWD affected palms and on an average 65% of these palms were superinfected with LRD; the incidence of LRD increasing with the intensity of RWD. Thus a strong inter-relationship of LRD with RWD incidence is evident. Young palms with RWD symptoms were readily attacked by LRD irrespective of soil types (Table 29).

Table 29. Incidence of LRD in relation to RWD

Soil type	Non-bearing (young) palms			Bearing (adult) palms		
	No of palms sampled	No diseased*	RWD with LRD (%)**	No of palms sampled	No diseased*	RWD with LRD (%)**
Sandy loam	354	126	85.71	1496	1316	50.61
Sandy	161	86	90.70	860	597	58.29
Alluvial	153	90	82.22	645	529	63.89
Clay	186	113	85.84	719	549	73.95
Laterite	105	47	93.62	806	589	74.36
Total	959	462	-	4526	3580	-

* Sum of RWD alone and RWD superinfected with LRD

** Out of diseased palms

Severe natural incidence of LRD in RWD infected seedlings in field conditions was observed (Anon., 1996). Inoculation experiment on two year old RWD affected seedlings showed severe infections under field conditions. The palms weakened by Phytoplasma, the causal agent of RWD, might result in the breakdown of defence machanism leading to susceptibility to LRD. The occurrence of LRD with RWD seems to be a distinct Phytoplasma - fungal disease complex, a phenomenon so far not known in other diseases of crop plants.

Symptomatology and Indexing

Symptoms

LRD appears initially as minute, water-soaked lesions on the emerging spindle with different shades of colour and shapes. These lesions enlarge, coalesce freely leading to extensive rotting. The rotten portions dry

Fig. 19. Leaf rot symptoms on the midrib

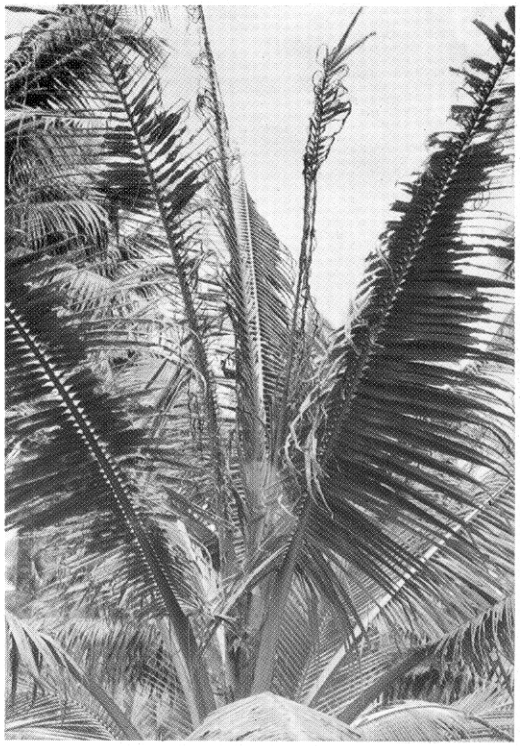

Fig. 18. Rotten spindle of an affected palm infected with leaf rot.

Fig. 20. Susceptibility of diseased palm to rhinoceros attack

Table 30. Symptoms of LRD observed in different whorls

Soil type	No palms	Palms exhibited LRD in different whorls/whorl combinations(%)*				
		Inner (I)	Outer (O)	I+M	I+O	I+M+O
Sandy loam	774	58.53	4.65	0.26	12.14	24.42
Sandy	426	26.06	18.54	-	12.44	42.96
Alluvial	412	32.53	16.50	-	18.69	32.28
Clay	503	19.48	29.62	-	13.12	37.78
Laterite	482	21.99	12.86	-	26.97	38.18
Total	2597	34.73	15.17	0.08	16.17	33.85

* Symptoms only in Middle (M) whorl or M+O whorl combination not observed

up, turn black and fall off. Tips of leaflets and mid-ribs often become blackish and shrivelled. The progress of rotting slows down with the maturity of leaflets (Fig. 18-20). The quantitative pattern of the LRD symptoms in different whorls of affected palms revealed that the innerwhorls of leaves are vulnerable to infection. Out of 2597 diseased palms sampled representing different soil types, 24 to 43% expressed the disease symptoms in the entire crown accounting approximately one third of LRD affected palms. These palms had contracted successive infection of freshly emerged leaves which resulted in varying degrees of rotting of the leaflets and hence the 'fan-like' appearance of all the leaves in the crown (Table 30) (Srinivasan and Gunasekaran, 1992). Younger leaves, especially the spindle, play a critical role in LRD incidence. In severely affected spindle tissues, mould growth is also commonly seen on the surface of the affected leaflets. (Srinivasan *et al.*, 1995) Rapid expansion of lesions relate to infection in the early period of spindle leaf emergence and the rotting may extend into the interior of the spindle. The symptoms of LRD on parts such as leaf petiole and mid-rib/mid-vein of leaflets was observed in naturally infected palms and such symptoms have been reproduced by artificial inoculations also (Anon., 1996).

Disease indexing

For quantification of disease intensity a four point grading system was adopted for LRD indexing (Srinivasan and Gunasekaran 1996a).

Disease grade	% leaf area infected
0	No infection
1	up to 25
2	26-50
3	51-75
4	Above 75

$$\text{Disease Index (DI)} = \frac{\text{Total Numerical ratings}}{\text{No of leaves x Max No. of grades}} \times 100$$

The above disease indexing method could be utilized for comparison of disease intensity. However, LRD indexing may be refined by using more grades with narrow intervals of disease ratings and also an intergrated disease indexing has to be evolved for LRD and RWD.

Yield Loss

Information on the loss due to leaf rot disease alone is not available as it is generally associated with the RWD. However, Menon and Nair (1948) estimated the loss at Rs. 5.6 million annually. This is besides the loss in quality of the leaves rendering them unfit for thatching and other purposes. Applying the above criteria the loss due to leaf rot may be computed at present as 461 million nuts annually as the area affected by the disease in Kerala extends to 0.41 million hacteres (Anon., 1985 b).

Etiology

McRae (1916) and Sundararaman (1925) isolated a salamon coloured *Penicillium* like fungus from diseased leaves in Kochi. *Helminthosporium halodes* (= *Bipolaris halodes*) *Gleosporium* sp., *Curvularia* sp., *Gliocladium roseum, Pestalotia* sp., and *Fusarium* sp. were isolated from the diseased leaves (Menon and Nair, 1948, Anon, 1985). Srinivasan and Gunasekaran (1993, 1996 b, c) made isolations from over 500 infected spindles and revealed the association of a number of fungal species, and attributed LRD to a fungal complex. The fungi isolated were identified at CAB International Mycological Institute, United Kingdom as *Colletotrichum gloeosporiodes* (Penzig) Penzig and Sacc., *Exserohilum rostratum* (Drechsler) Leonard and Suggs, *Gliocladium vermoeseni* (Biourge) Thom. (Srinivasan and Gunasekaran, 1994a), *Cylindrocladium scoparium* Morgan (Srinivasan and Gunasekaran, 1995a), *Fusarium solani* Martius (Sacc.), *F. moniliforme* Sheldon var. *intermedium* Neish and Legget (Srinivasan and Gunasekaran, 1998a), *Thielaviopsis paradoxa* (Dade) C. Moreau, *Rhizoctonia solani* Kuhn, *Mortierella elongata* Linnem, *Curvularia* sp., *Acremonium* sp., *Thielavia microspora* Mouch, *T. terricola* (J. Gilman and E.V. Abbott) Emmons and *Chaetomium brasiliense* Batista and Pont. (Srinivasan and Gunasekaran, 1994b).

Studies on the pathogenicity of the fungi were initiated by Menon and Nair (1948, 1951). Inoculating bits of tender leaves and leaflets of mature leaves with spore suspensions of *H. halodes, Gloeosporium* sp., *G. roseum* and *Pestalotia* sp., *in vitro*, they found that *H. halodes* induced infection with in 12 hours and the rest in 48 hours. They considered *H. halodes* as the most virulent and the rest only as secondary parasites aggravating the rotting initiated by *H. halodes*. They confirmed these findings through *in vivo* tests and established the pathogenicity of *H. halodes* using single and mixed inocula. Later Radha and Lal (1968) also confirmed the infectivity of *B. halodes* on coconut. Culture filtrate of *H. halodes* when applied on tender leaves of coconut failed to demonstrate any toxic effect (Anon., 1981). The pathogenicity of *C. gloeosporioides* (Cg), *E. rostratum* (Er), *G. vermoeseni* (Gv), *F. solani* (Fs), *F. moniliforme* var, *intermedium* (Fn), *T. paradoxa* (Tp), *R. solani* (Rs), *M. elongata* (Me) and *Curvularia* sp. was established (Srinivasan and Gunasekaran 1994a, b, 1995a, 1996d, 1998a). The fungi associated with LRD were grouped

into three categories (Group A, B and C) based on the frequency, pattern and relative association with the disease (Anon., 1994).

In diseased spindle tissues, profuse mycelial growth and spore masses are common especially in between infected leaflets. Hence mycoflora of spindles and older leaves of 120 palms were observed by leaf scrapings as well as isolations. The fungi were present either independently or in association with other species. *C. gloeosporioides, E. rostratum, G. vermoeseni, F. solani, F. moniliforme* var. *intermedium* and *T. paradoxa* were frequently recorded. *Pestalotiopsis palmarum* was isolated from older leaves only (Srinivasan *et al.*, 1995). Isolations from LRD affected spindles with different disease grades has also brought out the predominant species of fungi irrespective of the degree of symptoms and *C. gloeosporioides* was more frequently observed under various grades (Table 31) (Srinivasan and Gunasekaran, 1996b). From several attempts of *in vitro* isolations, presence of fungi singly or co-occurrence in individual palms was noticed.

Table 31. Fungi isolated from leaf rot affected coconut spindles under different grades of disease symptom

Fungi isolated (independently or in combination)	No. of leaflet pieces which developed fungal growth (out of 50 pieces per grade)*					
	August			December		
	Grades of symptom			Grades of symptom		
	I	II	III	I	II	III
Colletorichum gloeosporioides	9(16)	10(14)	7(13)	12(20)	10(17)	7(15)
Exserohilum rostratum	4(9)	2(4)	2(6)	6(9)	4(9)	3(6)
Fusarium spp.	3(5)	3(5)	2(4)	5(12)	8(14)	8(13)
Gliocladium vermoeseni	2(2)	3(3)	1(1)	3(3)	4(4)	2(2)
Thielaviopsis paradoxa	-	2	1	-	-	2
Mortierella elongata	-	-	1	-	-	-
Rhizoctonia solani	-	-	-	-	1	2
Acremonium sp.	-	-	1	-	-	-
Cylindrocladium scoparium	-	-	-	-	-	1
C. gloeosporioides + *Fusarium* spp.	5	2	4	2	3	3
C. gloeosporioides + *Fusarium* spp.	2	2	2	6	4	5
E. rostratum + *Fusarium* spp.	-	-	-	1	2	-
Total No. of leaflet pieces which developed fungal growth	25	24	21	35	36	33
Percentage of fungal recovery	50	48	42	70	72	66

Chi-square value 2.23 (Not significant) 1.29 (Not significant)

* Figures within the parantheses are pooled No. of leaflet pieces for concerned perdominant fungi subjected for chi-square analysis.

Table 32. ***In vitro*** **interaction among the likely incitants of LRD**

Characters of fungal colony	Fungal combinations	Status of inhibiton reaction
Colonies merged	Cg x Er, Cg x Tp, Cg x Cs, Cg x Rs, Cg x Me, Er x Fx, Er x Fm, Er x Cs, Er x Rs, Er x Me, Gv x Tp, Fs x Cs, Fm x Cs, Tp x Me, Cs x Me	No inhibition discerned between colonies under dual cultures of the fungal combinations
Colonies close	Cg x Gv, Cg x Fs, Cg x Fm, Er x Gv, Er x Tp, Gv x Fs, Gv x Fm, Gv x Cs, Gv x Fs, Gv x Me, fs x Fm, Fs x Rs, Fm x Rs, Tp x Rs, Cs x Rs	Extremely mild, mild or moderate inhibition only noticed in specific combinations; in Fs x Rs and Fm x Rs dual cultures moderate inhibition of Rs by Fs/Fm evident; similarly Gv by Cs
Colonies apart	Fs x Tp, Fs x me, Fm x Tp, Fm x Me, Tp x Cs, Rs x Me	Strong inhibitions detected; Tp and Me strongly inhibited by Fs and Fm; similarly Tp by Cs and Me by Rs

In vitro interaction among selected nine species of fungi of LRD was studied by dual culturing. The interaction varied in respect of colony merger, over growing capacity and inhibition zone (Table 32). The behaviour of the predominant fungi was seen to be synergistic than antagonistic, implying etiological significance of disease complex (Srinivasan and Gunasekaran, 1995b).

In certain RWD endemic areas, leaves of middle whorl of palms suddenly become yellow evenwhile the central shoot (spindle) and leaves of outerwhorl appear normal. Lesions/spots appear in such leaves which coalesce leading to severe blighting of lamina. *C. gloeosporioides* was found to be the most commonly associated fungus (90%) and it is likely that the fungus gains access to yellowed leaves of RWD affected palms (Srinivasan and Gunasekaran, unpublished).

Pathogenic behaviour of predominant species of LRD was further elucidated in potted seedlings and field palms, through artificial inoculations. In healthy palms (free from RWD), LRD fungi induced only restricted lesions as compared to severe rotting in RWD palms (Srinivasan and Gunasekaran, 1996d). Based on the frequency of occurrence, seasonal relationship, pathogenicity etc. *C. gloeosporioides* and *E. rostratum* are considered as the main pathogens of LRD (Fig. 21 & 22). The disease symptoms were consistently reproduced in the spindles of RWD affected seedlings in field with the main pathogens, individually and in combination. The inoculations with pathogens developed early lesions which subsequently resulted in the severe rotting of tissues. The disease lesions were observed on lamina, leaf petioles, and mid-veins.

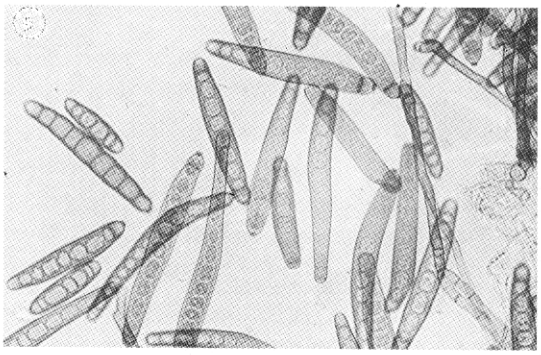

Fig 21. *E. rostratum*

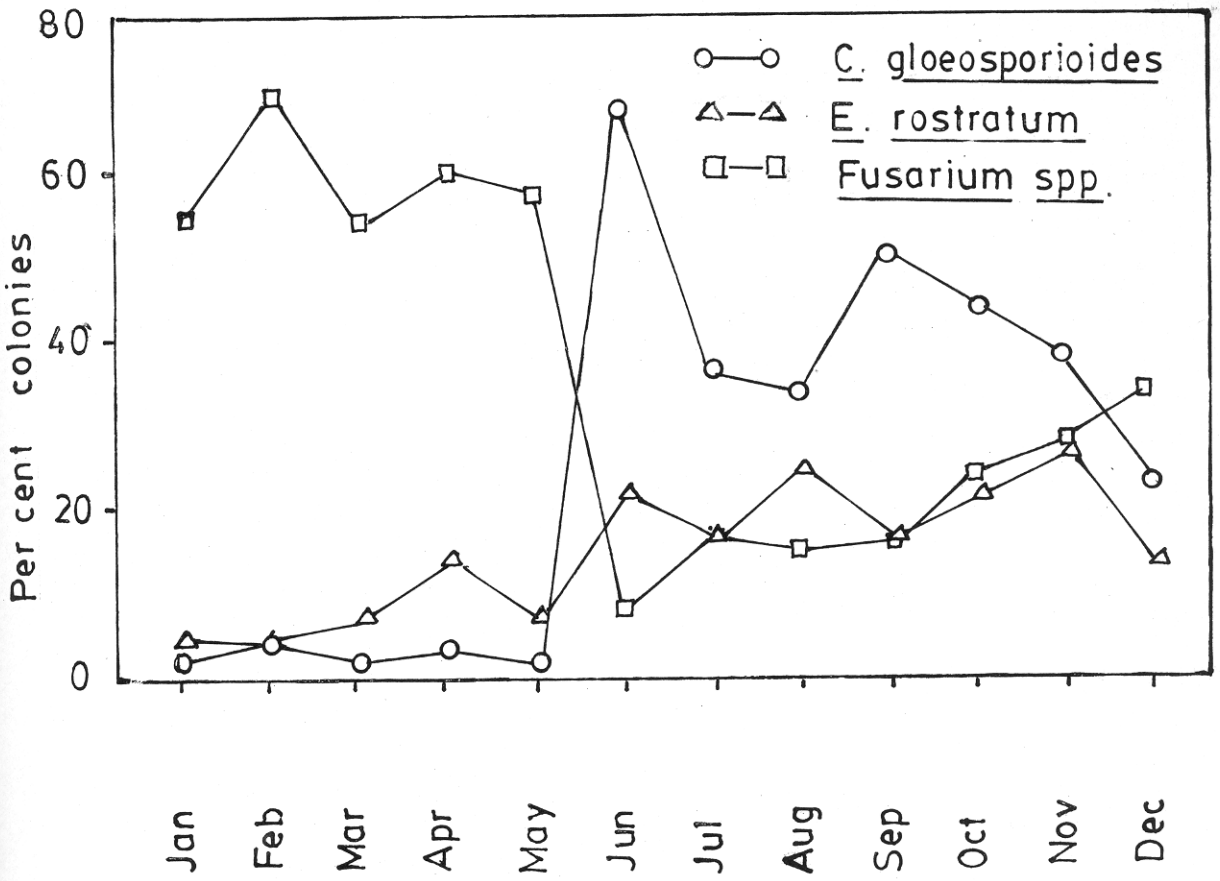

Fig 22. Seasonal variation in the isolation of major fungi from leaf rot affected samples

Symptoms were also noticed on spindles which emerged subsequent to the ones inoculated and thus the disease recurrence in successively emerging spindles was accomplished. Although LRD initially appears as a spot in spindle irrespective of the pathogen, later it results in rotting of tissues. A number of foliar diseases of coconut palms recorded world-wide causing leaf blight/leaf rot could be compared with LRD.

Epidemiology

Knowledge of dynamics of pathogen(s) is essential in view of the complex nature of the LRD. Leaf rot infection was found to be more severe during the seasons when atmospheric humidity was at its maximum (Menon and Nair 1951). Severity of leaf infection with *H. halodes*, *Gloeosporium* sp. and *G. roseum* was found correlated with high humidity and low temperatures prevalent during the monsoon period (Radha *et al.*, 1961). Monthly records of observations for 3 years revealed that period of high atmospheric humidity and low temperatures

are favourable for natural development of leaf rot disease (Radha and Lal 1968). The population dynamics of LRD pathogens in relation to weather variables was recorded by sequential monthly isolations from diseased palms (spindles) (Srinivasan and Gunasekaran, 1996c). The incidence of *C. gloeosporioides* was conspicuously higher in frequency and population during monsoons with a peak in June/July (Fig. 23). Its incidence was most strongly correlated with rainfall and relative humidity and negatively with maximum temperature and hours of sunshine. It was this fungus isolated from early lesions more frequently than advanced lesions (Fig. 24). Therefore, *C. gloeosporioides* was implicated as the principal pathogen of LRD during monsoons.

Incidence of *E. rostratum* was less frequent, erratic and its population was less strongly/consistently correlated with weather. In winter with low humidity and temperature, *C. gloeosporioides* incidence was subdued while that of *E. rostratum* was moderate. *Fusarium* spp. were isolated most commonly during the dry season of January - May (Fig. 22). Dry conditions

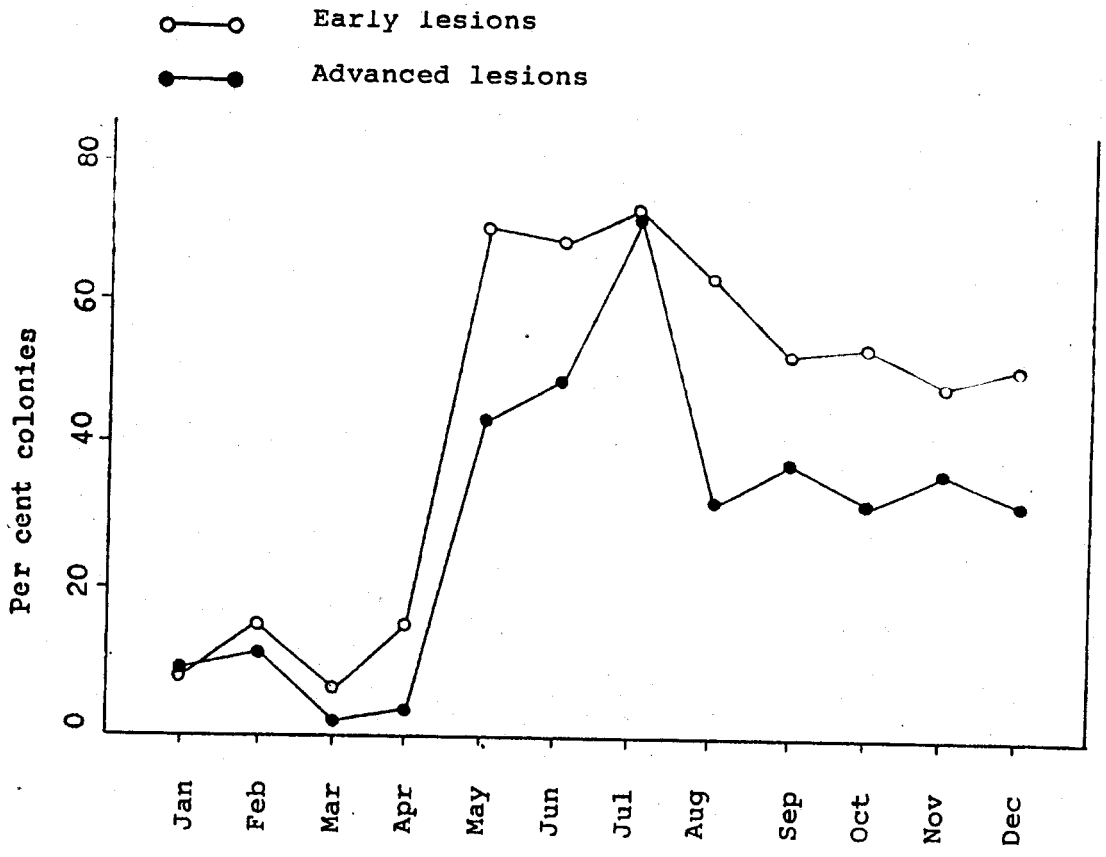

Fig 23. Isolation *C. gloesporioides* from early and advanced lesions during different seasons

generally favoured *R. solani* also. Fusarial presence throughout the year and their predominance in the dry period point out that such fungi could be potential pathogens, co-occurring with other fungi and perpetuating the disease in the dry period. They may also play a role in predisposing the palms to subsequent infection by other pathogens in favourable conditions. The incidence of other fungi was not influenced by specific weather conditions, but they may play some role in the disease in certain circumstances. The low incidence of *C. gloeosporioides* in dry season might be due to its quiescent phase. Aggressive re-emergence of the fungus with the onset of South West monsoon (with favourable weather) was linked to the severity of LRD during monsoons.

From LRD infected young palms also the incidence of *C. gloeosporioides* during the period (June-December) was consistent and significantly higher than *E. rostratum*. Hence aggressiveness of *C. gloeosporioides* in LRD during monsoon months (principal pathogen) was further ascertained (Anon, 1996). Though *E. rostratum* can vigorously induce LRD upon artificial inoculation, in nature *C. gloeosporioides* is distinctly active in the disease during wet season.

Disease Management

Control of LRD is considered important in view of its destructive potential to the palms. *In vitro* screening of fungicides against the pathogens of LRD and control studies were undertaken.

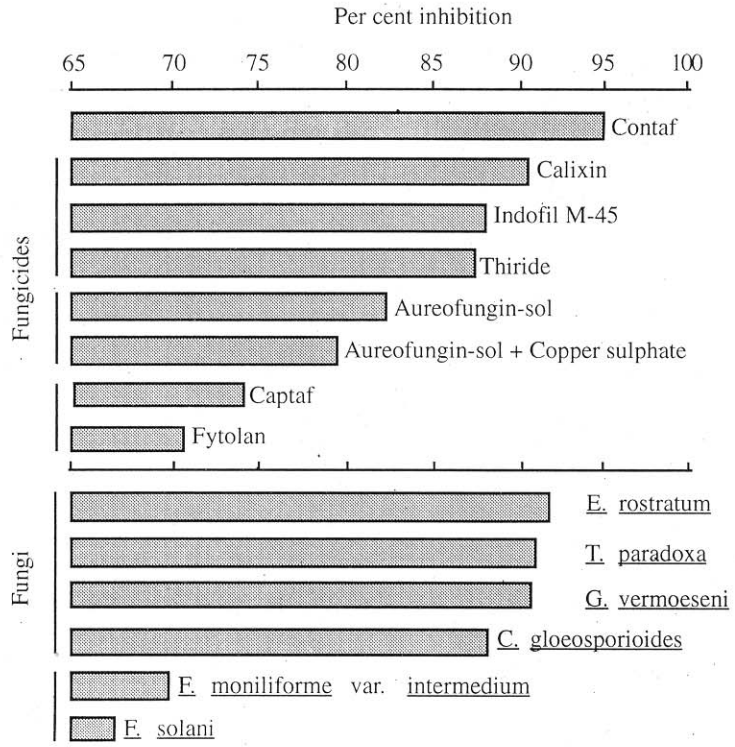

Fig 24. *In vitro* inhibition of major leaf rot fungi by different fungicides

In vitro assay

In vitro studies on the effect of different copper fungicides on the mycelial growth and spore germination of *H. halodes* showed that Bordeaux mixture (0.3%) completely inhibited the mycelial growth. (Prasannakumari *et al.* 1960). Assay of a few contact (Indofil M-45, Fytolan, Captan and Thiram) and systemic fungicides (Contaf, Calixin, Aureofungin -Sol) was done on solid medium against *C. gloeosporioides, E. rostratum* etc. by poisoned food technique (Srinivasan and Gunasekaran, 1998b). Varying degrees of inhibition of fungi by fungicides was observed. Contaf (hexaconazole) exhibited a broad-spectrum activity inhibiting all the pathogens of LRD (Fig. 24).

Field control

Attempts at controlling leaf rot disease were started as early as in 1951. Menon and Nair (1952) conducted spraying in the field using 1.0% and 0.5% of bordeaux mixture against leaf rot. Half strength of bordeaux mixture was almost as good as full strength. In a fungicial field trial using copper fungicides it was observed that Bordeaux mixture reduced the intensity by 74.5% followed by Kirti copper 65.6% and Fytolan 59.6% (Anon., 1963). Gregory (1960) felt that aerial spraying of oil based copper fungicide in Vadayar and Malankara estates was useful in controlling leaf rot. However, Samraj *et al.* (1966) observed that spray fluid did not adequately protect the spindle leaf. George and Samraj (1966). reported that coconut palms affected by leaf rot responded favourably by foliar application of boron, suggesting boron deficiency as the factor responsible for development of disease. Prophylactic basal application of systemic fungicides (Actidione, Bavistin, Benlate and MBC) at the rate of 4g per healthy palm twice a year failed to prevent incidence of leaf rot. (Anon., 1983). Sequential spraying of Bordeaux mixture 1%, Fytolan, 0.3% and Dithane M-45 0.3% on leaf rot affected palms in farmer's gardens resulted in control of the disease (Anon. 1985).

A field control trial on 20 year old infected palms was conducted. Treatments were imposed thrice a year by contact (Indofil M-45 0.3%, Fytolan 0.5%) and systemic fungicides (Calixin 1%). This was continued for three years. The method involved a simple method of pouring the chemical into axil around the spindle, besides spraying on to the crown and root feeding (Srinivasan and Gunasekaran, 1998a). Pouring of calixin and spraying of Indofil M-45 were beneficial as the LRD intensity reduced in newly emerged leaves (Fig. 25). Although the overall impact of the treatments in reducing the disease indices was moderate, the results pointed out the necessity of using a broadspectrum potential fungicide in LRD management and protecting the tender leaves, specially the spindles from infection.

Another field trial initiated in 1986 revealed higher level of disease suppression by Phystosanitation plus pouring of Contaf into leaf axil.

Biocontrol

Studies on biological control of LRD was also initiated. Antagonistic activity of an isolate of *Pseudomonas fluorescens* (TNAU, Coimbatore isolate) against *C.*

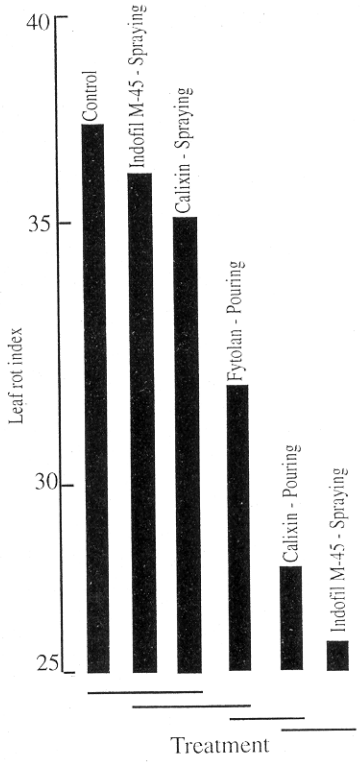

Fig 25. Effect of different fungicides on leaf rot disease

gloeosporioides E. rostratum etc. was tested *in vitro*. The growing bacterial culture as well as its culture filtrate were found to be inhibitory to the growth of all the fungi at different levels. Application of this bacterium with the inoculum of the main pathogens of LRD under *in vivo* conditions reduced the leaf rot onset/lesions development.

In order to search for native antagonistic bacteria, isolations were made from coconut phylloplane and rhizophere and 96 and 21 isolates from these zones respectively were purified and screened against the main pathogens of LRD (Anon, 1997). Assessment of these isolates lead to the detection of two isolates each from phylloplane and rhizosphere as effective native antagonists against both the pathogens.

Radha (1961) observed that Andaman Ordinary and New Guinea were more resistant than other varieties tested against leaf rot disease.

Occurrence of LRD restricted to the RWD endemic region, points to the interrelationship of these diseases. The control of LRD becomes significant because of vulnerability of RWD infected palms to fungal infection. A management system integrating phytosanitation (judicious pruning of infected spindle and a few leaves close to it from the initial stage of disease onset itself), and use of broad-spectrum systemic fungicide, biological agents etc. are of paramount importance (Srinivasan and Gunasekaran, 1998c).

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