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IDENTIFICATION OF *COLLETOTRICHUM GLOEOSPORIOIDES, EXSEROHILUM ROSTRATUM* AND *GLIOCLADIUM VERMOESENI* ASSOCIATED WITH LEAF ROT DISEASE OF COCONUT IN INDIA

By

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SUMMARY

Leaf rot is a common disease predominantly superimposed on root (wilt) affected coconut palms in India. Three fungi implicated in the disease have been found to be *Colletotrichum gloeosporioides, Exserohilum Yostratum and Gliocladium vermoeseni*. The cultural characters and morphological features of these fungi have been described. The pathogenic role of these fungi has been confirmed. The three fungi independently induced symptoms leading to leaf rot. *E. rostratum* developed the disease symptom relatively faster. Pin prick injury in leaf pieces generally favoured the fungi in infection and had pronounced effect for *G. vermoeseni*.

INTRODUCTION

Coconut leaf rot disease is rampant and generally superimposed on root (wilt) affected palms in southern districts of Kerala State, India. It appears on palms of all ages and intensity increase with age of the palms and advancement of root (wilt) disease (Radha and Lal, 1968; Srinivasan, 1991). The disease is important as it brings about rapid deterioration of affected

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palms. The annual yield loss due to leaf rot is estimated to be around 461 million nuts (Nair *et al.*, 1991). The disease in the early phase appears in spindles as tiny, brown, necrotic, angular and sunken lesions which enlarge, coalesce and develop into severe rotting of tender tissues (Joseph and Rawther, 1991; Srinivasan and Gunasekaran, 1992).

Fungal etiological nature of the leaf rot has been attributed. McRae (1916) was the earliest to isolate a fungus from leaf rot diseased tissues. Menon and Nair (1951) isolated *Helminthosporium halodes, Gloeosporium* sp., *Gliocladium roseum* and *Fusarium* sp. from the diseased tissues and found that except *Fusarium* sp. others were pathogenic. *H. halodes* was found to be the most virulent fungus, others being secondary invaders. Subsequent reports centred especially on *H. Hlodes (= Bipolaris halodes)* in the causal relationship with leaf rot disease (Radha and Lal, 1968; Anonymous, 1979; Lily, 1981). Since the isolations were done only from a few palms, it was felt that isolations may be done from a large number of palms in different seasons (Srinivasan and Gunasekaran, 1993). Moreover, the need for studying the role of each fungus in the disease incidence, the taxonomical and cultural characters of the fungi involved was felt. The results emanated from such a study is presented in this paper.

MATERIALS AND METHODS

Isolation and identification

Leaflets showing very early symptoms in affected spindles were cut into small bits (0.5 cm), surface sterilized with 0.1% mercuric chloride solution, rinsed in three sequential changes of sterile distilled water and plated onto Potato Dextrose Agar (PDA) in petriplates and incubated at 30 + 1^{o}C and observed for fungal growth. Leaf samples from different palms were used for isolation during different months. The fungi were subcultured, purified and grouped based on their

apparent cultural characters. Representative cultures of these fungi were sent to CAB-International Mycological Institute, England for identification.

Pathogenicity

Pathogenicity was tested using pieces of detached leaflet or whole leaflet taken from healthy spindles. The leaflets were cleaned with sterile distilled water and used for inoculation as follows:

1. *Leaflet pieces*: leaflet pieces of approximately 70 mm length were cut and placed in sterile petriplates (90 mm dia.) lined inside with moist filter paper. The leaflet pieces were inoculated by placing drops of inoculum containing spore suspension (approx. $4x10^5$ spores/ml) either after pin prick injury or without injury separately at four locations on each leaflet piece. Ten such leaflet pieces were used for inoculation by each fungus. Controls were maintained by placing drops of sterile distilled water. The petriplates were incubated at room temperature (approx. 30^oC) and development of disease symptoms monitored at 24 hr. intervals.

2. *Detached leaflets*: Individual healthy leaflet detached from spindle was placed in test-tube (150 x 25 mm) containing sterile distilled water with the upper portion of the leaflet projecting outside the test tube. The mouth of the tube was closed with sterile cotton wool to keep the leaflet in position. Thirty leaflets were spray inoculated per fungus. Mixed inoculum of all the three fungi was also used for spray-inoculation. Leaflets sprayed with sterile distilled water served as control. The tubes with leaflets were incubated inside bell jars at room temperature and symptom development monitored at 24 hr. intervals.

Reisolations of fungi involved were attempted when symptom development was noticed.

RESULTS AND DISCUSSION

Three fungi found predominant in the isolations were identified as *Colletotrichum gloeosporioides* (Penzig) Penzig & Sacc., *Exserohilum rostratum* (Drechsler) Leonard & Suggs and *Gliocladium vermoeseni* (Biourge) Thom. by IMI (IMI 349539, IMI 349538 and IMI 349540).

A. Cultural characters

1. *Colletotrichum gloeosporioides* (Penzig) & Sacc.

Colonies on PDA fast growing, greyish white with abundant aerial mycelium (Fig.1) and colony reverse bluish black. Growth was seen in concentric rings. The fungus consistently developed fructifications, acervuli in the medium with dark brown long setae. Conidia

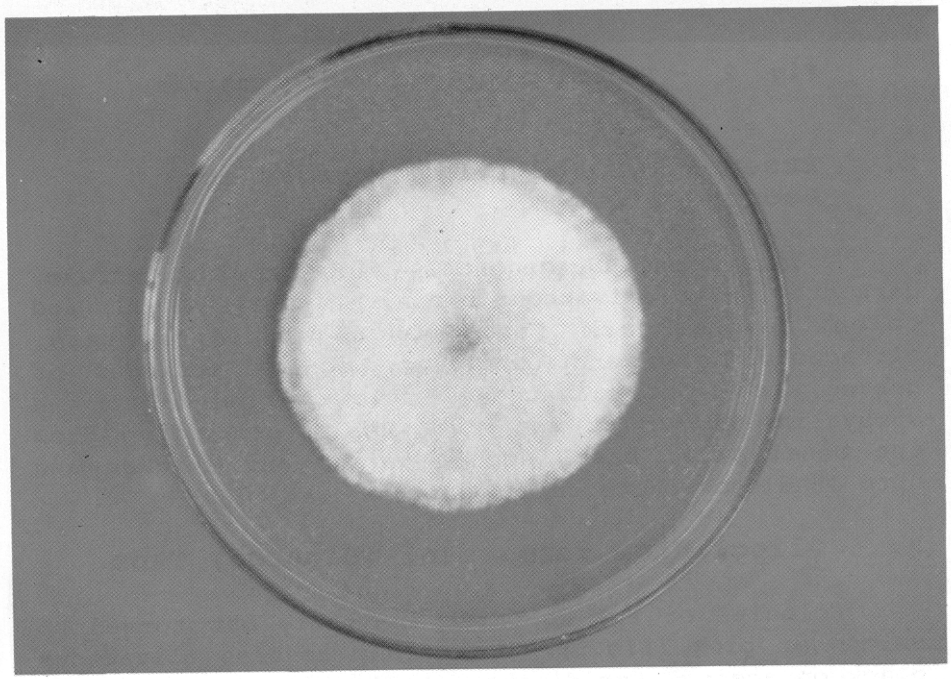

Fig. 1 - *Colletotrichum gloeosporioides* - culture

hyaline, aseptate, straight to slightly bent, slightly narrower in the middle and abtuse at the apices (Fig.2).

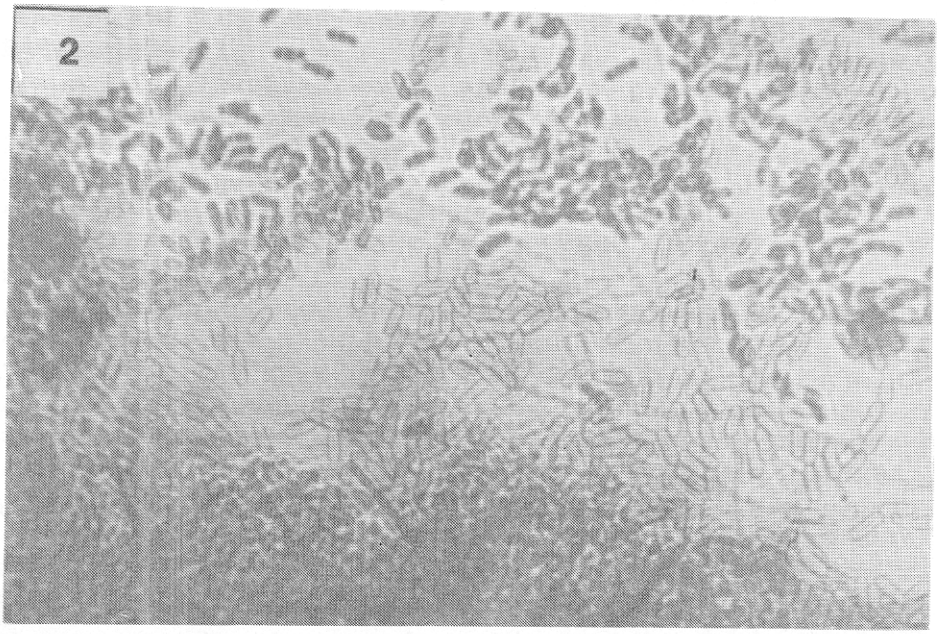

Fig. 2 - Photomicrograph showing *conidia*

2. Exserohilum rostratum (Drechsler) Leonard & Suggs

Colonies on PDA generally slow growing, effuse, dark greyish to olivaceous brown, velvetty (Fig.3) and colony reverse dark. Conidiophore simple, septate, olivaceous brown and geniculate conida straight or curved, brown to olivaceous, thick walled except in subhyaline region at the apex, hilum protruding from the basel cell, basal septum darker and thicker and upto 18 distoseptate (Fig.4).

3. Gliocladium vermoeseni (Biourge) Thom

Colonies on PDA whittish initially but turned to salmon or pink (Fig.5). The colony reverse olivaceous black on ageing. Conidiophore hyaline, septate, simple

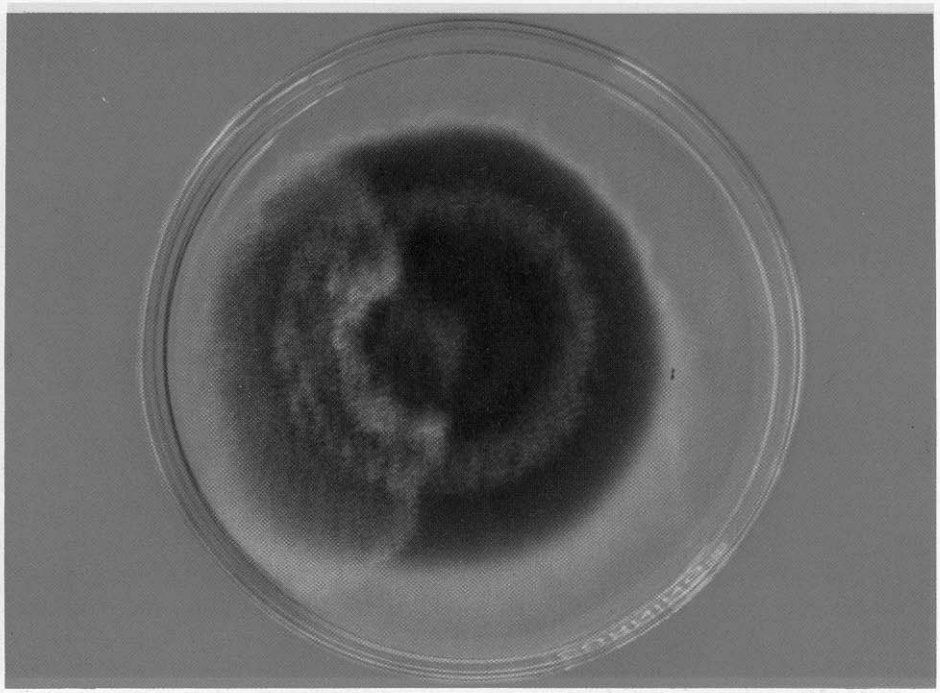

Fig. 3 *Exserohilum rostratum* culture

Fig. 4 Photomicrograph showing *conidiophores* and *conidia*

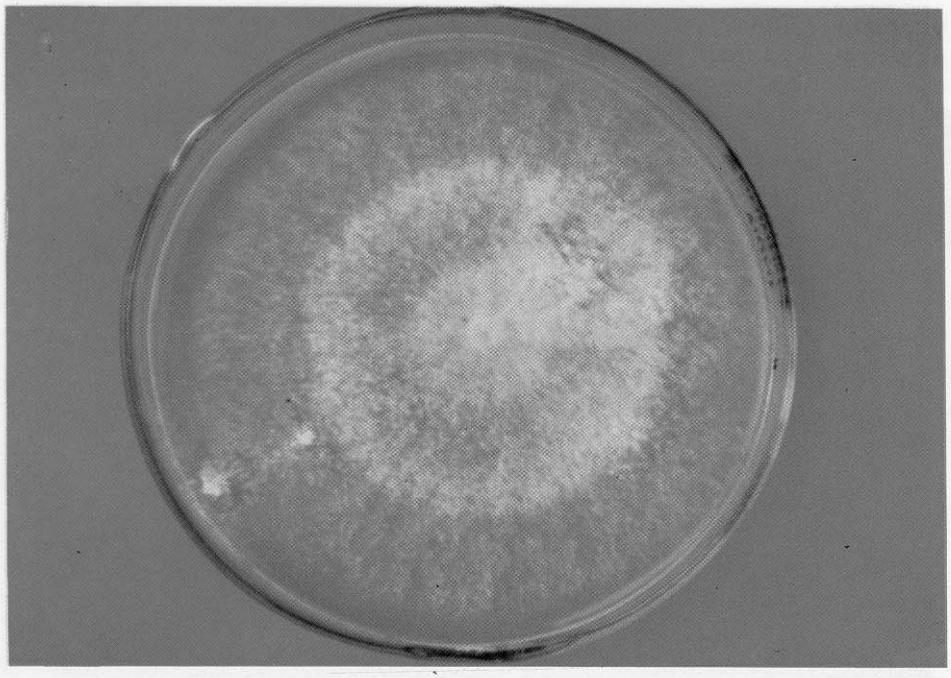

Fig. 5 *Gliocladium vermoeseni* - culture

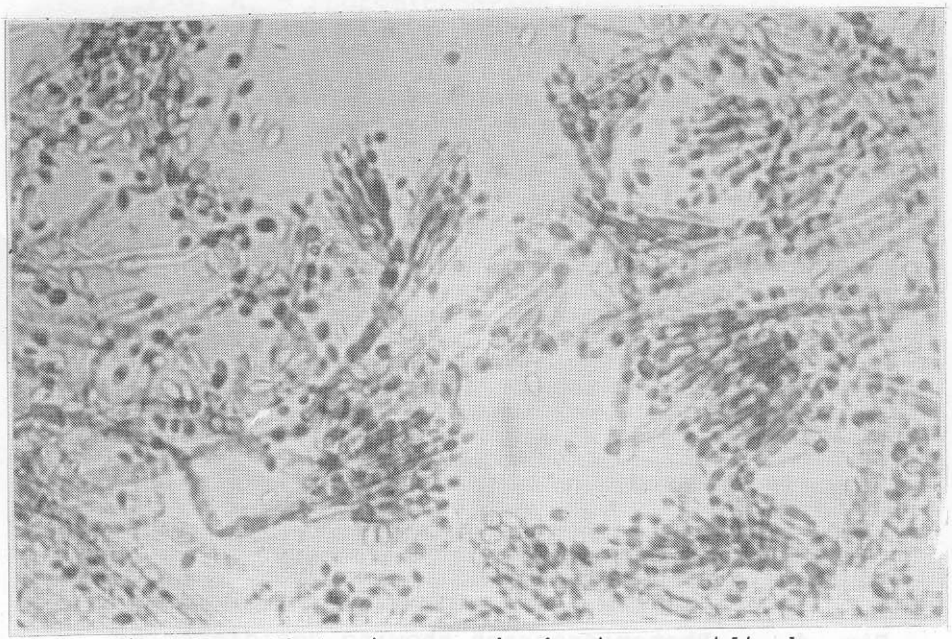

Fig. 6 Photomicrograph showing *conidiophores* and *conidia*

or penicillately branched terminating on phialids. Conidia hyaline, unicellular, oval to elliptical and pinkish or rose in masses (Fig.6).

B. Pathogenicity

The results of pathogenicity tests are presented in Tables 1 and 2.

All the three fungi viz., *C. gloeosporioides*, *E. rostratum* and *G. vermoeseni* induced 100% infection in leaflet pieces when inoculated with pin prick injury. The percentage recovery of the fungi from the lesions was 75,80 and 50 respectively (Table 1). When inoculation was done without pin prick the percentage infection was 40, 60 and 30 only. In the case of detached whole leaflets, the respective percentage of infection was 86.7, 96.7 and 66.7 while it was 100% with mixed inoculum (Table 2).

In the leaflet pieces inoculated with *C. gloeosporioides* the symptom appeared as necrotic lesions which soon expanded and tissue rotten (Fig.7).

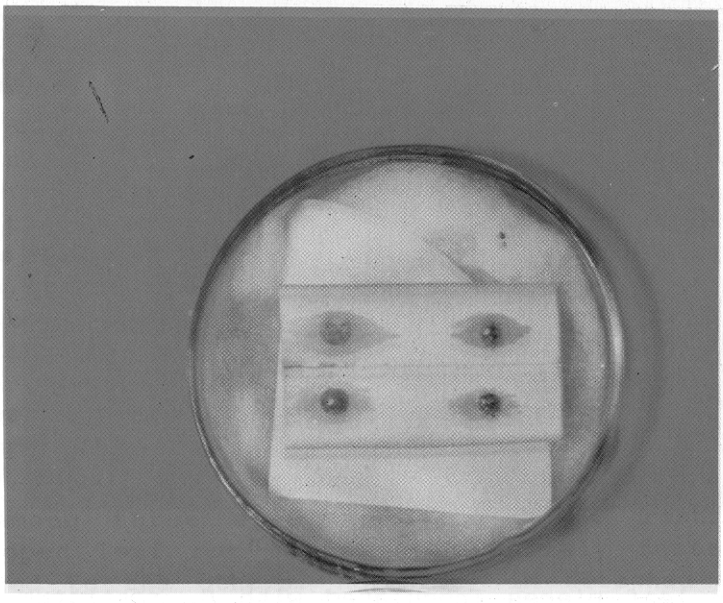

Fig. 7 Symptoms in leaflet pieces and detached leaflets (extreme leaf-healthy)

In detached leaflets the symptom appeared as minute, dark brown to greyish black, angular to irregular lesions. The early lesions appeared irrespective of margins, tips and intermittent areas of leaflets that coalesced to form large necrotic areas and leaf tissue rotten within 5 days (Fig.8).

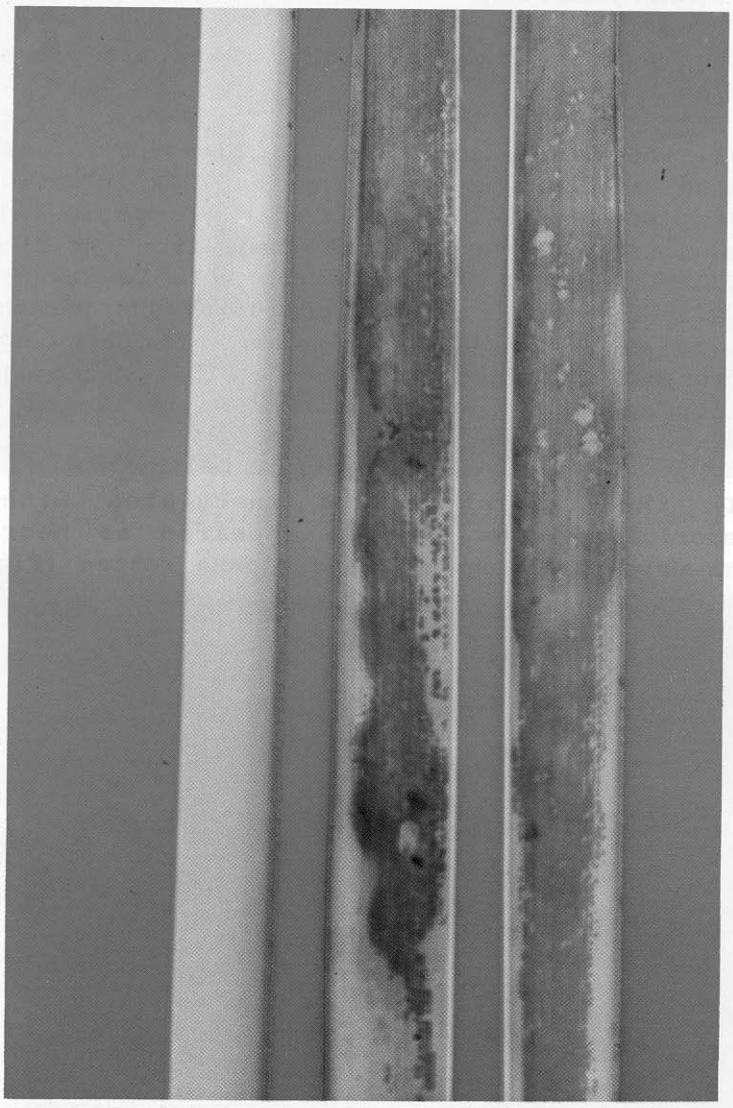

Fig. 8 Colletotrichum

In leaf pieces inoculated with *E. rostratum*, the fungus induced distinct necrotic lesions at points of inoculation. The lesions expanded faster resulting into

extensive rotting of tissues (Fig.9), In detached leaflets, the early symptom appeared as tiny, water soaked lesions turning dark brown to black, elliptical to oval spots with a halo around and orr-white area at the centre. The lesions readily coalesced leading to extensive rotting of leaflets within 5 days after inoculation (Fig.10). *E. rostratum* is distributed in different countries and in India its association with graminicolous (Sivanesan, 1987) and other hosts (Mohanan and Sharma, 1986) has been reported.

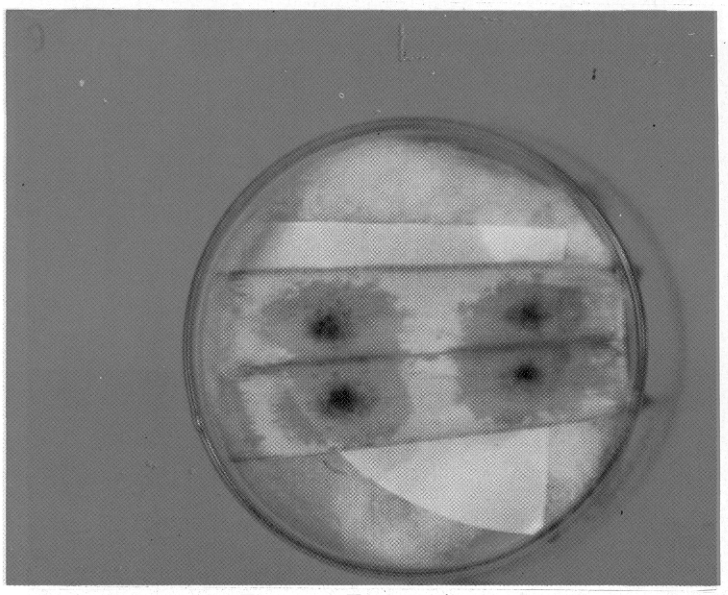

Fig. 9 Exserohilum

In leaflet pieces inoculated with *G. vermoeseni* the necrosis appeared at inoculated spots expanded to cause tissue rotting (Fig.11). In detached leaflets the fungus induced early symptoms as minute, necrotic specks, turned to circular to oval, brown spots with light brown margins. The lesions expanded and coalesced resulting in rotting of tissues (Fig.12). Reynolds (1964) reported *Gliocladium* disease of palm in Florida. Pathogenic association of *G. vermoeseni* with several palm species has also been reported by Atilango *et al.*, (1980), Hodel (1985) and Hahn and Nussbaum (1986).

Among three species of fungi *E. rostratum* developed leaf rot symptoms relatively faster as compared to the others. Moreover, in detached whole leaflets inoculated with spray inoculation of spores, maximum infection (96.7%) was obtained with *E.*

rostratum. When the mixed inoculum of the three fungi was inoculated into leaflets, the infection was cent per cent. In reisolations from these infected leaflets, out of 60 leaf bits *C. gloeosporioides* was recovered from 40 bits (70%) and *E. rostratum* from 36 bits (60%), either singly or in combinations. *G. Vermoeseni* could not be reisolated (Table 2). The results pointed out predominance of *E. rostratum* and *C. gloeosporioides* over *G. Vermoeseni*. The frequency of symptom development by *G. vermoeseni* in leaflet pieces without

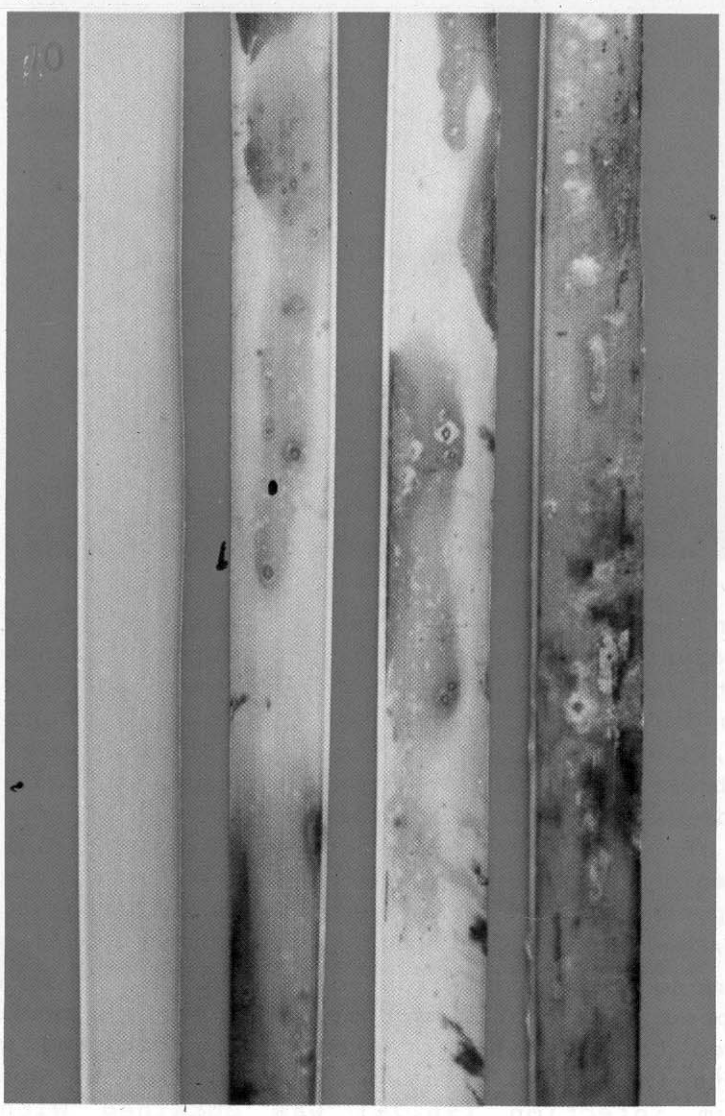

Fig. 10 Exserohilum rostratum

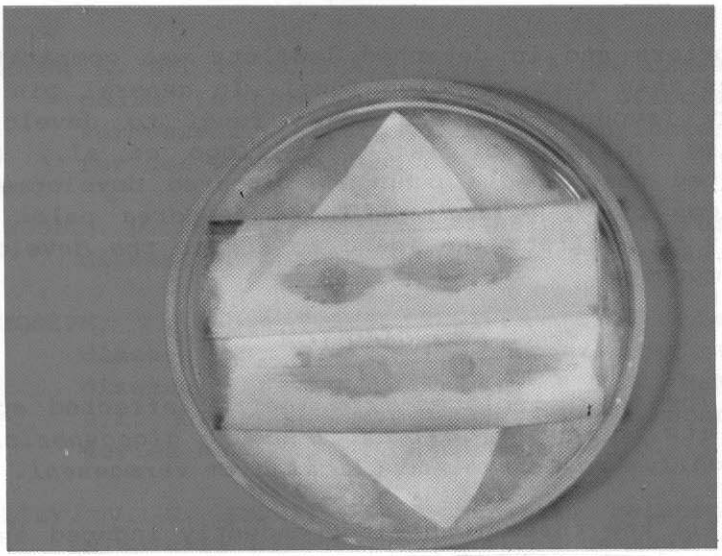

Fig. 11 Gliocladium

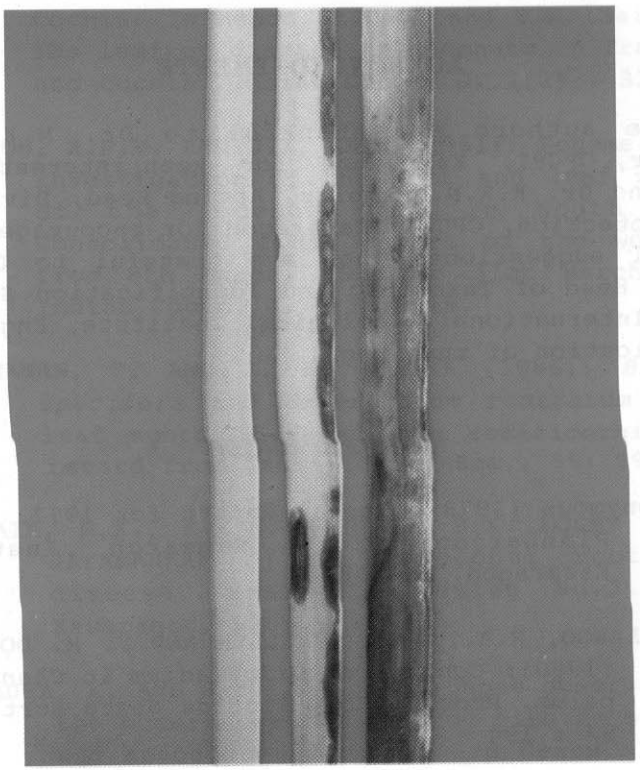

Fig. 12 Gliocladium vermoeseni

pin pricks and in detached leaflets was comparatively lesser than that by other fungi. In general pin prick injury favoured all the three fungi to develop the disease in leaflet pieces. Atilango *et al.*, (1980) reported that needle puncture favoured development of symptoms by *G. vermoeseni* in *Chanaedorea* palms since injury to leaflets was found to aid in the development of symptoms.

CONCLUSION

1. The fungi isolated from leafrot affected spindle leaflets are *Colletotrichum gloeosporioides*, *Exserohilum rostratum* and *Gliocladium vermoeseni*.

2. All the three fungi independently induced initial symptom leading to leaf rot. *E. rostratum* generally develop the disease vigorously and *G. vermoeseni* comparatively slower.

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Table 1. Development of leaf rot in leaflet pieces of coconut spindle leaf by different fungi and reisolations

S. No.	Fungi	Without pinprick inoculation											
		No. of leaflet pieces			Reisolations: No. of Leaflets bits			No. of leaf pieces					
		Inoculated	Developed	%	Inoculated into medium	Established fungal growth	%	Inoculated	Developed symptoms	%	Inoculated into medium	Established fungal growth	%
1.	Colletotrichum gloeosporioides	10	4	40	20	15	75	10	10	100	20	15	75
2.	Exserohilum restratum	10	6	60	20	14	70	10	10	100	20	16	80
3.	Gliocladium vermoeseni	10	3	30	20	8	40	10	10	100	20	10	50
4.	Control	10	0	0	-	-	-	10	0	0	-	-	-

Table 2. Development of leaf rot in detached leaflets of coconut spindle by different fungi and reisolations

S. No.	Fungi	No. of leaflets Inoculated	Developed symptom	%	Reisolation No. of leaflet bits Inoculated into medium	Established fungal growth	%
1.	Colletotrichum gloeosorioides(C)	30	26	86.67	70	50	71.43
2.	Exserohilum rostratum(E)	30	29	96.67	85	69	81.18
3.	Gliocladium vermoeseni(G)	30	20	66.67	40	25	62.50
4.	Mixedinoculum of all the above fungi	30	30	100.00	60	60	100.00
5.	Control	30	0	-	-	-	-