CHAPTER 4

RESULTS

4.1 Endophytes of mature teak leaves

Endophytes were isolated from mature teak leaves collected at Chulalongkorn University during January-December 1999. The total number of isolates obtained and the total range of species identified, together with their isolation frequency, are given in Table 4.1, Figure 4.1. A total of 9 genera and 25 species of fungi were identified during the year. Unidentified isolates were recorded as mycelia sterilia since they failed to develop either sexual or asexual structures or spores. Species of *Phomopsis* followed by members of the family Xylariaceae and mycelia sterilia were the most frequently isolated fungi during the year. There were, however, differences in the species isolated and, in particular, in the frequency of isolation, between the months.

In January-February 113 fungal endophytes belonging to 4 genera and representing 8 species were isolated. Species of *Phomopsis* (52.18%) followed by mycelia sterilia (30%) were the most frequently isolated fungi (Table 4.2, Figure 4.2).

In March-April, out of 74 fungal isolates, 2 genera and 3 species were recorded. *Phomopsis* was still dominant (43.24%). *Penicillium* sp. (27.02%) was the next most frequently isolated taxon followed by unknown no. 5. (16.21%) (Table 4.3, Figure 4.3).

In May-June period 102 fungal isolated were obtained and these were assigned to 4 genera and 9 species. *Phomopsis* spp., *Xylaria* spp. and mycelia sterilia represented 31.36%, 24.5% and 17.64% of the isolates, respectively (Table 4.4, Figure 4.4).

In July-August of the 107 fungal isolates obtained 5 genera and 13 species were recognised. *Xylaria* at 36.41% was the dominant genus followed by species of *Phomopsis* (24.28%) and then mycelia sterilia (11.21%) (Table 4.5, Figure 4.5).

In September-October, from the 121 fungal isolates obtained, 6 genera and 10 species were recorded. The most frequently recovered endophytic genus was

Phomopsis (47.91%). Others found were *Xylaria* (12.39%), *Colletotrichum* (9.91%) and the unknown no. 5 (9.91%) (Table 4.6, Figure 4.6).

In November-December, 5 genera consisting of a total of 7 species were recorded. A total of 122 fungal isolates were obtained make this the highest recovery for the 6 periods sampled. *Phomopsis* species at (38.51%), unknown no. 5 (16.39%), a *Daldinia* species (14.75%) and a *Colletotrichum* species (9.01%) were the most isolated endophytes (Table 4.7, Figure 4.7).

Table 4.1 Endophytic fungi from mature leaves of teak during January-December 1999

Species	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sep-Oct	Nov-Dec
Alternaria sp.	8	0	0	0	3	11
Colletotrichum sp. T1	7	0	10	10	12	16
Daldinia eschscholzii	0	0	0	0	0	18
Fusarium sp.	0	0	0	0	5	0
Nigrospora sp.	9	0	5	4	0	6
Penicillium sp.	0	20	0	0	0	0
Phomopsis sp. T1	21	15	0	10	20	9
Phomopsis sp. T2	0	0	15	12	24	24
Phomopsis sp. T3	7	0	6	4	10	0
Phomopsis sp. T4	13	17	0	0	0	14
Phomopsis sp. T5	0	0	11	0	0	0
Phomopsis sp. T6	5	0	0	0	0	0
Phomopsis sp. T7	9	0	0	0	4	0
Schizophyllum commune	0	0	0	6	5	0
Xylaria sp. 1	0	0	0	6	0	0
Xylaria sp. 2	0	0	0	5	0	0
Xylaria sp. 3	0	0	7	0	0	0
Xylaria sp. 4	0	0	0	0	10	0
Xylaria sp. 5	0	0	8	0	0	0
Xylaria sp. 6	0	0	0	0	5	0
Xylaria sp. 7	0	0	5	4	0	0
Xylaria sp. 8	0	0	0	5	0	0
Xylaria sp. 9	0	0	0	8	0	0
Xylaria sp. 10	0	0	0	3	0	0
Xylaria sp. 11	0	0	5	8	0	0
Mycelia sterilia	30	10	18	12	8	4
Unknown no. 3	0	0	4	0	0	0
Unknown no. 4	4	0	0	0	3	0
Unknown no. 5	0	12	8	10	12	20
total isolates	*113*	*74*	*102*	*107*	*121*	*122*

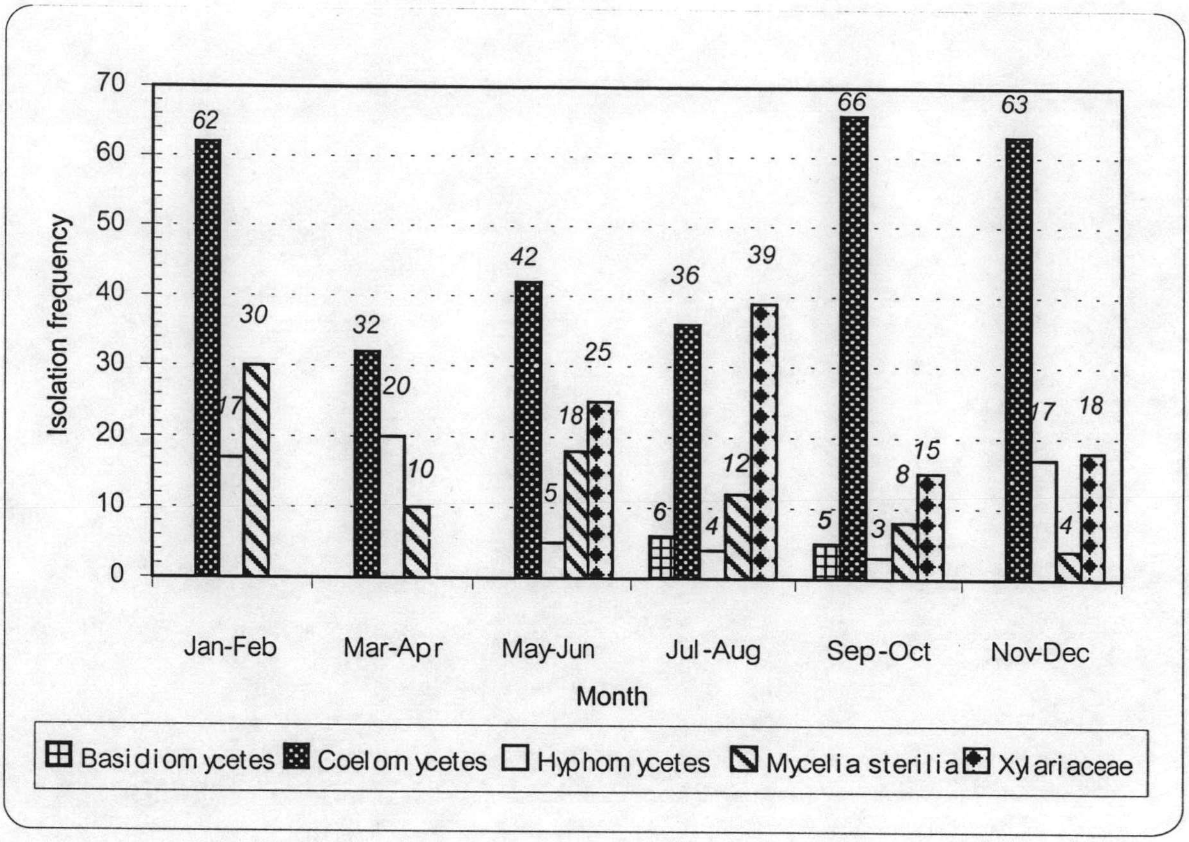

Figure 4.1 Endophytic fungi from mature teak leaves during January-December 1999.

Table 4.2 Endophytic fungi from mature teak leaves in January-February 1999

Species	Isolation frequency	Percentage	Species abbreviation
Alternaria sp.	8	7.07	*A*
Colletotrichum sp. T1	7	6.19	*B*
Nigrospora sp.	9	7.96	*C*
Phomopsis sp. T1	21	18.58	*D*
Phomopsis sp. T3	7	6.19	*E*
Phomopsis sp. T4	13	11.5	*F*
Phomopsis sp. T6	5	4.42	*G*
Phomopsis sp. T7	9	7.96	*H*
Mycelia sterilia	30	26.54	*I*
Unknown no. 4	4	3.53	*J*
Total isolates	*113*	*100*	

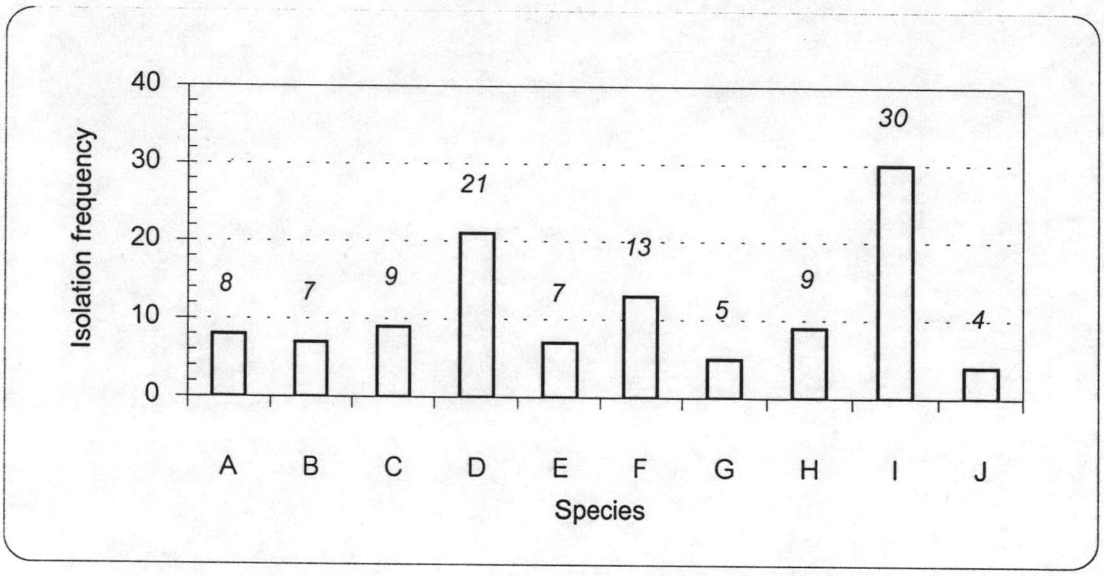

Figure 4.2 Endophytic fungi from mature teak leaves during January-February 1999

Table 4.3 Endophytic fungi from mature teak leaves during March-April 1999

Species	Isolation frequency	Percentage	Species abbreviation
Penicillium sp.	20	27.02	*A*
Phomopsis sp. T1	15	20.27	*B*
Phomopsis sp. T4	17	22.97	*C*
Mycelia sterilia	10	13.51	*D*
Unknown no. 5	12	16.21	*E*
Total isolates	*74*	*100*	

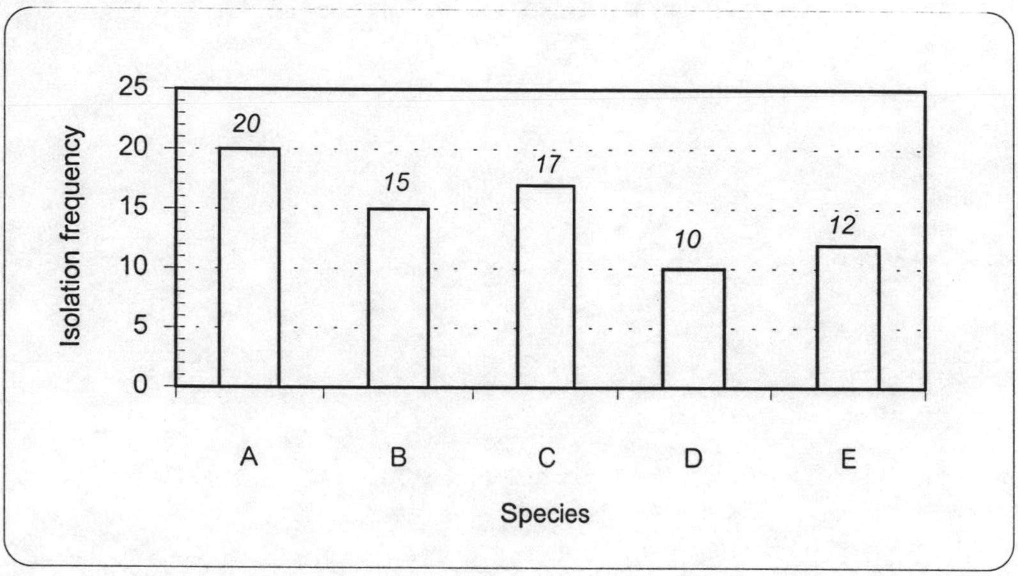

Figure 4.3 Endophytic fungi from mature teak leaves during March-April 1999

Table 4.4 Endophytic fungi from mature teak leaves during May-June 1999

Species	Isolation frequency	Percentage	Species abbreviation
Colletotrichum sp. T1	10	9.8	*A*
Nigrospora sp.	5	4.9	*B*
Phomopsis sp. T2	15	14.7	*C*
Phomopsis sp. T3	6	5.88	*D*
Phomopsis sp. T5	11	10.78	*E*
Xylaria sp. 3	7	6.86	*F*
Xylaria sp. 5	8	7.84	*G*
Xylaria sp. 7	5	4.9	*H*
Xylaria sp. 11	5	4.9	*I*
Mycelia sterilia	18	17.64	*J*
Unknown no.3	4	3.92	*K*
Unknown no.5	8	7.84	*L*
Total isolates	*102*	*100*	

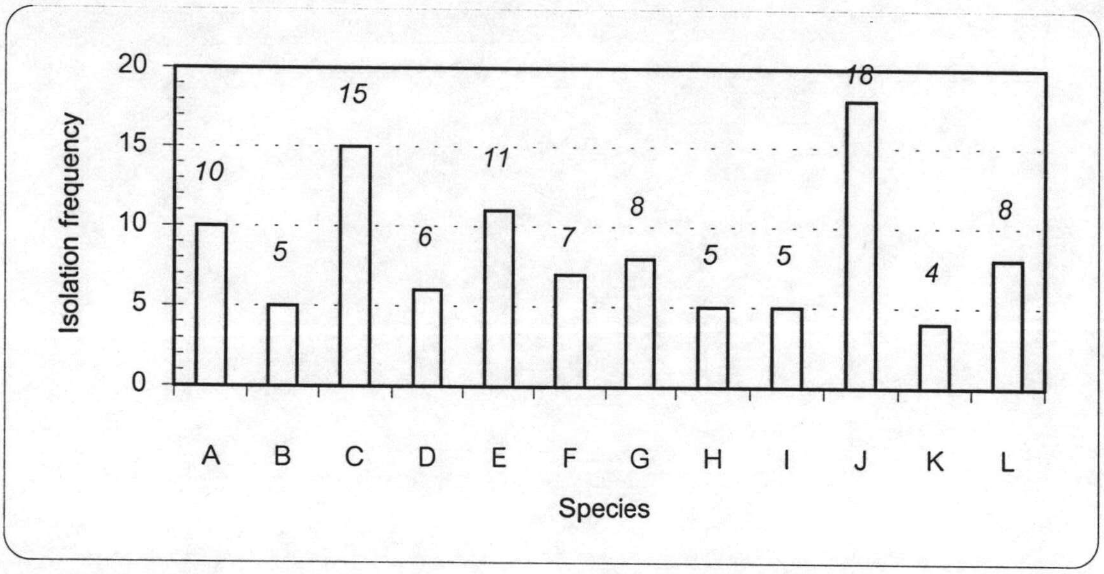

Figure 4.4 Endophytic fungi from mature teak leaves during May-June 1999

Table 4.5 Endophytic fungi from mature teak leaves during July-August 1999

Species	Isolation frequency	Percentage	Species abbreviation
Colletotrichum sp. T1	10	9.34	*A*
Nigrospora sp.	4	3.73	*B*
Phomopsis sp. T1	10	9.34	*C*
Phomopsis sp. T2	12	11.21	*D*
Phomopsis sp. T3	4	3.73	*E*
Schizophyllum communne	6	5.6	*F*
Xylaria sp. 1	6	5.6	*G*
Xylaria sp. 2	5	4.67	*H*
Xylaria sp. 7	4	3.73	*I*
Xylaria sp. 8	5	4.67	*J*
Xylaria sp. 9	8	7.47	*K*
Xylaria sp. 10	3	2.8	*L*
Xylaria sp. 11	8	7.47	*M*
Mycelia sterilia	12	11.21	*N*
Unknown no. 5	10	9.34	*O*
Total isolates	*107*	*100*	

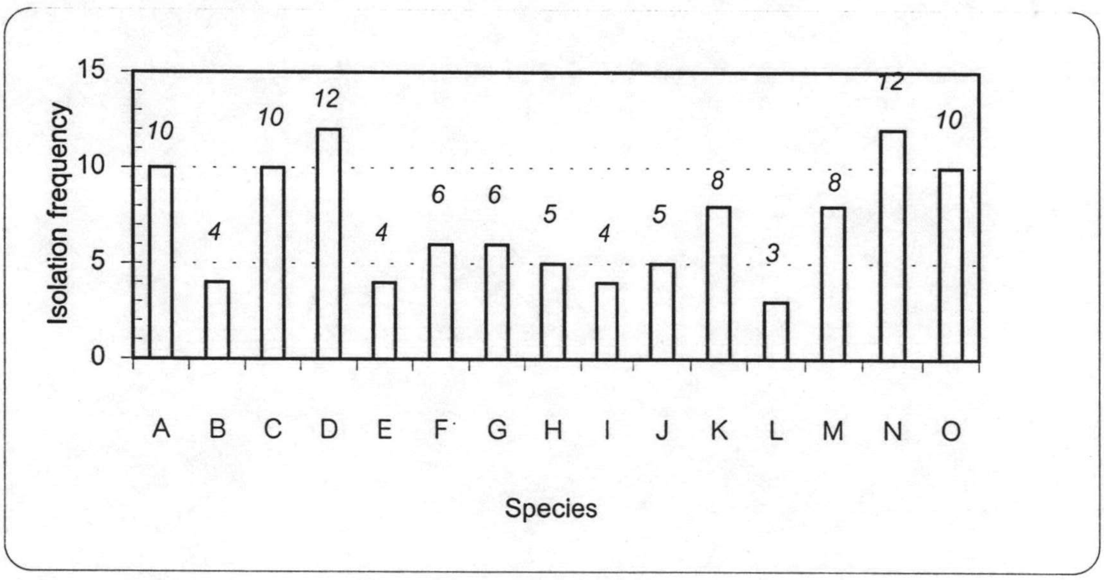

Figure 4.5 Endophytic fungi from mature teak leaves during July-August 1999

Table 4.6 Endophytic fungi from mature teak leaves during September-October 1999

Species	Isolation frequency	Percentage	Species abbreviation
Alternaria sp.	3	2.47	*A*
Colletotrichum sp. T1	12	9.91	*B*
Fusarium sp.	5	4.13	*C*
Phomopsis sp. T1	20	16.52	*D*
Phomopsis sp. T2	24	19.83	*E*
Phomopsis sp. T3	10	8.26	*F*
Phomopsis sp. T7	4	3.3	*G*
Schizophyllum commune	5	4.13	*H*
Xylaria sp. 4	10	8.26	*I*
Xylaria sp. 6	5	4.13	*J*
Mycelia sterilia	8	6.61	*K*
Unknown no. 4	3	2.47	*L*
Unknown no. 5	12	9.91	*M*
Total isolates	***121***	***100***	

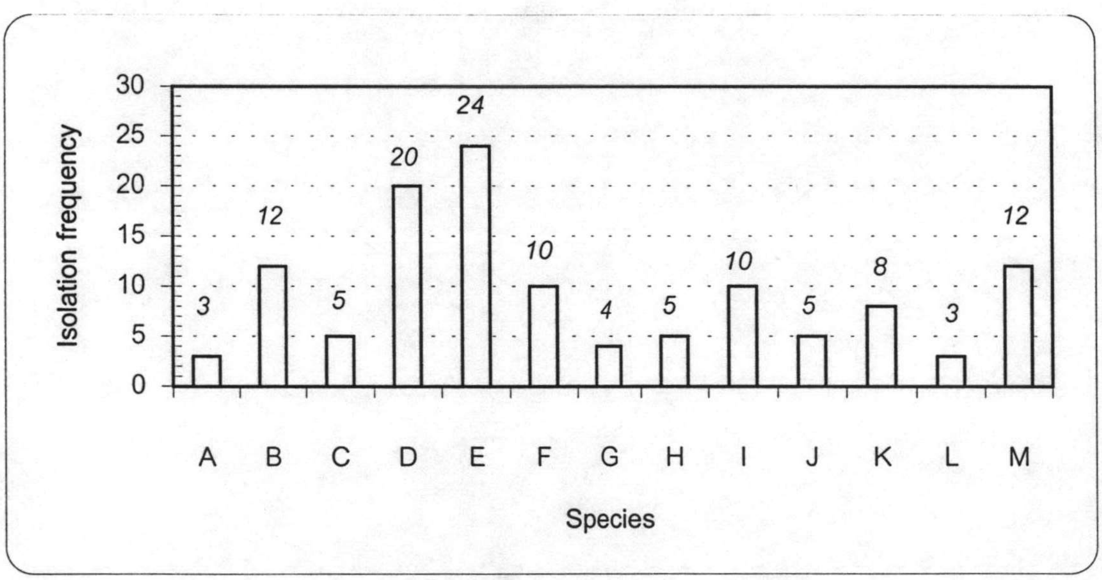

Figure 4.6 Endophytic fungi from mature teak leaves during September-October 1999

Table 4.7 Endophytic fungi from mature teak leaves during November-December 1999

Species	Isolation frequency	Percentage	Species abbreviation
Alternaria sp.	11	9.01	*A*
Colletotrichum sp. T1	16	13.11	*B*
Daldinia eschscholzii	18	14.75	*C*
Nigrospora sp.	6	4.91	*D*
Phomopsis sp. T1	9	7.37	*E*
Phomopsis sp. T2	24	19.67	*F*
Phomopsis sp. T4	14	11.47	*G*
Mycelia sterilia	4	3.27	*H*
Unknown no. 5	20	16.39	*I*
Total isolates	122	100	

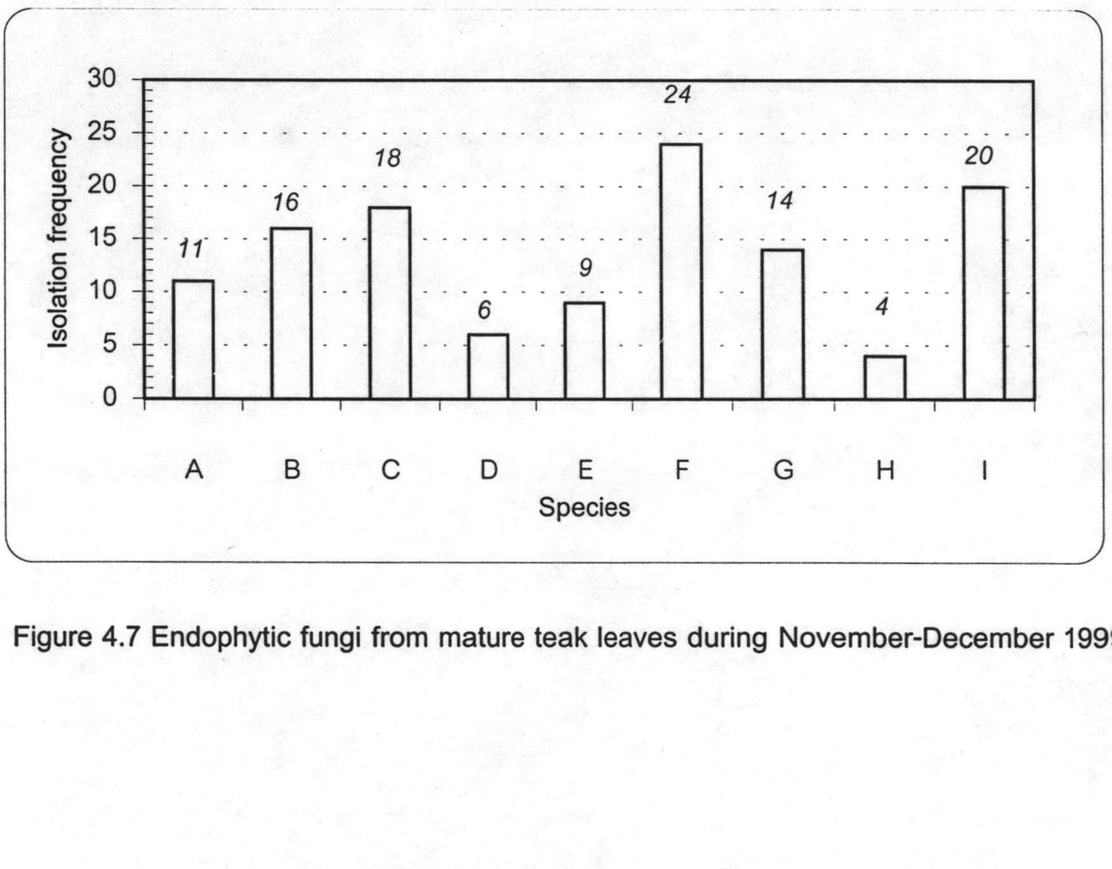

Figure 4.7 Endophytic fungi from mature teak leaves during November-December 1999

4.2 Endophytes of young teak leaves.

Examination of young leaves collected during January-December demonstrated that these have a much lower diversity of fungal species with only 7 species recorded. *Phomopsis* and mycelia sterilia were the most frequently isolated fungi. There were, however, differences in the species isolated, and particularly in the frequency of isolation, between the months (Table 4.8, Figure 4.8).

In January-February only 11 fungal isolates were obtained and these were recorded as an *Alternaria* species and as mycelia sterilia. In March-April, out of the 12 fungal isolates mycelia sterilia and *Phomopsis* sp. T1 were recognised (Table 4.8).

During the May-June period a total of 58 fungal isolates were recovered. Two genera and 4 species were identified. *Phomopsis* spp. with 44.44% was the most frequently recovered genus followed by mycelia sterilia (22.22%) and then unknown no. 5 (18.51%) (Table 4.9, Figure 4.9).

In July-August 24 fungal isolates were recorded and these represented 2 genera and 4 species. Again *Phomopsis* spp. at 58.33% isolation frequency was most dominant genus followed by a *Nigrospora* species (16.67%) and unknown no. 1 (16.67%) (Table 4.10, Figure 4.10).

Sampling in September-October resulted in a total of 31 fungal isolates representing 2 genera and 2 species. *Phomopsis* sp.T1 at 41.93% was the dominant isolate followed by unknown no. 5 (32.25%) (Table 4.11, Figure 4.11).

In November-December a total of 35 fungal isolates were obtained which represented 2 genera and 2 species. *Nigrospora* sp., *Phomopsis* sp. T1 and mycelia sterilia represented 34.29%, 34.29% and 22.85% of these isolates, respectively (Table 4.12 , Figure 4.12).

Comparison of species isolated, and the frequency of the total number of isolates between the young and mature teak leaves during January-December 1999 demonstrates that the number of isolates from mature leaves is greater than for young leaves (Figure 4.13). Futhermore the mature leaves had more genera of endophytic fungi than young leaves. The fungal genera found in young and mature leaves such as

Alternaria , *Colletotrichum*, *Nigrospora*, *Phomopsis* spp., Mycelia sterilia, unknown no. 2 and unknown no. 5 . *Phomopsis* proved to be the dominant genus in both young and mature leaves and was also dominant in relation to the number of isolates found during the sampling period. *Schizophyllum communne*, *Fusarium* sp., *Penicillium* sp. and members of the Xylariaceae were found only in mature leaves. In the January-February and March-April period, no Xylariaceous taxa were found (Table 4.1 and Table 4.8).

Table 4.8 Endophytic fungi from young leaves of teak during January-December 1999

Species	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sep-Oct	Nov-Dec
Alternaria sp.	6	0	0	0	0	0
Colletotrichum sp. T1	0	0	8	0	0	0
Nigrospora sp.	0	0	0	4	4	12
Phomopsis sp. T1	0	4	12	2	13	12
Phomopsis sp. T3	0	0	0	8	0	0
Phomopsis sp. T4	0	0	6	4	0	0
Phomopsis sp. T8	0	0	6	0	0	0
Mycelia sterilia	5	8	12	0	4	8
Unknown no.1	0	0	0	4	0	0
Unknown no.2	0	0	0	2	0	0
Unknown no.5	0	0	10	0	10	3
Total isolates	*11*	*12*	*58*	*24*	*31*	*35*

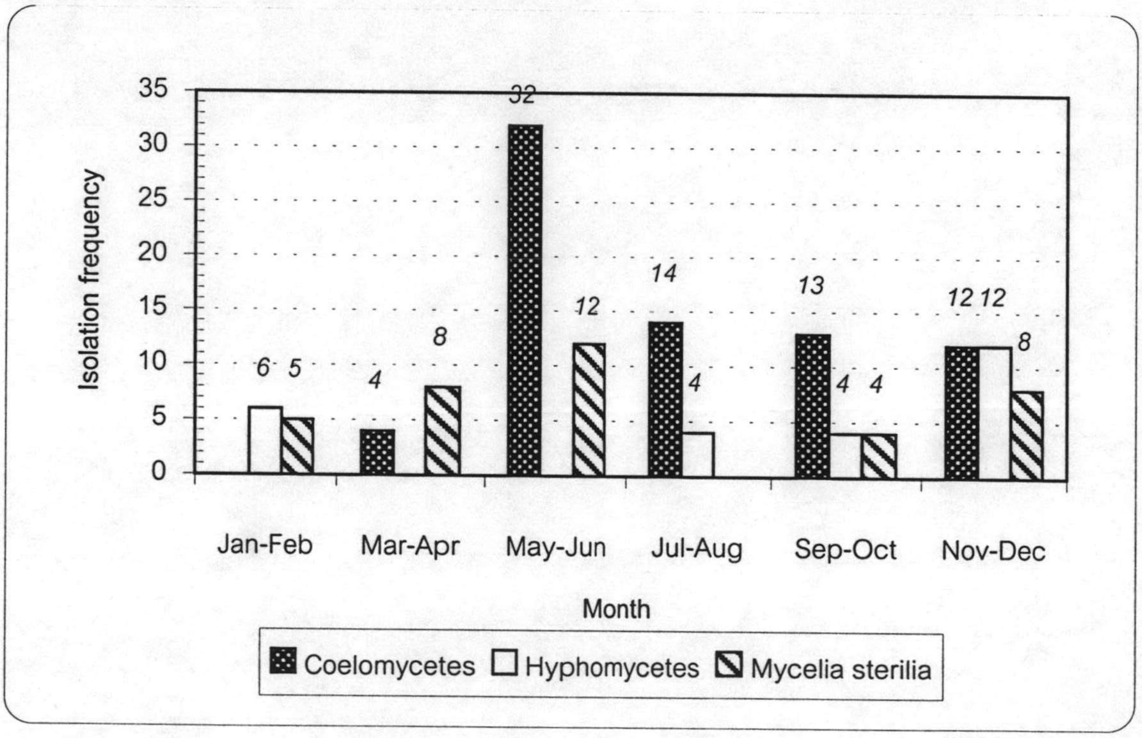

Figure 4.8 Endophytic fungi from young teak leaves during January-December 1999.

Table 4.9 Endophytic fungi from young teak leaves during May-June 1999

Species	Isolation frequency	Percentage	Species abbreviation
Colletotrichum sp. T1	8	14.81	*A*
Phomopsis sp. T1	12	22.22	*B*
Phomopsis sp. T4	6	11.11	*C*
Phomopsis sp. T8	6	11.11	*D*
Mycelia sterilia	12	22.22	*E*
Unknown no. 5	10	18.51	*F*
Total isolates	*58*	*100*	

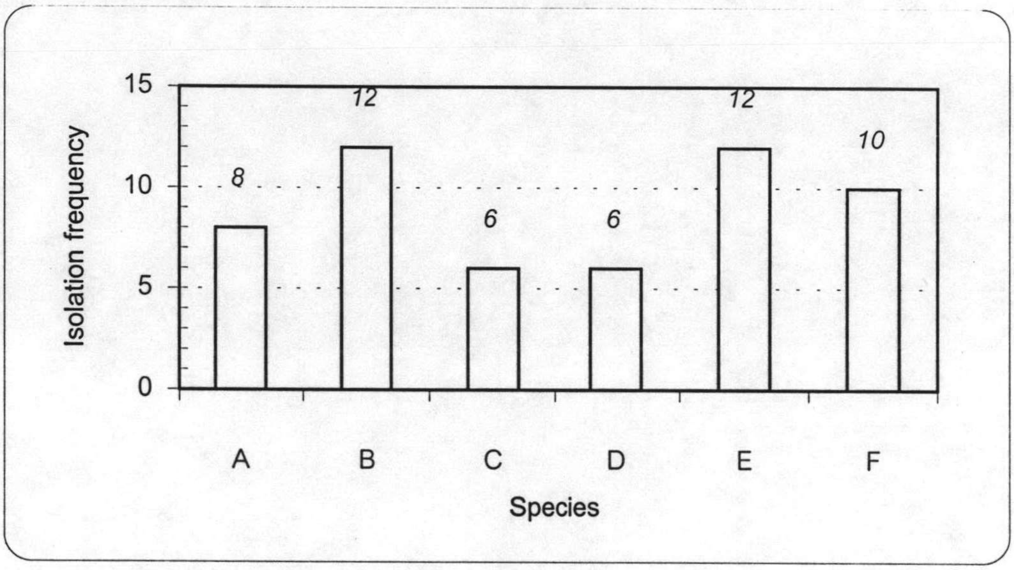

Figure 4.9 Endophytic fungi from young teak leaves during May-June 1999

Table 4.10 Endophytic fungi from young teak leaves during July-August 1999

Species	Isolation frequency	Percentage	Species abbreviation
Nigrospora sp.	4	16.67	*A*
Phomopsis sp. T1	2	8.33	*B*
Phomopsis sp. T3	8	33.33	*C*
Phomopsis sp. T4	4	16.67	*D*
Unknown no. 1	4	16.67	*E*
Unknown no. 2	2	8.33	*F*
Total isolates	*24*	*100*	

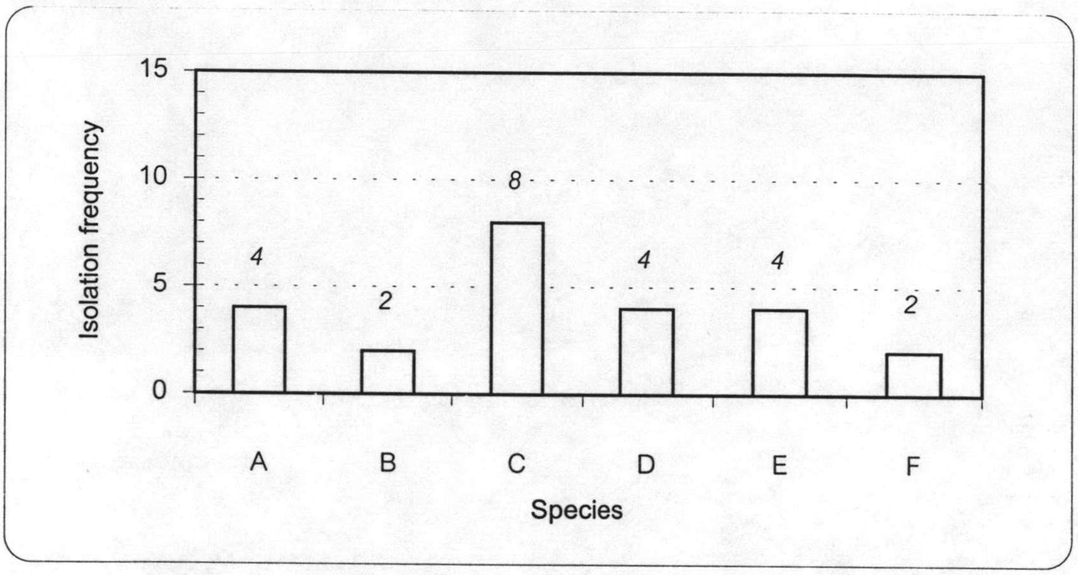

Figure 4.10 Endophytic fungi from young teak leaves during July-August 1999

Table 4.11 Endophytic fungi from young teak leaves during September-October 1999

Species	Isolation frequency	Percentage	Species abbreviation
Nigrospora sp.	4	12.9	*A*
Phomopsis sp. T1	13	41.93	*B*
Mycelia sterilia	4	12.9	*C*
Unknown no. 5	10	32.25	*D*
Total isolation	***31***	***100***	

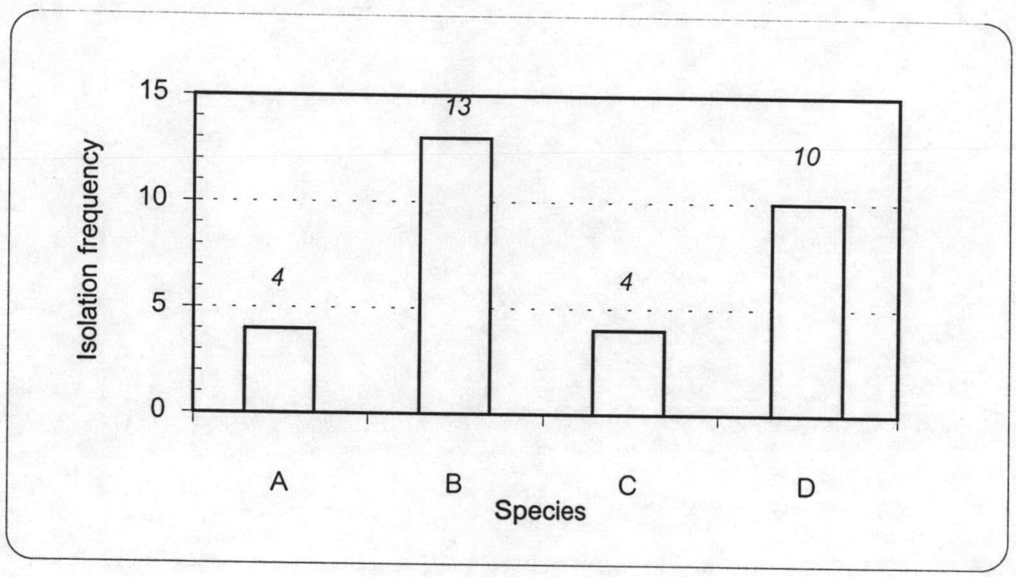

Figure 4.11 Endophytic fungi from young teak leaves during September-October 1999

Table 4.12 Endophytic fungi from young teak leaves during November-December 1999

Species	Isolation frequency	Percentage	Species abbreviation
Nigrospora sp.	12	34.29	*A*
Phomopsis sp. T1	12	34.29	*B*
Mycelia sterilia	8	22.85	*C*
Unknown no. 5	3	8.57	*D*
Total isolates	*35*	*100*	

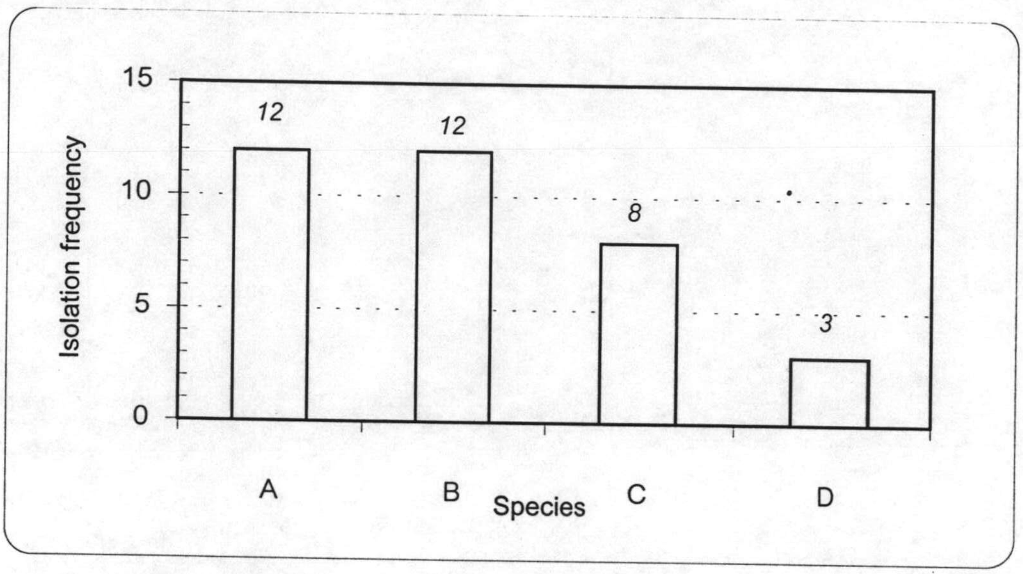

Figure 4.12 Endophytic fungi from young teak leaves during November-December 1999

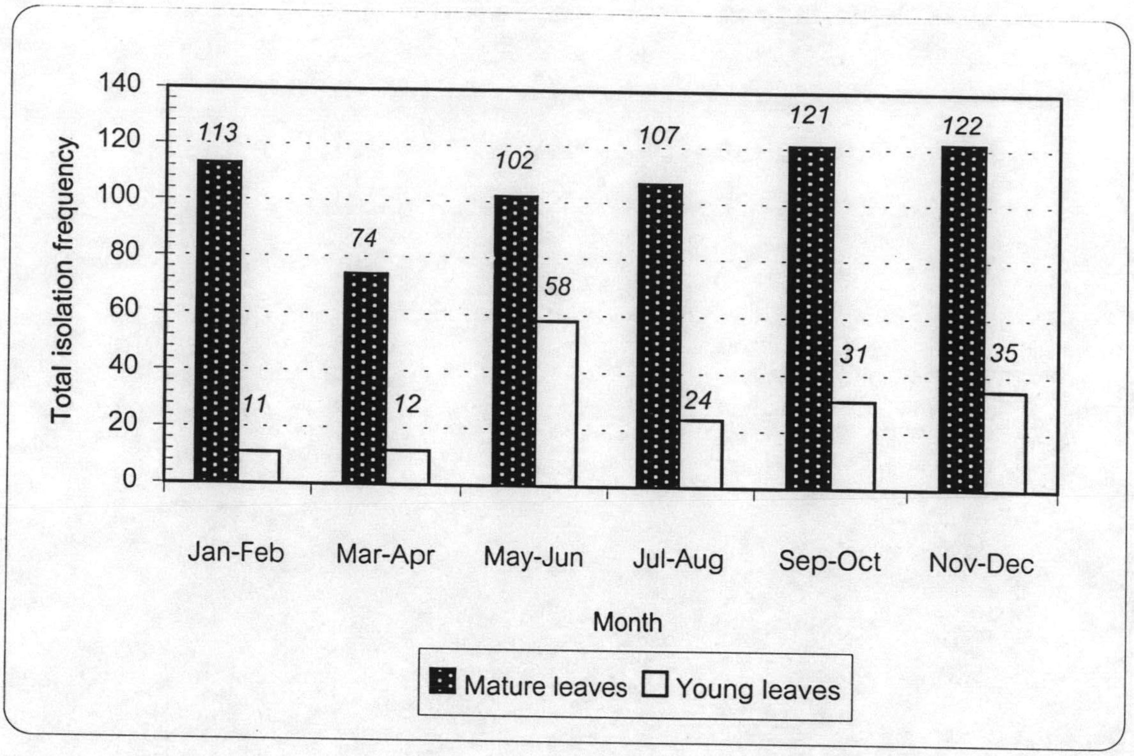

Figure 4.13 Comparison of total isolation frequency of endophytic fungi in mature and young teak leaves

4.3 Endophytes of mature rain tree leaves

Endophytes from mature rain tree leaves were isolated from leaves collected at Chulalongkorn University during January-December 1999. The frequency of the total isolates obtained and the total range of species identified, together with their isolation frequency, are given in Table 4.13 and Figure 4.14. Isolates were identified as belonging to three genera, *Colletotrichum*, *Penicillium*, and *Phomopsis*. Unidentified isolates were recorded as mycelia sterilia since they failed to develop either sexual or asexual structures or spores.

During the January-February period (Table 4.14, Figure 4.15) a total of 60 endophytic isolates belonging to 2 genera and representing 4 species were obtained. Mycelia sterilia at 33.33% was found to be the dominant isolate class followed by *Phomopsis* sp.S6 (30%) and then *Phomopsis* sp.S5 (20%).

The March-April sampling resulted in only 20 fungal isolates and these represented 2 genera with a total of 3 species. *Penicillium* sp., *Phomopsis* sp. S4 and *Phomopsis* sp. S5 represented 50%, 30% and 20% of the isolates, respectively (Table 4.15, Figure 4.16).

However the May-June period resulted in the isolation of 38 fungal isolates. There were found to belong to 2 genera and represented 4 distinct species. Mycelia sterilia with 10 isolates or 26.31% were the most frequently recovered fungi followed by *Phomopsis* sp. S5 with 23.68% as the most frequently recorded taxon (Table 4.16 , Figure 4.17).

In July-August, from the 64 fungal isolates obtained there were 2 genera and 4 species recognised. The most frequently isolated taxon was *Phomopsis* sp. S5 (31.25%), followed by *Phomopsis* sp. S4 (25%) with mycelia sterilia at 18.75% (Table 4.17, Figure 4.18).

In September-October, 2 genera and 5 species were recorded out of the total of 76 fungal isolates. *Phomopsis* sp. S6 (23.68%) was the dominant isolate followed by *Colletotrichum* sp. S1 (21.05%), *Phomopsis* sp. S4 (15.79%) and then *Phomopsis* sp. S5 (15.79%) (Table 4.18 , Figure 4.19).

In November-December, 88 fungal isolates were obtained and these represented 2 genera and 7 species. *Colletotrichum* sp. S1 (27.27%) was the most frequently isolated taxon. *Phomopsis* sp. S3 (20.45%) and *Phomopsis* sp. S5 (20.45%) were the next most frequently isolated taxa (Table 4.19, Figure 4.20).

Table 4.13 Endophytic fungi from mature rain tree leaves during January-December 1999

Species	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sep-Oct	Nov-Dec
Colletotrichum sp. S1	4	0	8	8	16	24
Penicillium sp.	0	10	0	0	0	0
Phomopsis sp. S1	0	0	0	0	0	9
Phomopsis sp. S2	0	0	0	0	0	6
Phomopsis sp. S3	0	0	5	8	10	18
Phomopsis sp. S4	6	6	6	16	12	6
Phomopsis sp. S5	12	4	9	20	12	18
Phomopsis sp. S6	18	0	0	0	18	0
Phomopsis sp. S7	0	0	0	0	0	7
Mycelia sterilia	20	0	10	12	8	0
Total isolates	60	20	38	64	76	88

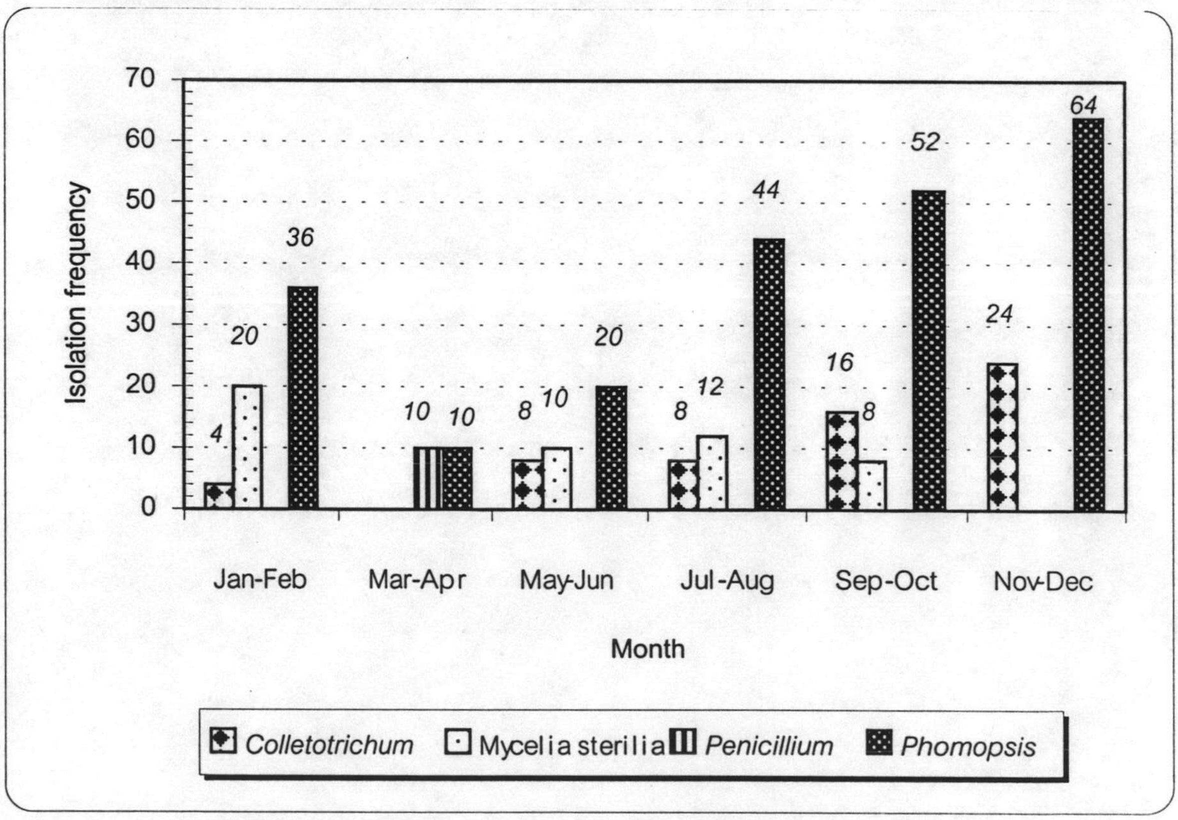

Figure 4.14 Endophytic fungi from mature rain treee leaves during January-December 1999.

Table 4.14 Endophytic fungi from mature rain tree leaves during January-February 1999

Species	Isolation frequency	Percentage	Species abbreviation
Colletotrichum sp. S1	4	6.67	*A*
Phomopsis sp. S4	6	10	*B*
Phomopsis sp. S5	12	20	*C*
Phomopsis sp. S6	18	30	*D*
Mycelia sterilia	20	33.33	*E*
Total isolates	*60*	*100*	

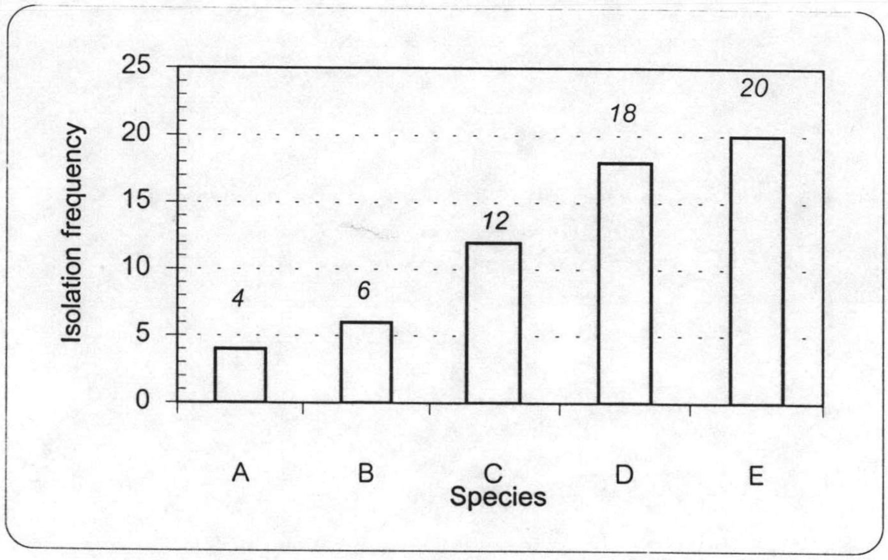

Figure 4.15 Endophytic fungi from mature rain tree leaves during January-February 1999

Table 4.15 Endophytic fungi from mature rain tree leaves during March-April 1999

Species	Isolation frequency	Percentage	Species abbreviation
Penicillium sp.	10	50	*A*
Phomopsis sp. S4	6	30	*B*
Phomopsis sp. S5	4	20	*C*
Total isolates	*20*	*100*	

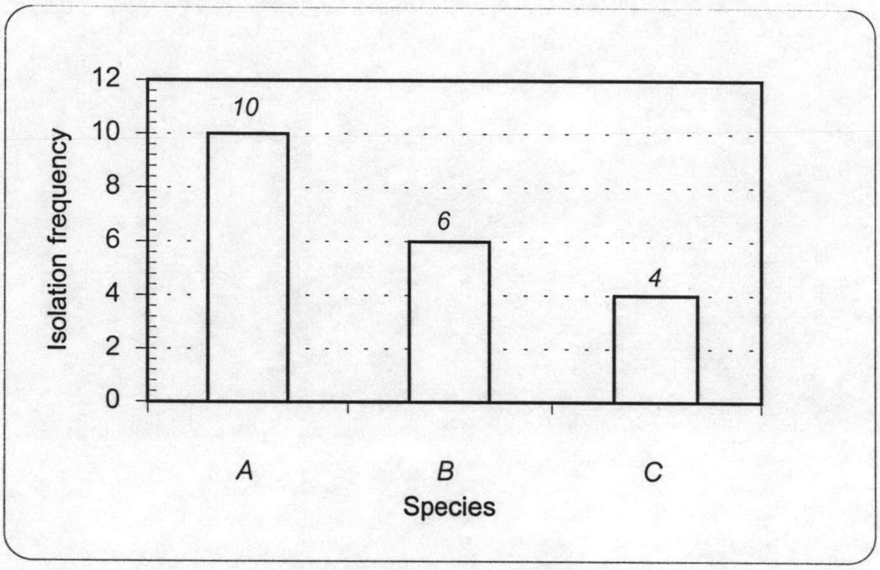

Figure 4.16 Endophytic fungi from mature rain tree leaves during March-April 1999

Table 4.16 Endophytic fungi from mature rain tree leaves during May-June 1999

Species	Isolation frequency	percentage	Species abbreviation
Colletotrichum sp. S1	8	21.05	*A*
Phomopsis sp. S3	5	13.15	*B*
Phomopsis sp. S4	6	15.78	*C*
Phomopsis sp. S5	9	23.68	*D*
Mycelia sterilia	10	26.31	*E*
Total isolates	*38*	*100*	

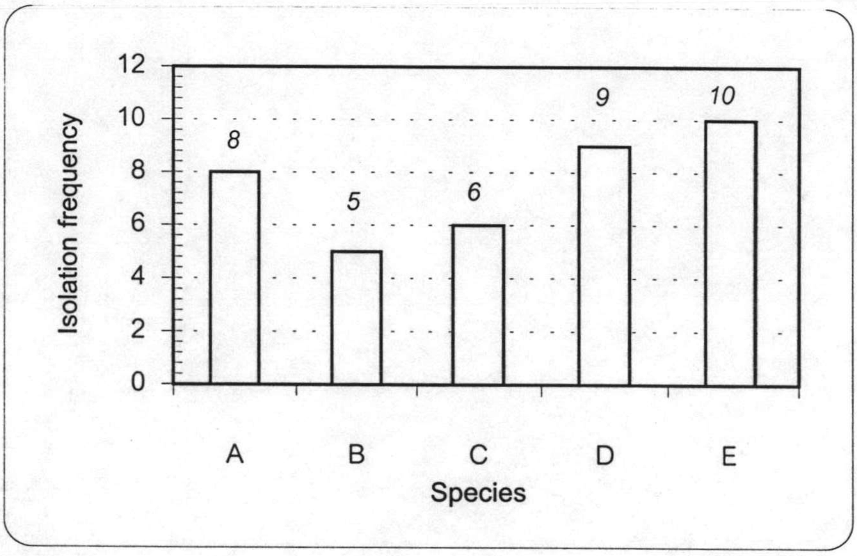

Figure 4.17 Endophytic fungi from mature rain tree leaves during May-June 1999

Table 4.17 Endophytic fungi from mature rain tree leaves during July-August 1999

Species	Isolation frequency	percentage	Species abbreviation
Colletotrichum sp. S1	8	12.5	*A*
Phomopsis sp. S3	8	12.5	*B*
Phomopsis sp. S4	16	25	*C*
Phomopsis sp. S5	20	31.25	*D*
Mycelia sterilia	12	18.75	*E*
Total isolates	*64*	*100*	

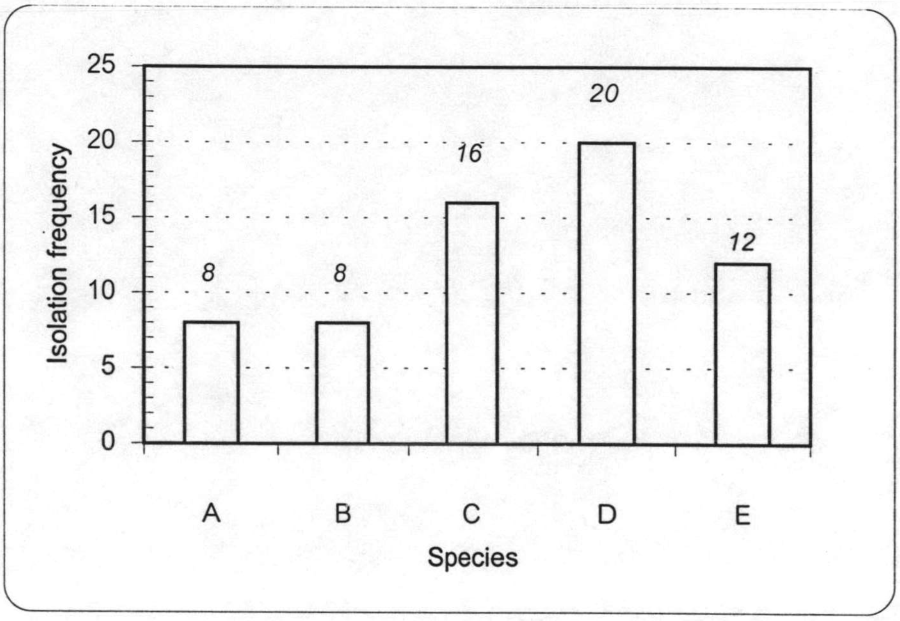

Figure 4.18 Endophytic fungi from mature rain tree leaves during July-August 1999

Table 4.18 Endophytic fungi from mature rain tree leaves during September-October 1999

Species	Isolation frequency	percentage	Species abbreviation
Colletotrichum sp. S1	16	21.05	*A*
Phomopsis sp. S3	10	13.16	*B*
Phomopsis sp. S4	12	15.79	*C*
Phomopsis sp. S5	12	15.79	*D*
Phomopsis sp. S6	18	23.68	*E*
Mycelia sterilia	8	10.52	*F*
Total isolates	*76*	*100*	

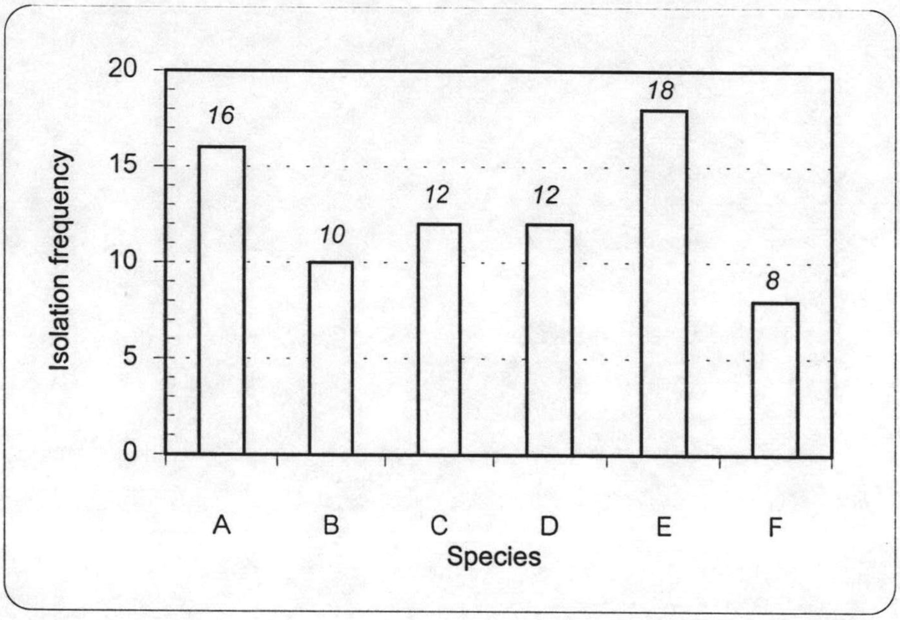

Figure 4.19 Endophytic fungi from mature rain tree leaves during September-October 1999

Table 4.19 Endophytic fungi from mature rain tree leaves during November-December 1999

Species	Isolation frequency	percentage	Species abbreviation
Colletotrichum sp. S1	24	27.27	*A*
Phomopsis sp. S1	9	10.22	*B*
Phomopsis sp. S2	6	6.81	*C*
Phomopsis sp. S3	18	20.45	*D*
Phomopsis sp. S4	6	6.81	*E*
Phomopsis sp. S5	18	20.45	*F*
Phomopsis sp. S7	7	7.95	*G*
Total isolates	*88*	*100*	

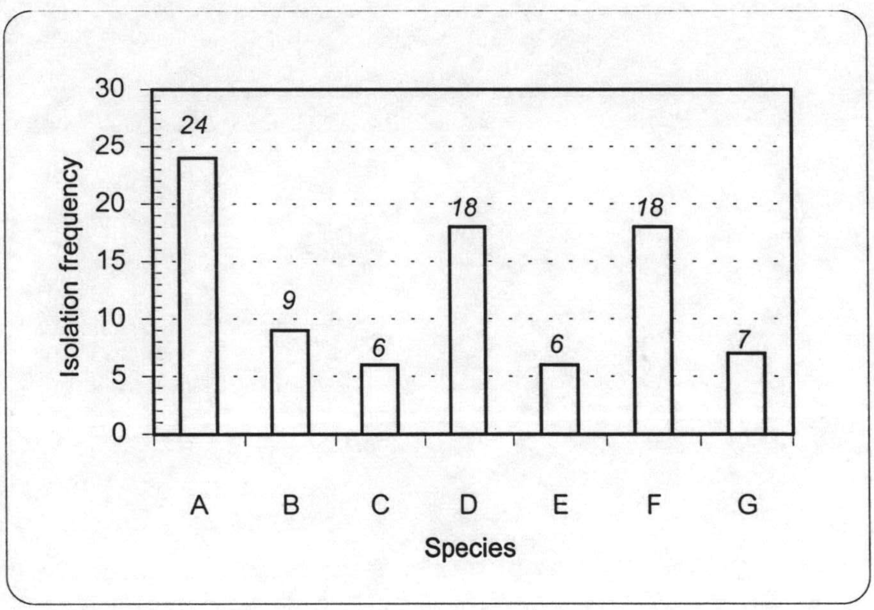

Figure 4.20 Endophytic fungi from mature rain tree leaves during November-December 1999

4.4 Endophytes of young rain tree leaves

The young rain tree leaves collected during January-December 1999 resulted in the identification of isolates belonging to the 2 genera, *Nigrospora* and *Phomopsis* together with isolates recorded as mycelia sterilia. The *Colletotrichum* species isolated from mature rain tree leaves was not found. No fungal endophytes were isolated during January-February and March-April (Table 4.20, Figure 4.21).

In May-June, 20 fungal endophytes were obtained which represented 2 genera and 3 species. Mycelia sterilia (40%) were the dominant isolates followed by *Nigrospora* sp. (20%) , *Phomopsis* sp. S2 (20%) and *Phomopsis* sp. S4 (20%) (Table 4.21, Figure 4.10).

The July-August sampling resulted in the isolation of only 4 fungal isolates which were identified as *Nigrospora* sp. (Table 4.20, Figure 4.21).

In September-October a total of 16 fungal isolates were obtained. *Phomopsis* sp. S3 (50%) was the most frequently isolated taxon followed by *Nigrospora* sp. (25%) and *Phomopsis* sp. S2 (25%) (Table 4.22, Figure 4.23).

The November-December sampling period resulted in the isolation of only 8 fungal isolates belonging to *Phomopsis* sp.S5 (50%) and *Phomopsis* sp. S6 (50%) (Table 4.20 , Figure 4.21).

Comparison of species isolated, and the frequency of the total number of isolates between the young and mature rain tree leaves during January-December 1999 demonstrates that the number of isolates from mature leaves is greater than for young leaves (Figure 4.24). Also the mature leaves had more genera than young leaves. *Colletotrichum* and *Penicillium* were found only in mature leaves, whereas *Nigrospora* was found only in young leaves. Species of *Phomopsis* and mycelia sterilia were found in both young and mature leaves (Table 4.13 and Table 4.20).

Table 4.20 Endophytic fungi from young rain tree leaves during January-December 1999

Species	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sep-Oct	Nov-Dec
Nigrospora sp.	0	0	4	4	1	0
Phomopsis sp. S2	0	0	4	0	1	0
Phomopsis sp. S3	0	0	0	0	8	0
Phomopsis sp. S4	0	0	4	0	0	0
Phomopsis sp. S5	0	0	0	0	0	4
Phomopsis sp. S6	0	0	0	0	0	4
Mycelia sterilia	0	0	8	0	0	0
Total isolates	0	0	20	4	16	8

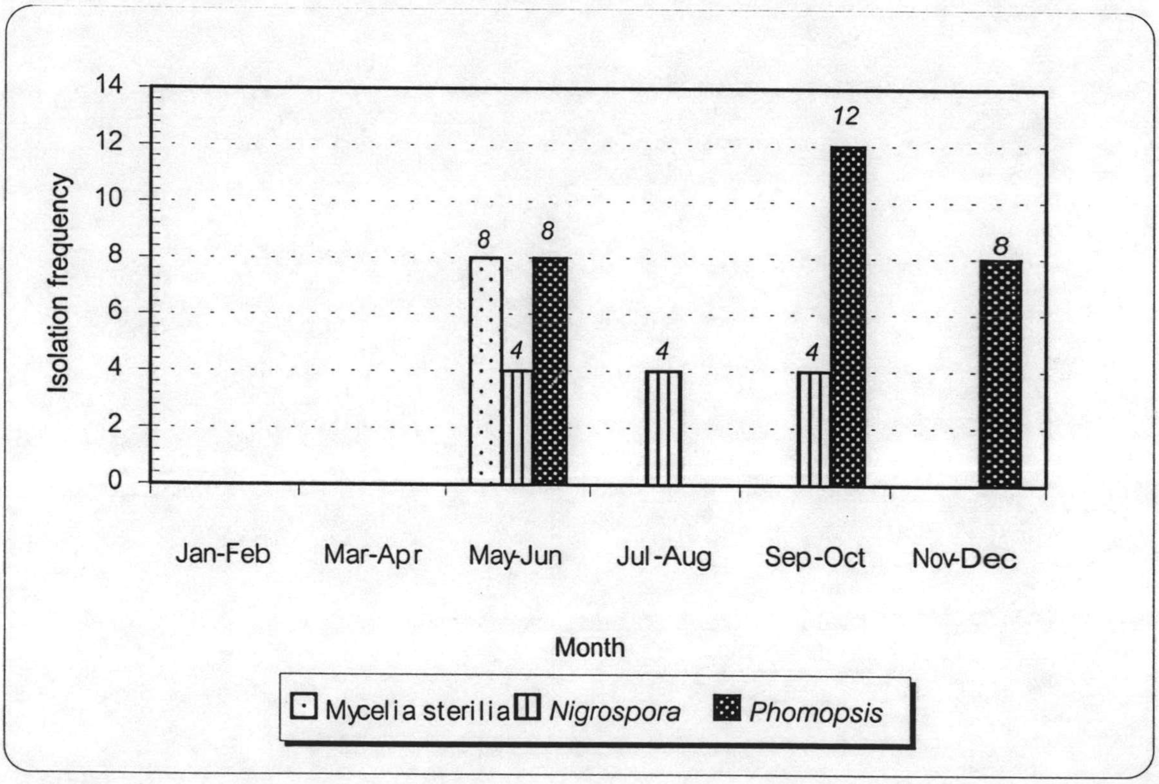

Figure 4.21 Endophytic fungi in young rain tree leaves during January-December 1999.

Table 4.21 Endophytic fungi from young rain tree leaves during May-June 1999

Species	Isolation frequency	percentage	Species abbreviation
Nigrospora sp.	4	20	*A*
Phomopsis sp. S2	4	20	*B*
Phomopsis sp. S4	4	20	*C*
Mycelia sterilia	8	40	*D*
Total isolates	*20*	*100*	

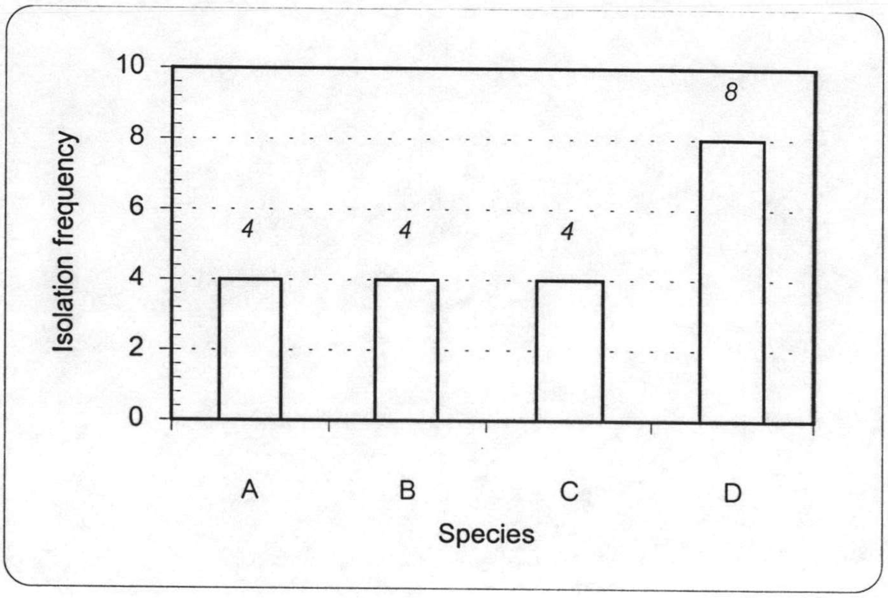

Figure 4.22 Endophytic fungi from young rain tree leaves during May-June 1999

Table 4.22 Endophytic fungi from young rain tree leaves during September-October 1999

Species	Isolation frequency	percentage	Species abbreviation
Nigrospora sp.	4	25	*A*
Phomopsis sp. S2	4	25	*B*
Phomopsis sp. S3	8	50	*C*
Tatal isolations	*16*	*100*	

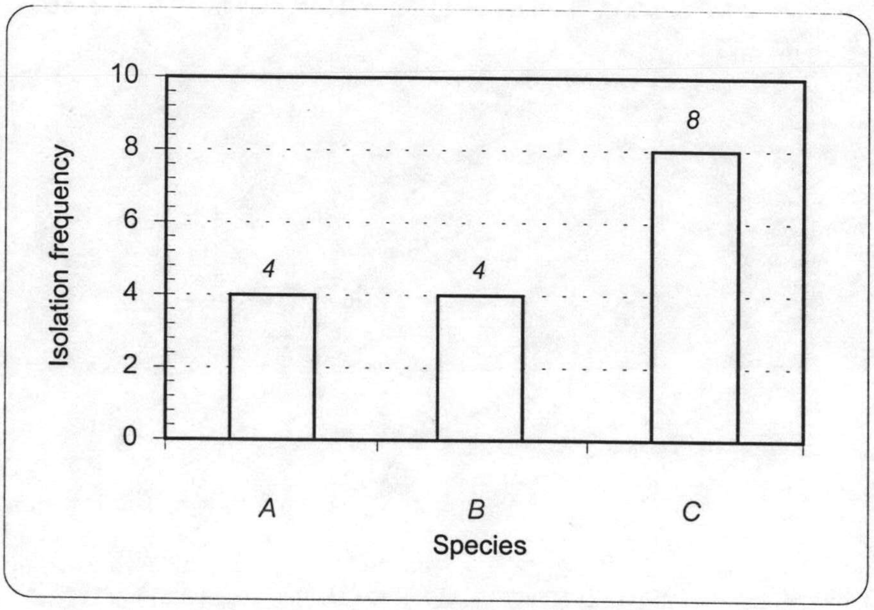

Figure 4.23 Endophytic fungi from young rain tree leaves during September-October 1999

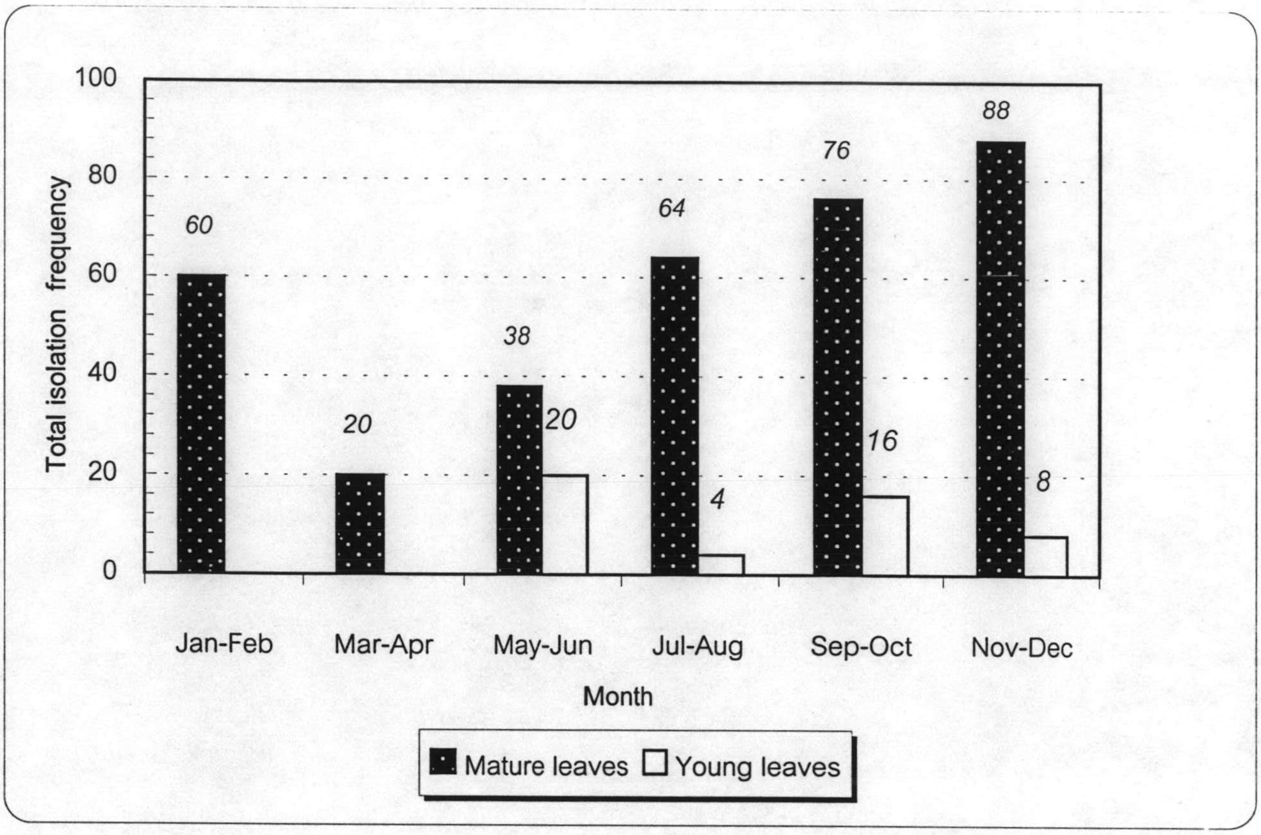

Figure 4.24 Comparison of total isolation frequency of endophytic fungi in mature and young rain tree leaves

4.5 Taxonomy of endophytic species

Descriptions of the genus

Alternaria Nees ex Fr. ; Nees, 1816, Syst. Pilze Schwamme: 72; Fries, 1821, Syst. Mycol. , 1: XL VL. Figure 4.25

Colonies effuse,usually grey, dark blackish brown or black. Mycelium all immersed of partly superficial ; hyphae colourless, olivaceous brown of brown. Stroma rarely formed. Setae and hyphopodia absent. Conidiophores macronematous, mononematous , simple or irregularly and loosely branched,pale brown or brown,solitary of in fascicles. Conidiogenous cells integrated,terminal becoming intercalary , polytretic , sympodial, or sometimes monotretic, cicatrized. Conidia catenate or solitary,dry,typically ovoid or obclavate,often rostrate, pale or mid olivaceous brown,or brown, smooth or verrucose, with transverse and frequently also oblique or longitudinal septa.

Colletotrichum Cda in Sturm, Deutschlands Flora 3: 41(1831-1832). Figure 4.27 and Figure 4.29

Mycelium immersed,branched,septate,hyaline,pale brown or dark brown. Conidiomata acervular, subcuticular, epidermal, subepidermal or peridermal, separate or confluent, composed of hyaline to dark brown, thin- or thick-walled textura angularis; dehiscence irregular. Sclerotia sometimes present in culture, dark brown to black, often confluent, occasionally setose. Setae in conidiomata or sclerotia, brown, smooth, septate, tapered to the apices. Conidiophores hyaline to brown, septate , branched only at the base, smooth , formed from the upper cells of the conidiomata. Conidiogenous cells enteroblastic,phialidic, hyaline , smooth , determinate, cylindrical ,integrated or discrete, channel minute but occasionally collarette and periclinal thickening quite prominent. Conidia hyaline , aseptate (except prior to germination), straight or falcate, smooth, thin-walled, sometimes guttulate , muticate or with the apex prolonged into a simple cellular appendage. Appressoria brown, entire or with crenate to irregular margins, simple or repeatedly germinating to produce complex columns of several closely connected appressoria.

Daldinia eschscholzii (Ehrenb.: Fr.) Rehm., Ann. Mycol. 2: 175. 1904. Figure 4.37

Stromata Turbinate to placentiform, sessile or with short, stout stipe, solitary to infrequently aggregated, smooth, 1.5-4 cm diam × 1-3 cm high; surface brown, vinaceous, dark brick, sepia, greyish sepia or vinaceous grey, blackened and varnished in age; dull reddish brown granules immediately beneath surface, with KOH-extractable pigments livid purple, dark livid or vinaceous purple; the tissue between perithecia brown, pithy to woody; the tissue below the perithecial layer composed of alternating zones. The darker zones dark brown, pithy to woody, 1-0.2 mm thick, the lighter zones white, grey or greyish brown, gelatinous and very hard when dry, becoming pithy to woody, persistent, 0.3-1 mm thick.

Perithecia tubular, 0.8-1.6 mm high × 0.3-0.4 mm diam

Ostioles obsolete or slightly papillate.

Asci 95-125 μm long, with apical apparatus, discoid, 0.5 μm high × 2-2.5 μm broad blueing in Melzer's iodine reagent.

Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 11.3-12.5 × 5.0-6.3 μm, with straight germ slit spore-length on convex side; perispore dehiscent in 10% KOH, conspicuous coil ornamentation; epispore smooth.

Cultural characteristics

Colonies on malt extract agar, under 12 h light and 12 h darkness, reaching edge of a 9 cm diam. Petri dish in 2 weeks; at first white to orange-white in the centre, becoming pale to dull yellow, floccose, finally in patches orange-grey to brownish grey in the centre, greyish yellow and olive-brown towards the margins, and in between, brown woolly with coarse texture. Reverse at first pale yellow to greyish yellow and finally fairly uniform dark greyish brown. Exudate orange to reddish brown. Agar staining yellowish brown to brown. Odour ether-like with a sweet component. Hyphae sparingly branched, septate, at first hyaline, later light brown, smooth or slightly verrucose, 2-3 µm diam. Conidiogenous structures formed after 3-4 days, at first in the centre, later abundant throughout the whole colony. Directly on the mycelium. Conidiophores mononematous, di-or trichotomously branched, especially towards the apex, regularly septate, at first hyaline and smooth, later light brown and verrucose, up to 280 µm long X 2-3 µm diam. Conidiogenous cells terminal, cylindrical, at first hyaline and smooth, later light brown and verrucose, 10-20 X 2-3 µm, bearing circular refractile to more or less denticulate conidial scars in the somewhat flattened apices. Conidia acrogenous, ellipsoid to obovoid, with a flattened circular abscission scar at the base, hyaline, smooth by L.M. (4)4.5-7(8) X 2-3(3.5) µm.

Fusarium Figure 4.26

Mycelium extensive and cotton-like in culture, often with some tinge of pink, purple, or yellow in the mycelium on medium; conidiophores variable, slender, and simple, or stout, short, branched irregularly or bearing a whorl of phialides, single or grouped into sporodochia; conidia (phialospores) hyaline, variable, principally of two kinds, often held in small moist heads; macroconidia several-called, slightly curved or bent at the pointed ends, typically canoe-shaped; microconidia 1-celled, ovoid or oblong, borne singly or in chains; some conidia intermediate, 2- or 3-celled, oblong or slightly curved; parasitic on higher plants or saprophytic on decaying plant material. A large and variable genus, sometimes placed in the Tuberculariaceae because some species produce sporodochia. Thick-walled chlamydospores common in some species.

Nigrospora Zimmermann, 1902, Zentbl. Bakt. ParasitKde, Abt. 2, 8: 220. Figure 4.28

Colonies at first white with small, shining black conidia easily visible under a low-power dissecting microscope, later brown or black when sporulation is abundant. Mycelium all immersed or partly superficial. Stroma none. Setae and hyphopodia absent. Conidiophores micronematous or semi-macronematous,branched,flexuous ,colourless to brown,smooth. Conidiogenous cells monoblastic,discrete, solitary , determinate, ampulliform or subsperical , colourless. Conidia solitary,with a violent discharge mechanism (fully described by Webster, 1952) , acrogenous , simple, spherical or broadly ellipsoidal, compressed dorsiventrally ,black,shining, smooth, O-septate

Phomopsis

Mycelium immersed, branched, septate, hyaline to pale brown. Conidiomata eustromatic, immersed, brown to dark brown, separate or aggregated and confluent, globose , ampulliform or applanate , unilocular , multilocular or convoluted, thick-walled; walls of brown, thin-or thick-walled textura angularis, often somewhat darker in the upper region, lined by a layer of smaller-celled tissue. Ostiole single, or several in complex conidiomata, circular, often papillate. Conidiophores branched and septate at the base and above, occasionally short and only 1-2 septate, more frequently multiseptate and filiform, hyaline,formed from the inner cells of the locular walls. Conidiogenous cells enteroblastic, phialidic, determinate, integrated, rarely discrete, hyaline, cylindrical, apertures apical on long or short lateral and main branches of the conidiophores , collarette, channel and periclinal thickening minute. Conidia of two basic types, but in some species with intermediates between the two: ∝-conidia hyaline, fusiform, straight, usually biguttulate (one guttule at each end) but sometimes with more guttules, aseptate; β-conidia hyaline,filiform,straight or more often hamate,eguttulate,aseptate.

Phomopsis isolates obtained from teak leaves isolations were seperated into 8 different groups depending on colony morphology, spore forming and spore size (Figure 4.30 and Figure 4.31). *Phomopsis* sp. T1 produce both ∝-conidia and β-conidia, ∝-conidia 5.0-7.5 μm × 2.0 μm, β-conidia 20-27.5×1.25 μm. *Phomopsis* sp. T2 produce only ∝-conidia 7.5-12.5 μm × 1-1.75 μm *Phomopsis* sp. T3 produce both ∝-conidia and β-conidia, ∝-conidia 5.5-8.75 μm × 2.0 μm, β-conidia 20-27.5×1.25 μm

Phomopsis sp. T4 produce both ∝-conidia and β-conidia, ∝-conidia 7.5-10 µm X 2.5 µm, β-conidia 17-25 X1.25 µm *Phomopsis* sp. T5 produce both ∝-conidia and β-conidia, ∝-conidia 5.0-7.5 µm X 2.5 µm, β-conidia 20-27.5 X1.25 µm Phomopsis sp. T6 produce both ∝-conidia and β-conidia, ∝-conidia 7.5-10µm X 2.0 µm, β-conidia 18X1.25 µm *Phomopsis* sp. T7 produce both ∝-conidia and β-conidia, ∝-conidia 5.25-7.5 µm X 2.5 µm, β-conidia 25-32.5 X1.25 µm *Phomopsis* sp. T8 produce both ∝-conidia and β-conidia, ∝-conidia 7.5-10.0 µm X 2.5 µm, β-conidia 22.5-25.0 X1.25 µm.

Phomopsis isolates obtained from rain tree leaves isolations were seperated into 7 different groups depending on colony morphology, spore forming and spore size (Figure 4.32 and Figure 4.34). *Phomopsis* sp. S1 produce both ∝-conidia and β-conidia, ∝-conidia 5.0-10.0 µm X 2.0 µm, β-conidia 12.5-22.5 µm.X1.25 µm. *Phomopsis* sp. S2 produce both ∝-conidia and β-conidia, ∝-conidia 6.25-7.25 µm X 2.5 µm, β-conidia 15-25 µm.X1.25 µm. *Phomopsis* sp. S3 produce both ∝-conidia and β-conidia, ∝-conidia 5.0-8.75 µm X 2.0 µm, β-conidia 17.5-25X1.25 µm. *Phomopsis* sp. S4 produce both ∝-conidia and β-conidia, ∝-conidia 7.5-12.5 µm X 2.0 µm, β-conidia 17.5-27.5 µm. X1.25 µm. *Phomopsis* sp. S5 produce both ∝-conidia and β-conidia, ∝-conidia 5.0-7.5 µm X 2.0 µm, β-conidia 15-25 µm.X1.25 µm. *Phomopsis* sp. S6 produce both ∝-conidia and β-conidia, ∝-conidia 7.5-10 µm X 2.5 µm, β-conidia 17.5-25 µm.X1.25 µm. *Phomopsis* sp. S7 produce both ∝-conidia and β-conidia, ∝-conidia 7-10 µm X 2.2 µm, β-conidia 20.75 µm.X1.25 µm.

Penicillium Figure 4.33

Conidiophores arising from the mycelium singly or less often in synnemata , branched near the apex, penicillate, ending in a group of phialides; conidia (phialospores) hyaline or brightly colored in mass, 1-celled, mostly globose or ovoid , in dry basipetal chains.

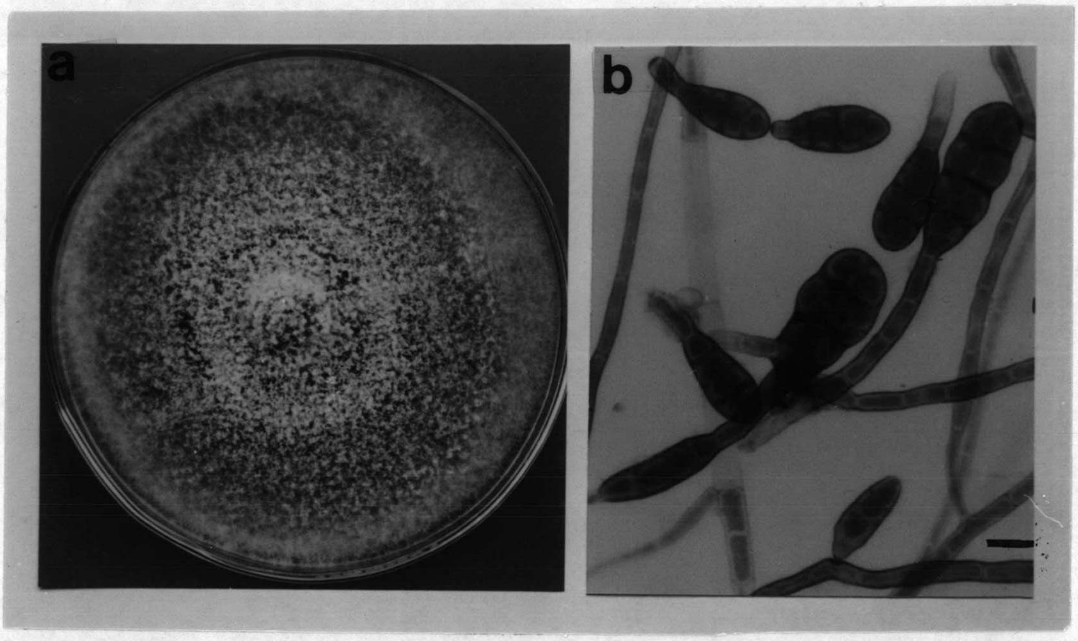

Figure 4.25 *Alternaria* sp. (a) culture on PDA (7-10 days) (b) conidia (Bar = 10 μm)

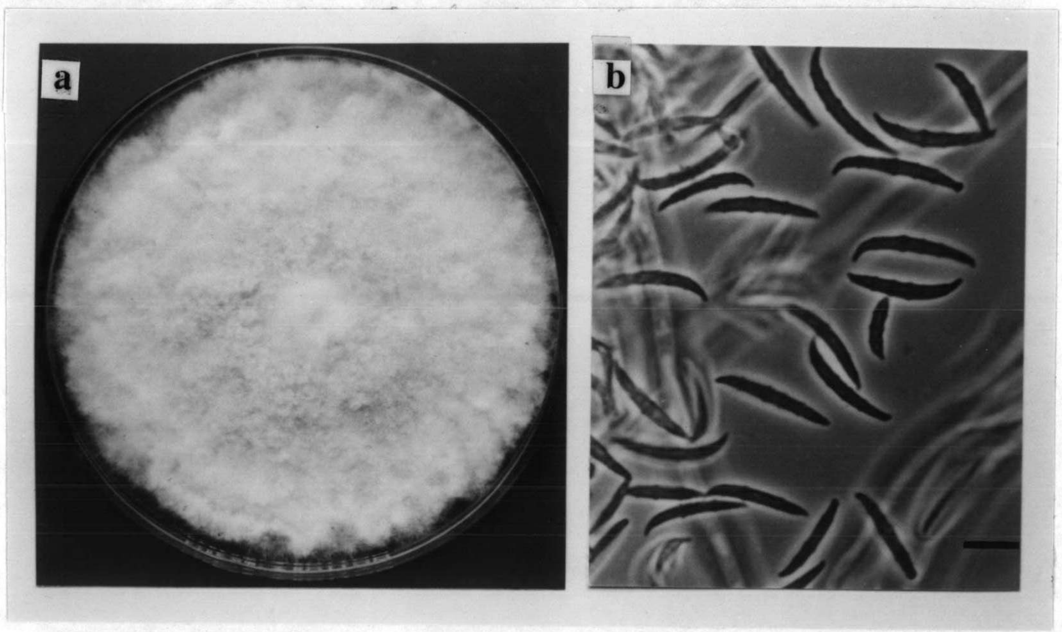

Figure 4.26 26 *Fusarium* sp. (a) culture on PDA (7-10 days) (b) conidia (Bar = 10 μm)

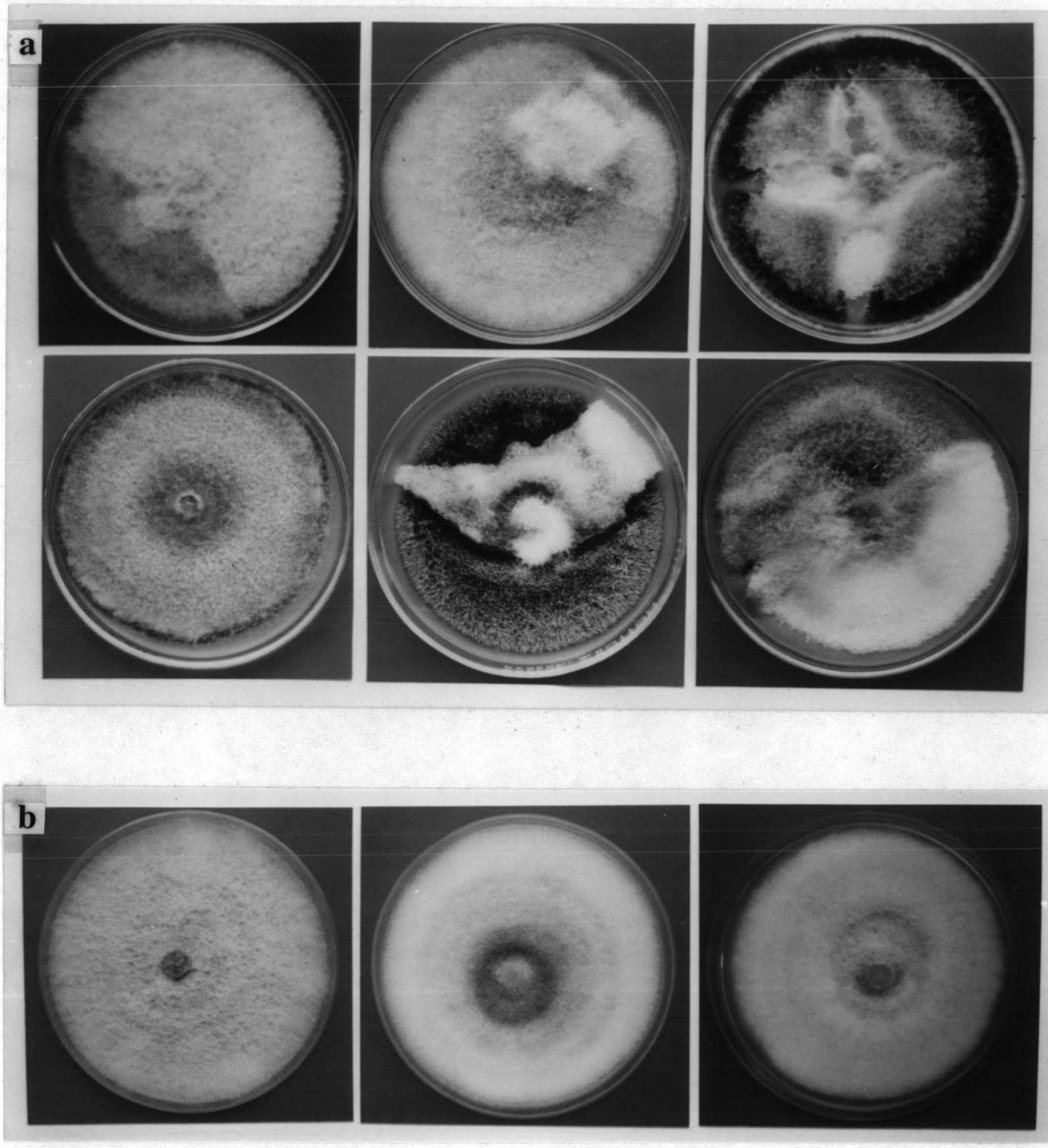

Figure 4.27 (a) Culture of *Colletotrichum* sp.T1 of teak leaves on PDA (5-7 days)

(b) Culture of *Colletotrichum* sp.S1 of rain tree leaves on PDA (5-7 days)

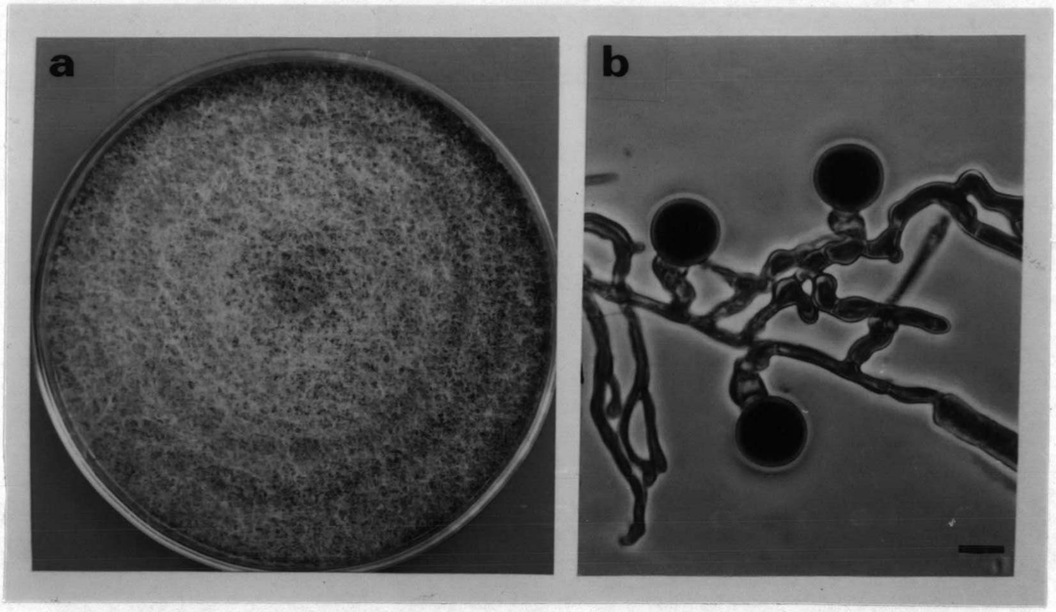

Figure 4.28 *Nigrospora* sp. (a) culture on PDA (7-10 days) (b) conidia (Bar = 10 μm)

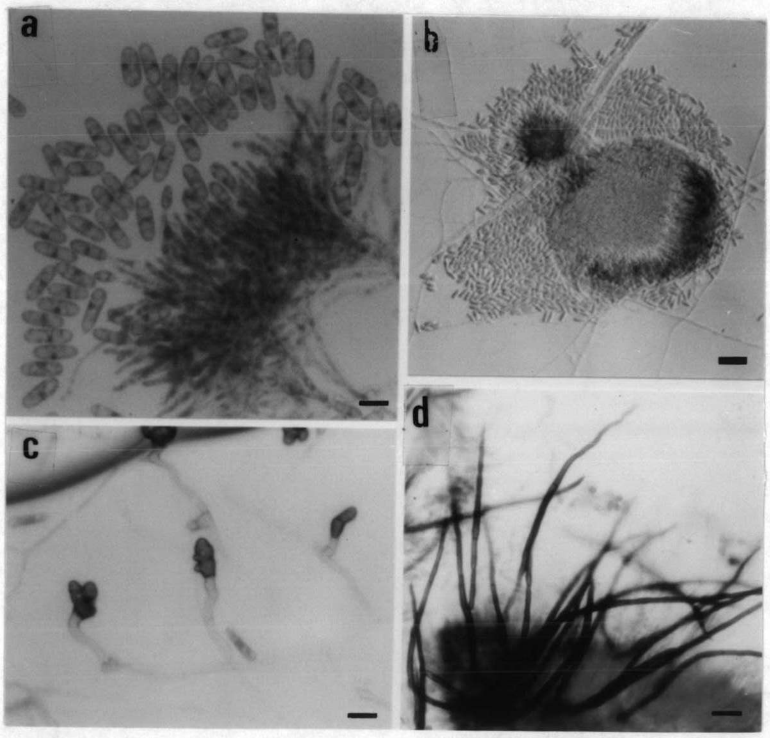

Figure 4.29 *Colletotrichum* sp. (a-b) conidiophore and conidia

(c) appressoria (d) setae (Bar = 10 μm)

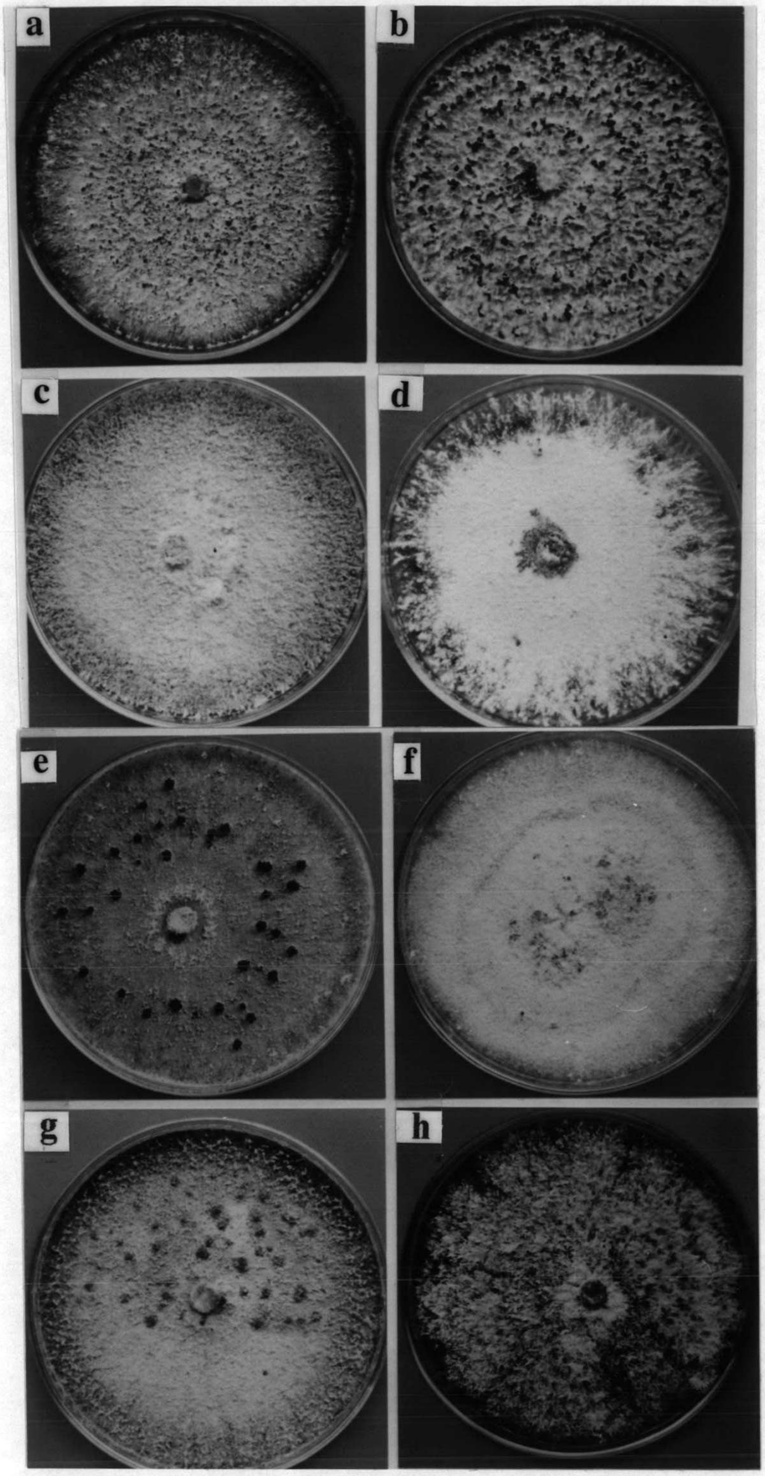

Figure 4.30 Culture on PDA of *Phomopsis* spp. from teak leaves (10-20 days)

(a) *Phomopsis* sp. T1 (b) *Phomopsis* sp. T2 (c) *Phomopsis* sp. T3

(d) *Phomopsis* sp. T4 (e) *Phomopsis* sp. T5 (f) *Phomopsis* sp. T6

(g) *Phomopsis* sp. T7 (h) *Phomopsis* sp. T8

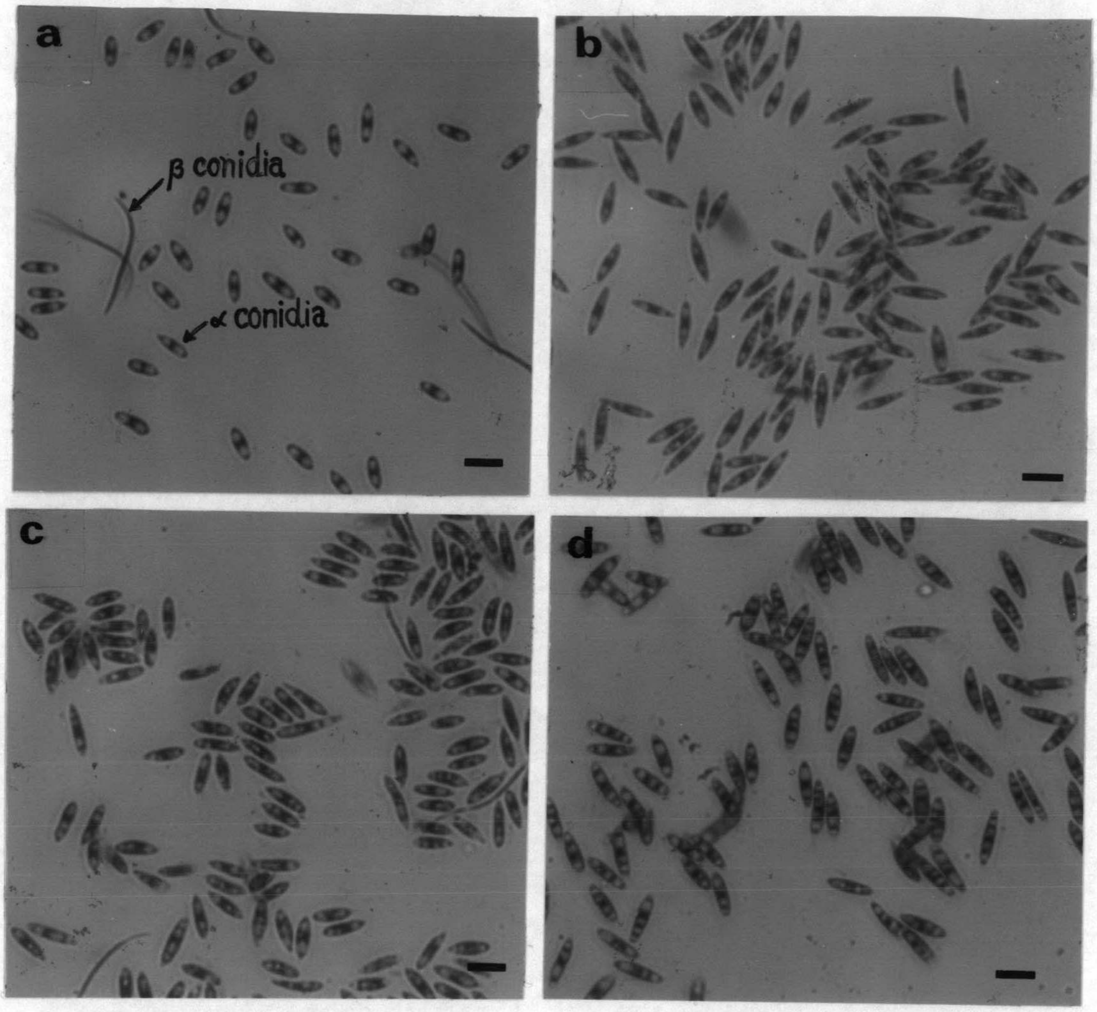

Figure 4.31 Conidia of *Phomopsis* spp. from teak leaves (Bar = 10 μm)

(a) α and β conidia of *Phomopsis* sp. T1

(b) α conidia of *Phomopsis* sp. T2

(c) α and β conidia of *Phomopsis* sp. T3

(d) α conidia of *Phomopsis* sp. T4

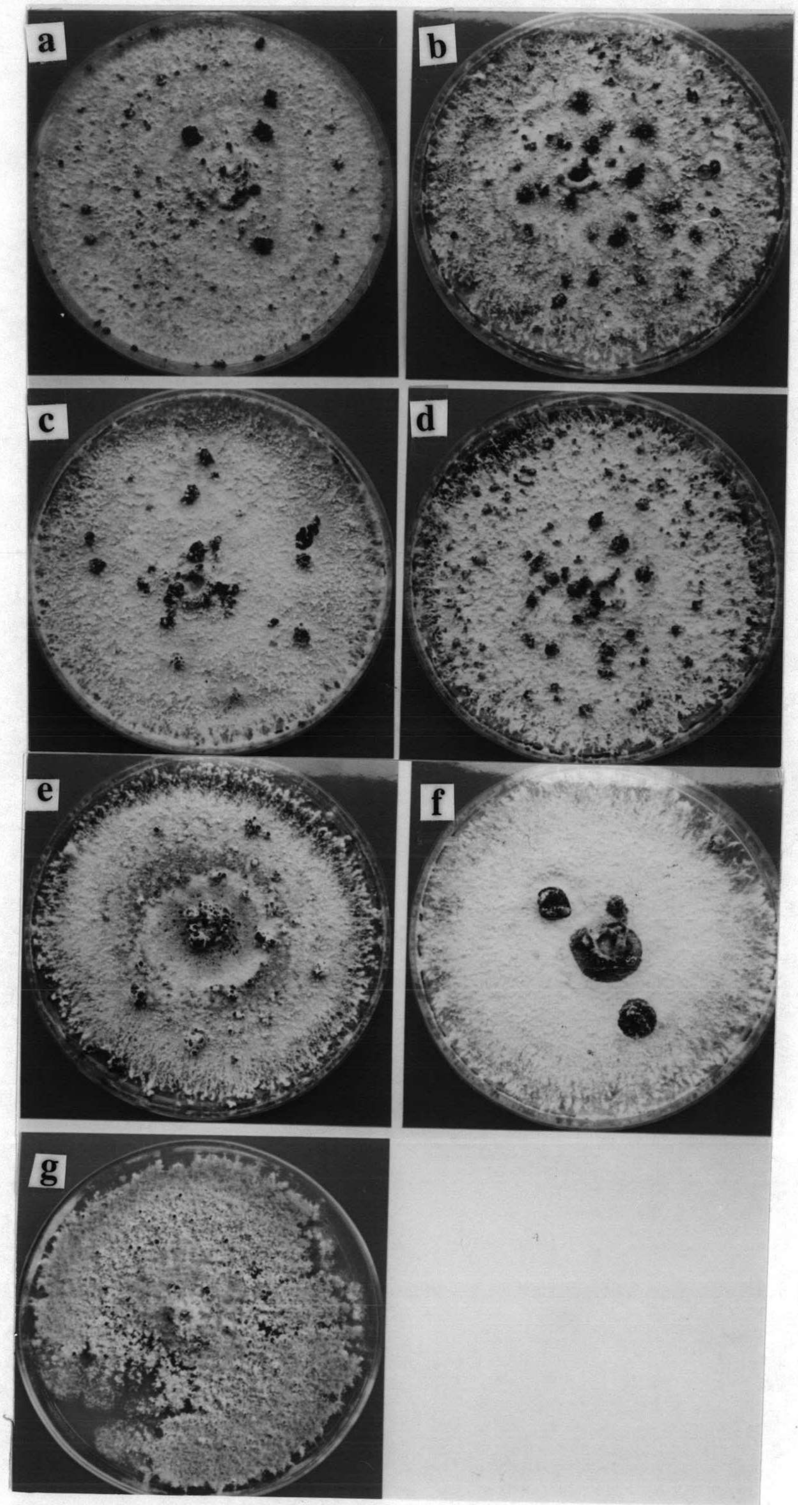

Figure 4.32 Culture on PDA of Phomopsis spp. from rain tree leaves (10-20 days)

(a) *Phomopsis* sp. S1 (b) *Phomopsis* sp. S2 (c) *Phomopsis* sp. S3

(d) *Phomopsis* sp. S4 (e) *Phomopsis* sp. S5 (f) *Phomopsis* sp. S6

(g) *Phomopsis* sp. S7

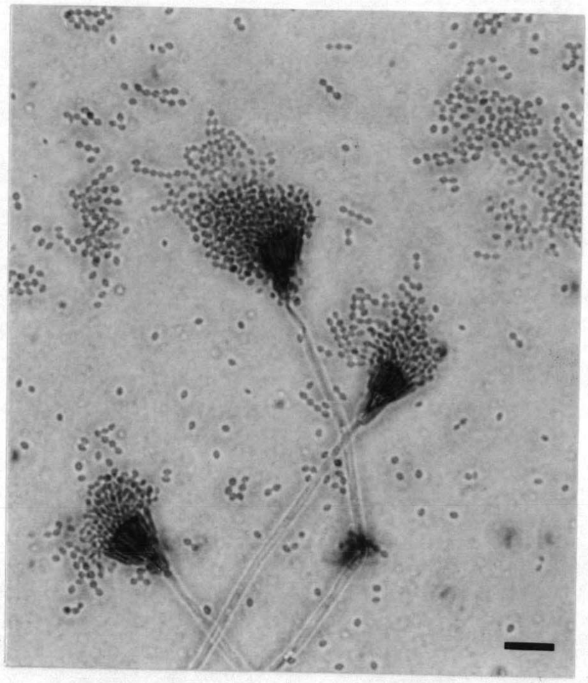

Figure 4.33 Conidial head and conidia of *Penicillium* sp. (Bar = 10 μm)

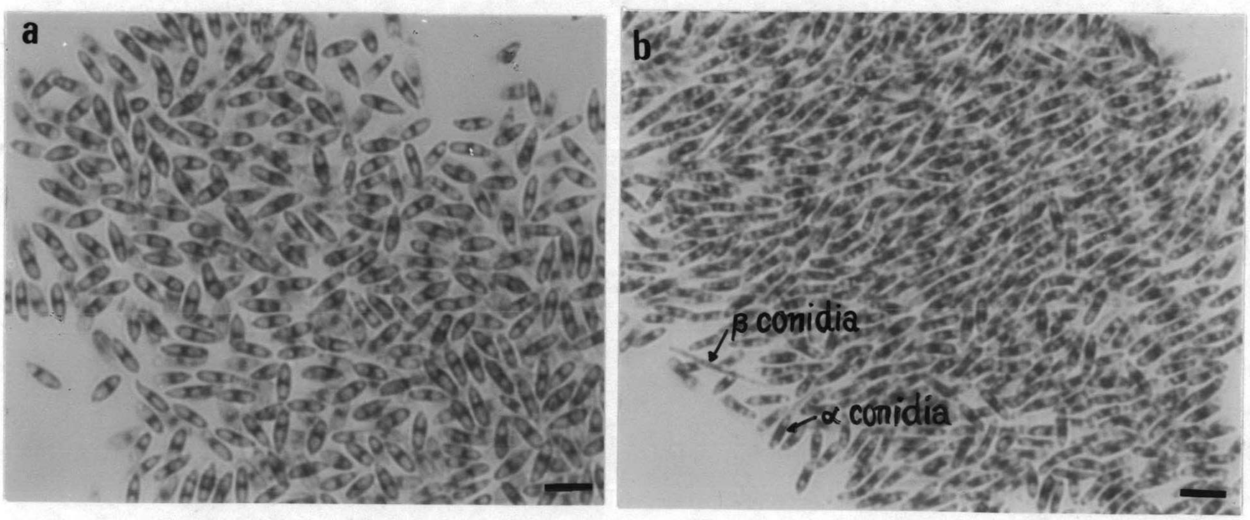

Figure 4.34 Conidia of Phomopsis spp. from rain tree leaves (Bar = 10 μm)

(a) α conidia of *Phomopsis* sp. S3

(b) α and β conidia of *Phomopsis* sp. S5

4.6 Induction of teleomorph formation

All isolates considered to be xylariaceous were used to induce teleomorph formation (Figure 4.40). The pure cultures of individual xylariaceous isolates were grown on PDA in glass bottles 11 cm tall and 6 cm diam until the surface of the agar was completely covered by the fungus. Pieces of cut twigs approx. 8 cm long X 3 cm diam. from teak and rain tree were introduced following sterilization. After appropriate incubation the twigs were removed and subjected to special environmetally devised incubation to allow teleomorph formation. *Xylaria* sp. 1 produced its teleomorph stage within 35 weeks but failed to developed to maturity (Figure 4.35) whereas, *Daldinia eschscholzii* developed to maturity within 18-20 weeks (Figure 4.36). *Xylaria* sp. 4 (Figure 4.37), *Xylaria* sp. 8 (Figure 4.38). , *Xylaria* sp. 11 (Figure 4.39) also produced stromata on twigs. Other *Xylaria* sp. failed to produce any stromata on twigs.

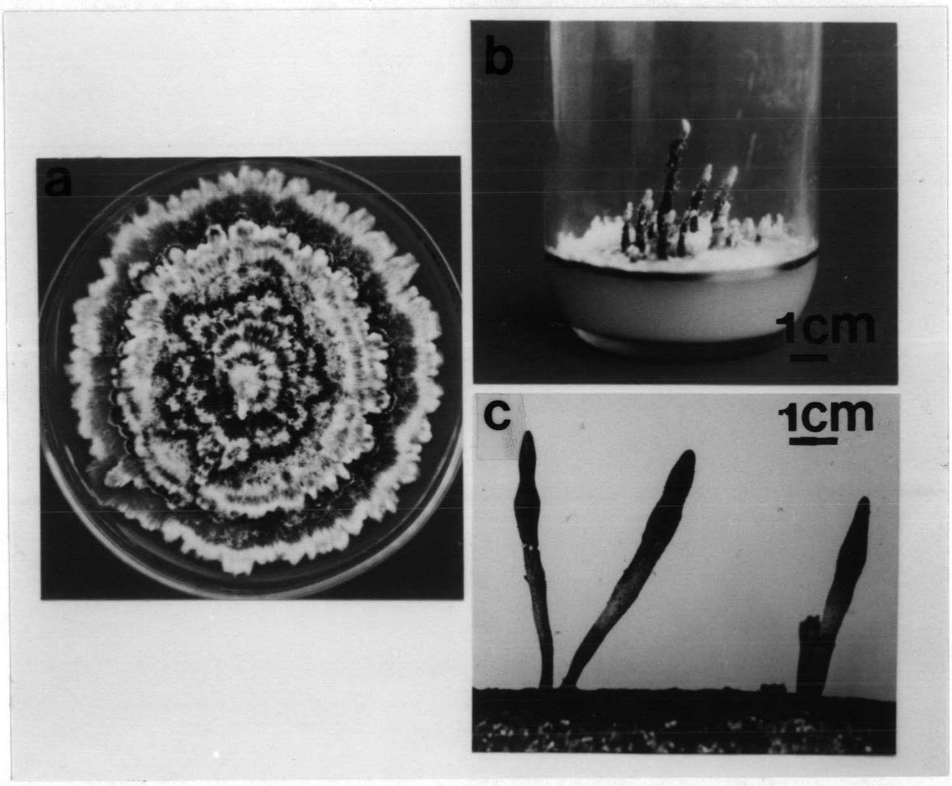

Figure 4.35 *Xylaria* sp.1 (a) culture on PDA (10 days) (b) Developing stromata on agar surface (c) Immature teleomorphic stromata on twig

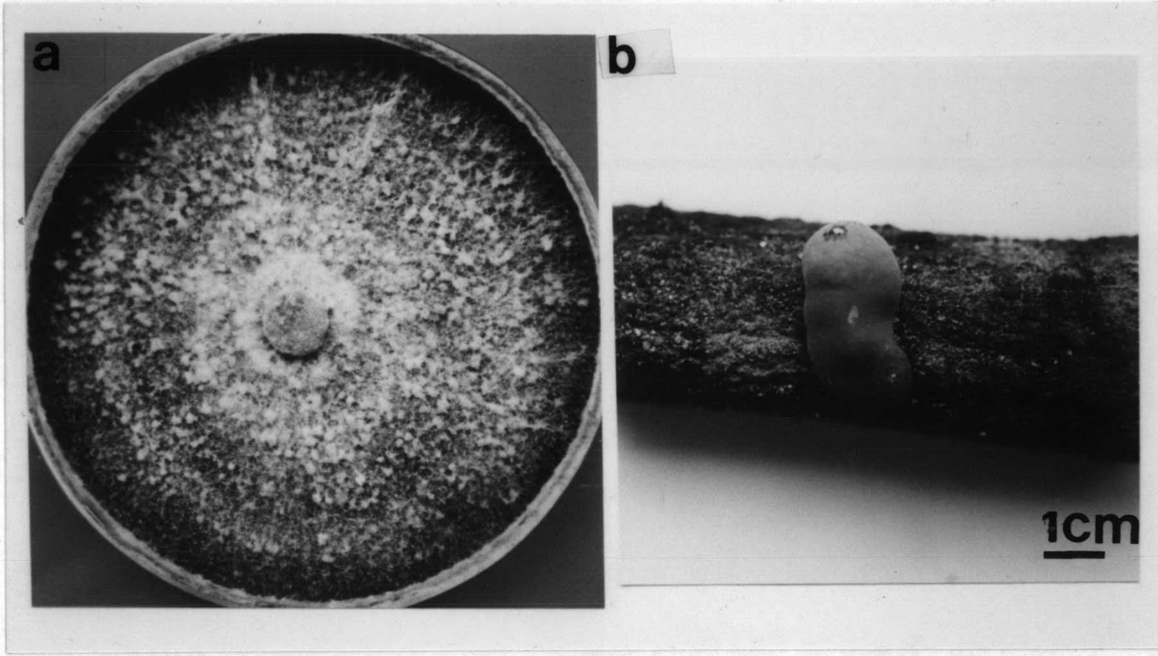

Figure 4.36 *Daldinia eschscholzii* (a) Culture on PDA (10 days) (b) Mature teleomorphic stromata on twig

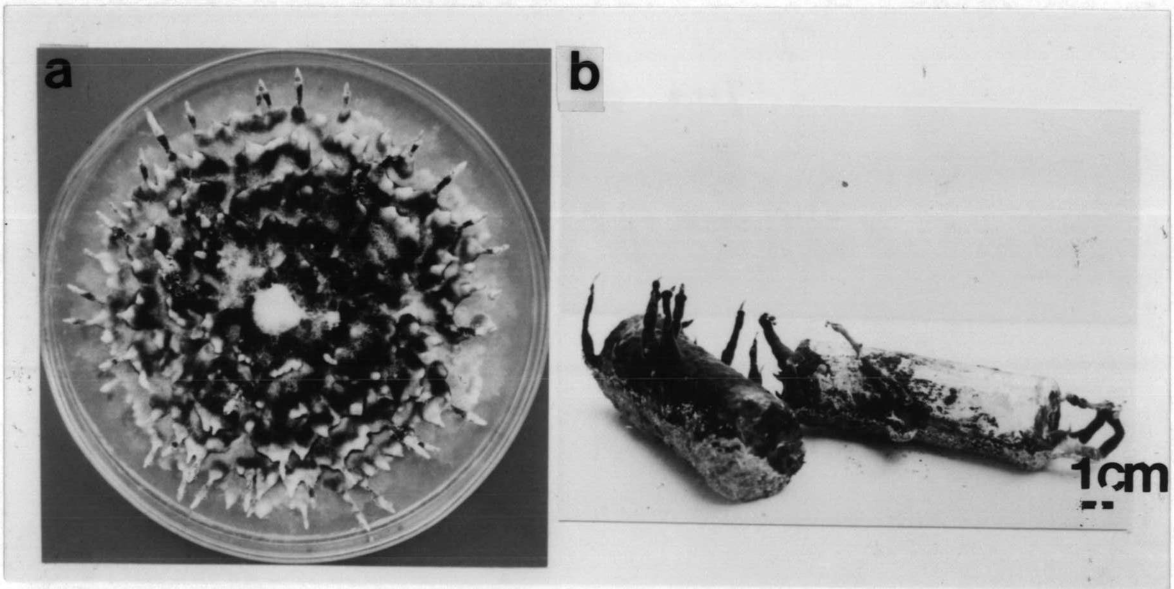

Figure 4.37 *Xylaria* sp. 4 (a) Culture on PDA (10 days) (b) Anamorphic stromata on twig

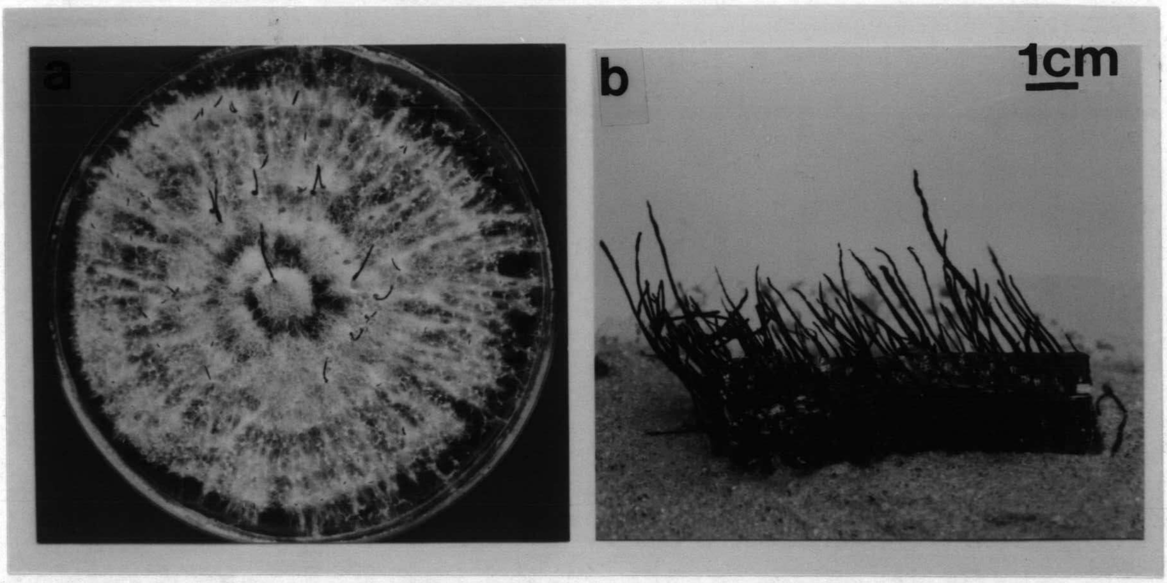

Figure 4.38 *Xylaria* sp. 8 (a) Culture on PDA (10 days) (b) Anamorphic stromata on twig

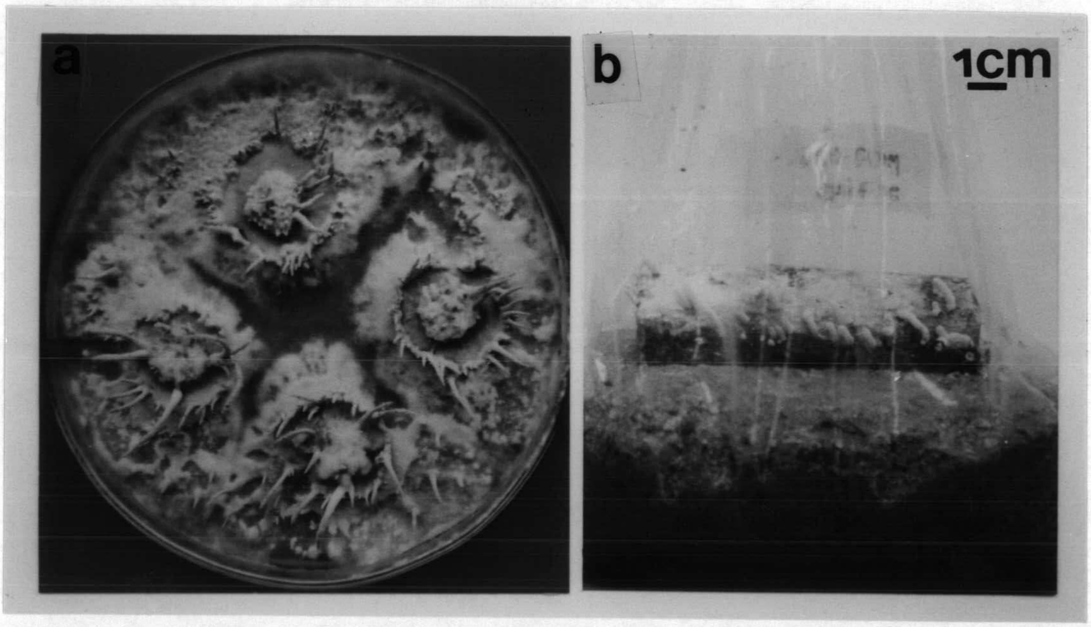

Figure 4.39 *Xylaria* sp.11 (a) Culture on PDA (10 days) (b) Anamorphic stromata on twig

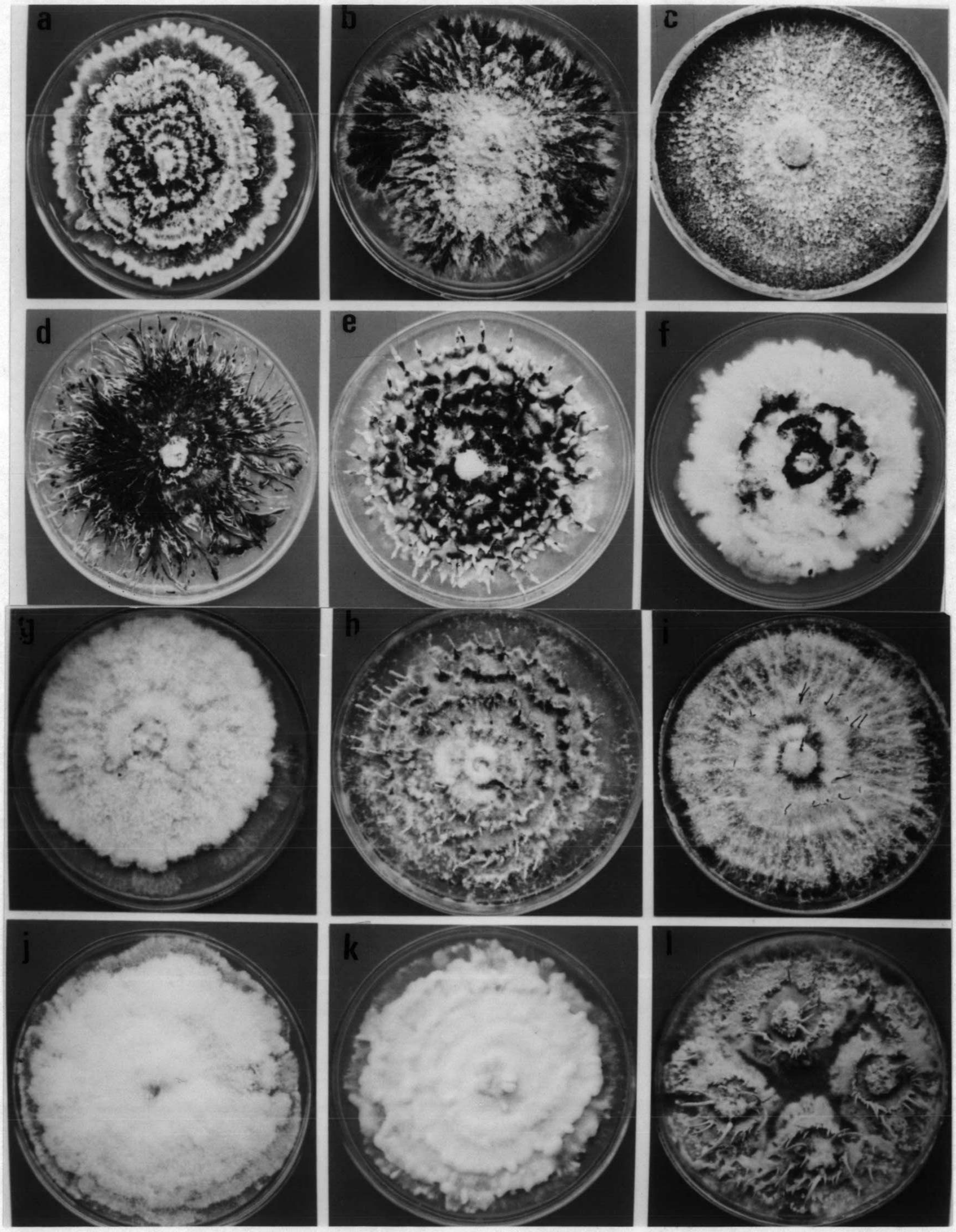

Figure 4.40 Culture on PDA of Xylariaceae (10-15 days) (a) *Xylaria* sp. 1 (b) *Xylaria* sp. 2 (c) *Daldinia eschscholzii* (d) *Xylaria* sp. 3 (e) *Xylaria* sp. 4 (f) *Xylaria* sp. 5 (g) *Xylaria* sp. 6 (h) *Xylaria* sp. 7 (i) *Xylaria* sp. 8 (j) *Xylaria* sp. 9 (k) *Xylaria* sp. 10 (l) *Xylaria* sp. 11

4.7 Antimicrobial testing

A total of 37 strains of endophytic fungi isolated from teak leaves and rain tree leaves (Table 4.23) were tested for antimicrobial activity, as an indication of their capability to produce secondary metabolites of potential therapeutic interest. There were 19 fungal strains could produce inhibitory substances which inhibited growth of bacteria and 3 fungal strains inhibited growth of *C.albicans*. Most of the antimicrobial activities were specific for Gram-positive bacteria. Only 2 fungal strains were found to produce inhibitors of the resistant Gram-negative bacteria selected. Almost all of the fungal strains that exhibited any antimicrobial activity inhibited the growth of *B.subtilis* and *S.aureus*, whereas no fungal strains inhibited the growth of *P.aeruginosa*. *E. coli* was inhibited only by the compounds produced by *Xylaria* sp. 5 and *Xylaria* sp. 7 For *C.albican*, there were only three fungal strains which produced compound able to inhibit its growth. These were *Daldinia eschscholzii*, *Xylaria* sp. 1 and *Xylaria* sp. 2 were detected.

Table 4.23 Antimicrobial activities of fungal isolates from plants.

Species	Host	Test organisms				
		B. subtilis (G+) ATCC6633	*S. aureus* (G+) ATCC25923	*E. coli* (G-) ATCC25922	*P. aeruginosa* (G-) ATCC27853	*C. albican*
1. *Alternaria* sp.	*T.grandis*	-	-	-	-	-
2. *Colletotrichum* sp. T1	*T.grandis*	-	++	-	-	-
3. *Daldinia eschscholzii*	*T.grandis*	-	++	-	-	+
4. *Fusarium* sp.	*T.grandis*	+	+	-	-	-
5. *Nigrospora* sp.	*T.grandis*	-	-	-	-	-
6. *Phomopsis* sp. T1	*T.grandis*	-	+	-	-	-
7. *Phomopsis* sp. T2	*T.grandis*	-	-	-	-	-
8. *Phomopsis* sp. T3	*T.grandis*	-	-	-	-	-
9. *Phomopsis* sp. T4	*T.grandis*	++	++	-	-	-
10. *Phomopsis* sp. T5	*T.grandis*	-	-	-	-	-
11. *Phomopsis* sp. T6	*T.grandis*	-	-	-	-	-
12. *Phomopsis* sp. T7	*T.grandis*	-	-	-	-	-
13. *Phomopsis* sp. T8	*T.grandis*	+	-	-	-	-
14. *Xylaria* sp. 1	*T.grandis*	+	-	-	-	+
15. *Xylaria* sp. 2	*T.grandis*	++	++	-	-	+

Activities were classfied according to the diameter of the inhibition zones around the point of application of the sample ++ , more than 15 mm ; +, less than 15 mm ; -, no inhibition.

Table 4.23 (continued)

Species	Host	Test organisms				
		B. subtilis (G+) ATCC6633	*S. aureus* (G+) ATCC25923	*E. coli* (G-) ATCC25922	*P. aeruginosa* (G-) ATCC27853	*C. albican*
16. *Xylaria* sp. 3	*T.grandis*	-	-	-	-	-
17. *Xylaria* sp. 4	*T.grandis*	-	-	-	-	-
18. *Xylaria* sp. 5	*T.grandis*	+	+	+	-	-
19. *Xylaria* sp. 6	*T.grandis*	-	-	-	-	-
20. *Xylaria* sp. 7	*T.grandis*	+	+	+	-	-
21. *Xylaria* sp. 8	*T.grandis*	+	-	-	-	-
22. *Xylaria* sp. 9	*T.grandis*	-	-	-	-	-
23. *Xylaria* sp. 10	*T.grandis*	++	+	-	-	-
24. *Xylaria* sp. 11	*T.grandis*	+	-	-	-	-
25. Unknown no. 1	*T.grandis*	-	-	-	-	-
26. Unknown no. 2	*T.grandis*	-	++	-	-	-
27. Unknown no. 3	*T.grandis*	-	-	-	-	-
28. Unknown no. 4	*T.grandis*	-	-	-	-	-
29. Unknown no. 5	*T.grandis*	-	-	-	-	-

Activities were classfied according to the diameter of the inhibition zones around the point of application of the sample ++ , more than 15 mm ; +, less than 15 mm ; -, no inhibition.

Table 4.23 (continued)

Species	Host	Test organisms				
		B. subtilis (G+) ATCC6633	*S. aureus* (G+) ATCC25923	*E. coli* (G-) ATCC25922	*P. aeruginosa* (G-) ATCC27853	*C. albican*
30. *Colletotrichum* sp. S1	*S.saman*	+	++	-	-	-
31. *Phomopsis* sp. S1	*S.saman*	-	-	-	-	-
32. *Phomopsis* sp. S2	*S.saman*	+	++	-	-	-
33. *Phomopsis* sp. S3	*S.saman*	-	-	-	-	-
34. *Phomopsis* sp. S4	*S.saman*	-	-	-	-	-
35. *Phomopsis* sp. S5	*S.saman*	+	++	-	-	-
36. *Phomopsis* sp. S6	*S.saman*	-	+	-	-	-
37. *Phomopsis* sp. S7	*S.saman*	++	-	-	-	-

Activities were classfied according to the diameter of the inhibition zones around the point of application of the sample ++ , more than 15 mm ; +, less than 15 mm ; -, no inhibition.