CHAPTER 3

SAPROBIC AND PATHOGENIC FUNGI ON DRACAENA AND PANDANUS

3.1 INTRODUCTION

The majority of previous studies on fungal associated with species of *Dracaena* and the Pandanaceae have concentrated on the discovery or descriptions of the diversity on their hosts (Hedjaroude, 1968; Shoemaker and Babcock, 1985; Dulymamode *et al.*, 1998a, b, c, d, e, 2001b; Whitton, 1999; Whitton *et al.*, 1999a, b, 2000a, b; Câmara *et al.*, 2001; Thongkantha *et al.*, 2003). There have been no previous detailed studies of the saprobic and pathogenic fungi on *Dracaena*. This chapter presents diversity and ecology data of saprobes and pathogens on *Dracaena lourieri* and three selected *Pandanus* species in Thailand, with additional data on fungal succession on *Pandanus penetrans* leaves.

3.2 MATERIALS AND METHODS

3.2.1 Diversity and ecology of saprobic fungi on *Dracaena* and *Pandanus*

Ten dead plant tissues were randomly collected from 10 plants of each host at different sites during 3 seasons; cool dry (November-January), hot dry (March-May) and hot wet (July-September). *Dracaena loureiri* was collected from Chiang Dao National Park, Chiang Mai (at around 400 m attitude), *Pandanus amaryllifolius* from one site at Medicinal Plant Garden in Doi Suthep Pui National Park, Chiang Mai (950 m attitude) and one site in Rayong Province, *P. odoratissimus* from coast in Rayong (300 m attitude) and, *P. penetrans* from one site in Doi Suthep Pui National Park (950 m attitude) and one site at the foothill of Kardthee Village in Phayao Province (300 m attitude). The collection details are listed in Table 3.1.

Table 3.1 Collection details of saprobic fungi study.

Host	Code	Site	Tissues*	Collection date
Dracaena lourieri	D1	Chiang Mai	Leaves	12/7/2002-Hot wet
(Wild species)	D2	Chiang Mai	Leaves	1/11/2005-Cool dry
Pandanus amaryllifolius	Pa1	Chiang Mai	Leaves	14/8/2005-Hot wet
(Cultivated species)	Pa2	Rayong	Leaves	10/8/2005-Hot wet
Pandanus odoratissimus	Po1	Rayong	Leaves	11/4/2004-Hot dry
(Possibly cultivated	Po2	Rayong	Leaves	6/12/2004-Cool dry
species)	Po3	Rayong	Prop roots	6/12/2004-Cool dry
	Po4	Rayong	Seeds	6/12/2004-Cool dry
Pandanus penetrans	Pp1	Phayao	Leaves	27/4/2004-Hot dry
(Wild species)	Pp2	Chiang Mai	Leaves	24/4/2004-Hot dry
	Pp3	Chiang Mai	Leaf sheaths	9/4/2004-Hot dry
	Pp4	Chiang Mai	Leaves	3/8/2003-Hot wet
	Pp5	Chiang Mai	Leaves	19/1/2004-Cool dry
	Pp6 or D0	Chiang Mai	Green leaves	19/1/2003-Cool dry, in succession study at day 0
	Pp7 or M12	Chiang Mai	Leaves	19/1/2004-Cool dry, in succession study at month 12
	Pp8 or M18	Chiang Mai	Leaves	19/6/2004-Hot wet, in succession study at 18

* Ten samples were collected for each collection.

All samples (ca 30 cm long) were placed in separate plastic bags with tissue paper, then sprayed with sterile water to create humid conditions and incubated at room temperature. The fungi present on the samples were examined and recorded within 1-4 weeks of incubation. Each fungus was identified according to taxonomic keys (e.g. Ellis, 1971, 1976; Carmichael, 1980; Sutton, 1980; Von Arx, 1981; Fröhlich and Hyde, 2000; Hyde *et al.*, 2000), and a species list with frequency of occurrence is presented for each host. Saprobic fungi were isolated by single spore methods (Choi *et al.*, 1999), and grown on ½ strength PDA. Small sections of the samples containing the fungi were cut out, dried and prepared as herbarium specimens. Correspondence analyses were performed to test whether the species composition of the trials are statistically different.

3.2.2 Fungal succession on *Pandanus penetrans* leaves

Mature leaves of *Pandanus penetrans* were cut from plants in Doi Suthep-Pui National Park at around 950 m attitude (the same site as those of the natural samples).

Eleven randomly selected plants were used in this experiment (included some spare trees and leaves). Twelve mature green leaves were cut from each of the eleven selected *Pandanus* trees. Ten of these leaves were randomly selected as a day 0 sample. The other leaves were tied with nylon string to the host plants to prevent the leaves being washed away by high rainfall or winds. At each sampling time ten decaying bait leaves were randomly collected from the eleven trees. It was planned to collect the bait leaves at week 1, and months 1, 2, 4, 6, 12, 18 and 24. However, sampling was stopped when the leaves had completely decayed at 18 months. Samples were placed in separate plastic bags in the forest and brought back to the laboratory. They were incubated individually in plastic bags, with an addition of tissue paper moistened with sterilized water. All leaves were examined under a microscope for the presence of fungi after one day of incubation and then periodically for up to two weeks. Squash mounts of sporulating fungi were made in water and/or other suitable mountains for examination with differential interference contrast microscopy. Fungi were isolated by single spore isolation (Choi *et al.*, 1999). Herbarium specimens of fungi were prepared. The percentage occurrence and a correspondence analysis were performed to examine the difference in fungal communities at different times of decay.

The percentage occurrence was calculated as follow

$$\text{Percentage occurrence} = \frac{\text{number of samples which fungus was detected} \times 100}{\text{total number of samples examined at each sampling time}}$$

A 2- or 3-dimensional correspondence analysis was performed to examine the difference in fungal communities on different collections.

Similar communities are positioned closely together in the same ordination space (Anonymous, 1995). Sorenson index was also used to measure similarity between species diversity on different hosts.

Similarity index = 2c/a+b

a = the number of species in host 1

b = the number of species in host 2

c = the number of species in common in both hosts

Species area curves were used to determine the adequacy of the sampling size.

Shannon index (H') was used to express species diversity of a community (Shannon and Weaver, 1949).

Shannon index (H') = $-\Sigma p_i \ln p_i$

p_i = the proportion of occurrence of taxon i^{th} and occurrence of all taxa

3.2.3 Pathogenic fungi on *Dracaena* and *Pandanus* leaves

Dracaena loureiri and *Pandanus* spp. plants with leaf spots or other disease symptoms were collected in the same sites as those in the saprobe study and returned to the laboratory. Fungi on the diseased tissue were isolated and identified. Some of them were tested for their pathogenicity according to Koch's postulates (Figure 3.1). In this way a collection of pathogens on these hosts were established.

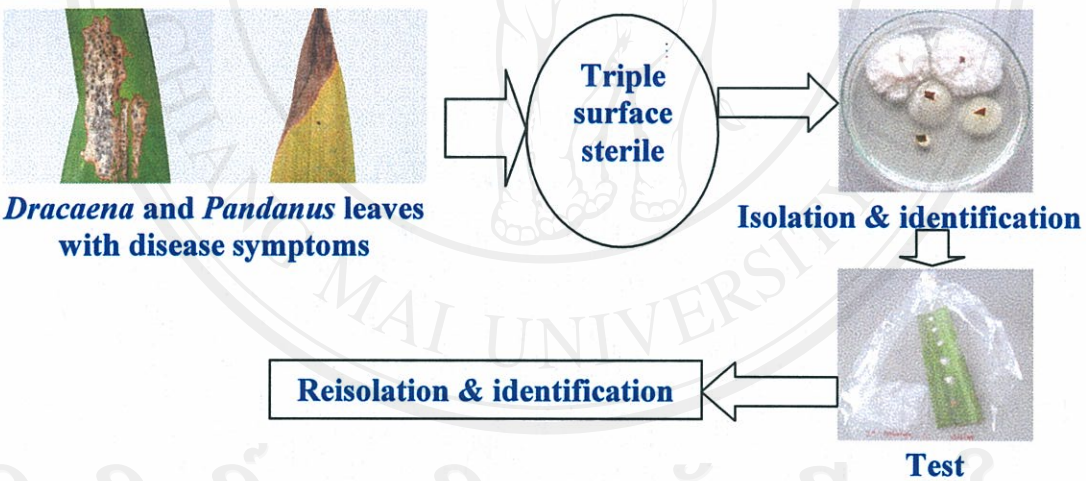


Figure 3.1 Isolation and pathogenicity test of fungal pathogens on *Dracaena loureiri* and *Pandanus* spp. leaves.

Isolates of parasitic and saprobic fungi which have previously been reported as plant pathogens and a few isolates of *Xylaria* as endophytes were tested for their pathogenicity to *Dracaena loureiri*, *Pandanus amaryllifolius* and *P. penetrans* leaves. The selected fungal isolates (*Acremonium*, *Cladosporium*, *Colletotrichum*, *Curvularia*, *Fusarium*, *Guignardia*, *Oxydothis*, *Phomopsis* and *Xylaria*) were grown on ½ strength PDA for 1-4 weeks depending on their growth rate. Pathogenicity

testing was determined by inoculating healthy leaves in the plastic bag with the mycelium of the pathogen. In one treatment the leaves were wounded with a sterile needle, while the others treatment the leaves were unwounded. For the control, the same procedure was followed, using disks of sterile ½ strength PDA. Any lesions on the leaves were determined after 10-15 days of incubation. Necrotic lesions were removed and inoculated on ½ strength PDA for recovery of the infected strains.

3.3 RESULTS

3.3.1 Diversity and ecology of saprobes

3.3.1A Determination of sample size

The species area curve for almost all sampling of saprobes on *Dracaena loureiri* and *Pandanus* spp. reached asymptote (Figure 3.2). Therefore the number of samples of about 10 (from 10 plants) was large enough to obtain a highly representative result. The species area curve for *D. loureiri* leaves during the cool dry season (D2) almost reached asymptote because the slopes of the curves were declining with the increase of sample size and at about 10 samples the slopes were near zero. Although the curve did not completely level off, the number of samples was also large enough.

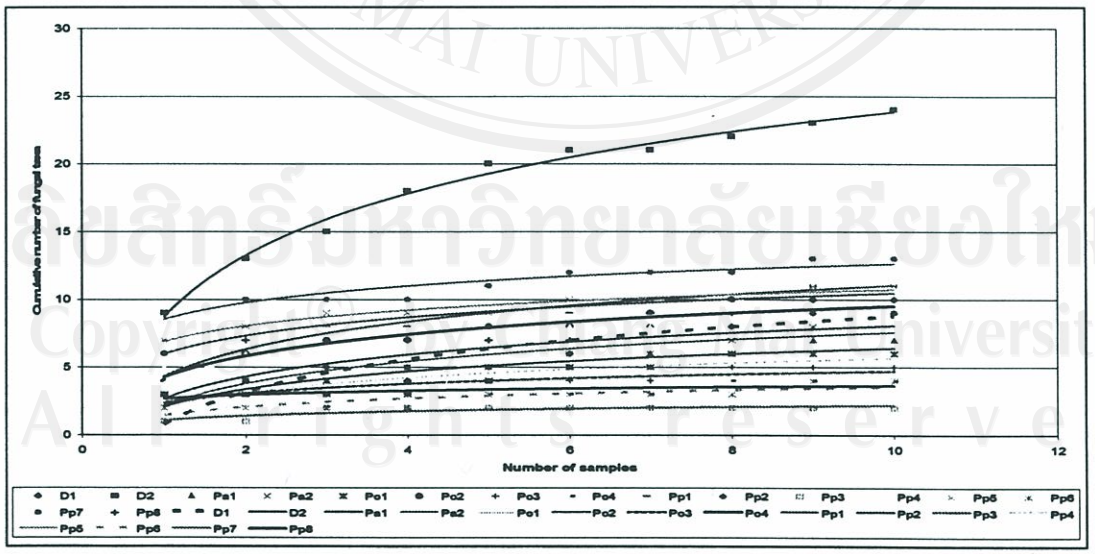


Figure 3.2 Species area curve for fungi collected on *Dracaena loureiri* and *Pandanus* spp. at each sampling times.

3.3.1B Fungal taxonomic composition

The fungi occurring on *Dracaena loureiri* and *Pandanus* spp. at each collection are listed and summarized in Table 3.2. One-hundred and twenty-six taxa were recorded, comprising 39 ascomycetes, 1 basidiomycete and 86 anamorphic fungi.

The most common taxa occurring on dead leaves of *Dracaena loureiri* were *Stachybotrys chartarum* (75%), *Botryodiplodia theobromae* (65%) and *Zygosporium dracinicola* sp. nov. (65%). *Aspergillus parasiticus*, *Colletotrichum gloeosporioides*, *Microthyrium* sp. 1, *Phomopsis archeri* and *Trichothecium roseum* were found on 50-55% of samples. *Cladosporium cucumerinum*, *Cryptophiale*-like, *Fusarium oxysporum*, Hyphomycete 5, *Monodictys* sp. 2, *Nectria*-like 2 and *Stachybotrys theobromae* usually occurred on 40-45% of samples (Table 3.2).

Fungal taxa found on decaying leaves of *Pandanus amaryllifolius* with high overall percentage occurrences at 100%, 75%, 70%, 65%, 55% and 35% were *Acremonium* sp. 6, *Nectria*-like 3, *Phoma* sp., *Botryodiplodia theobromae*, *Zygosporium oscheoides* and *Nigrospora oryzae* (Table 3.2).

Dead tissues of *Pandanus odoratissimus* were frequently colonized by *Acremonium* sp. 3 (50%), *Aspergillus parasiticus* (67.5%), *Botryodiplodia theobromae* (40%), *Monodictys* sp. 1 (52.5%), *Phomopsis* sp.1 (35%), *Cladosporium cucumerinum* (30%), *Linocarpon lammiae* (27.5%) and *Ophiostoma* sp. (25%) (Table 3.2).

The taxa occurring on *Pandanus penetrans* leaves with at least 20% of samples both from natural samples (Pp1, Pp2, Pp4 and Pp5) and baits (Pp6, Pp7 and Pp8) were compared and ranked in Table 3.3. *Myrothecium pandanicola* sp. nov., *Nectria*-like 1, *Oxydothis linospadicis*, *Phaeosphaeria*-like, *Sporidesmium ghanaense* and *Trichoderma* sp. were overlapping taxa and frequently found on both samples (20-63.3% of samples).

Table 3.2 Frequency and overall percentage occurrence of fungal taxa occurred on *Dracaena lourieri* (D1-D2), *Pandanus amaryllifolius* (Pa1-Pa2), *P. odoratissimus* (Po1-Po4) and *P. penetrans* (Pp1-Pp8).

No.	Code	Taxa	D1	D2	%	Pa1	Pa2	%	Po1	Po2	Po3	Po4	%	Pp1	Pp2	Pp3	Pp4	Pp5	%	Pp6	Pp7	Pp8	%
1	S013h	*Acremonium* sp. 1	1	6	35																		
2	S043h	*Acremonium* sp. 2												4			1		10		1		3.3
3	S104h	*Acremonium* sp. 3							9	3	8		50										
4	S145h	*Acremonium* sp. 4												1	1		1	1	8				
5	S106h	*Acremonium* sp. 5							1	2			7.5										
6	S188h	*Acremonium* sp. 6		7	35	10	10	100															
7	S202h	*Acremonium* sp. 7		3	15																		
8	S135h	*Alternaria alternata*												1					2				
9	S004a	*Anthostomella tomicoides*	1		5																		
10	S014a	Ascomycete 1[#]	1		5																		
11	S095a	Ascomycete 2															1		2				
12	S105a	Ascomycete 3							1	4			12.5										
13	S178a	Ascomycete 4								5			12.5										
14	S020h	*Aspergillus niger*		6	30																		
15	S102h	*Aspergillus parasiticus*		10	50				5	4	10	8	67.5										
16	S107h	*Aspergillus* sp. 1							1	5			15										
17	S122h	*Aspergillus* sp. 2													1			1	4		1		3.3
18	S143h	*Aspergillus* sp. 3												8					16				
19	S126a	*Astrosphaeriella tornata*													3	10		3	32		3		10
20	B001b	Basidiomycete													1				2				
21	S054h	*Berkleasmium* sp.																		2			6.7
22	S082c	*Botryodiplodia theobromae*	3	10	65	6	7	65		3	6	7	40										
23	S108a	*Byssosphaeria*-like							2	2			10										
24	S152h	*Canalisporium exiguum*												2	1		2	2	14		3	9	40
25	S196h	*Canalisporium variabile*		5	25																		
26	S119a	*Chaetomium globosum*								6			15		3			1	8		1		3.3
27	S069h	*Chloridium virescens*		3	15									5									

Table 3.2 continued

No.	Code	Taxa	D1	D2	%	Pa1	Pa2	%	Po1	Po2	Po3	Po4	%	Pp1	Pp2	Pp3	Pp4	Pp5	%	Pp6	Pp7	Pp8	%
28	S103h	*Cladosporium cucumerinum*		8	40				7	5			30	2					4				
29	S197c	Coelomycete 1		4	20																		
30	S075c	*Colletotrichum gloeosporioides*	3	8	55									1					2				
31	S186c	*Colletotrichum* sp.		2	10																		
32	S084h	*Cryptophiale*-like		9	45																		
33	S169h	*Curvularia eragrostidis*								3			7.5										
34	S128h	*Curvularia lunata*												7					14				
35	S185h	*Cylindrocarpon* sp.		4	20																		
36	S118h	*Cylindrocladium* sp. 1													1		2	1	8		1		3.3
37	S200h	*Cylindrocladium* sp. 2		2	10																		
38	S205h	*Dactylaria purpurella*					4	20															
39	S046h	*Dictyochaeta fertilis*																		1			3.3
40	S034h	*Dictyosporium heptasporum*		1	5										1		1		4				
41	S045h	*Ellisembia adscendens*													1			3	8	3	3	7	43.3
42	S172a	*Emericella nidulan*								1			2.5										
43	S180a	*Emericella* sp.								2			5										
44	S174h	*Exserohilum* sp.#								2			5										
45	S129h	*Fusarium oxysporum*	4	5	45									5					10				
46	S006h	*Fusicladium* sp.	4		20															1			3.3
47	S076a	*Glomerella cingulata*	2	2	20									5					10				
48	S139a	*Glomerella* sp. 1												5					10				
49	S156a	*Glomerella* sp. 2															4		8			3	10
50	S080a	*Guignardia* sp.	3		15																		
51	S160h	*Helicosporium* sp.																			1	1	6.7
52	S117h	Hyphomycete 1							1				2.5										
53	S142h	Hyphomycete 2												3					6				
54	S151h	Hyphomycete 3																				1	3.3
55	S171h	Hyphomycete 4								3			7.5										
56	S192h	Hyphomycete 5		3	15																		

Table 3.2 continued

No.	Code	Taxa	D1	D2	%	Pa1	Pa2	%	Po1	Po2	Po3	Po4	%	Pp1	Pp2	Pp3	Pp4	Pp5	%	Pp6	Pp7	Pp8	%
57	S206h	Hyphomycete 6					6	30															
58	S071h	Hyphomycete (synnematous) 1															2		4				
59	S124h	Hyphomycete (synnematous) 2													1				2				
60	S149h	Hyphomycete (synnematous) 3																				8	26.7
61	S029a	*Hyponectria* sp.															3		6				
62	S112a	*Leptosphaeria*-like							1				2.5										
63	S101a	*Linocarpon lammiae*							4	7			27.5									1	3.3
64	S033a	*Linocarpon livistonae*													1		2		6				
65	S157a	*Linocarpon siamensis* sp. nov.*															4	2	12		2	1	10
66	S153a	*Melanochaeta hemipsila*														8	4		24		5	5	33.3
67	S110h	*Memnoniella echinata*	3		15	4	1	25	3	1			10		2			3	10		2		6.7
68	S183h	*Memnoniella* sp.								2	4		15										
69	S007a	*Microthyrium* sp. 1	1	10	55																		
70	S048a	*Microthyrium* sp. 2					4	20												3			10
71	S109h	*Monodictys* sp. 1							5	8		8	52.5										
72	S194h	*Monodictys* sp. 2		8	40																		
73	S038h	*Myrothecium pandanicola* sp. nov.#													9		4	5	36	2	5		23.3
74	S164a	*Nectria*-like 1	3		15										1		6	5	24		6		20
75	S201a	*Nectria*-like 2	3	5	40																		
76	S207a	*Nectria*-like 3				8	7	75															
77	S134h	*Nigrospora oryzae*	3		15	4	3	35		6			15	8				1	18		1	1	6.7
78	S184h	*Oidiodendron griseum*		5	25																		
79	S011a	*Ophioceras chiangdaoensis* sp. nov.#	3	3	30																		
80	S031a	*Ophioceras leptosporum*													1		1		4				
81	S204a	*Ophiostoma* sp.										10	25										
82	S159a	*Ornatispora* sp.#																2	4		4	1	16.7
83	S024a	*Oxydothis linospadicis*												1	10		9	10	60		10	4	46.7
84	S141a	*Oxydothis siamensis* sp. nov.#												4					8				
85	S146h	*Paecilomyces varioti*												4					8				

Table 3.2 continued

No.	Code	Taxa	D1	D2	%	Pa1	Pa2	%	Po1	Po2	Po3	Po4	%	Pp1	Pp2	Pp3	Pp4	Pp5	%	Pp6	Pp7	Pp8	%
86	S015a	*Paraphaeosphaeria obtusispora*	1		5																		
87	S131h	*Penicillium chrysogenum*												10	3			10	46				
88	S125h	*Penicillium* sp. 1																		2	10		40
89	S176h	*Penicillium* sp. 2								2			5										
90	S050h	*Periconia cookei*	3		15										1			7	16	1	8		30
91	S056h	*Periconia minutissima*																		1			3.3
92	S179h	*Periconia* sp.[#]								2			5										
93	S049c	*Pestalotiopsis guepinii*	3	2	25															2			6.7
94	S032a	*Phaeonectriella pandani* sp. nov.[#]															1		2				
95	S127a	*Phaeosphaeria*-like[#]													3		4	8	30		10	9	63.3
96	S148h	*Phaeostalagmus cyclosporus*																4	8		5	8	43.3
97	S025h	*Phialocephala bactrospora*															5		10				
98	S163h	*Phialocephala* sp.													5				10		2		6.7
99	S182c	*Phoma destructiva*								2			5										
100	S208h	*Phoma* sp.				4	10	70															
101	S027a	*Phomatospora* sp. 1															2	8	20		9	2	36.7
102	S035a	*Phomatospora* sp. 2															1		2				
103	S203c	*Phomopsis archeri*	3	8	55																		
104	S008c	*Phomopsis* sp. 1	4		20	3																	
105	S094c	*Phomopsis* sp. 2												1	2		6	1	20				
106	S111h	*Phomopsis* sp. 3							3	3	8		35										
107	S199a	*Pseudohalonectria suthepensis*		1	5																		
108	S155c	*Pyrenochaeta* sp.																			1	2	10
109	S189h	*Ramichloridium subulatum*		8	40																		
110	S140a	*Sordaria fimicola*												2					4				
111	S047h	*Sporidesmium ghanaense*												2	3		2	3	20	7	2	10	63.3
112	S001h	*Stachybotrys chartarum*	5	10	75																		
113	S195h	*Stachybotrys theobromae*		8	40																		
114	S042h	*Stachylidium bicolor*													1		2	4	14		5		16.7

Table 3.2 continued

No.	Code	Taxa	D1	D2	%	Pa1	Pa2	%	Po1	Po2	Po3	Po4	%	Pp1	Pp2	Pp3	Pp4	Pp5	%	Pp6	Pp7	Pp8	%
115	S063h	Sterile mycelia 1																		2			6.7
116	S120h	*Trichoderma* sp.												5	4			5	28		6		20
117	S191h	*Trichothecium roseum*		10	50										1				2				
118	S067h	*Tubercularia lateritia*																		1	1		6.7
119	S150a	*Tubeufia cerea*																				3	10
120	S115h	*Veronaea botryosa*							1				2.5										
121	S086h	*Verticicladiella* sp.															2		4				
122	S060h	*Verticillium tenerum*												3			2		10				
123	S173h	*Verticillium* sp.				4		20		4			10										
124	S165h	*Volutella* sp.															3		6				
125	S085h	*Zygosporium dracinicola* sp. nov.[#]	3	10	65																		
126	S073h	*Zygosporium oscheoides*				6	5	55							1			4	10		4	1	16.7
Total number of fungal records (1109)			60	186	246	49	57	106	44	87	36	33	200	89	62	18	77	94	340	28	112	77	217
Anamorphic fungi (86)			13	27		8	8		10	18	5	3		18	18	0	14	16		12	19	10	
Ascomycetes (39)			9	5		1	2		4	7	0	1		5	7	2	13	8		1	9	9	
Basidiomycetes (1)			0	0		0	0		0	0	0	0		0	1	0	0	0		0	0	0	
Total taxa (126)			22	32		9	10		14	25	5	4		23	26	2	27	24		13	28	19	

* New species known only from *P. penetrans* with the description in Chapter 4 and Thongkantha *et al.*, 2003 or presently inpress.

[#] New species known only from *D. loureiri*, *Pandanus odoratissimus*, *P. penetrans* with the description in Chapter 4 or in preparation.

D1 = 10 leaves of *D. lourieri* collected from Chiangdao National Park in Chiang Mai during hot wet season

D1 = 10 leaves of *D. lourieri* collected from Chiangdao National Park in Chiang Mai during cool dry season

Pa1 = 10 leaves of *P. amaryllifolius* collected from Medicinal Plant Garden in Chiang Mai during hot wet season

Pa2 = 10 leaves of *P. amaryllifolius* collected from a garden in Rayong during hot wet season

Po1 = 10 leaves of *P. odoratissimus* collected from Nang Rum Beach in Rayong during hot dry season

Po2 = 10 leaves of *P. odoratissimus* collected from Nang Rum Beach in Rayong during cool dry season

Po3 = 10 prop roots of *P. odoratissimus* collected from Nang Rum Beach in Rayong during cool dry season

Po4 = 10 seeds of *P. odoratissimus* collected from Nang Rum Beach in Rayong during cool dry season

Pp1 = 10 leaves of *P. penetrans* collected from the foothill of Kardthee Village in Phayao during hot dry season

Pp2 = 10 leaves of *P. penetrans* collected from Doi Suthep Pui National Park in Chiang Mai during hot dry season

Pp3 = 10 leaf sheaths of *P. penetrans* collected from Doi Suthep Pui National Park in Chiang Mai during hot dry season

Pp4 = 10 leaves of *P. penetrans* collected from Doi Suthep Pui National Park in Chiang Mai during hot wet season

Pp5 = 10 leaves of *P. penetrans* collected from Doi Suthep Pui National Park in Chiang Mai during cool dry season

Pp6 = 10 leaf baits of *P. penetrans* collected at day 0

Pp7 = 10 leaf baits of *P. penetrans* collected at month 12

Pp8 = 10 leaf baits of *P. penetrans* collected at month 18

Table 3.3 Comparison of most common fungal taxa recorded on samples in nature and baits (in partial succession study) of *Pandanus penetrans* leaves.

Taxa	Overall percentage occurrence (rank)	
	Natural samples (Pp1-Pp5)	Baits (Pp6-Pp8)
Canalisporium exiguum		40 (4)
Ellisembia adscendens		43 (3)
Hyphomycete (synnematous) 3		27 (8)
Melanochaeta hemipsila		33 (6)
Myrothecium pandanicola sp. nov.*	36 (3)	23 (9)
Nectria-like 1	24 (6)	20 (10)
Oxydothis linospadicis	60 (1)	47 (2)
Penicillium chrysogenum	46 (2)	
Penicillium sp. 1		40 (4)
Periconia cookei		30 (7)
Phaeosphaeria-like*	30 (4)	63 (1)
Phaeostalagmus cyclosporus		43 (3)
Phomatospora sp. 1	20 (7)	37 (5)
Phomopsis sp.1	20 (7)	
Sporidesmium ghanaense	20 (7)	63 (1)
Trichoderma sp.	28 (5)	20 (10)

* New taxa known only from *Pandanus penetrans* with description presently in press and in chapter 4 or in preparation.

3.3.1C Effect of hosts and their habitats on fungal communities

Fungal community composition was influenced by the host plant. Figure 3.3 shown distinct fungal community occurrence on *Dracaena lourieri* (2 times collections), *Pandanus amaryllifolius* (2), *P. odoratissimus* (4) and *P. penetrans* (8). A percentage of total variance explained by the model of three dimensional corresponding analyses is 37.94%.

In comparison to samples from different habitats, fungal communities on *P. odoratissimus* from the beach in Rayong Province were distinct and were not similar with those of *D. lourieri* and *P. penetrans* from rainforests in Chiang Mai. The highest number of fungal taxa occurred on collections of *D. lourieri, P. amaryllifolius*, *P. odoratissimus*, *P. penetrans* occurring on samples in nature and *P. penetrans* in succession study samples (baits) and were 32, 10, 25, 27 and 28 respectively (Table 3.2). *Acremonium*, *Aspergillus*, *Botryodiplodia*; *Cladosporium*, *Memnoniella*, *Nigrospora*, *Phomopsis* and *Zygosporium* were the overlap genera found on *Dracaena* and *Pandanus* and some of them were also common on their hosts (Table

3.2). The Sorensen indices also show that fungal taxa occurring on samples between *D. lourieri* and *P. penetrans* (40-50%) were more similar than samples between *P. amaryllifolius*, *P. odoratissimus* and *P. penetrans* (10-20%) (Table 3.4). The similarities of fungi discovered from naturally occurring samples and baits (day 0 plus month 12 and month 18) of *P. penetrans* was high (50%).

Table 3.4 Similarity of fungal taxa composition between *Dracaena loureiri* and *Pandanus* species.

Sorenson index (%)	***D. loureiri***	***P. amaryllifolius***	***P. odoratissimus***	***P. penetrans*** (Natural samples)
P. amaryllifolius	20			
P. odoratissimus	10	20		
P. penetrans (Natural samples)	40	10	10	
P. penetrans (baits in day 0, month 12 and 18)	10	20	10	50

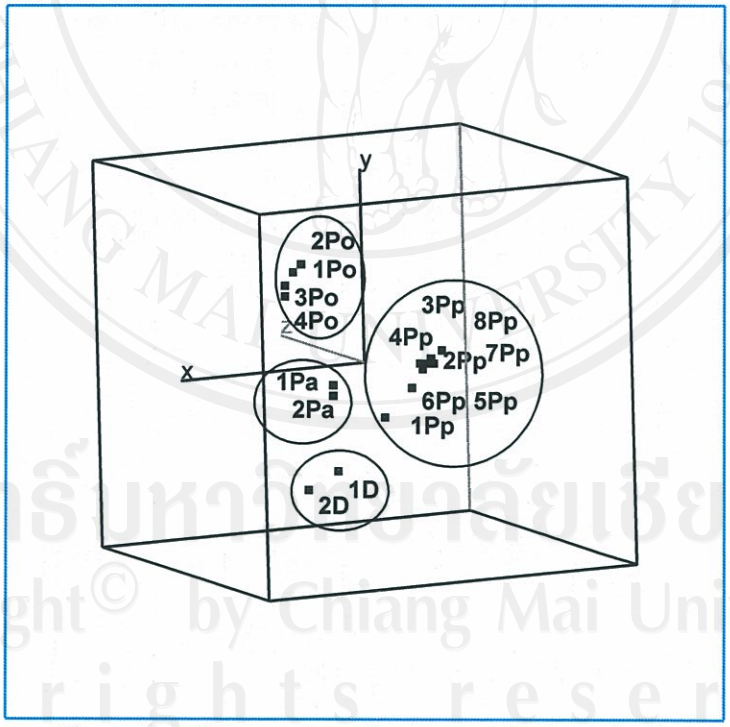


Figure 3.3 Three-dimensional correspondence analysis of fungal communities recorded from *Dracaena lourieri* (D), *Pandanus amaryllifolius* (Pa), *P. odoratissimus* (Po) and *P. penetrans* (Pp). A percentage of total variance explained by the model is 37.94%.

3.3.1D Fungal occurrence on *Pandanus penetrans* leaves from different sites and effect of stages of decay

Three-dimensional correspondence analysis plots of fungal communities on *Pandanus penetrans* leaves collected from different sites and stages of decay are presented in Figure 3.4. A percentage of total variance explained by the model is 72.82%. Distinct fungal communities were found on the collection of *Pandanus penetrans* leaves from different sites and at different stages of decay.

The site effect indicated by the separation of fungal communities found on sample collections from Chiang Mai and Phayao with few overlap fungi including *Oxydothis linospadicis*, *Penicillium chrysogenum*, *Phomopsis* sp.1 and *Sporidesmium ghanaense* (Pp1-Pp2, Pp4-Pp8 in Table 3.2).

Collections of *Pandanus penetrans* from Chiang Mai representing stages of decay at day 0 (green leaves), month 12 (mature stage) and month 18 (later stage) show that the fungal community at day 0 differed from those of mature and later stages (Figure 3.4). The number fungal taxa occurring at day 0, mature and later stages were 13, 28 and 19 respectively, and only *Ellisembia adscendens* (43.3% of samples) and *Sporidesmium ghanaense* (63.3%) were found at all stages of decay (Pp1-Pp2, Pp4-Pp8 in Table 3.2).

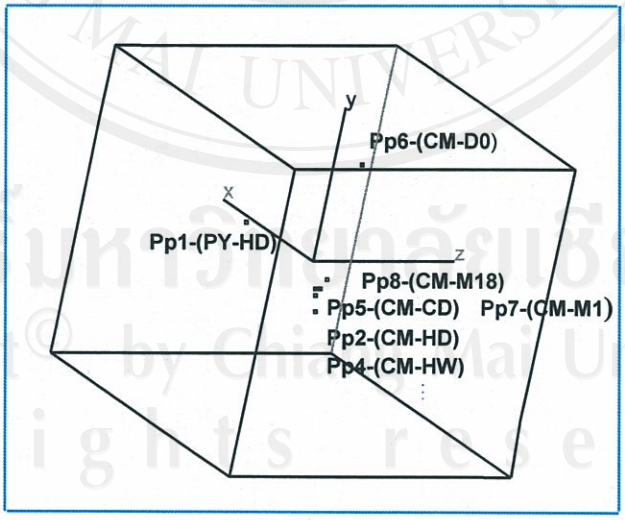


Figure 3.4 Three-dimensional correspondence ordinations of fungal communities occurring on *Pandanus penetrans* leaves that were collected from Chiang Mai (CM) and Phayao (PY) during 3 seasons of cool dry (CD), hot dry (HD) and hot wet (HW) or during succession study at day 0 (D0), month 12 (M12) and month 18 (M18). A percentage of total variance explained by the model is 72.82%.

3.3.1E Fungal occurrence on different parts of *Pandanus odoratissimus* and *P. penetrans*

Plant tissues affected fungal occurrence. Fungal communities on leaves of *Pandanus odoratissimus* from the coast in Rayong Province were different from those on prop roots and seeds. That is indicated by the principal-coordinate axes c1 and c2 which separate into distinct clusters of species in two-dimensional correspondence analysis. Axis c1 separate the fungal communities on leaves from those of prop roots and seeds (Figure 3.5). The number of fungi found on prop roots and seeds were also less than those on leaves, and the fungal taxa commonly found on all tissues were *Aspergillus parasiticus* and *Botryodiplodia theobromae* (Table 3.5). In *P. penetrans* from the rainforest at Doi Suthep Pui National Park during hot dry season, there were more taxa occurring on decaying leaves (26 taxa) than on the leaf sheaths (2) (Table 3.2).

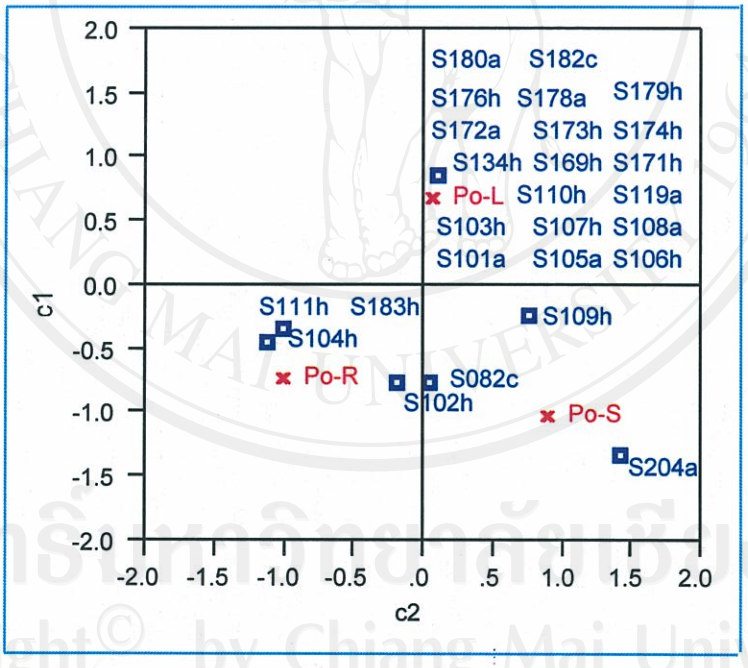


Figure 3.5 Two-dimensional correspondence analysis of fungal communities occurred on leaves (L), prop roots (P) and seeds (S) of *Pandanus odoratissimus* (Po) in Rayong Province during cool dry season. This plot accounts for 100% of the variance in the data set.

Table 3.5 Fungal occurrence on different tissues of *Pandanus odoratissimus*.

Code	Taxa	Percentage occurrence			Overall percentage
		10 Leaves	10 Prop roots	10 Seeds	occurrence (rank)
S104h	*Acremonium* sp. 3	30 (6)	80 (1)		37 (3)
S106h	*Acremonium* sp. 5	20 (7)			7 (13)
S105a	Ascomycete 3	40 (5)			13 (11)
S178a	Ascomycete 5	50 (4)			17 (10)
S102h	*Aspergillus parasiticus*	40 (5)	10 (4)	80 (2)	73 (1)
S107h	*Aspergillus* sp.1	50 (4)			17 (9)
S082c	*Botryodiplodia theobromae*	30 (6)	60 (2)	70 (3)	53 (4)
S108a	*Byssosphaeria*-like	20 (7)			7 (13)
S119a	*Chaetomium globosum*	60 (3)			20 (8)
S103h	*Cladosporium cucumerinum*	50 (4)			17 (9)
S169h	*Curvularia eragrostidis*	30 (6)			10 (12)
S180a	*Emericella* sp.	20 (7)			7 (13)
S174h	*Exserohilum* sp.*	20 (7)			7 (13)
S171h	Hyphomycete 4	30 (6)			10 (12)
S101a	*Linocarpon lammiae*	70 (2)			23 (7)
S110h	*Memnoniella echinata*	10 (8)			3 (14)
S183h	*Memnoniella* sp.	20 (7)	40 (3)		20 (8)
S109h	*Monodictys* sp. 1	80 (1)		80 (2)	53 (2)
S134h	*Nigrospora oryzae*	60 (3)			20 (8)
S204a	*Ophiostoma* sp.			100 (1)	33 (6)
S176h	*Penicillium* sp. 2	20 (7)			7 (13)
S179h	*Periconia* sp.*	20 (7)			7 (13)
S182c	*Phoma destructiva*	20 (7)			7 (13)
S111h	*Phomopsis* sp.1	30 (6)	80 (1)		37 (5)
S173h	*Verticillium* sp.	40 (5)			13 (11)
S172a	*Emericella nidulan*	10 (8)			3 (14)

*Probably new taxa known only on *Pandanus odoratissimus*.

3.3.1F Seasonal pattern of fungal occurrences on *Pandanus penetrans* leaves

Figure 3.6 shows three-dimensional correspondence ordinations of fungal communities occurring on *Pandanus penetrans* leaves in a tropical rainforest at Doi Suthep Pui National Park including the natural samples that were collected during 3 seasons (cool dry, hot dry and hot wet), and the succession study samples at 3 stages of decay (day 0, month 12 and month 18). A percentage of total variance explained by the model is 82.14%. The results show no seasonal patterns of fungal occurrences on *Pandanus penetrans* leaves both in natural samples and succession study samples (Figure 3.6). The number of fungal taxa occurring on randomly selecting leaves collections during the cool dry, hot dry and hot wet seasons were 24, 26 and 27

respectively. *Myrothecium pandanicola* sp. nov., *Nectria*-like 1, *Ornatispora* sp., *Oxydothis linospadicis, Penicillium* sp. 1, *Periconia cookie*, *Phaeosphaeria*-like, *Phaeostalagmus cyclosporus*, *Phomatospora* sp. 1, *Sporidesmium ghanaense*, *Stachylidium bicolor*, *Trichoderma* sp. and *Zygosporium oscheoides* were abundant in both of natural leaves and baits (Table 3.2).

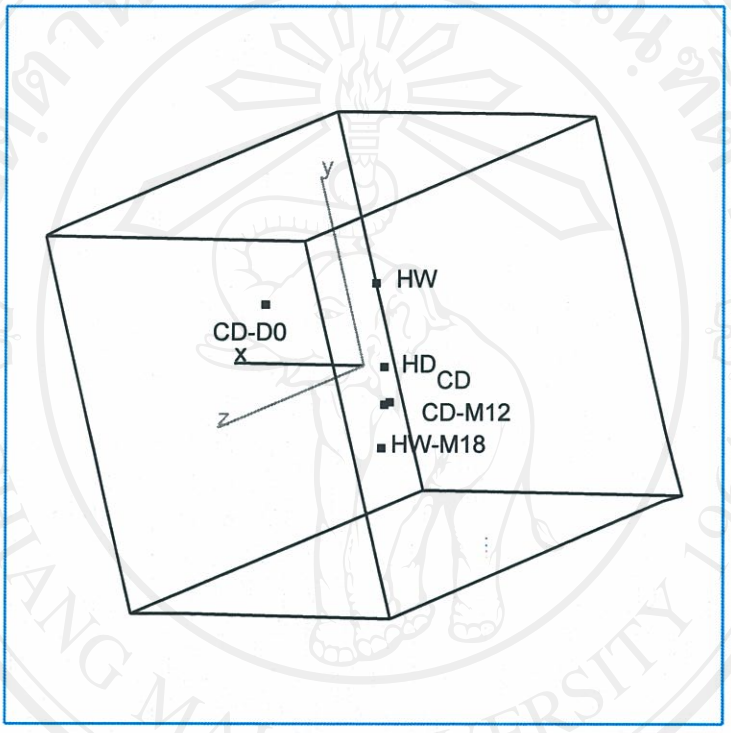


Figure 3.6 Three dimensional correspondence ordinations of fungal communities occurred on *Pandanus penetrans* leaves from Suthep Pui National Park that were collected during 3 season of cool dry (CD), hot dry (HD) and hot wet (HW) as naturally samples, and samples collected during succession stage of decayed at day 0 (D0), month 12 (M12) and month 18 (M18). A percentage of total variance explained by the model is 82.14%.

3.3.2 Fungal succession on *Pandanus penetrans* leaves

3.3.2A Determination of sample size

The species area curve for each stage of fungi on *Pandanus penetrans* leaves during the succession period almost reached asymptote because the slopes of the curves were declining with the increase of sample size and at about 10 samples the slopes were near zero (Figure 3.7). Although the curve did not completely level off, the number of samples was large enough to obtain a highly representative result.

3.3.2B Fungal taxonomic composition on *Pandanus penetrans* leaves and effect of stages of decay

Fifty-five taxa were identified on *Pandanus penetrans* leaves during the succession process and their percentage occurrence are listed in Table 3.6. The overall common taxa were *Sporidesmium ghanaense* (52.5%), *Phaeosphaeria*-like (46.3%), *Oxydothis linospadicis* (33.75%), *Ellisembia adscendens* (32.5%), *Acremonium* sp. 4 (27.5%), *Chloridium virescens* (21.25%), *Myrothecium pandanicola* sp. nov. (20%), *Nectria*-like (20%), *Stachylidium bicolor* (17.5%), *Phaeostalagmus cyclosporus* (16.25%), *Canalisporium exiguum* (15%), *Penicillium chrysogenum* (15%) and *Phialocephala* sp. (15%) (Table 3.6).

Three-dimensional correspondence analysis of fungal communities on leaves of *Pandanus penetrans* showed that fungal compositions were distinct at each stage of succession (Figure 3.8). The values of Shannon index are varied between 2.1-2.4 during the beginning of the study period (0-180 days). The peaks reached 3.1 at about 300-365 days (Figure 3.9). The overall numbers of fungi found at each sampling time are shown in Figure 3.10. Dominant species at each stage of decay are distinct (Figure 3.11).

Almost all fungi recorded on green leaves at day 0, such as *Ellisembia adscendens*, *Myrothecium pandanicola* sp. nov. and *Sporidesmium ghanaense* were associated with brown spots. Pioneer (day 0 - month 6), mature (month 7-12) and impoverished communities (month 13-18) were observed. There were 12-16 taxa found in the pioneer community stage, with *S. ghanaense* having the highest frequency. In the mature community, the diversity was high (28 taxa) and had peaked with a number of taxa with low percentage occurrence. *Oxydothis linospadicis*,

Penicillium chrysogenum, Periconia cookie, *Phaeosphaeria*-like and *Phomatospora* sp. 1 had a high level of occurrence. In the impoverished stage, the diversity and number of taxa declined. Dominant taxa were *Canalisporium exiguum* *Phaeosphaeria*-like and *S. ghanaense*

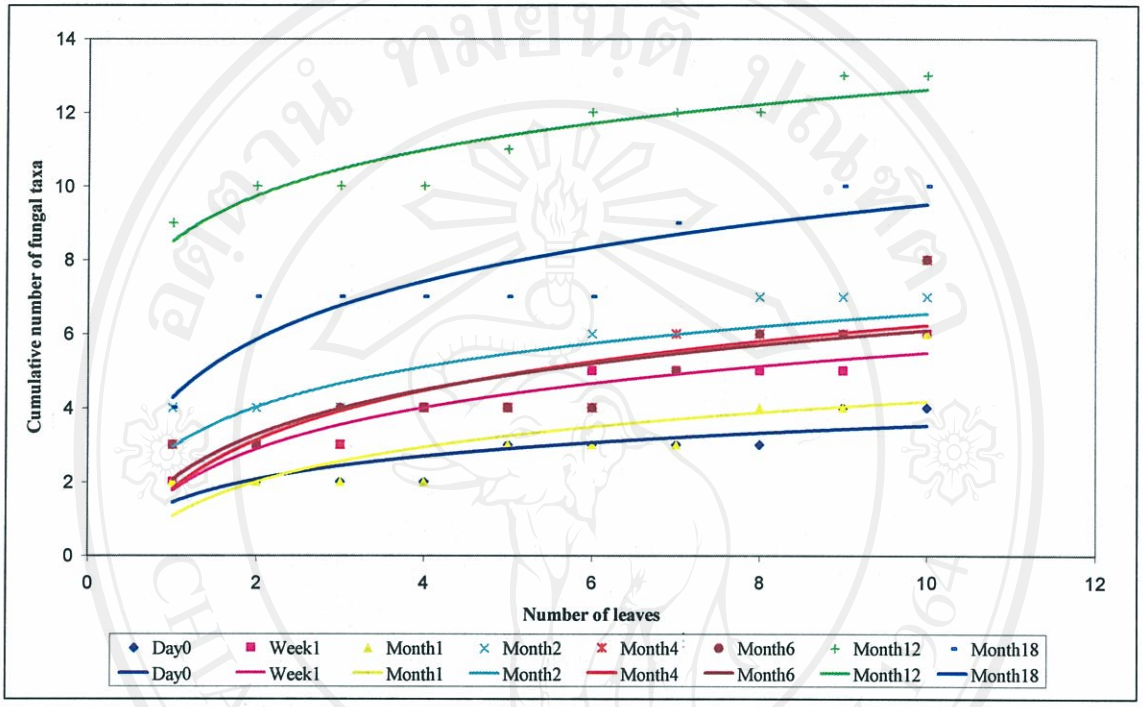


Figure 3.7 Species area curve for fungi collected on *Pandanus penetrans* leaves at different stages of succession.

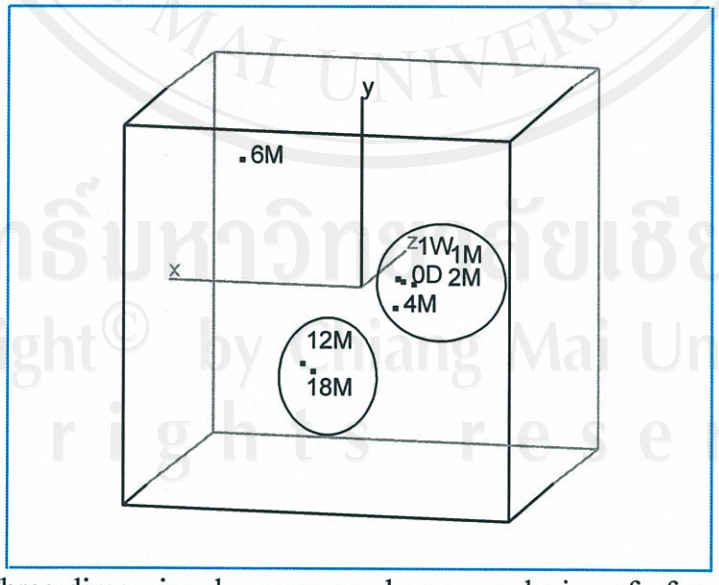


Figure 3.8 Three-dimensional correspondence analysis of fungal samples of *Pandanus penetrans*. 0, 1, 2, 4, 6, 12, 18: sampling times and stages of succession (day, week and month). Percentages of total variance is 64.79%.

Table 3.6 Frequency and overall percentage occurrence of fungal taxa on *Pandanus penetrans* leaves during the succession process.

Code	Taxa	Number of leaves which fungus was detected								Overall percentage occurrence
		19/1/2003 Day0 10 leaves	26/1/2003 Week1 10 leaves	19/2/2003 Month1 10 leaves	19/3/2003 Month2 10 leaves	19/5/2003 Month4 10 leaves	20/7/2003 Month6 10 leaves	19/1/2004 Month12 10 leaves	19/6/2004 Month18 10 leaves	
S043h	*Acremonium* sp. 2			1				1		2.5
S061h	*Acremonium* sp. 4		7	2	4	9				27.5
S122h	*Aspergillus* sp. 2							1		1.3
S126a	*Astrosphaeriella tornata*							3		3.8
S054h	*Berkleasmium* sp.	2			1					3.8
S152h	*Canalisporium exiguum*							3	9	15.0
S167h	*Cepharosporiopsis* sp.						2			2.5
S119a	*Chaetomium globosum*		2					1		3.8
S162a	*Chaetosphaeria* sp.						3			3.8
S069h	*Chloridium virescens*				8	9				21.3
S064c	Coelomycete 2		1							1.3
S065c	Coelomycete 3			1						1.3
S059c	*Colletotrichum gloeosporioides*		1							1.3
S118h	*Cylindrocladium* sp. 1							1		1.3
S046h	*Dictyochaeta fertilis*	1								1.3
S045h	*Ellisembia adscendens*	3		5	7	1		3	7	32.5
S055h	*Fusicladium* sp.	1	2		1					5.0
S156a	*Glomerella* sp.						2		3	6.3
S160h	*Helicosporium* sp.							1	1	2.5
S151h	Hyphomycete 3								1	1.3
S041h	Hyphomycete 9					2				2.5

Table 3.6 Continued.

Code	Taxa	Number of leaves which fungus was detected								Overall percentage occurrence
		19/1/2003 Day0 10 leaves	26/1/2003 Week1 10 leaves	19/2/2003 Month1 10 leaves	19/3/2003 Month2 10 leaves	19/5/2003 Month4 10 leaves	20/7/2003 Month6 10 leaves	19/1/2004 Month12 10 leaves	19/6/2004 Month18 10 leaves	
S071h	Hyphomycete (synnematous) 1					1				1.3
S149h	Hyphomycete (synnematous) 3						2		8	12.5
S101a	*Linocarpon lammiae*								1	1.3
S157a	*Linocarpon siamensis* sp. nov.							2	1	3.8
S153a	*Melanochaeta hemipsila* (*Sporochrisma saccardoi*)							5	5	12.5
S123h	*Memnoniella echinata*							2		2.5
S048a	*Microthyrium* sp. 2	3	1	4	1					11.3
S038h	*Myrothecium pandanicola* sp.nov.	2	4		1		4	5		20.0
S164a	*Nectria*-like 1						10	6		20.0
S134h	*Nigrospora oryzae*							1	1	2.5
S159a	*Ornatispora* sp.*							4	1	6.3
S024a	*Oxydothis linospadicis*		5	1	1	4	2	10	4	33.8
S161a	*Oxydothis oraniopsis*						2			2.5
S125h	*Penicillium* sp. 1	2						10		15.0
S050h	*Periconia cookie*	1			2			8		13.8
S056h	*Periconia minutissima*	1								1.3
S166h	*Periconiella daphniphylli*						1			1.3
S049c	*Pestalotiopsis guepinii*	2		1	1					5.0
S058a	*Phaeosphaeria*-like*		5	1	2	10		10	9	46.3
S148h	*Phaeostalagmus cyclosporus*							5	8	16.3

Table 3.6 Continued.

Code	Taxa	Number of leaves which fungus was detected								Overall percentage occurrence
		19/1/2003 Day0	26/1/2003 Week1	19/2/2003 Month1	19/3/2003 Month2	19/5/2003 Month4	20/7/2003 Month6	19/1/2004 Month12	19/6/2004 Month18	
S163h	*Phialocephala* sp.						10	2		15.0
S158a	*Phomatospora berkeleyi*							9	2	13.8
S155c	*Pyrenochaeta* sp.					1		1	2	5.0
S047h	*Sporidesmium ghanaense*	7	6	5	7	1	4	2	10	52.5
S042h	*Stachylidium bicolor*			2		7		5		17.5
S120h	*Trichoderma* sp.							6		7.5
S066h	*Trichothecium roseum*			2	1					3.8
S067h	*Tubercularia* sp.	1		1	1			1		5.0
S150a	*Tubeufia cerea*								3	3.8
S063h	Sterile mycelia 1	2	5	4						13.8
S070h	Sterile mycelia 2				9					11.3
S060h.	*Verticillium* sp. 1		3	1	6	1				13.8
S165h	*Volutella* sp.						5			6.3
S073h	*Zygosporium oscheoides*					1		4	1	7.5
Total number of fungal records (437)		**28**	**42**	**31**	**53**	**47**	**47**	**112**	**77**	
Anamorphic fungi (40)		12	8	11	13	10	7	19	10	
Ascomycetes (15)		1	4	4	3	2	5	10	9	
Total taxa (55)		**13**	**12**	**14**	**16**	**12**	**12**	**28**	**19**	

*possibly new taxa

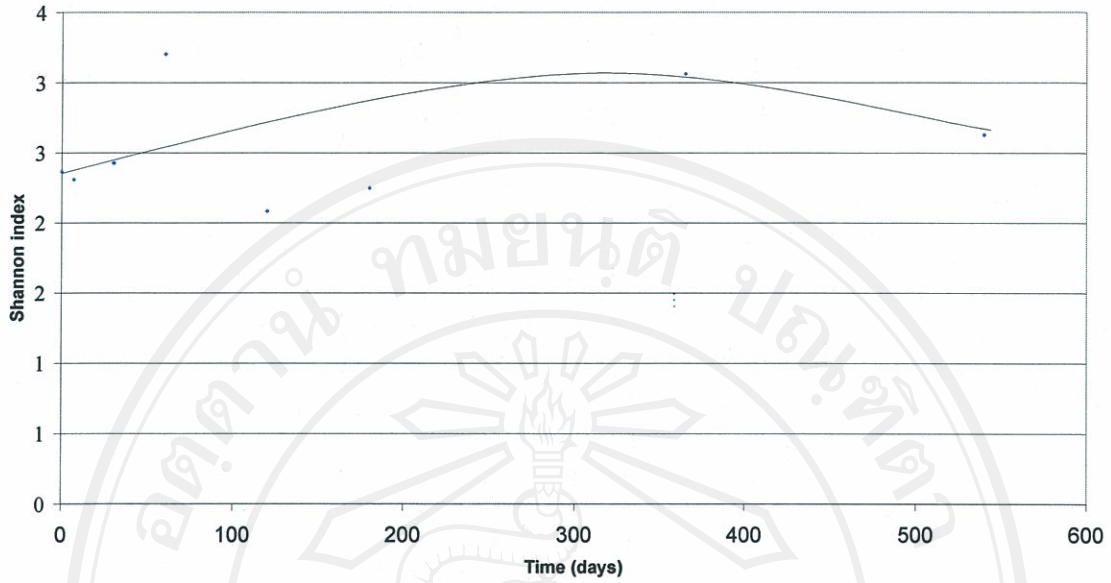


Figure 3.9 Shannon indices for *Pandanus penetrans* leaves throughout the experiment.

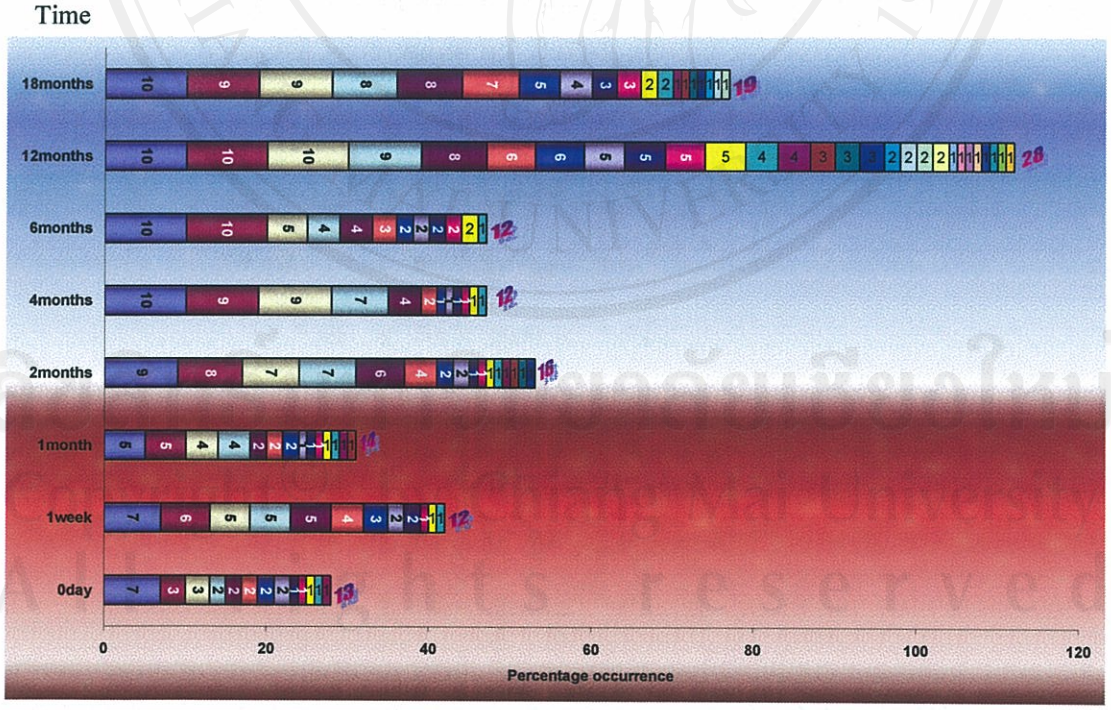
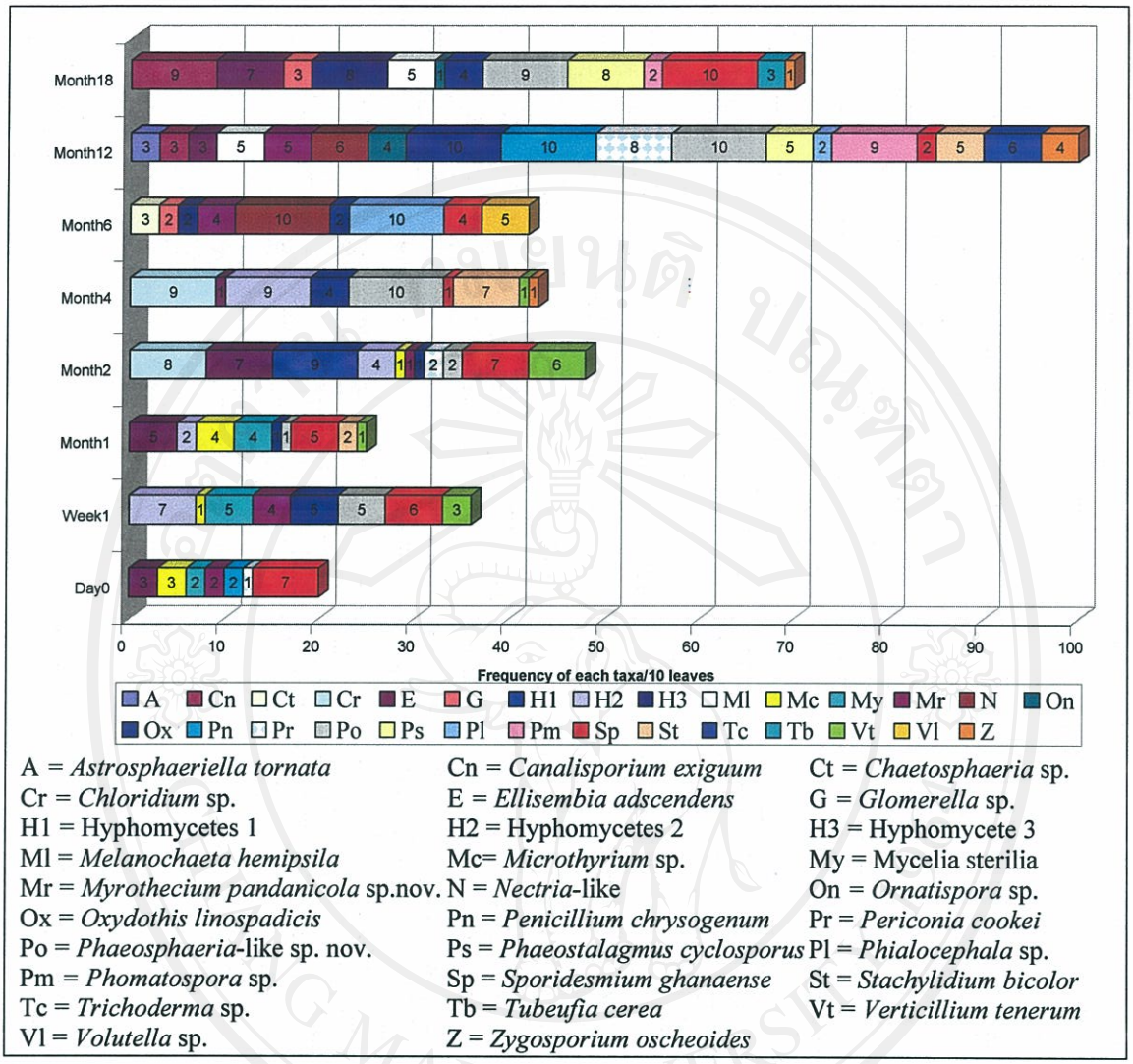


Figure 3.10 Number of fungal taxa occurring on samples during different stages of decay. Taxa recorded at each sampling time are ordered with the most abundant to the left to the least abundant to the right.



A = *Astrosphaeriella tornata* Cn = *Canalisporium exiguum* Ct = *Chaetosphaeria* sp.
Cr = *Chloridium* sp. E = *Ellisembia adscendens* G = *Glomerella* sp.
H1 = Hyphomycetes 1 H2 = Hyphomycetes 2 H3 = Hyphomycete 3
Ml = *Melanochaeta hemipsila* Mc= *Microthyrium* sp. My = Mycelia sterilia
Mr = *Myrothecium pandanicola* sp.nov. N = *Nectria*-like On = *Ornatispora* sp.
Ox = *Oxydothis linospadicis* Pn = *Penicillium chrysogenum* Pr = *Periconia cookei*
Po = *Phaeosphaeria*-like sp. nov. Ps = *Phaeostalagmus cyclosporus* Pl = *Phialocephala* sp.
Pm = *Phomatospora* sp. Sp = *Sporidesmium ghanaense* St = *Stachylidium bicolor*
Tc = *Trichoderma* sp. Tb = *Tubeufia cerea* Vt = *Verticillium tenerum*
Vl = *Volutella* sp. Z = *Zygosporium oscheoides*

Figure 3.11 Frequency of dominant fungi occurring on leaves at each sampling time.

3.3.3 Pathogenic fungi on *Dracaena* and *Pandanus* leaves

3.3.3A Collection of *Dracaena* and *Pandanus* diseases

Twenty-three fungi were identified from samples of *Dracaena loureiri* and species of *Pandanus* showing symptoms of anthracnose on leaves, speckle or leaf spot (Table 3.7). The disease symptoms are described and illustrated.

Table 3.7 A comparisonal of the pathogenic taxa recovered from *Dracaena loureiri*, *Pandanus amaryllifolius* and *P. penetrans*.

Fungal Name	*D. loureiri* Chiang Dao 1/6/05	*P. amaryllifolius* Phayao 20/2/05	*P. amaryllifolius* Suthep Pui 12/4/05	*P. penetrans* Suthep Pui 12/4/05
Acremonium sp.				+
Aspergillus sp.				+
Cercospora sp.			++	
Cladosporium oxysporum	++	++		
Colletotrichum gloeosporioides (*Glomerella cingulata* have been shown in culture)	++	+		+++
Curvularia lunata				+
Fusarium oxysporum			++	+
Guignardia sp.	++	+		+
Herpomyces sp.				+
Gliocladium roseum				+
Myrothecium pandanicola sp. nov.				+
Nigrospora oryzae	+	+		
Oxydothis sp.				+
Penicillium sp.				+
Phomopsis sp.				+
Ramichloridium sp. 1				+
Ramichloridium sp. 2				+
Sporidesmium ghanaense				+++
Sterile mycelia (grey 1)	+			
Sterile mycelia (grey 2)				++
Sterile mycelia (grey 3)				+
Sterile mycelia (white 1)			+	
Sterile mycelia (white 2)				+
Total taxa	5	3	3	18

\+ = low frequency of occurrence or found on only 1 sample

++ = moderate frequency of occurrence or found on more than 2 samples

+++ = high frequency of occurrence or found on more than 5 samples

1. *Acremonium* leaf spot

Causal agent: *Acremonium* sp. (Figure 3.12)

Host: *Pandanus penetrans*.

Disease symptoms: The first symptom is the appearance on leaf surface of numerous small yellow or pale brown spots. Brown spots later increase in size to form an extensive reddish-brown lesion (Figure 3.12).

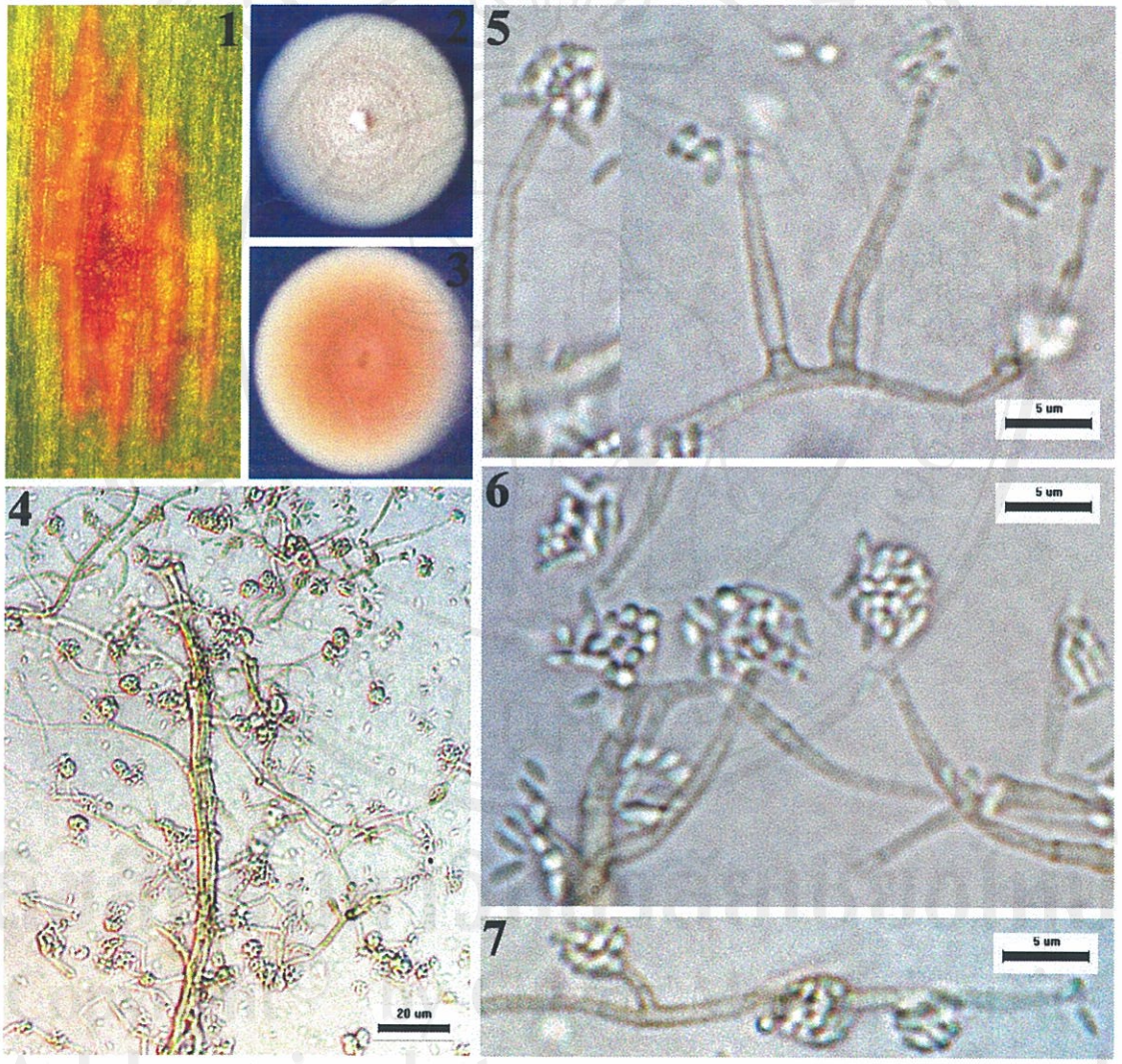


Figure 3.12 1. Appearance of disease symptoms on *Pandanus penetrans* leaves. 2-7. ***Acremonium* sp.** 2-3. Colony. 4-7. Mycelium, conidiophores and conidia. Scale bar: 4 = 20 μm; 5-7 = 10 μm.

2. Anthracnose

Causal agent: *Colletotrichum gloeosporioides* (Figure 3.13).

Host: *Dracaena loureiri*, *Pandanus amaryllifolius* and *P. penetrans*.

Disease symptoms: The first symptom is a small circular speck on the leaf surface. Brown spots later increase in size and coalesce to form an extensive area of sunken grey to brown tissue with reddish brown margins. Sometime the disease starts at the leaf margins or leaf tip and later enlarges along the leaf laminar (Figure 3.13).

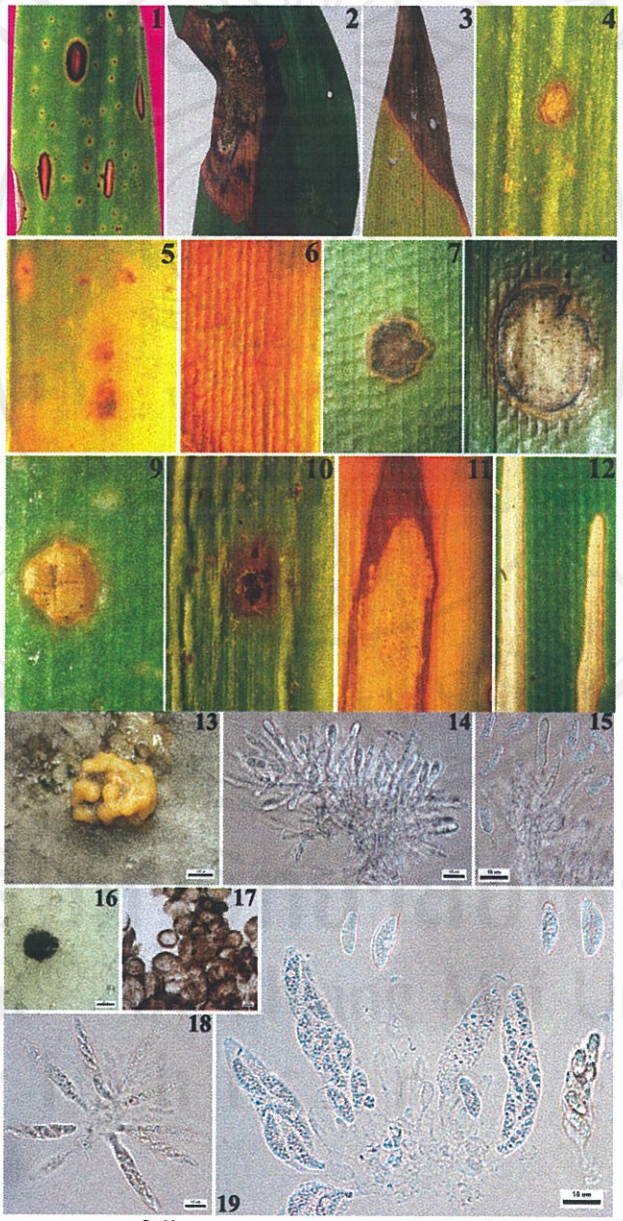


Figure 3.13 1-12. Appearance of disease symptom on the host leaves: 1-2 ***Dracaena loureiri***; 3 ***Pandanus amaryllifolius***; 4-12 ***P. penetrans***. 13-15. Conidia ooze, conidiophores and conidia of ***Colletotrichum gloeosporioides***. 16-19. Ascomata, asci and ascospores of ***Glomerella cingulata***. Scale bar: 13, 16 = 1000 µm; 17 = 20 µm; 14-15, 18-19 = 10 µm.

3. *Aspergillus* leaf spot

Causal agent: *Aspergillus* sp. (Figure 3.14)

Host: *Pandanus penetrans*.

Disease symptoms: In the early stage symptoms appear as brownish spots, later the spot elongate along leaf length and brake when aging (Figure 3.14).

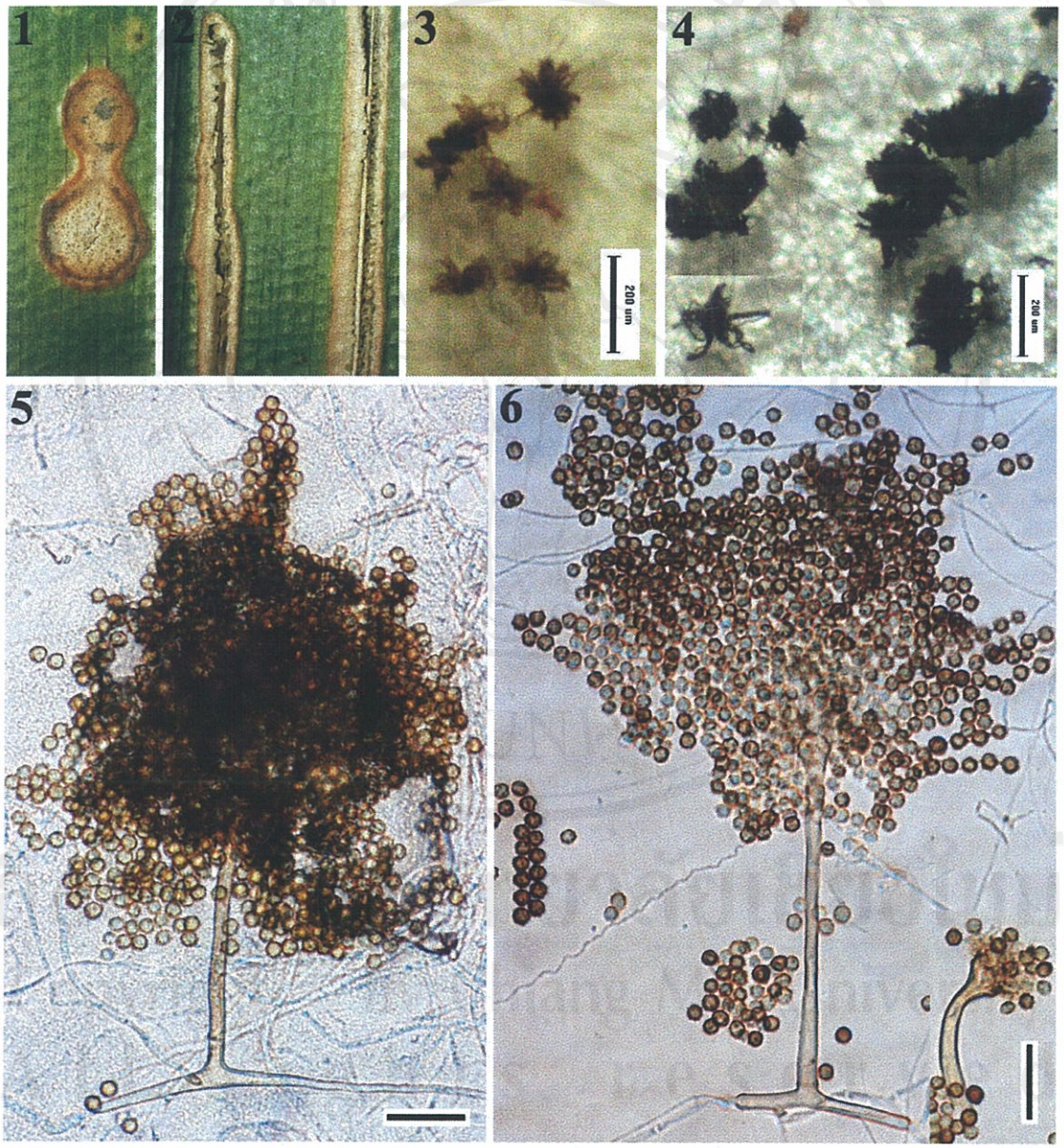


Figure 3.14 1-2. Appearance of disease symptom on the host leaves. 3-6. ***Aspergillus* sp.**: 3-4 Conidiophores with brown and black conidia appearance on culture; 5-6 Conidiophores, conidia and foot-cells. Scale bar: 3-4 = 200 μm; 5-6 = 10 μm.

4. *Cladosporium* leaf spot

Causal agent: *Cladosporium oxysporum* (Figure 3.15).

Host: *Dracaena loureiri*, *Pandanus amaryllifolius*.

Disease symptoms: Spots occurred on the surface of host leaf. White spots later increase in size and coalesce to form an extensive area with reddish-brown margins. The disease lesion was also covered with black conidial mass of *Nigrospora oryzae* (Figure 3.15).

Figure 3.15 1. Appearance of disease symptom on leaf of ***Dracaena loureiri***. 2. Conidiophores and conidia of ***Cladosporium oxysporum.*** Scale bar = 10 μm.

5. *Curvularia* leaf spot

Causal agent: *Curvularia lunata* (Figure 3.16).

Host: *Pandanus penetrans*.

Disease symptoms: The first symptom is a small brown spot with greenish-brown margins on the leaf surface, the lesion later increase in size (Figure 3.16).

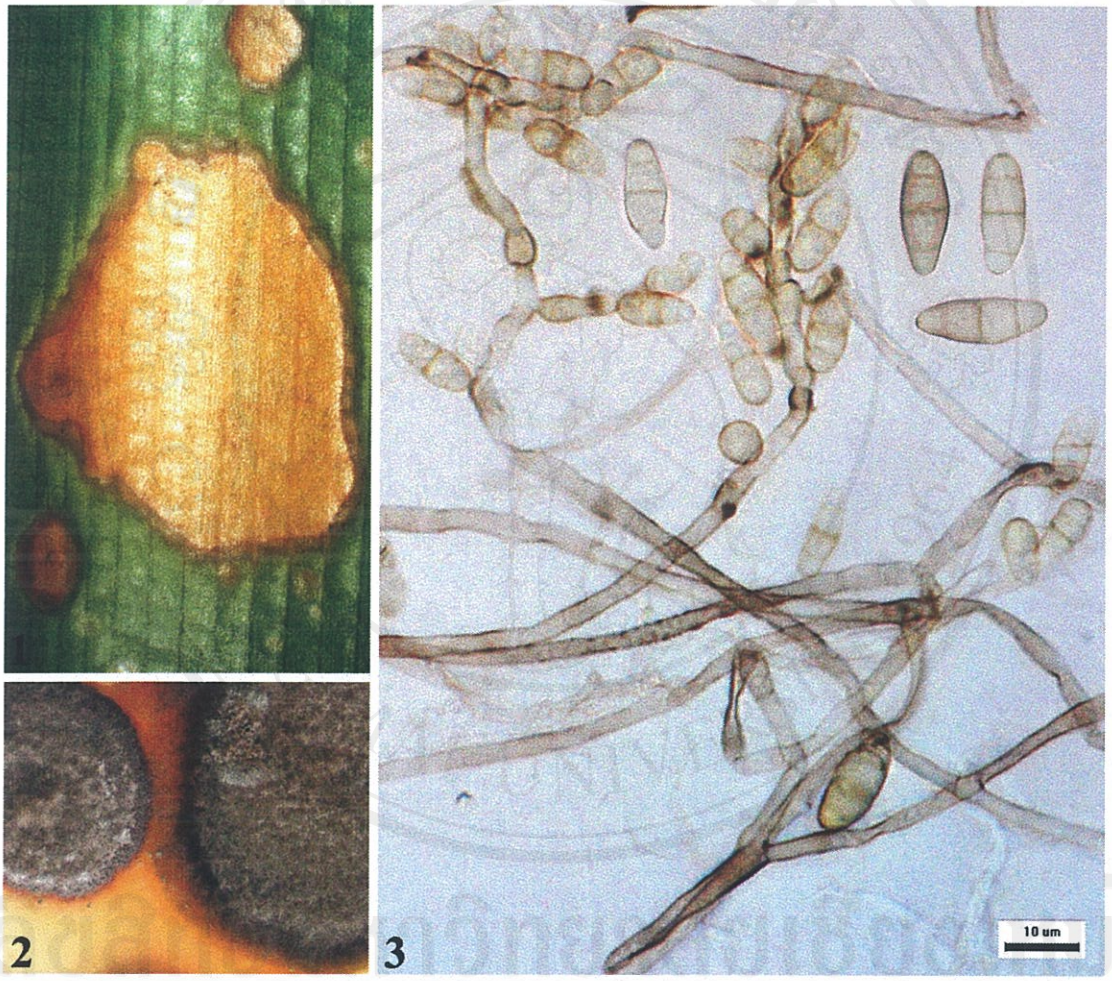


Figure 3.16 1. Appearance of disease symptoms on ***Pandanus penetrans*** leaf. 2-3. ***Curvularia lunata***: 2 Colony on ½ strength PDA; 3 Conidiophores and conidia. Scale bar = 10 μm.

6. *Fusarium* rot

Causal agent: *Fusarium oxysporum* (Figure 3.17).

Host: *Dracaena loureiri*, *Pandanus amaryllifolius* and *P. penetrans*.

Disease symptoms: In the early stage symptoms develop pale yellow or whitish, soft and small raised areas on the host surface. The lesion later increases in size and is surround by a dark brown to black zone (Figure 3.17).

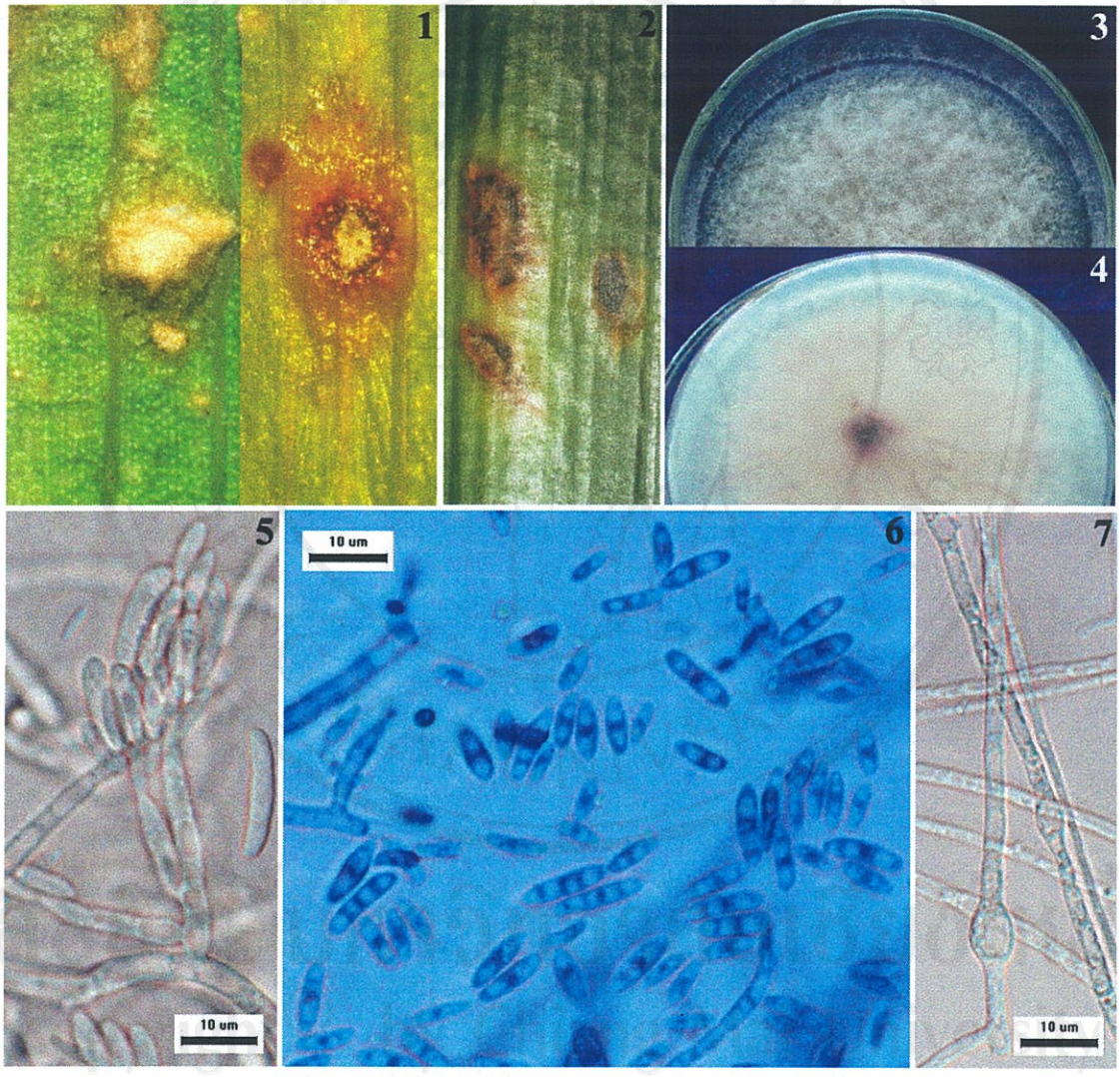


Figure 3.17 1-2. Appearance of disease symptoms on the host leaves: 1 ***Pandanus amaryllifolius***; 2 ***P. penetrans***. 3-7. ***Fusarium oxysporum***: 3-4 Colony on ½ strength PDA; 5-6 Conidiophore and conidia; 7 Chlamydospore. Scale bar = 10 μm.

7. Guignardia leaf spot

Causal agent: *Guignardia* sp. (Figure 3.18)

Host: *Dracaena loureiri*, *Pandanus amaryllifolius* and *P. penetrans*.

Disease symptoms: The first symptom appears as small yellowish or brownish spots on the leaf surface. The spots later increase in size and coalesce to form an extensive area of dark brown tissue with yellowish margins (Figure 3.18). Sometime the disease lesion could be also infected by the other causal agent such as species of *Cladosporium*, *Colletotrichum* and *Fusarium*.

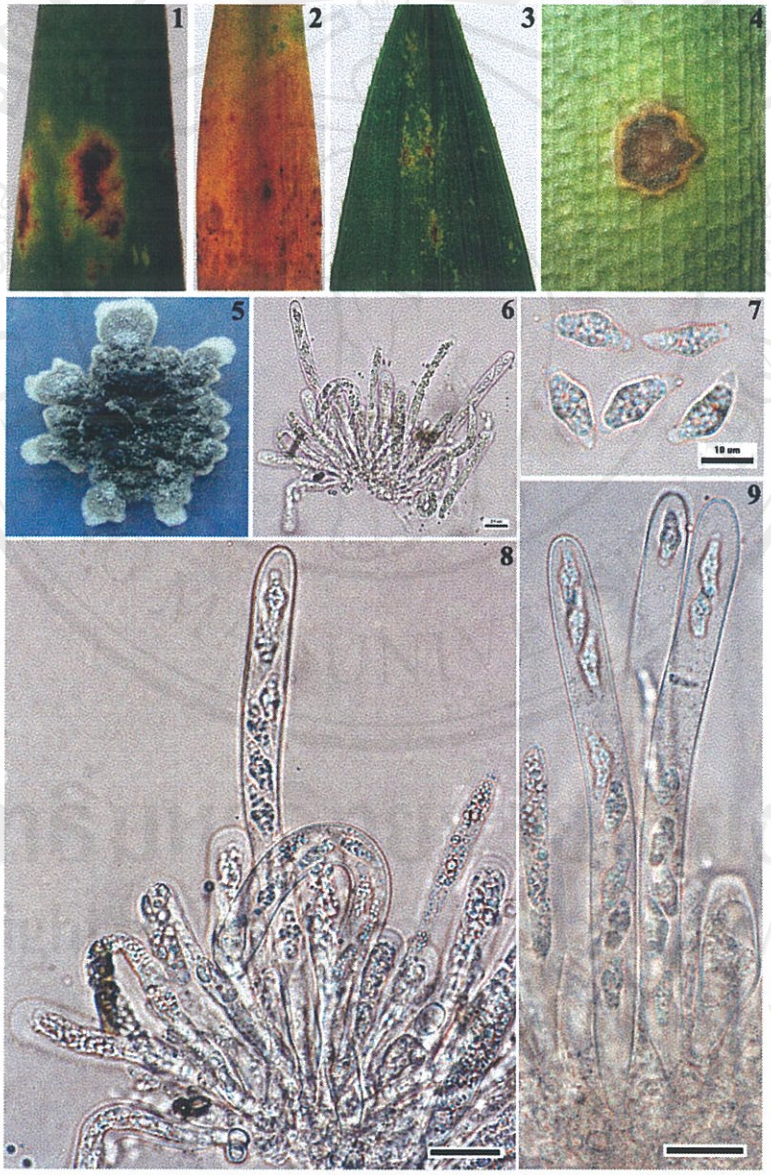


Figure 3.18 1-4. Appearance of disease symptoms on the host leaves: 1-2 ***Dracaena loureiri***; 3 ***Pandanus amaryllifolius***; 4 ***P. penetrans***. 5-9. ***Guignardia* sp.**: 5 Colony on ½ strength PDA; 6, 8-9 Asci; 7. Ascospores. Scale bar: 6, 8-9 = 20 µm; 7 = 10 µm.

8. *Oxydothis* leaf spot

Causal agent: *Oxydothis* sp. (Figure 3.19)

Host: *Pandanus penetrans*.

Disease symptoms: The ascomata of causal agent develop in darkened, raised areas on the host surface with distinct eccentric ostiole (Figure 3.19).

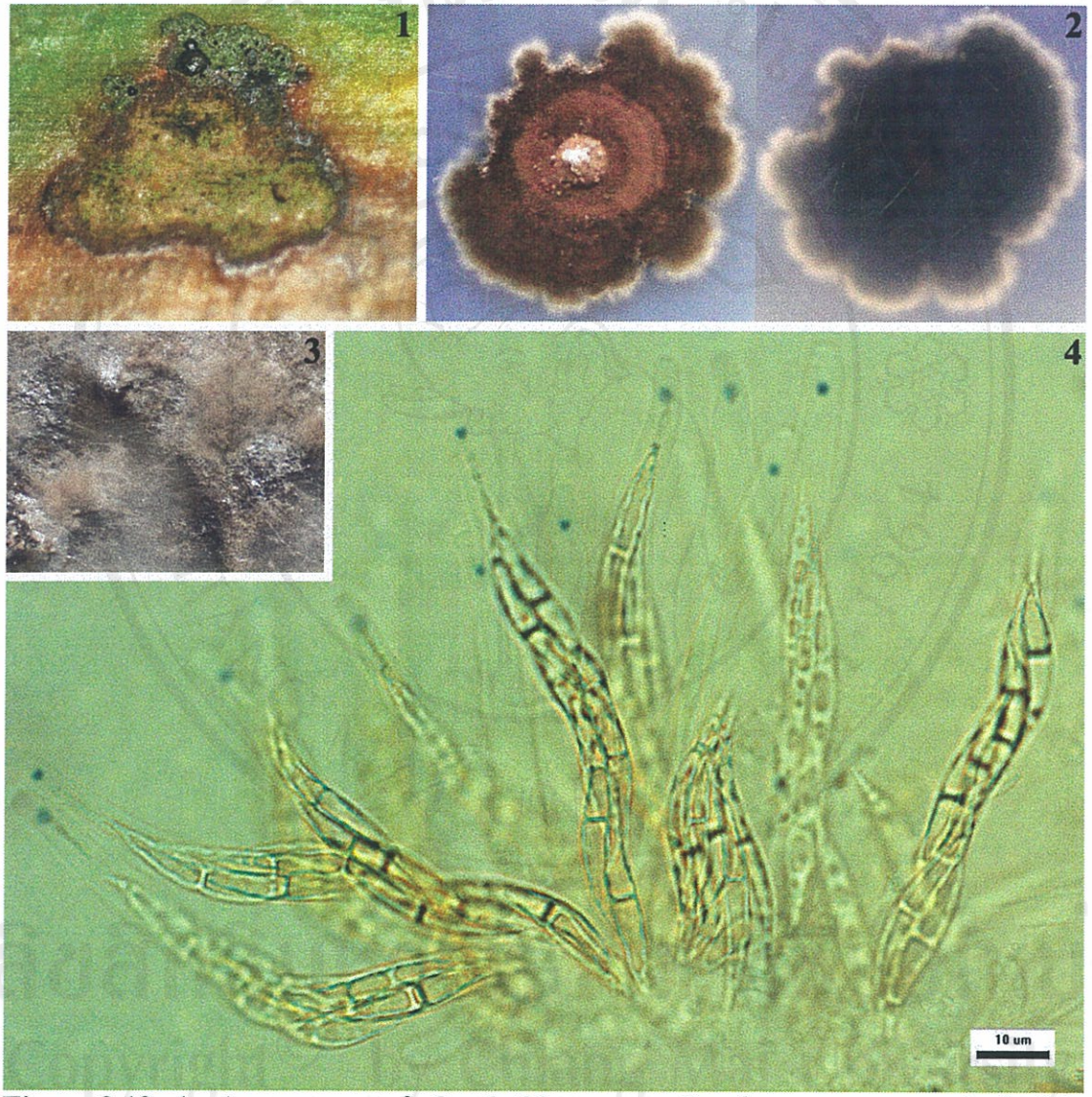


Figure 3.19 1. Appearance of ***Oxydothis* sp.** on *Pandanus penetrans* leaf. 2-3. Colony on ½ strength PDA. 4. Asci and ascospores. Scale bar: = 10 µm.

9. *Penicillium* leaf spot

Causal agent: *Penicillium* sp. (Figure 3.20)

Host: *Pandanus penetrans*.

Disease symptoms: The leaf surface is covered by numerous of very small spots which later become dark grey to black spots (Figure 3.20).

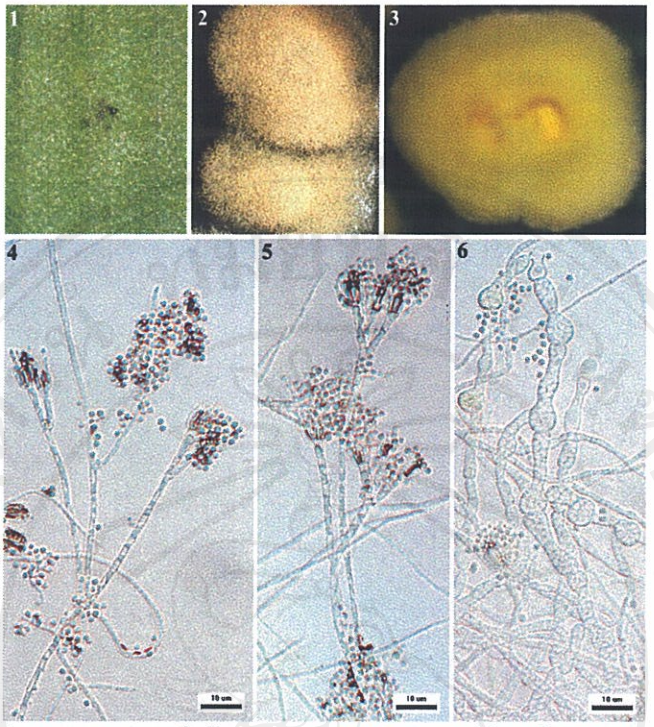


Figure 3.20 1. Appearance of disease symptom on ***Pandanus penetrans*** leaf. 2-6. ***Penicillium* sp.**: 2-3 Colonies on ½ strength PDA; 4-5 Conidiophores and conidia; 6 Chlamydospores. Scale bar: 4 = 10 µm.

3.3.3B Pathogenicity tests

Twenty isolates of endophytes (3), pathogens (13), and saprobes (4) from *Dracaena* and *Pandanus* were tested for pathogenicity (Table 3.8). The isolates of *Colletotrichum gloeosporioides*, *Curvularia lunata*, *Fusarium oxysporum*, *Guignardia* sp. and *Phomopsis* sp. caused disease lesions on the host leaves and could be reisolated (Figures 3.21-3.23). The controls produced no disease symptoms.

Table 3.8 Results of pathogenicity test of fungal taxa isolated as endophytes, saprobes or pathogens.

Fungal Name	Disease lesion (pathogen/saprobe), 0 or +		
	Dracaena loureiri	***Pandanus amaryllifolius***	***Pandanus penetrans***
Acremonium sp.	-	-	0/0
Cladosporium oxysporum	0/-	0/-	-
Colletotrichum gloeosporioides	+/+	+/-	+/+
Curvularia lunata	-	-	+/+
Fusarium oxysporum	-	+/-	+/-
Guignardia sp.	-	+/-	+/-
Oxydothis sp.	-	-	0/-
Phomopsis sp.	-	-	+/+
Xylaria sp. 1 (endophyte)	0	-	-
Xylaria sp. 2 (endophyte)	0	-	-
Xylaria sp. 3 (endophyte)	-	-	0

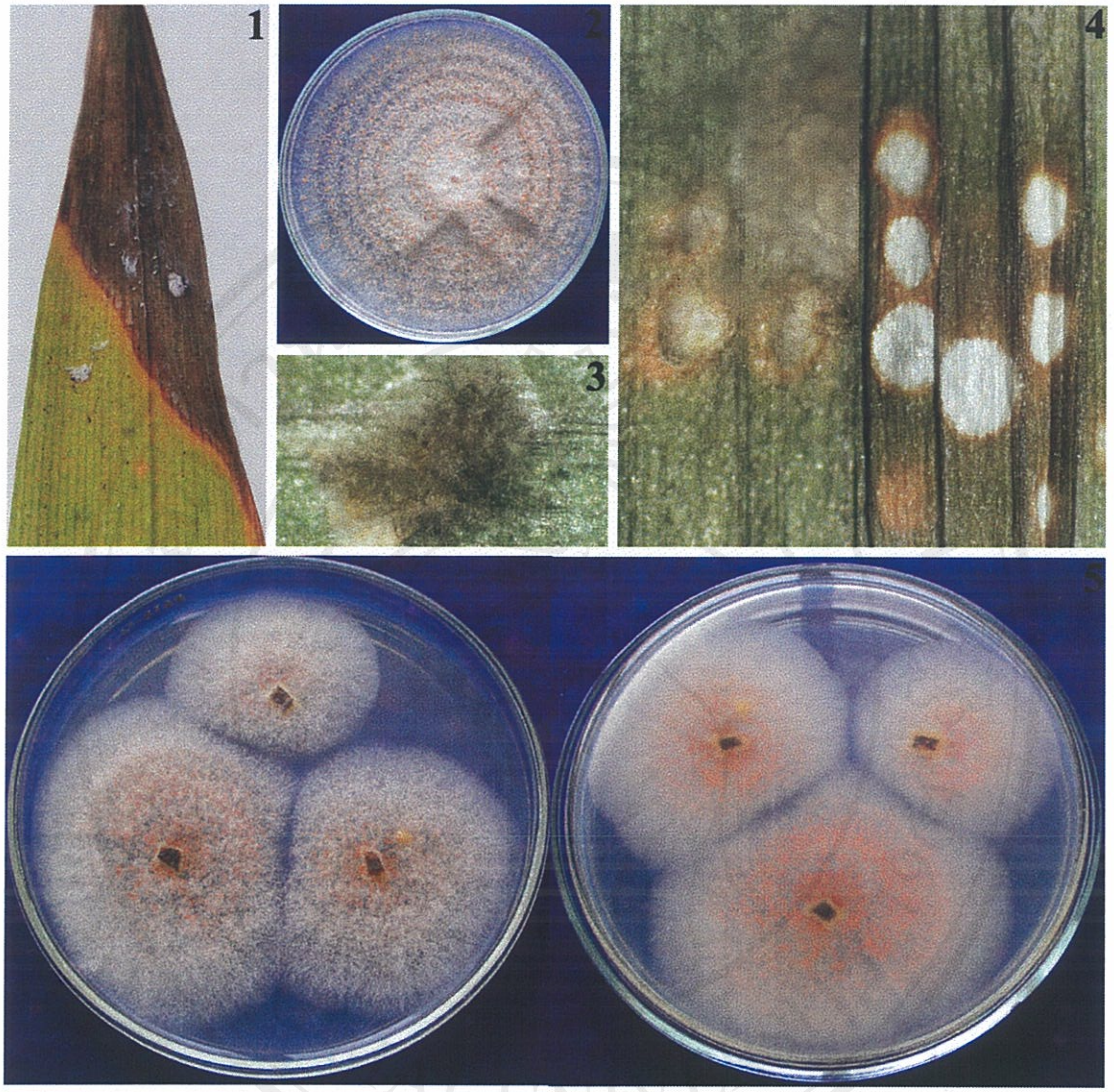


Figure 3.21 1. Appearance of disease symptoms on ***Pandanus amaryllifolius*** leaf. 2. Isolated culture of ***Colletotrichum gloeosporioides***; 3 No disease symptom on tested leaves without wound; 4 Disease symptoms on wounds of tested leaf; 5 Reisolation of fungus from diseased lesions.

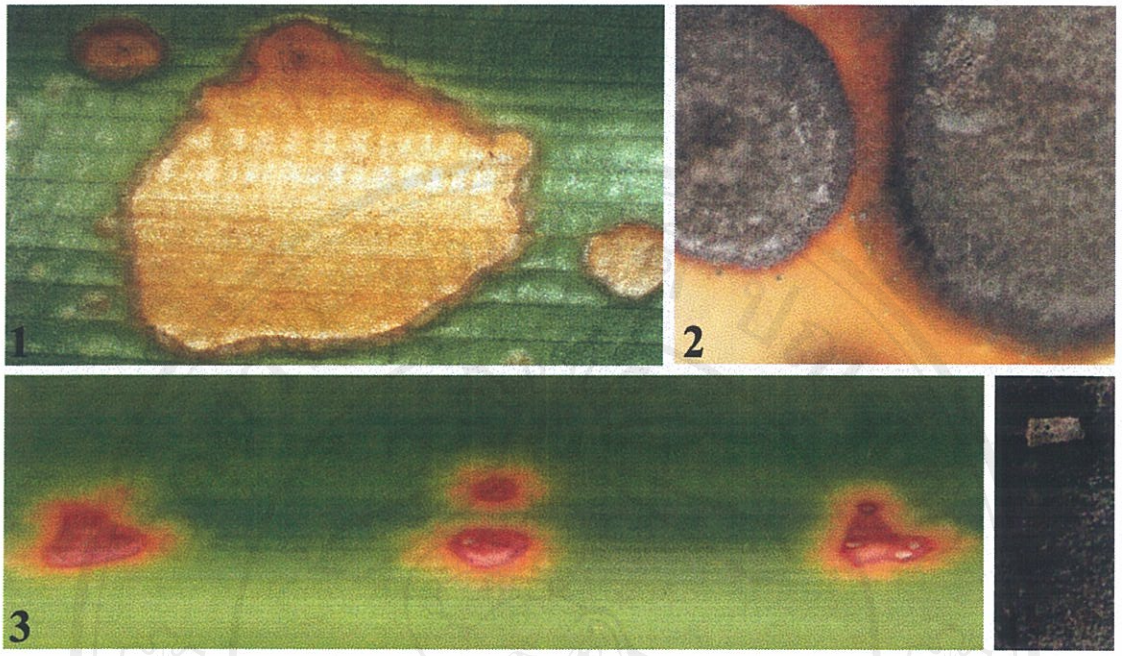


Figure 3.22 1. Appearance of disease symptom on ***Pandanus penetrans*** leaf. 2. Isolated culture of ***Curvularia lunata***. 3. Disease symptom on wounds of tested leaf. 4. Reisolation of fungus from diseased lesions.

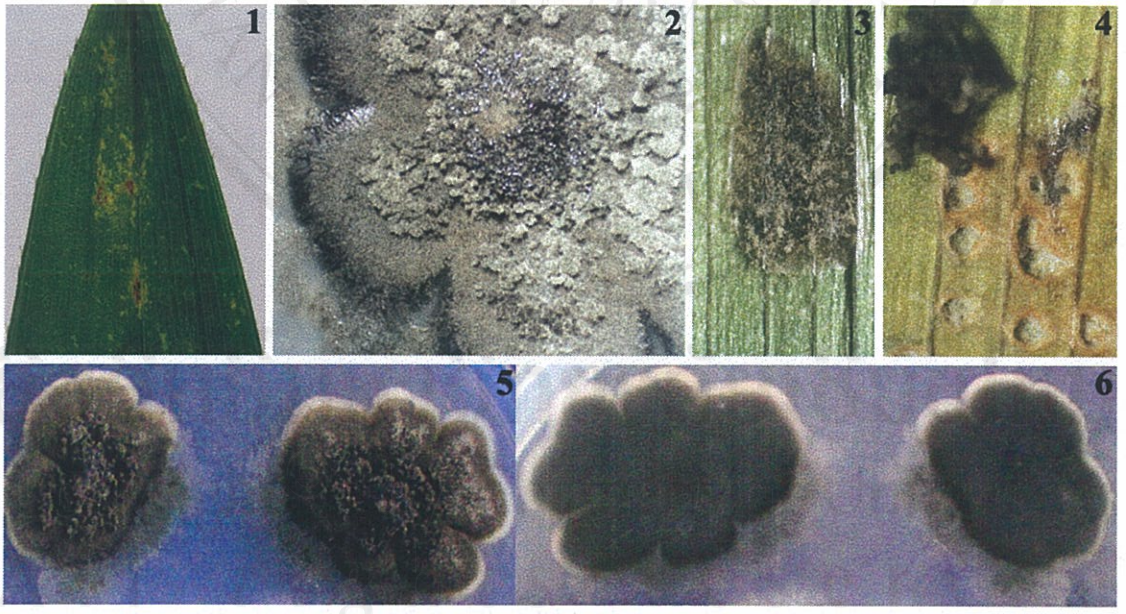


Figure 3.23 1. Appearance of disease symptom on ***Pandanus amaryllifolius*** leaf. 2. Isolated culture of ***Guignardia* sp.** 3. No disease symptom on tested leaves without wounding. 4. Disease symptom on wounds of tested leaf. 5. Reisolation of fungi from diseased lesions.

3.4 DISCUSSION

In this study, the fungal communities among similar leaves of monocotyledonous plants in Thailand were documented including *Dracaena loureiri*, *Pandanus amaryllifolius*, *P. odoratissimus* and *P. penetrans*. The plants support a variety of saprobic and pathogenic fungi. The results indicate that saprobes and pathogens may be specific to different plants species. Almost all of these plants found supported a high number of fungal taxa. The results confirm that the fungi on *Dracaena* and *Pandanus* are diverse, and could be a source for more undescribed fungi (McKenzie and Hyde, 1997; Whitton, 1999; Dulymamode *et al.*, 2001a; McKenzie *et al.*, 2002). In addition the present study elaborate some factors which may effect the occurrence of fungi on the hosts (natural samples or baits, seasons, sites, stages of decayed and tissues). Eleven new taxa were found in this study. Two of them have recently been described, *Linocarpon siamensis*, and *L. suthepensis* (Thongkantha *et al.*, 2003). *Myrothecium pandanicola* sp. nov., *Ophioceras chiangdaoensis* sp. nov., *Oxydothis siamensis* sp. nov., *Phaeonectriella pandani* sp. nov. and *Zygosporium dracinicola* sp. nov. are represently in press, and their descriptions are provided in Chapter 4, while 4 have yet to be validly described.

3.4.1 Bioiversity of saprobic fungi on *Dracaena loureiri* and *Pandanus* spp.

In the present study, high numbers of fungal taxa were found (126 fungi were identified from 160 samples of the wild plants of *D. loureiri* and *P. penetrans* and the two of cultivated *Pandanus* species). Although previous studies have concentrated on the saprobic fungi on Pandanaceae, 205 species mainly anamorphic fungi and ascomycetes have been reported from members of the Pandanaceae (McKenzie and Hyde, 1996; McKenzie *et al.*, 2002). Surprisingly no species of these fungi (1996-2002) was found in the present study. However, *Linocarpon lamiae* which have recently been described from *Pandanus tectorius* by Whitton (1999) was only one species that also occurred on *P. odoratissimus* in this study (see in Thongkantha *et al.*, 2003). The high diversity of saprobes on *D. loureiri, P. odoratissimus* and *P. penetrans* shown in the current study are similar to those of previous studies on five *Pandanus* species from Mauritius (Dulymamode *et al.*, 2001a) and some other monocotyledons from tropical rainforests such as bamboo (Hyde *et al.*, 2001, 2002a,

b), grasses (Wong and Hyde, 2001) and palms (Fröhlich and Hyde, 2000; Yanna *et al.*, 2001b, 2002). The occurrence of saprobes recorded in the current study can be compared with those recorded on various hosts from rainforests in Thailand. Somrithipol *et al.* (2002) identified 70 fungi from 130 samples of *Delonix regia* pods in Khao Yai National Park, with an overlap with the present study of 11%. At Suthep Pui National Park, Photita *et al.* (2003a) recorded 80 fungi on decaying tissues of *Musa acuminata* from 5 sites, with an overlap with this study of 15%; Bussaban *et al.* (2004) and Bussaban 2005 reported 130 fungal taxa from 320 samples of ginger (*Alpinia malaccensis* and *A. siamense*), with an overlap with the present study of 12%; and Promputtha *et al.* (2004b) reported 37 taxa from 90 dead leaves of *Magnolia liliifera*, with an overlap of 10% with the present study. The overlap of fungi occurring on *Dracaena* and *Pandanus* species with those recorded in both dicotyledonous and monocotyledonous hosts is thus low. This may be influenced by geographical distribution and host-recurrence (Dulymamode *et al.*, 2001a; Yanna *et al.*, 2001b; Zhou and Hyde, 2001).

The fungal communities on the leaf bases are richer (particularly of Ascomycete taxa) than the leaf apices, especially on those of *D. loureiri*, *P. odoratissimus* and *P. penetrans*. It is possible the leaf bases act as sites of storage for nutrient reserves, which overwinter and support early growth in spring (Isaac, 1992). They are also stouter and may decay more slowly. The overall fungal community on *P. tectorius* (88 species) is greater than that on *P. furcatus* (45) with a smaller number of overlapping species, which may be due to in *Pandanus furcatus* has a relatively thin cuticle on its leaves, no trunk or branches and is typically found along the edges of streams in areas covered by forests. Conversely *P. tectorius* has a distinct trunk, many branches, with a thicker cuticle on the leaves and is found along the coastline (Whitton, 1999; McKenzie *et al.*, 2002). Species low diversity has also been found on leaves of banana (Photita *et al.*, 2003a) and *P. amaryllifolius* (in this study). In *D. loureiri P. odoratissimus* and *P. penetrans* (in the present study) and palms (Yanna *et al.*, 2001a, b) that offering more durable, strongly sclerenchymatous substrata tended to support a higher fungal diversity.

The diversity of fungi known among various hosts in the previous studies are likely less fastidious, more ubiquitous species. For example McKenzie *et al.*, (2002)

found that fungal diversity on *Freycinetia* appears to differ from that on *Pandanus*. Of the 133 species known from *Freycinetia*, 44 species are known also to inhabit *Pandanus*, giving a species composition overlap of 33%. Of the overlapping species, 36 are known from other substrata besides the Pandanaceae.

In this study, the similarity of fungi occurring on *D. lourieri* (from the rainforest), *P. amaryllifolius* (from gardens) and *P. odoratissimus* (the coastline) are low. The similarity of fungi on *D. lourieri* and *P. penetrans* (both natural and baits in the rainforest) are high. Distinct fungal communities were also found on the collection of *P. penetrans* leaves from different sites. The results indicate that the fungal communities may be affected by the host habitats which differ in humidity, rainfall and winds. Fungal diversity may also be related to disturbance. Fungi assemblage on palms in a tropical pristine forest was more diverse than those on agricultural palms or palm in botanical gardens (Tsui *et al.*, 1998). Taylor (1998) found that the fungal communities in natural stands of palm, *Archontophoenix alexandrae* from rainforests of north Queensland were significantly richer than those of palms outside their natural habitat, e.g. naturalized in new habitats in Hong Kong. In the present study, decaying leaves of *D. lourieri* from garden plants supported only a few taxa of *Colletotrichum*, *Fusarium* and *Phomopsis* (Thongkantha, pers. obs.). Saprobic fungi are unlikely to be host specific (Shivas and Hyde, 1997). Therefore, host specificity is unlikely to influence saprobic fungal diversity. An important factor that may influence the biodiversity of saprobic fungi on various hosts is therefore host-recurrence. To allow for a better understanding of host recurrence and specificity, Lodge (1997) suggested that comparisons must be made between samples of the same species located at different sites, or samples from different species located at the same site. The reasons as to why fungi may occur recurrently on certain hosts is not understood, but may be related to the presence of these fungi as endophytes. Unfortunately there has been no study on the endophytic fungi on *Dracaena* and *Pandanus*. In addition, various studies of endophytes on other hosts found a large number of unidentified taxa especially sterile mycelia, coelomycetes and Xylariaceae (Rodrigues and Samuels, 1990; Rodrigues, 1994; Photita *et al.*, 2001; Bussaban *et al.*, 2004). Further studies are needed to isolate and identify most of the representative endophytes and saprobes

on *Dracaena* and *Pandanus* in particular more samples with different tissue of each host from the same and different sites should be examined.

3.4.2 Fungal occurrence on different tissues of *Pandanus odoratissimus* and *P. penetrans*

In this study, fungal communities found on different dead tissues of *Pandanus odoratissimus* (leaves, prop roots and seeds) and *P. penetrans* (leaves and leaf sheaths) are low overlapped. The recurrence of fungi on certain tissue types has also been noted in banana (Photita *et al.*, 2003a), *Magnolia liliifera* (Promputtha *et al.*, 2004b) and palms (Yanna *et al.*, 2001a, b). The structural differences of plant tissues may account for the fungi being confined to specific tissues as some fungi may have enzyme systems that can degrade the sclerenchyma tissues containing lignin, while others only degrade cellulose. Poon and Hyde (1998) found that fungi on *Phragmites australis* were vertically distributed, and have been influenced by moisture as the bases were submerged. The ascomycete taxa were distinct on the lower culm tissues comprising sclerenchyma, while the anamorphic taxa were rich on the upper herbaceous tissues. Sadaba *et al.* (1995) found different fungal communities on herbaceous and woody parts of *Acanthus ilicifolius* (Acanthaceae), more ascomycetes occurring on the lower woody part and more anamorphic taxa on the upper herbaceous parts. The recurrence of certain fungi on different tissue types may be due to differences in nutritional requirements, or the ability of the fungi to utilize different substrates (Adaskaveg *et al.*, 1991; Isaac, 1992; Ingold and Hudson, 1993). Alternatively, it may be related to the distribution of endophytes, a theory which requires testing. The enzymatic activities of fungi also warrants further investigation in order to understand whether their abundance on specific tissue types is due to differing enzymatic capabilities (Yanna *et al.*, 2001a, b).

3.4.3 Seasonal pattern of fungi on *Pandanus penetrans*

There is no seasonal pattern of fungal occurrence on *Pandanus penetrans* collected from tropical rainforests of Thailand. This may have been due to collection sites in Thailand show less fluctuation in temperature, humidity and rainfall (Appendix B). No seasonal differences were also observed between the fungi isolated from palms in the tropics, in both endophytes (Fröhlich *et al.*, 2000) and saprobes (Yanna *et al.*, 2001b). The effect of seasonality may be more acute in temperate

regions where greater fluctuations in temperature, humidity and rainfall usually occur. Further studies are, however, required to elucidate the effect of seasonality.

3.4.4 Fungal succession on *Pandanus penetrans* leaves

Succession of fungi on leaf litter of temperate and tropical monocotyledonous trees (e.g. grass, palms, pines) by using direct and indirect methods have been studied by Kendrick and Burges (1962), Sandhu and Sidhu, (1980), Hurst *et al.* (1983), Tokumasu *et al.* (1994), Hyde and Alias (2000), Yanna *et al.* (2001b, 2002), and Tokumasu and Aoiki (2002). There have also been extensive studies of fungal succession on leaf litter of dicotyledonous trees (Saito, 1956; Hering, 1965; Hogg and Hudson, 1966; Pasqualetti *et al.*, 1999; Promputtha *et al.*, 2002). Fungal communities change over time during the decay process of naturally dead bamboo and bamboo baits in Hong Kong (Zhou and Hyde, 2002). In the present study changes in fungal composition throughout the decay process on *Pandanus penetrans* leaf baits were directly observed after incubation in a moist chamber. Three stages of fungal succession were recognizable including the pioneer stage, mature stage and impoverished or later stage that are similar to those of previous studies (e.g. Dix and Webster, 1985; Promputtha *et al.*, 2002; Yanna *et al.*, 2002). Early colonizers may be endophytes (Bacon and White, 2000) or latent pathogens (Hudson, 1980). Fungal communities occurring on banana leaves as pioneer colonizers were *Deightoniella torulosa*, *Nigrospora* spp. and *Verticillium theobromae*, and were later replaced by species of *Alternaria*, *Aspergillus*, *Cephalosporium*, *Cladosporium*, *Fusarium*, *Paecilomyces* and *Penicillium* with time (Meredith, 1962). *Asterina clasterosporium*, *Cylindrocladium brasiliense*, *Endomelanconium phoenicicola*, *Pestalotiopsis palmurum* and *Sporidesmium pedunculatum* were common on green leaves of *Phoenix hanceana* palm in first stage of decay studied by, and found (Yanna *et al.*, 2002). The mature community at months 2-6 frequently comprised *Codinaea intermedia*, *Diaporthe phoenicis*, *Thozetella effusa* and *Tubakia* sp.

The time of fungal decomposition of plant material varies among plant species, the study area, tissues types and the thickness of samples. For instance, pine needles may take ten years to decompose completely in a cool temperate pine forest (Hudson, 1980). Decomposition time of couch grass stems (Hudson and Webster, 1958), sugarcane and pineapple leaves (Hudson, 1962; Tiwari *et al.*, 1994) in tropical areas

have been shown to be relatively short about 19, 14 and 24 months respectively. Sugarcane bagasse and palm (*Phoenix hanceana*) leaves needed only 7 and 4 months respectively to completely decay (Sandhu and Sidhu, 1980; Yanna *et al.*, 2002). Decomposition of senescent leaves of *Manglietia garrettii* in a rain forest in Thailand was completed within two months (Promputtha *et al.*, 2002). In contrast mature green leaves of *Pandanus penetrans* in this study were found to be completely decayed after 18 months although the samples were collected and incubated at the same site to those investigated by Promputtha *et al.* (2002).

3.4.5 Pathogenic fungi on leaves of *Dracaena lourieri* and *Pandanus* spp.

There are relatively few fungi that have been described or recorded as pathogens of *Dracaena* and *Pandanus.* For example, *Annellolacinia pandanicola* J. Fröhl, *Diplococcium pandani* B. Huguenin, *Echidnodes pandani* (Rostr.) Han, *Meliola* spp. *Phyllosticta pandanicola* E. Young, and *Volutellaria fuliginea* I. Hino & Katum were reported from species of *Pandanus* (McKenzie and Hyde, 1997). In the present study species of *Acremonium*, *Aspergillus*, *Cercospora, Colleotrichum, Curvularia*, *Fusarium*, *Gliocladium*, *Herpomyces*, *Myrothecium, Oxydothis*, *Penicillium*, *Phomopsis*, *Ramichloridium* and *Sporidesmium* were observed from anthracnose on leaves or leaf spots of *Pandanus penetrans* in rainforests of Thailand. Green and older leaves of *Dracaena lourieri* and *P. amaryllifolius* were frequently covered with anthracnose and leaf spots caused by *Colletotrichum gloeosporioides* and *Guignardia* sp. respectively. *Cladosporium oxysporum* and *Nigrospora oryzae* were found in large necrotic lesions on leaves of *D. lourieri* and *P. amaryllifolius* from garden plants. All these fungi have been previously reported as plant pathogens in various hosts world wide (Clay, 1988; Smith *et al.*, 1989; Farr *et al.*, 1989; Jones, 2000; Brooks, 2002) and Thailand (Sontirat *et al.*, 1994; Photita *et al.*, 2001, 2004; Bussaban, 2005).

Almost all of the fungi associated with leaf disease of *D. lourieri* and *Pandanus* spp. in this study were also recovered as saprobes on dead leaves. Taylor (1998) found that dead samples of garden palms contained a few typical palm fungi as *Lasiodiplodia* and *Pestalotiopsis* which are believed to be plant pathogens. Some saprobes can also be facultative parasites (Photita *et al.*, 2004).