CHAPTER 5

PATHOGENS, PATHOGENICITY AND ANTAGONISTIC TESTS

5.1 Introduction

The current study initially aimed to identify the diseases that occur on the zingiberaceous plants under study. However, it evolved into a wider study that involved testing the pathogenicity against tissue cultured ginger by endophytic, saprobic, or pathogenic fungi (genera which have been previously reported as zingiberaceous pathogens, e.g., *Colletotrichum*, *Fusarium*, *Phomopsis*, *Phyllosticta* and *Pyricularia*), and to test endophytic and saprobic fungi for their antagonism against *Pyricularia costina*—an important ginger pathogen caused blast disease on various ginger hosts especially on cutting flower ginger varieties.

Endophytes are fungi that colonize healthy plant tissues without causing any immediate symptoms of infection (Hirsch and Braun, 1992). Endophytes have been often isolated by plant pathologists and described as weak pathogens of little consequence, except under unusual conditions in which the host plant is subject to physiological stress (Kulik, 1984; Brown *et al*., 1998; Romero *et al*., 2001; Photita *et al*., 2004). Latent infection is a situation in which a pathogen infects a host, but the plant does not show disease symptoms. The pathogen persists, and later produces signs or symptoms of disease, prompted by environmental or nutritional conditions, or by the state of maturity of the host or pathogen (Verhoeff, 1974; Agrios, 1988). Endophytes are thought to become pathogenic when the host plant is stressed,

however, some endophytes are thought to benefit the host plants by enhancing absorption of soil nutrients such as phosphorus, conferring drought tolerance, providing protection from insect attack, and/or may also inhibit the development of plant pathogens (Thomson *et al*., 1986; Breen, 1993, 1994; Latch, 1993; Stone *et al.*, 2000; Dingle and McGee, 2003; Rubini *et al*., 2005).

5.2 Materials and methods

5.2.1 Collection of zingiberaceous diseases

Zingiberaceous plants with leaf spots or other disease symptoms were collected from the same host species in the same sites as that of the endophytic and saprobic studies (Chapters 2, 3). Diseased zingiberaceous tissue was collected and returned to the laboratory. The infected parts of the plants showing symptoms of disease were examined and, where possible, the causal agent identified under the microscope. In some cases, the specimens were incubated in moist chambers in order to induce sporulation of the fungi for identification. Disease symptoms were also described and the causal agents were isolated.

5.2.2 Isolation of zingiberaceous pathogens

Small disks (ca. 3 mm^2) were cut from the margin of diseased tissue. Each piece of tissue was sterilized by dipping in 3% Clorox for 3 min, washing in two change of sterile water, and then placing separately on PDA plates. The plates were incubated at room temperature (27–30 °C) and observed periodically. The growing edges of any colonies developing from the leaf disks were then transferred aseptically

to PDA agar slants. If the pathogen sporulated on the leaf, it was isolated using single spore methods as described in Chapter 4.

5.2.3 Pathogenicity tests

Selected isolates of endophytic, saprobic or pathogenic fungal genera which have previously been reported as zingiberaceous pathogens, were tested for their pathogenicity against tissue cultured ginger of commercial (cutting flower) species *Curcuma* sp. The selected fungal isolates (*Alternaria*, *Colletotrichum*, *Fusarium*, *Phomopsis*, *Phyllosticta* and *Pyricularia*) were grown on PDA for 1–4 weeks depending on their sporulation. A spore suspension of each isolates was then prepared in sterile water and the spore concentration adjusted to 10^5 conidia/ml. Pathogenicity was determined by inoculating 20 *Curcuma* plants (6 weeks old) grown in the tissue culture containers (10 plants per container), with 10 ml of spore suspension. For the control, 10 ml of sterile water was dropped instead of the spore suspension. The treated plants were incubated at room temperature in the tissue culture containers and any lesions or death of plants were noted after 1 week. Samples of the lesions were removed and placed on PDA for recovery of the inoculated fungal strain. Surviving plants were transplanted to plastic pots and incubated in a greenhouse (Figure 5.1). Any lesions or death were determined after a further 4 weeks.

Figure 5.1 Surviving *Curcuma* plants transplanted to plastic pots after pathogenicity test.

5.2.4 Antagonistic tests

Fifty seven strains of endophytic and 36 saprobic fungi isolated from zingiberaceous plants as previously described (Chapters 3, 4) were tested for their antagonistic ability against a selected pathogen, *Pyricularia costina*, by dual culture test (Jackson *et al*., 1991). A 5 mm diameter disk from the growing edge of *P. costina* was placed at one side of a Petri dish containing PDA while a 5 mm diameter disk from the growing edge of the endophytic or saprobic fungal isolate were placed on the other side of the dish. For the control a disk from the growing edge of *P. costina* was placed alone at one side of a Petri dish. Each test was replicated on four plates. The plates were incubated at room temperature and any inhibition by the test fungi were scored after 13 days and ranked to determine antagonistic activity (Figure 5.2).

$$\text{Percentage inhibition} = \frac{r_1 - r_2}{r_1} \times 100$$

r_1: radius of pathogen in control plate
r_2: radius of pathogen in dual culture plate

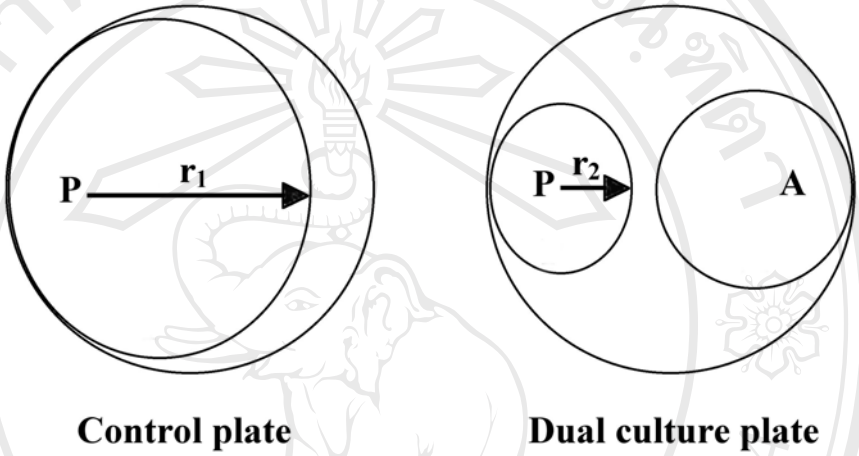

Figure 5.2 Percentage inhibition in dual culture test: P = pathogen, A = endophytic or saprobic fungi, r_1 = radius of pathogen in control plate, r_2 = radius of pathogen in dual culture plate.

The data generated from this experiment was analyzed using SPSS v. 10 package for one-way analysis of variance (ANOVA). The inhibitory activity of radial growth of the pathogen by endophytic and saprobic fungi was also evaluated as high, moderate or low antagonistic activity, where percentage inhibition was > 60%, 50–60% and < 50%, respectively. Morphological characters of *P. costina* were noted and its mycelium was examined using scanning electron microscopy (SEM).

5.3 Results

5.3.1 Collection of zingiberaceous diseases

Five fungi, *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Phomopsis* sp., *Phyllosticta capitalenis* and *Pyricularia costina* were identified from samples of ginger showing symptoms of anthracnose on leaves, leaf blast or leaf spot (Table 5.1). The disease symptoms are described below and illustrated in Figure 5.3.

1) Anthracnose

Causal agent: *Colletotrichum gloeosporioides*

Hosts: *Alpinia galanga*, *A. malaccensis*, *Amomum siamense*, *Etlingera elatior*, *E. littoralis*

Disease symptoms: The disease starts as a small circular speck on the upper 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 (Figure 5.3b, f). Sometime the disease starts at the leaf margins or leaf tip and later enlarges along the leaf larmina (Figure 5.3d).

2) Leaf blast

Causal agent: *Pyricularia costina*

Hosts: *Alpinia malaccensis*, *Amomum siamense*

Disease symptoms: The first symptoms are small reddish brown spots surrounded by distinct yellow halos on the upper leaf surface. On *Alpinia malaccensis* lesions enlarge with irregular margins and concentric zonation often with a white or grey center and a paler reddish brown border. Adjacent lesions often coalesce to form

necrotic areas (Figure 5.3c). On *Amomum siamense* oval or eye shaped lesions may grow to several centimeters in length and are characterized by concentric zonation often with a grey center and a dark brown border surrounded by a bright yellow halo (Figure 5.3g).

3) Leaf spot

Causal agent: *Alternaria alternata*

Hosts: *Alpinia galanga*

Disease symptoms: The disease begins as small brown spots on the upper leaf surface. The fungus repeatedly produce concentric rings of spores masses and the spots increase in size to approximately 1–3 cm long surrounded by a reddish brown margin and yellow halo (Figure 5.3a).

4) Leaf spot

Causal agent: *Phomopsis* sp.

Hosts: *Amomum siamense*

Disease symptoms: The first signs are a few scattered groups of black raised spots that appear on the upper leaf surface. The spots enlarge and turn necrotic, surrounded by a reddish brown margins and a yellow halo (Figure 5.3e).

5) Leaf spot

Causal agent: *Phyllosticta capitalensis*

Hosts: *Etlingera elatior*, *Zingiber officinale*

Disease symptoms: The disease starts as a water soaked spot and later turns to a white or light brown spot surrounded by a dark brown margin and a yellow halo. The lesions enlarge and may coalesce to form necrotic areas (Figure 5.3h, i).

Figure 5.3 Disease symptoms found on Zingiberaceae: a, b. *Alpinia galanga*, a. Leaf spot caused by *Alternaria alternata*, b. Anthracnose caused by *Colletotrichum gloeosporioides*, c, d. *Alpinia malaccensis*, c. Leaf blast caused by *Pyricularia costina*, d. Anthracnose caused by *Colletotrichum gloeosporioides*, e–g. *Amomum siamense*, e. Leaf spot caused by *Phomopsis* sp., f. Anthracnose caused by *C. gloeosporioides*, g. Leaf blast caused by *Pyricularia costina*, h. Leaf spot of *Etlingera elatior* caused by *Phyllosticta capitalensis*, i. Leaf spot of *Zingiber officinale* caused by *Phyllosticta capitalenis*.

Table 5.1 Diseases identified from wild and cultivated Zingiberaceae.

Host	Disease	Pathogen	Collection date	Location
Wild species				
Alpinia malaccensis	Leaf spot	*Colletotrichum gloeosporioides*	19 February 2003	Huay Kok Ma
	Leaf blast	*Pyricularia costina*	5 June 2001	Doi Pui
Amomum siamense	Leaf spot	*Colletotrichum gloeosporioides*	19 February 2003	Huay Kok Ma, Medicinal Plant Garden
	Leaf spot	*Phomopsis* sp.	19 February 2003	Huay Kok Ma, Medicinal Plant Garden
	Leaf blast	*Pyricularia costina*	19 February 2003	Huay Kok Ma, Medicinal Plant Garden Lampang
Etlingera elatior	Leaf spot	*Colletotrichum gloeosporioides*	23 September 2003	Chiang Mai University
	Leaf spot	*Phyllosticta capitalensis*	21 September 2003	Queen Sirikit Botanic Garden
Etlingera littoralis	Leaf spot	*Colletotrichum gloeosporioides*	19 February 2003	Huay Kok Ma, Medicinal Plant Garden
Cultivated species				
Alpinia galanga	Leaf spot	*Alternaria alternata*	19 October 2002	Chiang Mai (Hangdong), Lampang (Muang)
	Leaf spot	*Colletotrichum gloeosporioides*	26 October 2002	Chiang Mai (Hangdong), Lampang (Muang)
Zingiber officinale	Leaf spot	*Phyllosticta capitalensis*	3 September 2001	Phayao (commercial)

5.3.2 Pathogenicity tests

Twenty isolates of pathogens were tested for pathogenicity (Table 5.2). All isolates of *Alternaria alternata*, *Colletotrichum gloeosporioides*, and *Pyricularia costina*, and some isolates of *Phomopsis* sp. (4) and *Phyllosticta capitalensis* (1) caused disease lesions and death on the gingers either in the tissue culture containers or after transplanting to plastic pots. Three isolates of *Phomopsis* sp. and one isolate of *Phyllosticta capitalensis*, caused some disease lesions but the tissue cultured gingers survived after transplanting to plastic pots.

Twenty two isolates of endophytes and two isolates of saprobes were also tested for pathogenicity against tissue cultured gingers (Table 5.2). All isolates of *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Glomerella* sp. and *Pyricularia costina*, and some isolates of *Fusarium* sp. (3), *Phomopsis* sp. (1) and *Phyllosticta capitalensis* (1), caused disease lesions and death on the gingers either in the tissue culture containers or after transplanting to plastic pots. One isolate of *Fusarium* sp., three isolates of *Phomopsis* sp. and four isolate of *Phyllosticta capitalensis* caused some disease lesions but the gingers survived after transplanting to plastic pots, and the plants infected by these endophytes grew better (longer leaves and roots) than the control.

In the inoculated containers all tested fungi formed spore masses or hyphae on the tissue cultured gingers (Figure 5.4) while the control produced no disease symptoms. These fungi were reisolated from these symptoms (Figure 5.5).

Table 5.2 Results of pathogenicity testing of fungal taxa isolated as endophytes, saprobes or pathogens, and inoculated onto tissue cultured gingers (*Curcuma* sp.).

Isolate number (CMU)	Taxon	Original host	Disease[a]
Endophytes			
ZE0114	*Alternaria alternata*	*Alpinia malaccensis*	3
ZE0016	*Colletotrichum gloeosporioides*	*Amomum siamense*	3
ZE0028	*Colletotrichum gloeosporioides*	*Amomum siamense*	3
ZE0550	*Colletotrichum gloeosporioides*	*Zingiber officinale*	3
ZE0551	*Colletotrichum gloeosporioides*	*Alpinia galanga*	3
ZE0552	*Colletotrichum gloeosporioides*	*Alpinia galanga*	3
ZE0384	*Fusarium* sp.	*Alpinia malaccensis*	3
ZE0388	*Fusarium* sp.	*Amomum siamense*	3
ZE0390	*Fusarium* sp.	*Amomum siamense*	2
ZE0396	*Fusarium* sp.	*Amomum siamense*	1
ZE0091	*Glomerella* sp.	*Alpinia malaccensis*	3
ZE0071	*Phomopsis* sp.	*Amomum siamense*	1
ZE0141	*Phomopsis* sp.	*Amomum siamense*	0
ZE0110	*Phomopsis* sp.	*Amomum siamense*	3
ZE0232	*Phomopsis* sp.	*Etlingera littoralis*	1
ZE0443	*Phyllosticta capitalensis*	*Amomum siamense*	1
ZE0444	*Phyllosticta capitalensis*	*Amomum siamense*	1
ZE0445	*Phyllosticta capitalensis*	*Zingiber officinale*	1
ZE0446	*Phyllosticta capitalensis*	*Zingiber officinale*	2
ZE0451	*Phyllosticta capitalensis*	*Alpinia malaccensis*	1
ZE0003	*Pyricularia costina*	*Amomum siamense*	2
ZE0004	*Pyricularia costina*	*Amomum siamense*	2
Saprobes			
ZS187	*Fusarium oxysporum*	*Zingiber officinale*	2
ZS174	*Pyricularia costina*	*Amomum siamense*	3
Pathogens			
ZP0037	*Alternaria alternata*	*Alpinia galanga*	2
ZP0038	*Alternaria alternata*	*Alpinia galanga*	3
ZP0012	*Colletotrichum gloeosporioides*	*Etlingera littoralis*	3
ZP0016	*Colletotrichum gloeosporioides*	*Alpinia malaccensis*	3
ZP0018	*Colletotrichum gloeosporioides*	*Amomum siamense*	3
ZP0021	*Colletotrichum gloeosporioides*	*Amomum siamense*	3
ZP0025	*Colletotrichum gloeosporioides*	*Amomum siamense*	3
ZP0009	*Phomopsis* sp.	*Amomum siamense*	1
ZP0011	*Phomopsis* sp.	*Amomum siamense*	2
ZP0014	*Phomopsis* sp.	*Alpinia malaccensis*	1
ZP0015	*Phomopsis* sp.	*Alpinia malaccensis*	2
ZP0028	*Phomopsis* sp.	*Amomum siamense*	1
ZP0034	*Phomopsis* sp.	*Amomum siamense*	2
ZP0039	*Phomopsis* sp.	*Amomum siamense*	3
ZP0029	*Phyllosticta capitalensis*	*Etlingera elatior*	2
ZP0036	*Phyllosticta capitalensis*	*Zingiber officinale*	1
ZP0003	*Pyricularia costina*	*Amomum siamense*	2
ZP0004	*Pyricularia costina*	*Amomum siamense*	2
ZP0005	*Pyricularia costina*	*Alpinia malaccensis*	3
ZP0006	*Pyricularia costina*	*Alpinia malaccensis*	3
Control			
Sterile water			0

[a]Disease severity was based on symptom development or death of tissue cultured *Curcuma* sp.: 3 = died in container after inoculation for 1 week: 2 = died after transplanting to plastic pots for 4 weeks, 1 = survived with some necrotic lesions: 0 = survived without necrotic lesions

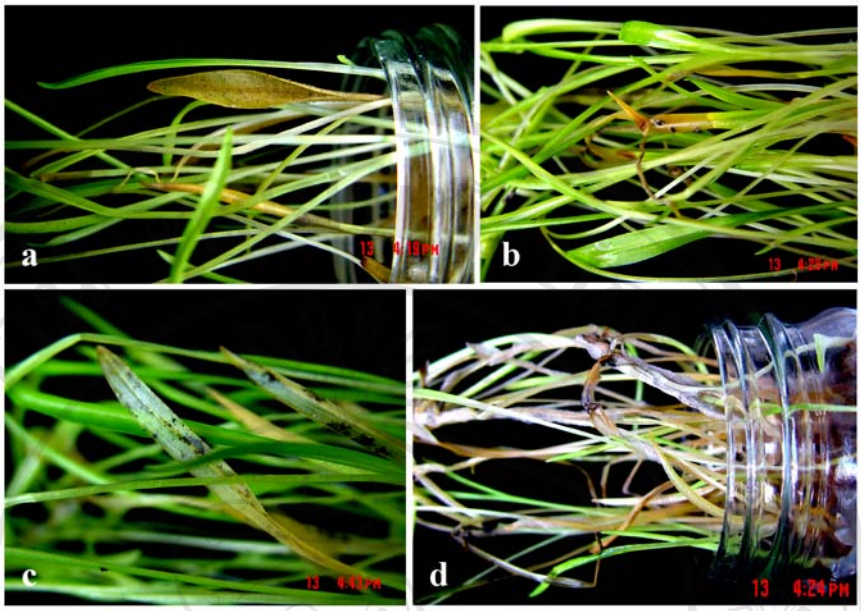

Figure 5.4 Disease symptoms on tissue cultured gingers (*Curcuma* sp.): a. *Colletotrichum gloeosporioides* (CMUZE0550), b. *Phomopsis* sp. (CMUZE0071), c. *Phyllosticta capitalensis* (CMUZE0446), d. *Pyricularia costina* (CMUZE0003).

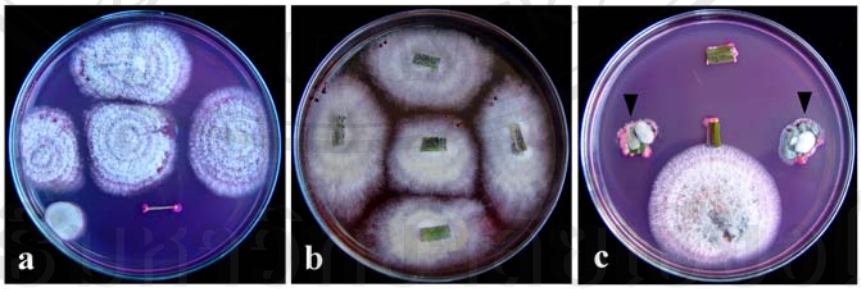

Figure 5.5 Reisolation of fungi from disease lesions on tissue cultured gingers (*Curcuma* sp.): a. *Colletotrichum gloeosporioides* (CMUZE0550), b. *Phomopsis* sp. (CMUZE0071), c. *Phyllosticta capitalensis* (CMUZE0446, arrowed).

5.3.3 Antagonistic tests

All selected isolates of fungal endophytes and saprobes from zingiberaceous plants inhibited the growth of the pathogen *Pyricularia costina*. However, there was no significant difference in the percentage inhibition of radial growth of the pathogen by *Berkleasmium sutheppuiense* (CMUZS043-1) and *Helicomyces* sp. (CMUZS104-2), relative to the control. Twenty seven isolates tested had a high antagonistic activity (> 60% inhibition), 10 isolates had a moderate antagonistic activity (51–60% inhibition), and 56 isolates had a low antagonistic activity (< 50% inhibition) against *P. costina* (Table 5.3).

Fusarium sp. (CMUZE0396) and *Papulaspora* sp. (CMUZE0116) were the most effective fungi in reducing the radial mycelium growth of the pathogen when paired in cultured, with a percentage inhibition of 74.4% and 72.9%, respectively (Table 5.3). The morphological characters of the pathogen in dual culture plates were changed, the growth was very slow, the colonies were extremely dwarfed (Figure 5.6) and mycelium was distorted (Figure 5.7).

Table 5.3 Percentage inhibition of radial growth of *Pyricularia costina* by endophytic and saprobic fungi after dual growth on PDA for 13 days.

Isolate number (CMU)	Taxa	Percentage inhibition
Endophytes		
ZE0132	*Chaetomium globosum*	65.4
ZE0127	*Colletotrichum gloeosporioides*	65.0
ZE0148	*Cylindrocarpon* sp.	18.0
ZE0149	*Cylindrocladium* sp.	66.7
ZE0150	*Cylindrocladium* sp.	69.9
ZE0151	*Eupenicillium crustaceum*	53.4
ZE0152	*Eupenicillium crustaceum*	24.0
ZE0155	*Eupenicillium crustaceum*	36.1
ZE0156	*Eupenicillium crustaceum*	15.0
ZE0161	*Eupenicillium crustaceum*	48.9
ZE0389	*Fusarium* sp.	70.7
ZE0393	*Fusarium* sp.	43.0
ZE0394	*Fusarium* sp.	70.6
ZE0396	*Fusarium* sp.	74.4
ZE0400	*Fusarium* sp.	19.0
ZE0401	*Fusarium* sp.	54.1
ZE0402	*Fusarium* sp.	16.0
ZE0403	*Fusarium* sp.	62.4
ZE0405	*Fusarium* sp.	48.9
ZE0407	*Fusarium* sp.	21.0
ZE0408	*Fusarium* sp.	17.0
ZE0117	*Fusicoccum* sp.	66.1
ZE0002	*Gaeumannomyces amomi*	60.0
ZE0100	*Gaeumannomyces amomi*	70.7
ZE0101	*Gaeumannomyces amomi*	59.0
ZE0008	*Geniculosporium* sp. 1	70.7
ZE0009	*Geniculosporium* sp. 2	66.0
ZE0065	*Glomerella* sp.	59.4
ZE0066	*Glomerella* sp.	50.0
ZE0011	*Humicola fuscoatra*	22.0
ZE0228	*Humicola fuscoatra*	59.0
ZE0102	*Leiosphaerella amomi*	60.2
ZE0116	*Papulaspora* sp.	72.9
ZE0162	*Penicillium* sp.	71.0
ZE0238	*Penicillium* sp.	64.0
ZE0133	*Phoma* sp.	62.0
ZE0134	*Phoma* sp.	70.0
ZE0141	*Phomopsis* sp.	69.9
ZE0232	*Phomopsis* sp.	38.0
ZE0012	Sterile mycelium	62.0
ZE0013	Sterile mycelium	72.0
ZE0120	Sterile mycelium	23.0
ZE0122	Sterile mycelium	56.4
ZE0123	Sterile mycelium	72.3
ZE0124	Sterile mycelium	33.0
ZE0126	Sterile mycelium	63.2
ZE0131	Sterile mycelium	67.7
ZE0145	Sterile mycelium	59.0
ZE0146	Sterile mycelium	51.0

Table 5.3 (Continued).

Isolate number (CMU)	Taxa	Percentage inhibition
Endophytes		
ZE0252	Sterile mycelium	22.0
ZE0179	*Talaromyces flavus*	39.0
ZE0195	*Talaromyces flavus*	34.0
ZE0199	*Talaromyces flavus*	57.9
ZE0200	*Talaromyces flavus*	8.0
ZE0201	*Talaromyces flavus*	8.0
ZE0416	*Xylaria* sp.	12.0
ZE0418	*Xylaria* sp.	64.7
Saprobes		
ZS150	*Agaricus* sp.	25.6
ZS112-1	*Bahusandhika sundarum*	31.0
ZS149	Basidiomycete 1	19.0
ZS153	Basidiomycete 2	67.7
ZS160	Basidiomycete 4	23.0
ZS167	Basidiomycete 6	27.8
ZS043-1	*Berkleasmium sutheppuiense*	6.5[ns]
ZS129-1	*Chloridium* sp.	10.1
ZS129-2	*Chloridium* sp.	14.3
ZS139	*Crepidotus* sp. 1	25.6
ZS145	*Crepidotus* sp. 2	70.7
ZS148-1	*Cyathus* sp.	49.4
ZS147	Discomycete	39.1
ZS104-2	*Helicomyces* sp.	6.0[ns]
ZS126-1	Hyphomycete 5	49.4
ZS130-1	Hyphomycete 6	26.2
ZS130-2	Hyphomycete 6	28.0
ZS154-1	*Hypocrea* sp.	44.0
ZS154-2	*Hypocrea* sp.	45.2
ZS144	*Marasmius* sp. 2	41.7
ZS143	*Marasmius* sp. 3	29.2
ZS162	*Marasmius* sp. 4	47.0
ZS121-1	*Memnoniella* sp.	28.0
ZS121-2	*Memnoniella* sp.	19.0
ZS133-1	*Memnoniella subsimplex*	25.0
ZS133-2	*Memnoniella subsimplex*	30.4
ZS048	*Myrothecium cinctum*	23.3
ZS050-1	*Pyricularia longispora*	42.3
ZS117-2	*Pyricularia longispora*	41.1
ZS026-1	*Solosympodiella* sp.	17.9
ZS026-2	*Solosympodiella* sp.	12.5
ZS136-1	*Synematous* sp.	15.5
ZS004-1	*Torula* sp. 2	21.1
ZS023-1	*Verticillium* sp. 1	35.1
ZS023-3	*Verticillium* sp. 1	31.0
ZS074	*Xenosporium intermedium*	11.3
Control		
ZP0006	*Pyricularia costina*	0.0

[ns] no significant difference between the percentage inhibition of treatment and control

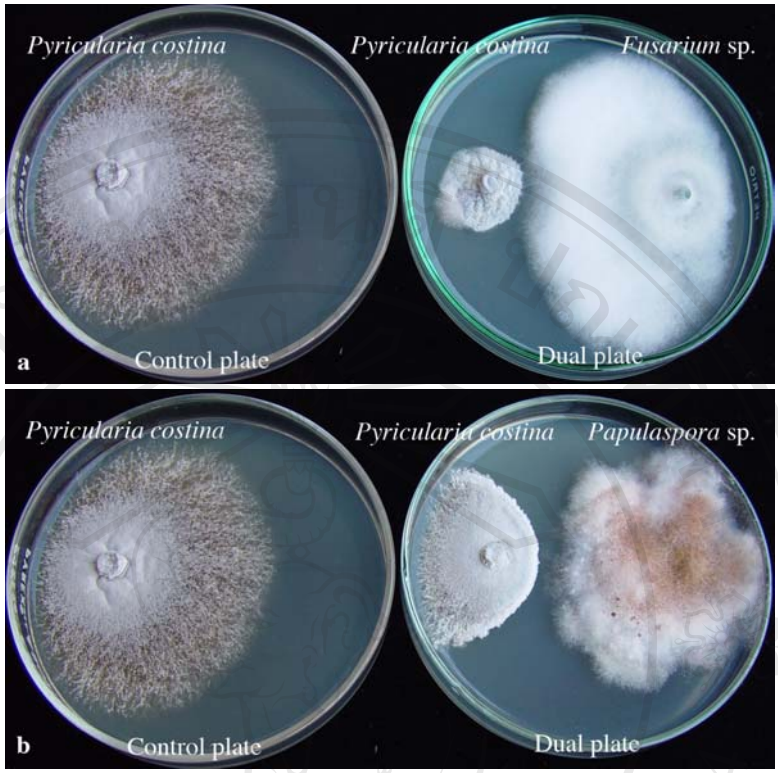

Figure 5.6 *Fusarium* sp. (CMUZE0396) (a) and *Papulaspora* sp. (CMUZE0116) (b) showing inhibition in growth of *Pyricularia costina* on PDA.

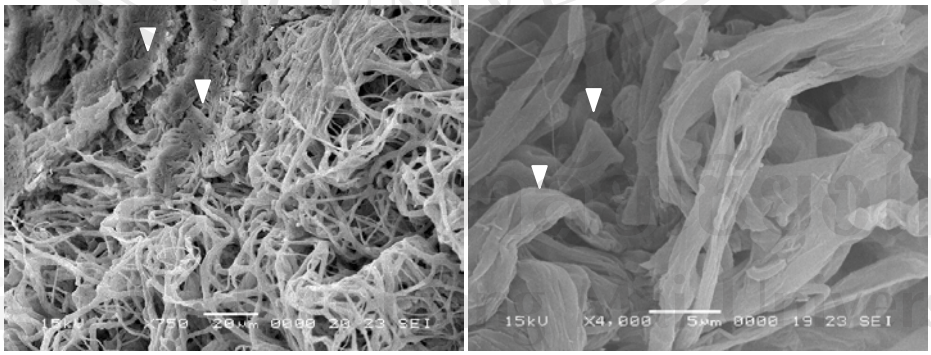

Figure 5.7 *Pyricularia costina* in dual culture plate inhibited by *Fusarium* sp. (CMUZE0396) showed distorted mycelium (arrowed).

5.4 Discussion

5.4.1 Pathogens on Zingiberaceae

Many fungi are known to be pathogens of Zingiberaceae (Bussaban *et al*., 2002) including *Colletotrichum capsici*, *C. gloeospoioides*, *Fusarium oxysporum* f. sp. *zingiberi*, *F. solani*, *Phomopsis* sp., *Phyllosticta zingiberis* and *Pyricularia curcumae* (Pavgi and Upadhyay, 1986; Farr *et al*., 1989; Sontirat *et al*., 1994). In present study five fungi were observed, collected and identified from samples of ginger with symptoms of anthracnose on leaves, leaf blast or leaf spot. *Colletotrichum gloeosporioides* and *Phyllosticta capitalensis* were found on both the wild and cultivated gingers, while blast fungus *Pyricularia costina* was recorded on the wild gingers, *Alpinia malaccensis* and *Amomum siamense*. Alternaria leaf spot was found only on cultivated ginger, *Alpinia galanga*.

Colletotrichum gloeosporioides is found throughout the world and has been recorded from a wide and disparate range of hosts, e.g., anthracnose of tropical fruits (Brown, 1975; Quimio and Quimio, 1975; Dodd *et al*., 1992), anthracnose of legume, *Stylosanthes* (Manner *et al*., 1992) and secondary leaf fall of rubber trees (Senechal *et al*., 1987). *Pyricularia* species are typically plant pathogens, e.g., *P. oryzae* is the serious rice blast pathogen (Ou, 1987), *Pyricularia grisea* is the cause of gray leaf spot of St Augustine grass (Malca and Owen, 1957), and other *Pyricularia* species cause diseases on members of Cannaceae, Commelinaceae, Costaceae, Marantaceae and Musaceae (Meredith, 1963; Asuyama, 1965; Hashioka, 1971, 1973; Kotani and Kurata, 1992; Pappas and Paplomatas, 1998). *Pyricularia costina*, *P. curcumae*, *P. distorta* and *P. zingiberis* have been previously reported as pathogenic on Zingiberaceae (Nishikado, 1917; Hashioka, 1971; Rathaiah, 1980; Sarbajna, 1990).

All of the fungi that were associated with leaf diseases of Zingiberaceae on the plants sampled in the present study were also isolated as endophytes from healthy wild and cultivated gingers (Chapter 3) and recovered from dead tissues of these plants (Chapter 4).

5.4.2 Can endophytes and saprobes of Zingiberaceae become pathogens?

The evidence that an endophytic stage may occur in the life cycle of some pathogens (e.g., banana pathogens) has been discussed (Brown *et al*., 1998; Photita *et al*., 2004). By definition, an endophyte cannot be considered as causing disease (Sinclair and Cerkauskas, 1996), however, genera and species that cause disease are regularly isolated and identified as endophytes. The distinction between a pathogen and endophyte is not always clear (Sinclair and Cerkauskas, 1996). The purpose of this study was not to differentiate between pathogens and endophytes, but to establish if any fungi regularly recovered as endophytes or saprobes from Zingiberaceae have the ability to be pathogenic (i.e. latent pathogens).

Other fungi associated with leaf diseases of Zingiberaceae in the present study, *Colletotrichum gloeosporioides*, *Phomopsis* sp., *Phyllosticta capitalensis* and *Pyricularia costina* were also commonly isolated as endophytes in healthy wild and cultivated gingers (Chapter 3) and recovered from dead tissues of the hosts (Chapter 4). In the current study, fungal genera that have been previously reported as zingiberaceous pathogens and isolated as endophytes, saprobes, or pathogens were tested for their pathogenicity against tissue cultured ginger. The results showed that all isolates of *Alternaria alternata*, *C. gloeosporioides*, *Glomerella* sp. and *P. costina*, and some isolates of *Fusarium* sp., *Phomopsis* sp. and *P. capitalensis*, caused disease

symptoms or death of tissue cultured ginger. The pathogenicity of these fungi was also confirmed by following Koch's postulates. Bussaban *et al.* (2005) employed molecular technique (ITS and 5.8 S rRNA sequences) to prove identification and determine the phylogenetic relationship of *Pyricularia* species. Their results confirmed that endophytic and pathogenic isolates of *Pyricularia costina* had close similarity. It is, therefore, quite feasible that some fungi recovered as saprobes, endophytes and pathogens of the Zingiberaceae may be the same strain. This has important implications for global fungal diversity numbers, which rely heavily on a host-fungus ratio (Hawksworth, 2001; Hyde *et al*., 2001). If saprobes have potential endophytic or pathogenic life modes, they are much more likely to be host-specific or host-recurrent due to evolutionary relationships developed with the host plants.

5.4.3 Are zingiberaceous fungi antagonistic to other fungi?

Endophytes may be beneficial to the host because of competition with, or chemical inhibition of pathogens, or by activating host defense mechanisms (Brown *et al*., 2003). It has been proposed that fungal endophytes could be potential biological control agents, particularly for control of latent pathogens (Petrini, 1993; Photita, 2003; Rubini *et al*., 2005). They are also thought to induce resistance against disease through their ability to alter the allelochemical defenses of a plant (Clay, 1992; Wilson, 1993). The role of antibiosis as a mechanism in biological control is well documented in pathogen/biocontrol interactions. Antagonists produce toxic metabolites near the infection site and thereby inhibit growth of a pathogen by antibiosis (Singh *et al*., 2003). Endophyte cultures have been tested by *in vitro* assays for antagonism against pathogenic fungi in leaves and stems, with significant

inhibition recorded for many isolates (Brown *et al*., 2003). Two isolates of *Phialocephala fortinii*, endophytes from root of eggplant and Chinese cabbage almost completely suppressed the effects of post-inoculated virulent strains of *Verticillium dahliae* and *V. longisporum* that cause Verticillium yellows in Chinese cabbage (Narisawa *et al*., 2003). *Gliocladium catenulatum*, an endophyte from branches of cacao trees (*Theobroma cacao*) strongly reduced the incidence of witches' broom disease in cacao seedlings (Rubini *et al*., 2005). These studies illustrate the ability of fungal endophytes to act as antagonistic agents, and demonstrate their potential for use in the biological control of certain plant pathogens.

In the present study all zingiberaceous fungi tested for antagonistic activity produced growth inhibition of the pathogen *Pyricularia costina*, with about one-third of isolates tested having a high antagonistic activity (> 60% inhibition). In earlier studies the dual culture method has demonstrated that fungal endophytes and saprobes can inhibit the growth of certain fungal pathogens (Jackson *et al*., 1997; Benhamou and Brodeur, 2000; Park *et al*., 2002; Tian *et al*., 2004; Rubini *et al*., 2005). Although antagonistic action of fungi on agar media is convenient and provides evidence regarding antagonism against the growth of a test organism, dual culture is only a preliminary method to indicate antagonistic activity. Further research is needed to assess any inhibition of pathogens in the host. There is often little or no correlation between antibiosis *in vitro* and disease suppression *in planta* (Fravel, 1988; Jackson *et al*., 1997; Rubini *et al*., 2005). Tian *et al*. (2004) demonstrated that some endophytic fungi from rice showed evident antagonism in the dual culture test, but their metabolites showed no or little activity. This may imply that no diffusable antibiotics were produced when they interacted with the pathogens, and that other antimicrobial

mechanisms may be involved. Biocontrol efficacy depends on various factors, e.g., the environment and equilibrium between pathogens and biocontrol agents (Schulz *et al*., 1999; Rodrigues *et al*., 2000).