CHAPTER 4

RESULT

4.1 Sample collection

Ten healthy fronds of Thai dwarf fishtail palm were randomly collected from Huay Kog Ma, Doi Suthep – Pui National park, Chiang Mai in May, 2007. All samples were cut and surface sterilized within 24 hours after the collection. Total of 270 plant segments (90 segments of leaflet, 90 segments of primary rachis and 90 segments of secondary rachis) were used for endophytic fungi isolation.

4.2 Isolation of endophytic fungi

All of 270 plant segments were deposited on PDA and PDA containing rose bengal. When endophytic fungi mycelium was presented then removed the tip part of mycelia and subcultured onto and other plate of PDA. Finally all obtained isolates were transferred to PDA slant as stock culture.

Total of 191 endophytic fungi were obtained from 270 healthy plant segments of Thai dwarf fishtail palm (Table 3). One hundred and one isolates (52.87%) were obtained from the isolation using PDA and 90 isolates (47.13%) were obtained from the isolation with PDA amended with rose bengal. The total number of isolate from leaflet, primary rachis and secondary rachis were 62 (32.46%), 66 (33.56%) and 63 (32.98%) respectively. The total number of isolate from each part of leaflet; distal, central and basal part of leaflet were 20 (10.47%), 25 (13.09%) and 17 (8.90%) respectively (Table 3). The overall colonization rate of fungal endophyte was

71.48%. Colonization rate on PDA (74.81%) was greater than PDA containing rose bengal (65.92%). The colonization rates from three part of Thai dwarf fishtail palm, secondary rachis (77.00%) showed the highest colonization and central leaflet (73.33%), primary rachis (70.00%), distal leaflet (63.33%) and basal leaflet (63.33%), respectively (Table 4). However, ANOVA analysis of colonization percentage and isolation rates from different parts of frond was not significantly different. Similar result was concluded when the colonization percentage and isolation rate from different media were analyzed by using ANOVA (Table 4).

Table 3 Number of endophytic fungi isolated from Thai dwarf fishtail palm fronds.

Part of frond	Number of isolates		
	PDA	PDA+Rose Bengal	Total (%)
1. Leaflet	36	26	62 (32.46%)
1.1 Distal	(12)	(8)	(20 (10.47%))
1.2 Central	(13)	(12)	(25 (13.09%))
1.3 Basal	(11)	(6)	(17 (8.90%))
2. Primary rachis	33	33	66 (33.56%)
3. Secondary rachis	32	31	63 (32.98%)
Total	101 (52.87%)	90 (47.13%)	191 (100%)

Table 4 Colonization percentage and isolation rates of endophytic fungi from Thai dwarf fishtail palm.

	PDA		PDA+ Rose Bengal		Total	
	Colonization percentage (%)	Isolation rate	Colonization percentage (%)	Isolation rate	Colonization percentage (%)	Isolation rate
Leaflet						
- distal	73.33	0.73	53.33	0.53	63.33	0.63
- central	66.66	0.66	80.00	0.80	73.33	0.73
- basal	73.33	0.73	53.33	0.53	63.33	0.63
Primary rachis	80.00	0.80	60.00	0.60	70.00	0.70
Secondary rachis	73.33	0.73	75.55	0.82	77.00	0.77
CV 23.09*						

* The data was analyzed for variance (ANOVA) by using program SXW and the data was transformed ($\sqrt{\text{arcsine (isolation rate)}}$) (Gomez and Gomez, 1984)

4.3 Identification of endophytic fungi

Six genera of endophytic fungi; *Chaetomium globosum*, *Colletotrichum* spp., *Nigrospora* spp., *Phoma* sp., *Phomopsis* sp., *Trichobotrys* sp.were successfully indentified from total of 83 isolates that produced conidia on PDA and MEA. However, 108 isolates failed to produce conidia on PDA and MEA. Therefore, these isolates were subjected to be studied on medium contain sterilized filter paper. This method successfully induced 19 isolates to produced fruiting bodies on the medium, which were identified as; *Diplodia* sp., *Nodulisporium* sp., *Phaeoisaria* sp., *Phomopsis* sp. and *Coelomycetes* species 1. However, 5 isolates that produced young fruiting body could not be identified.

Total of 94 isolates including 5 isolates that produced young fruiting body were classified into 30 morpho type by using colony characteristic such as; color, texture margin and growth rate. Therefore all of 191 isolates were identified and classified into 10 genera, unidentified ceolomycetes and 30 morphotypes.

1. *Chaetomium globosum*

Colonies effuse and woolly, white margin and brown to olivaceous at the middle, crenate border (Figure 7A). Mycelium septate. Perithecia are visualized, large, globose to flask shape, dark brown to black, perithecial hairs (Figure 7D). Ascospores 9-12 x 8-10 µm., one- celled, hyaline and lemon shape (Figure 7C).

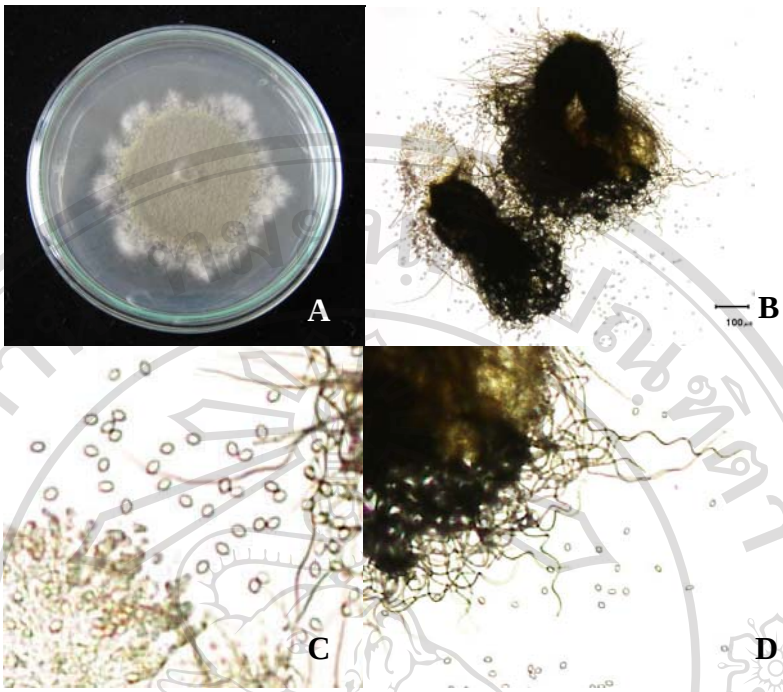

Figure 7 Characteristics of *Chaetomium globosum*; A: colony on PDA, B: perithecium, C: ascospore and D: coil appendages.

2. *Colletotrichum* sp.

Colony effuse, cottony, white to grayish brown. Mycelium immersed, branched, septate, hyaline, pale brown or dark brown (Figure 8A,8B). Sclerotia sometime present in culture, dark brown to black, often confluent, occasionally setose. Setae or sclerotia, brown, smooth, septate, tapered to the apices. Conidiophore hyaline to brown, septate, branched only at the base, smooth, formed from the upper cells of the conidiomata. Conidiogenous cells enteroblastic, phialidic, hyaline, smooth, determinate, cylindrical, integrated or discrete, channel minute but occasionally collarette and periclinal thickening quite prominent. Conidia 2.5-12.3 x 7.5-20.0 µm, hyaline, smooth, thin-walled, sometimes guttulate, (Figure 8D). Appressoria brown,

entire or with crenate to irregular margins, simple or simple or repeatedly germinating to produce complex columns of several closely connected appressoria (Figure 7C).

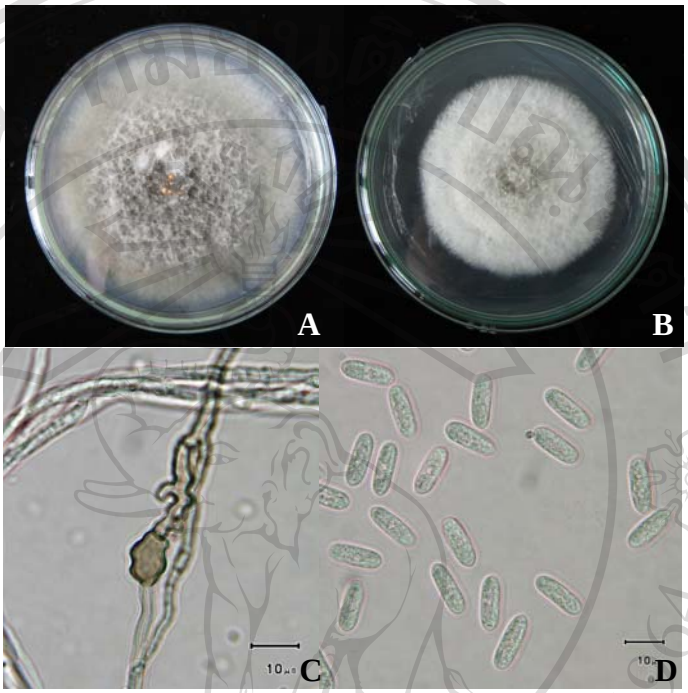

Figure 8 Characteristics of *Colletotrichum* sp.; A,B: colonies on PDA, C: appressorium and D: conidia.

3. *Diplodia* sp.

Colonies effuse, white, gray to brown. Mycelium immersed, branched, septate, dark brown and slow growing (Figure 9A). Conidiomata pycnidia, septate or aggregated, globose, dark brown to black, immersed, unilocular, thick-walled cells and an inner layer of thin walled, hyaline cells. Conidiophores hyaline, branched and septate above and at the base, smooth, cylindrical, formed from the inner cells of the pycnidial wall. Conidiogenous cells holoblastic, intregated or discrete, determinate, cylindrical, hyaline, smooth, forming a single apical conidium. Conidia at first hyaline

with a central guttule, 22-25 x 9.5-11 µm, thick-walled (>0.5 µm), ellipsoid to cylindrical, smooth, later become dark brown and medianly 1 euseptate, apex obtuse, base truncate (Figure 9B). Successful to produce conidia using filter paper method (Figure 9C).

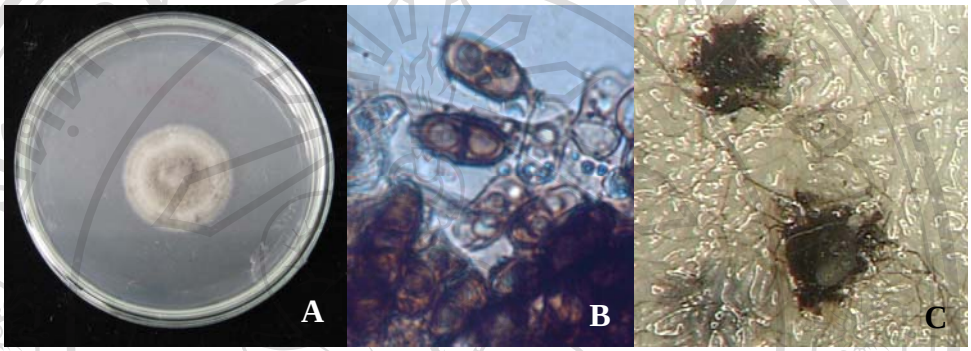

Figure 9 Characteristics of *Diplodia* sp.; A: colony on PDA, B: conidia, C: black pustules on filter paper.

4. *Nigrospora* sp.

Colonies effuse, white to cream with small shining black conidia easily visible under a low-power dissecting microscopic (Figure 10A). Conidiophore 10-28 x 4-12 µm, micronematous or semi- macronematous, branched, flexuous, colorless to brown, smooth. Stroma none. Setae absent. Conidiogenous cells 6-8 x 6-7 µm, monoblastic, discrete, ampulliform (Figure 10B). Conidia 14-17 x 10-13 µm. solitary, acrogenous, simple, spherical or broadly ellipsoidal, black, shining, smooth and aseptate (Figure 10B).

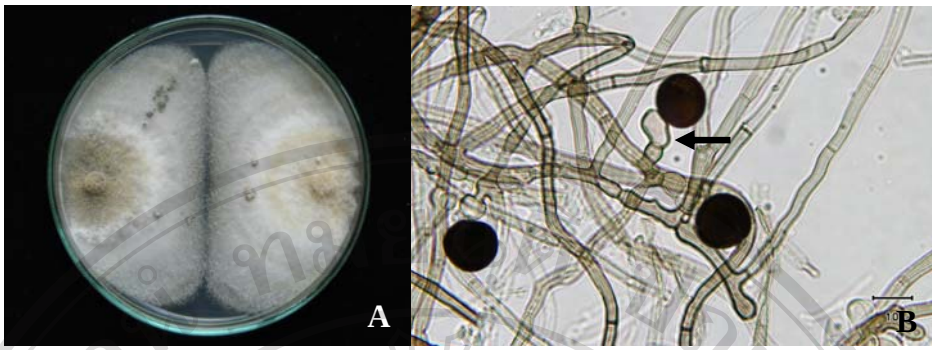

Figure 10 Characteristics of *Nigrospora* sp.; A: colony on PDA, B: conidiogenous cell (arrow) and conidia.

5. *Nodulisporium* sp.

Colonies effuse, gray, brown or blackish brown, velvety (Figure 11A, 12A, 13A). Conidiophores 40-45 x 1.5-15 µm, macronematous, mononematous individual threads often much branched especially towards the apex, flexous, pale to dark brown or olivaceous brown, smooth or verrucose. Conidiogenous cell 14-16 x 2-3 µm, polyblstic, solitary or arranged penicillately, sympodial, cylindrical to clavate, denticulate; denticles generally very short, fragile (Figure 11B, 12B, 13B). Stroma none. Setae absent. Conidia 3-4.5 x 1.5-2.5 µm, solitary, simple, ellipsoidal or obovoid, hyaline to brown or olivaceous brown, smooth, aseptate (Figure 11B, 12B, 13B). Successful to produce conidia using filter paper method (Figure 13C).

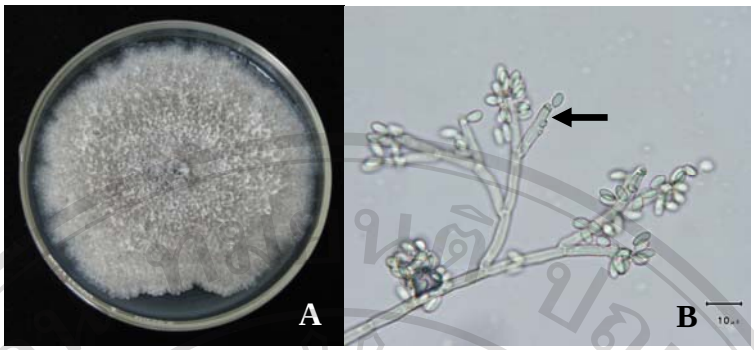

Figure 11 Characteristics of *Nodulisporium* sp. 1; A: colony on PDA and B: conidiophores, connidiogenous cell (arrow) and conidia.

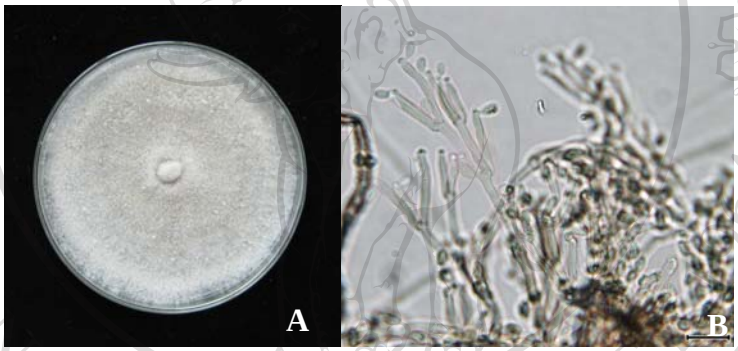

Figure 12 Characteristics of *Nodulisporium* sp. 2; A: colony on PDA, B: conidiophores and conidia.

Figure 13 Characteristics of *Nodulisporium* sp. 3; A: colony on PDA , B: conidiophores and C: condense of mycelium on filter paper.

6. *Phaeoisaria* sp.

Colony effuse, loose, white (Figure 14A). Setae absent. Conidiophore macronematous, synnematous, dark brown, smooth, straight (Figure 14B). Conidiogenous cells 10-12 x 2-3 µm, polyblastic, intregated and terminal. Conidia 5-10 x 2-3 µm, solitary, dry, acropleurogenous, simple, fusiform, ellipsoidal or subspherical, pale to brown, smooth, aseptate.

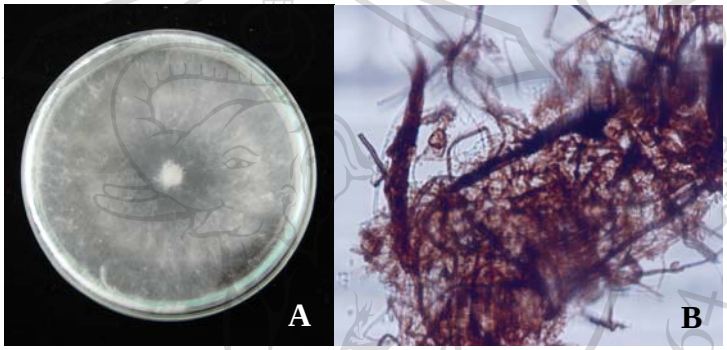

Figure 14 Characteristics of *Phaeoisaria* sp.; A: colony on PDA, B: conidiophores.

7. *Phoma* sp.

Colonies effuse, gray, velvety (Figure 15A). Conidiophores septate and branched or short irregularly branched and ramified respectively. Conidiogenous cells enteroblastic, phialidic, integraded or discrete, ampulliform to doliiform, hyaline, smooth, collarette aperture minute, periclinal wall markedly thicked. Conidia hyaline, aseptate, thin-walled, often guttalate, ellipsoid, cylindrical, fusiform, pyriform or globose (Figure 15B). Successful to produce conidia using filter paper method (Figure 15C).

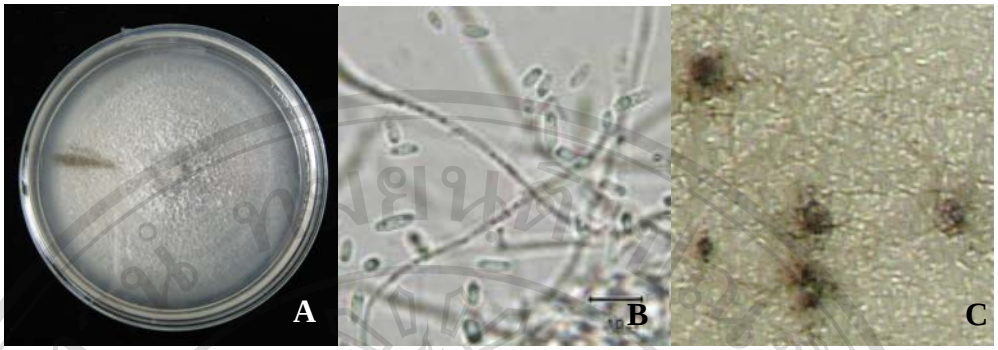

Figure 15 Characteristics of *Phoma* sp.; A: colony on PDA, B: conidia, C: black pustules on filter paper.

8. *Phomopsis* sp.

Colonies effuse, white to gray, fluffy (Figure 16A). Mycelium immersed, branched, septate, hyaline to pale brown. Conidiophores branched, septate, multiseptate and filiform, hyaline. Conidiogenous cells enteroblastic, phialidic, determinate, intregated, rarely discrete, hyaline, cylindrical, collarette, channel and periclinal thickening minute. Conidia of two basic types but in some species with intermediated between the two: α-conidia hyaline, fusiform, straight usually biguttulate (one guttule at each end), aseptate; *β*- conidia hyaline, filiform, straight, eguttulate (Figure 16B), aseptate. Successful to produce conidia using filter paper method (Figure 16C).

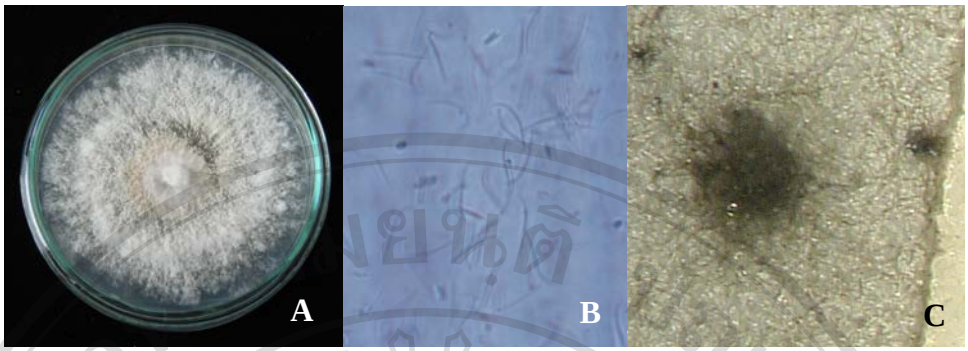

Figure 16 Characteristics of *Phomopsis* sp.; A: colony on PDA, B: α-conidia and β-conidia and C: black pustules on filter paper.

9. *Trichobotrys* sp.

Colonies effuse, white to orange, velvety, slow growing (Figure 17A). Stroma none. Setae absent. Conidiophores macronematous, mononematous, long, narrow, straight or flexuous, mid to dark brown, verruculose or echinulate. Conidiogenous cell 50-75 x 6.5-8 µm, polyblastic, integrated and terminal or discrete on branches, ellipsoidal, spherical or sub-spherical. Conidia 8.5-12.5 x 6.5-8 µm, brown, chains, spherical, verruculose, aseptate (Figure 17B).

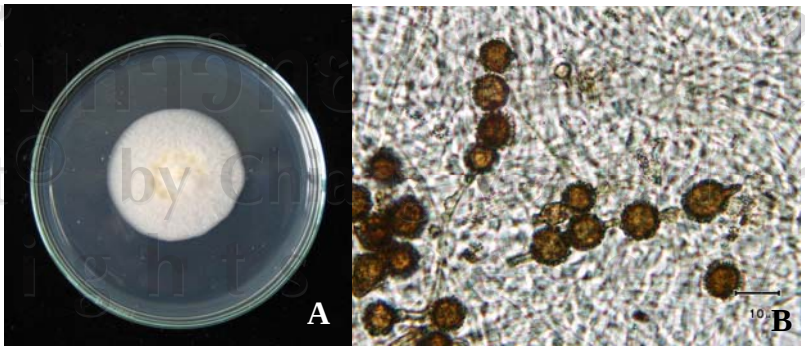

Figure 17 Characteristics of *Trichobotrys* sp.; A: colony on PDA, B: conidiophores and conidia.

10. Coelomycetes species.1

Colonies effuse, zonate, gray to brown, velvety (Figure 18A). Conidia 12-14.5 x 4-5.5 µm, cylindrical, 2-3 euseptate, thin- walled, smooth, hyaline to light purple (Figure 18B). Successful to produce conidia using filter paper method (Figure 18C).

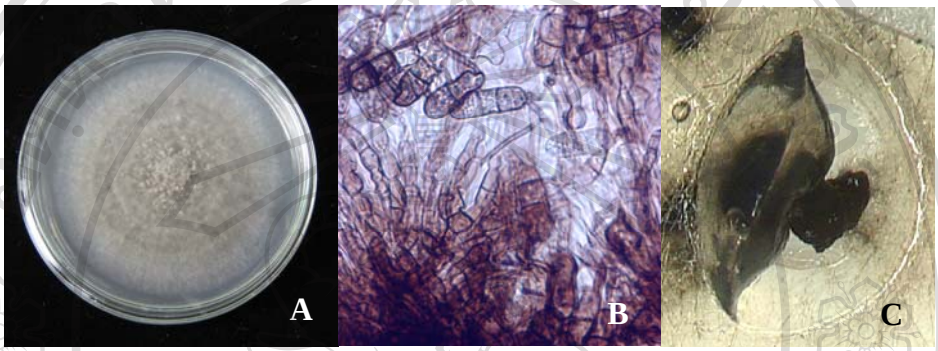

Figure 18 Characteristics of Coelomycetes species 1; A: colony on PDA, B: conidia and C: black pustules in agar.

11. Morphotype 1: Colonies effuse, white gray black, fluffy and cobwebby (Figure 19A-C)

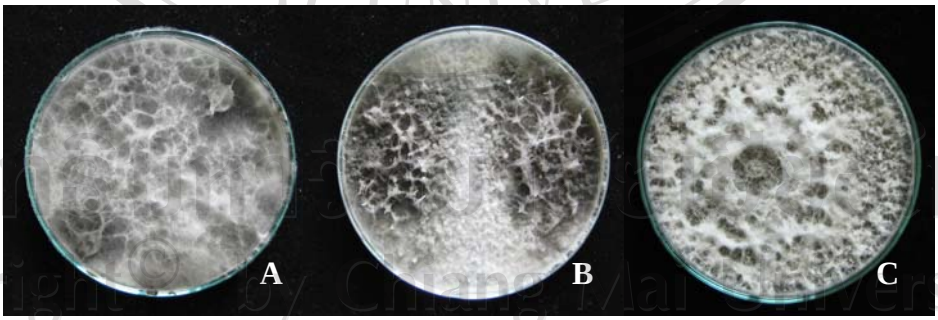

Figure 19 Characteristics of morphotype 1; A: isolate P5, B: P38 and C: R22 on PDA.

12. Morphotype 2: Colonies effuse, woolly and pulverulent, white to gray (Figure 20).

Figure 20 Characteristics of morphotype 2; isolate R2 on PDA.

13. Morphotype 3: Colonies effuse, granular, white grayish, raised in the middle with brown mycelium with spin, fimbriate boreder (Figure 21).

Figure 21 Characteristics of morphotype 3; isolate P3 on PDA.

14. Morphotype 4: Colonies effuse, velvety, first white become gray to brown, slightly zonate and sectoring, erose boreder (Figure 22A-C).

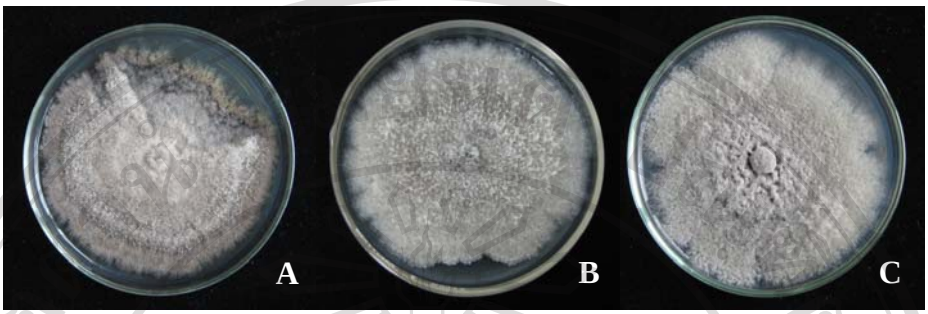

Figure 22 Characteristics of morphotype 4; A: isolate R5, B: P1 and C: R25 on PDA.

15. Morphotype 5: Colonies flat, velvety, white to cream, smooth border (Figure 23A-C)

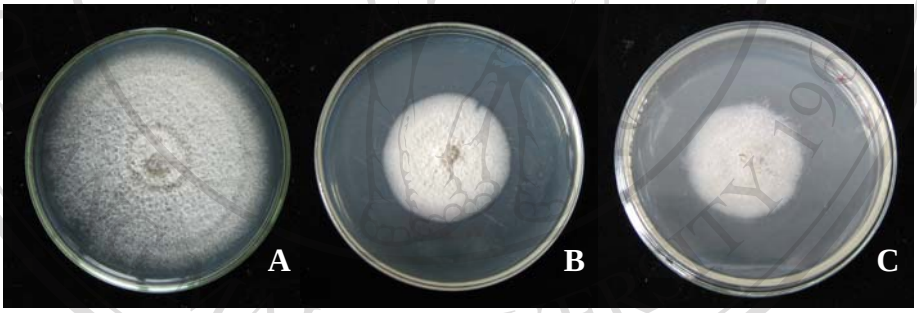

Figure 23 Characteristics of morphotype 5; A: isolate P19, B: P62 and C: P95 on PDA.

16. Morphotype 6: Colonies flat, thin, effuse, white gray at the middle brown, clear zonate (Figure 24).

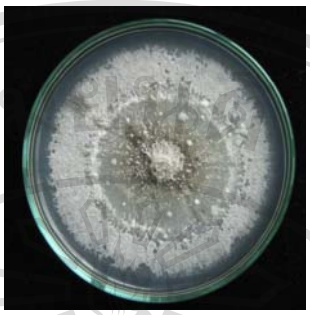

Figure 24 Characteristics of morphotype 6; isolate R34 on PDA

17. Morphotype 7: Colonies imbricated, white, granular to velvety, labate border (Figure 25A-C).

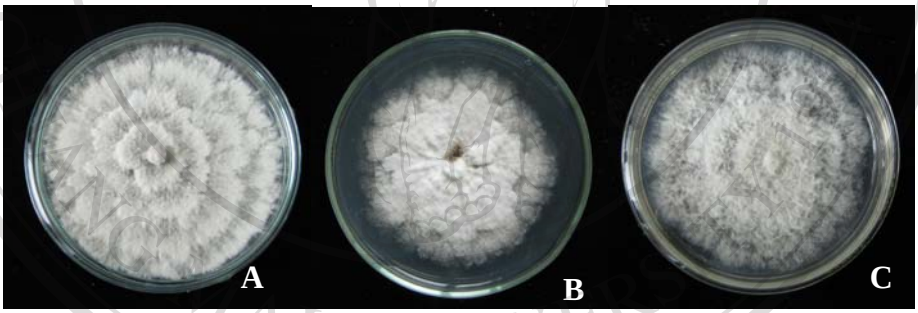

Figure 25 Characteristics of morphotype 7, A: isolate R103 , B: R50 and C: R73 on PDA

18. Morphotype 8: Colonies effuse, velvety, white to brown, notate with plaited, some fluffy at the middle (Figure 26A-C).

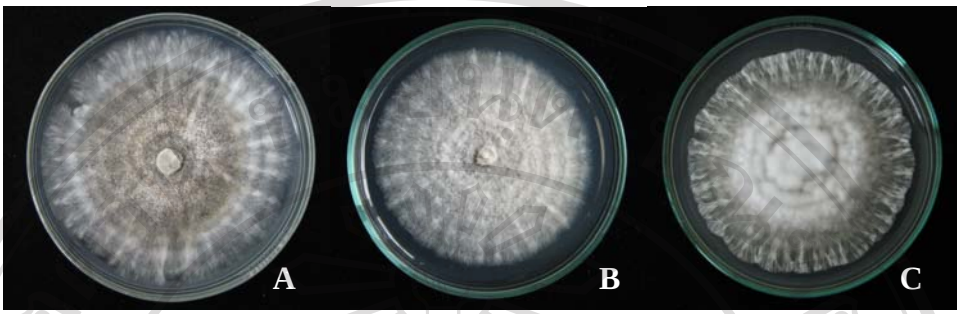

Figure 26 Characteristics of morphotype 8, A: isolate R32, B: R11 and C: R30 on PDA

19. Morphotype 9: Colonies effuse, pulverulent, white to cream, notate, erose border (Figure 27A-C).

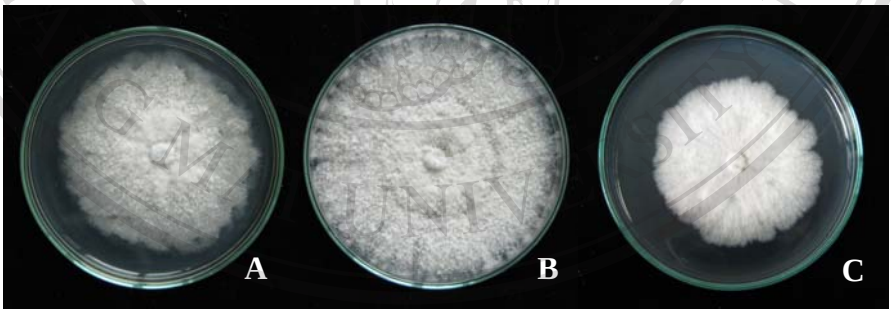

Figure 27 Characteristics of morphotype 9; A: isolate P37 (A), B: P35 and C: P63 on PDA

20. Morphotype 10: Colonies cottony, white, gray, black, smooth border (Figure 28A-C).

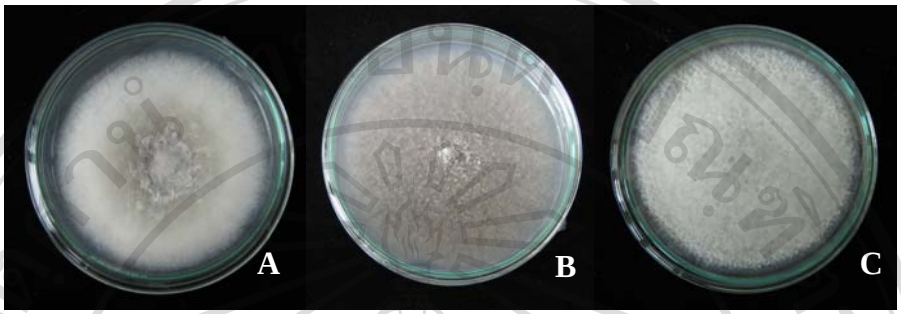

Figure 28 Characteristics of morphotype 10; A: isolate P69, B: R75 and C: R70 on PDA

21. Morphotype 11: Colonies slow growing, cottony, at middle granular, white to brown, smooth border (Figure 29A-C).

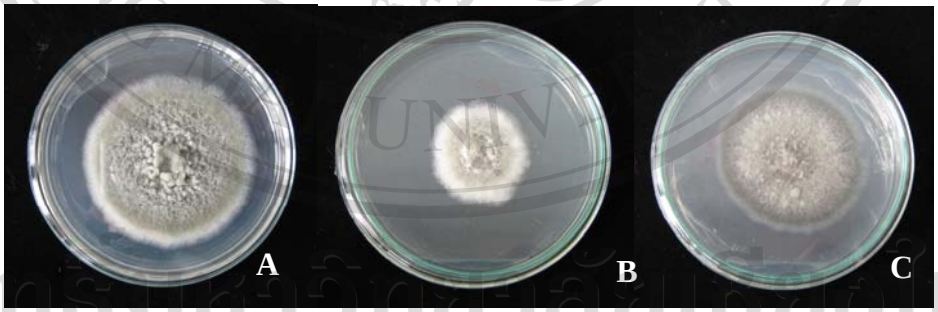

Figure 29 Characteristics of morphotype 11; A: isolate P88, B: R76 and C: R77 on PDA.

22. Morphotype 12: Colonies slow growing, loose, effuse, white to cream, smooth border (Figure 30A-B).

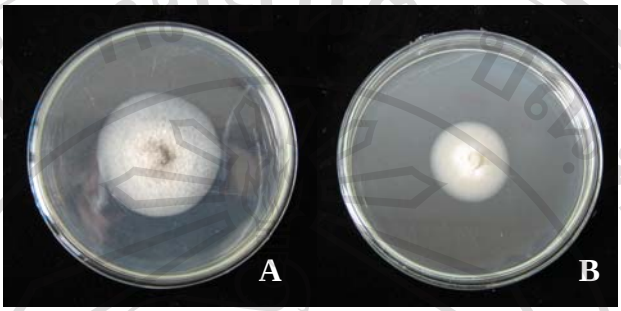

Figure 30 Characteristics of morphotype 12; A: isolate P73 and B: R83 on PDA

23. Morphotype 13: Colonies effuse, velvety and pulverulent, white to gray (Figure 31A-C).

Figure 31 Characteristics of morphotype 13 isolate; A: P2, B: R39 and C: R45 on PDA.

24. Morphotype 14: Colonies effuse velvety and pulverulent, white to gray, crenate border (Figure 32).

Figure 32 Characteristics of morphotype 6; isolate P4 on PDA

25. Morphotype 15: Colonies effuse, imbricate, white brown (Figure 33).

Figure 33 Characteristics of morphotype 15; isolate P12 on PDA

26. Morphotype 16: Colonies effuse and fluffy, lobed border, white to light brown (Figure 34A-C)

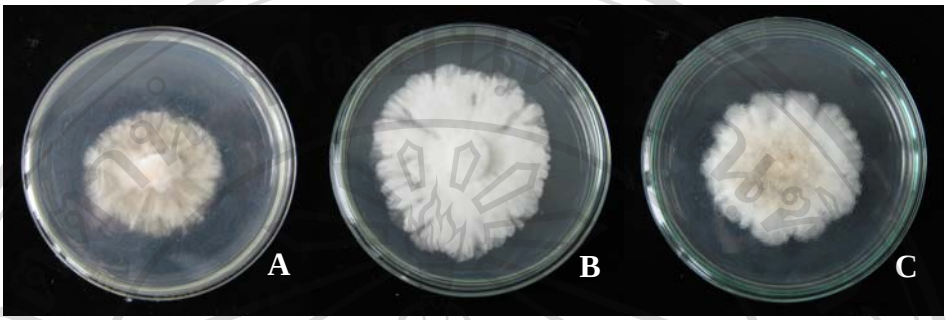

Figure 34 Characteristics of morphotype 16; A: isolate P90 (A), B: R18 and C: R80 on PDA

27. Morphotype 17: Colony loose, powdery and pulverulent, white (Figure 35).

Figure 35 Characteristics of morphotype 17; isolate P17 on PDA

28. Morphotype 18: Colony loose, thin and gray (Figure 36).

Figure 36 Characteristics of morphotype 18; isolate R12 on PDA

29. Morphotype 19: Colonies effuse, flat, white to cream, ornamented fibrils (Figure 37A-C).

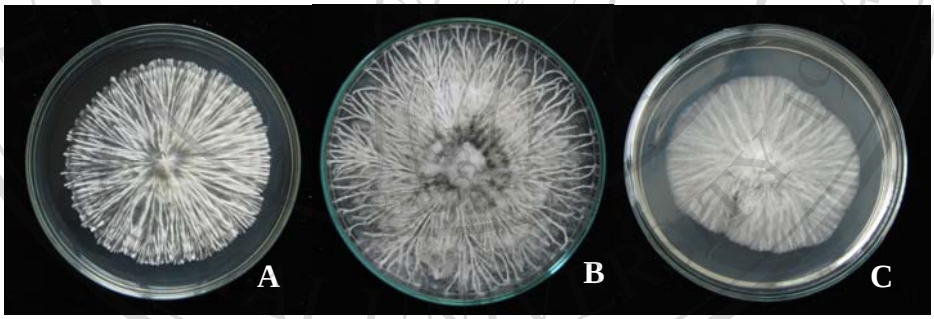

Figure 37 Characteristics of morphotype 19 ; A: isolate, P18, B: R28 and C: R94 on PDA.

30. Morphotype 20: Colonies fluffy, white grey, cobwebby, fimbriate border (Figure 38).

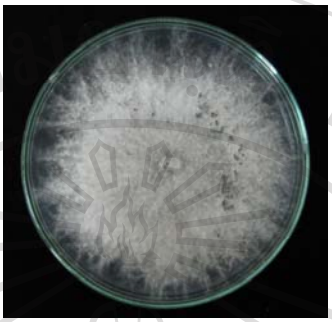

Figure 38 Characteristics of morphotype 20; isolate P20 on PDA

31. Morphotype 21: Colonies effuse, flat, zonate, brown with white crenate border (Figure 39A-B)

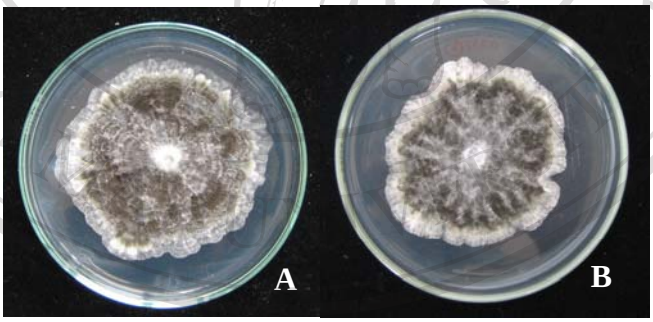

Figure 39 Characteristics of morphotype 21; A: isolate P24 and B: P33 on PDA

32. Morphotype 22: Colonies effuse, flat, white, zonate with ornamented fibrils, cottony colony raised at the middle (Figure 40A-B)

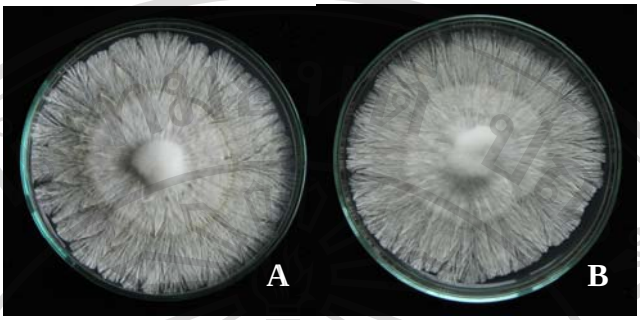

Figure 40 Characteristics of morphotype 22; A: isolate P45 and B: R37 on PDA.

33. Morphotype 23 (P47; 1 iso) colonies effuse, flat, loose at the middle and denser at the border, white (Figure 41).

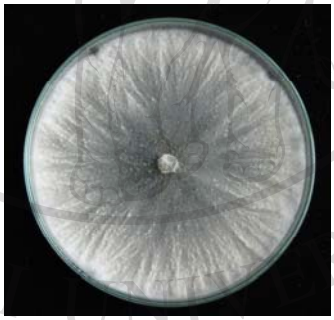

Figure 41 Characteristics of morphotype 23; isolate P47 on PDA.

34. Morphotype 24 (P52, R51; 2 iso) colonies effuse, velvety, granular, zonate, white gray (Figure 42A-B)

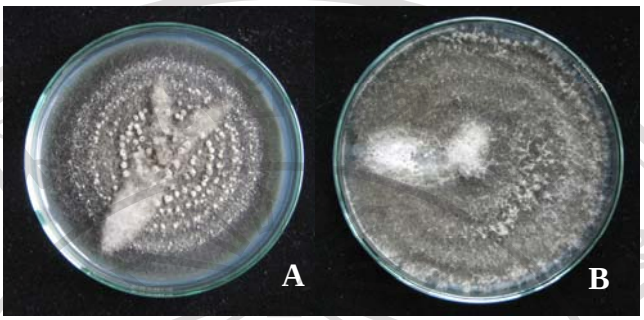

Figure 42 Characteristics of morphotype 24; A: isolate P52 and B: R51 on PDA.

35. Morphotype 25: Colony effuse, velvety, loose at the middle and denser at the border, white to cream (Figure 43)

Figure 43 Characteristics of morphotype 25; isolate P54 on PDA.

36. Morphotype 26: Colonies effuse, velvety, zonate, white to brown (Figure 44A-B)

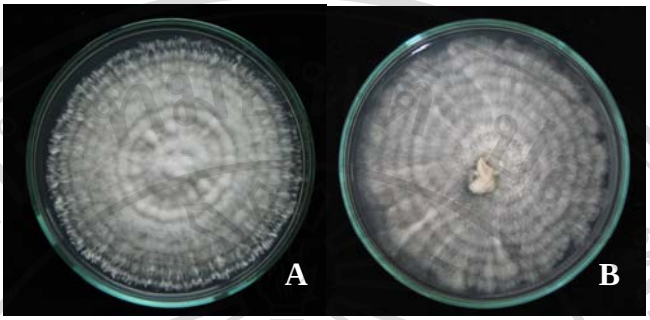

Figure 44 Characteristics of morphotype 26; A: isolate P56 and B: R57 on PDA.

37. Morphotype 27: Colonies effuse, flat, velvety, zonate, white, yellow, light brown, labate border (Figure 45A-B).

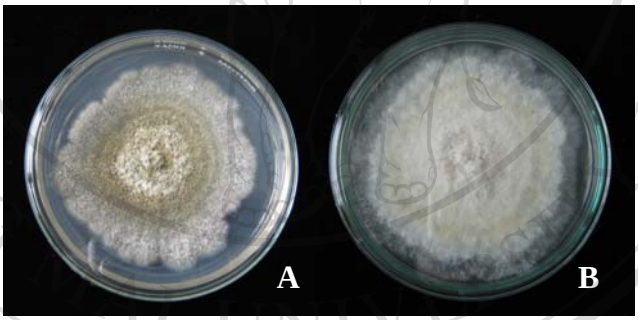

Figure 45 Characteristics of morphotype 27, A: isolate P61 and B: P84 on PDA

38. Morphotype 28: Colonies effuse, velvety, flat, white to cream, fimbriate border (Figure 46A-B)

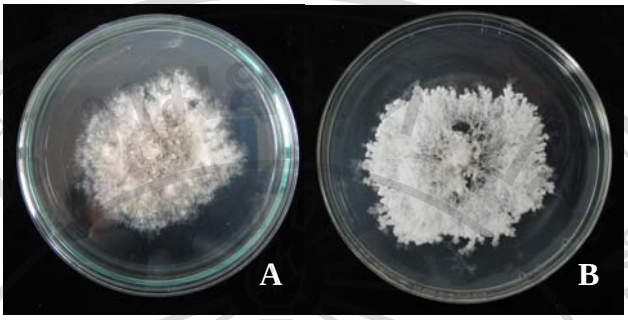

Figure 46 Characteristics of morphotype 28; A: isolate P79 and B: R26 on PDA

39. Morphotype 29: Colonies effuse, velvety, brown at the middle with white margin (Figure 47)

Figure 47 Characteristics of morphotype 29; isolate P100 on PDA

40. Morphotype 30; Colonies effuse, flat, imbricate, black with white margin (Figure 48A-B)

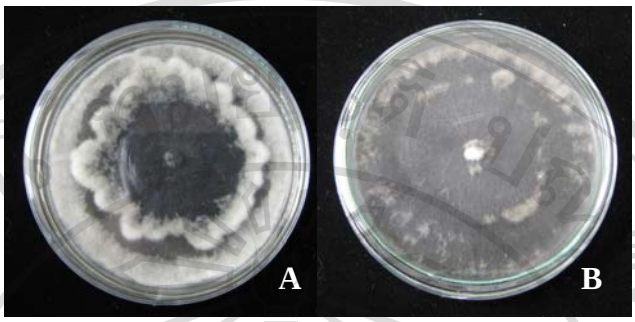

Figure 48 Characteristics of morphotype 30; A: isolate P94 and B: P49 on PDA

Base on the identificationresult of all the isolates, 21 genera/morphotype were produced from leaflet, 25 genera/morphotype from primary rachis and 24 genera/morphotypes from secondary rachis respectively (Table 5). The number of genera/morphotypes isolated from rachis was greater than leaflet. Leaflet, distal and central parts yielded more genera/morphotypes than basal part.

Table 5 Number of fungal endophyte genus/morpho type from Thai dwarf fishtail palm fronds.

Part of frond	Number of Genus/ Morpho type
1 Leaflet	21
1.1 Distal	(12)
1.2 Central	(13)
1.3 Basal	(9)
2. Primary rachis	25
3. Secondary rachis	24

All of 191 isolate of endophytic fungi could be grouped into 41 groups (Table 6). The dominant genus is *Collectotrichum* sp. (21.9%) followed by morphotype 10 (8.90%), 8 (8.37%), 7 (7.85%) and 16 (7.32%), respectively. (Table 6). *Collectotrichum* sp., morphotype 9 and morphotype 10 were commonly isolated from every part of frond. Several genera/morphotypes were isolated only from specific part (Table 6) such as *Chaetomium globosum* which was isolated from both primary and secondary rachis. *Diplodia* sp. was only isolated from secondary rachis, morphotype 20 was isolated from central leaflet. *Phaeoisaria* sp., *Trichobotrys* sp., Coelomycetes species 1, morphotype 15, morphotype 23, morphotype 24, morphotype 25, morphotype 29 and morphotype 30 were only isolated from primary rachis. *Phoma* sp., *Phomopsis* sp., morphotype 2, morphotype 3, morphotype 14 and morphotype 27 were only found in secondary rachis.

Table 6 Distribution of endophytic fungi in different part of Thai dwarf fishtail palm.

Genus/Morphotype	Leaflet			Primary rachis	Secondary rachis	Total isolate (%)
	Distal	Central	Basal			
1.*Chaetomium globosum*				1	1	2 (1.04)
2. *Colletotrichum* sp.	6	7	2	13	14	42 (21.9)
3. *Diplodia* sp.					1	1 (0.05)
4. *Melanographium* sp.		1				1(0.05)
5. *Nigrospora* sp.	1				1	2(1.04)
6. *Nodulisporium* sp.				2	3	5 (2.61)
7. *Phaeoisaria* sp.				1		1(0.05)
8. *Phoma* sp.					1	1(0.05)
9. *Phomopsis* sp.					1	1(0.05)
10. *Trichobotrys* sp.				1		1(0.05)
11. Coelomycetes sp1.				2	1	3(1.57)
12. Morphotype 1		1			2	3(1.57)
13. Morphotype 2					1	1(0.05)
14. Morphotype 3					1	1(0.05)
15. Morphotype 4	1	1		3		5(2.61)
16. Morphotype 5	1	1	1		1	4(2.09)
17. Morphotype 6			1			1(0.05)
18. Morphotype 7	2		4	5	4	15(7.85)
19. Morphotype 8	1	2		7	6	16(8.37)
20. Morphotype 9	1	1	1	1	3	7(3.66)
21. Morphotype 10	2	4	4	3	4	17(8.90)
22. Morphotype 11		3		3	2	8(4.18)
23. Morphotype 12		1		1	1	3(1.57)
24. Morphotype 13	1	1		2	5	9(4.71)
25. Morphotype 14					1	1(0.05)
26. Morphotype 15				2		2(1.04)
27. Morphotype 16	2			6	6	14(7.32)
28. Morphotype 17	1					1(0.05)
29. Morphotype 18				2		2(1.04)
30. Morphotype 19	1			1		2(1.04)
31. Morphotype 20		1				1(0.05)
32. Morphotype 21		1	1			2(1.04)
33. Morphotype 22			1		1	2(1.04)
34. Morphotype 23				1		1(0.05)
35. Morphotype 24				2		2(1.04)
36. Morphotype 25				1		1(0.05)
37. Morphotype 26				3	1	4(2.09)
38. Morphotype 27					1	1(0.05)
39. Morphotype 28			1	1		2(1.04)
40. Morphotype 29				1		1(0.05)
41. Morphotype 30				2		2(1.04)
Total	20	25	17	66	63	191

4.4 Antagonistic activity test

One hundred and ninety one isolates were tested against 3 plant pathogenic fungi; *Sclerotium* sp., *Phytophthora* sp. and *Curvularia senegalensis* by using dual culture technique. The tested pathogens were obtained from the culture collection of Molecular Laboratory, Plant Pathology Department, Chiang Mai University.

The result showed 5 isolates were potential against *Sclerotium* sp., 37 isolates against *Phytophthora* sp. and 23 isolates against *Curvularia senegalensis* (Table 7) by showing very high potential activity. Furthermore, 3 isolates showed the very high potential to control all 3 tested pathogens were P59 (*Phaeoisaria* sp.) (Figure 49D-F), R12 (Morphotype18) (Figure 49G-I) and R14 (*Colletotrichum* sp.) (Figure 49J-L) (Table 7). The maximum inhibition percentage of *Sclerotium* sp. weas presented in P59 (86.75%), R17 (85.41%) and P86 (82.50%), respectively (Figure 50A-C). The antagonistic test against *Phytophthora* sp. showed the highest inhibition percentage were R22 (92.08%), R45 (89.58%) and R7 (88.74%), respectively (Figure 50D-F). The maximum inhibition percentage of *Curvularia senegalensis* were showed in P86 (98.24%), P45 (87.50%) and P5 (85.42%), respectively (Figure 50G-I).

Table 7 Number of potential isolates in controlling 3 tested pathogens

	Very high: Vh	High: H	Moderate: M	Low: L
Sclerotium sp.	5	7	26	153
Phytophthora sp.	37	72	33	49
Curvularia senegalensis	23	114	34	20

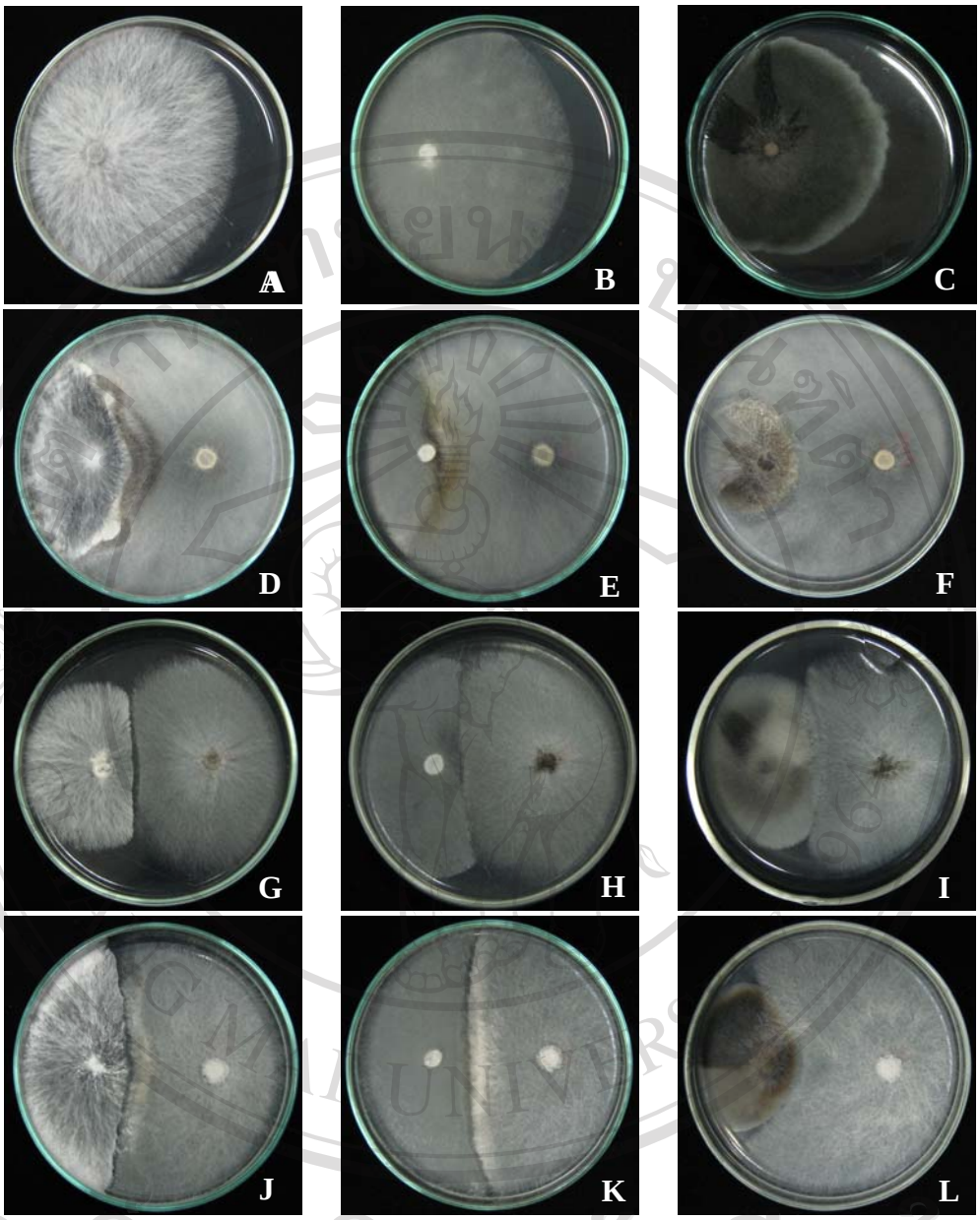

Figure 49 Very high potential isolates against 3 tested pathogens; A-C: control plate of *Sclerotium* sp., *Phytopthora* sp. and *Curvularia senegalensis*, respectively. D-F: P59 against *Sclerotium* sp, *Phytopthora* sp. and *C. senegalensis*, respectively. G-I; R12 against *Sclerotium* sp, *Phytopthora* sp. and *C. senegalensis*, respectively. and J-L: R14 against *Sclerotium* sp, *Phytopthora* sp. and *C. senegalensis*, respectively.(Left colony= tested pathogen, right colony= fungal endophyte)

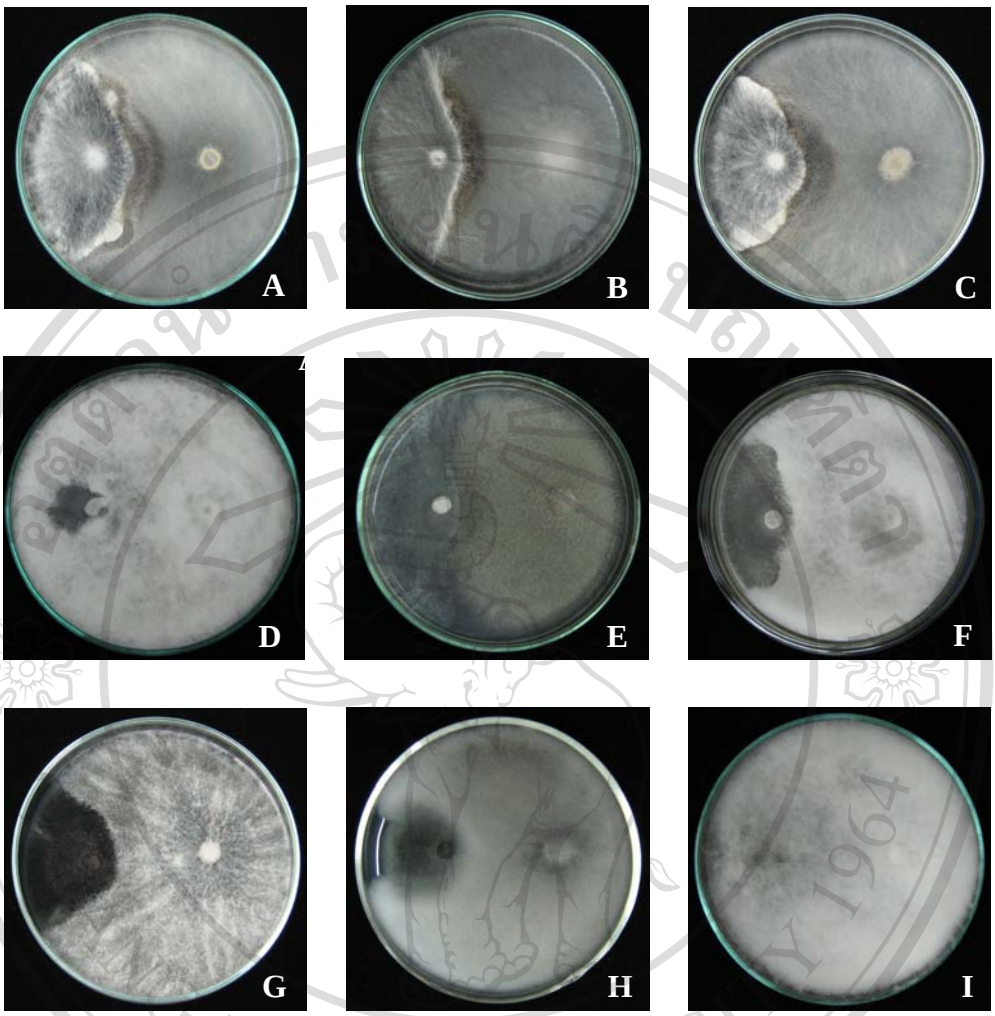

Figure 50 Very high potential isolates against each tested pathogens; A-C: *Sclerotium* sp. against P59, R17 and P86, respectively. D-F: *Phytophthora* sp. against R22, R45 and R7, respectively. G-I: *Curvularia senegalensis* against P86, P5 and R22, respectively. (Left colony= tested pathogen, right colony= fungal endophyte)

Interactions between endophytic fungi and *Sclerotium* sp. were showed in two type of competitive interaction; deadlock with distance and replacement (Table 8), consisting in the inhibition of one organism, followed by partial growth (Figure 50) or complete over growth (Figure 50E-G). Replacement was considerably more frequent (95.81+1.57) than dead lock (2.62). Replacement of endophytic by pathogen was much more frequent (95.81) than replacement of pathogen by endophytic fungi (1.57) (Table 8).

Table 8 Frequency of type and sub-type of interactions between mycelium of endophytic fungi and *Sclerotium* sp. using dual culture technique on PDA.

Dead lock		Replacement of pathogen by endophytic fungi		Replacement of endophytic by pathogen	
Sub-type	%	Sub-type	%	Sub-type	%
A	0	C	0.53	C	58.13
B	2.62	CA1	0	CA1	5.23
		CA2	1.04	CA2	25.65
		CB1	0	CB1	0
		CB2	0	CB2	6.80
Total	2.62		1.57		95.81

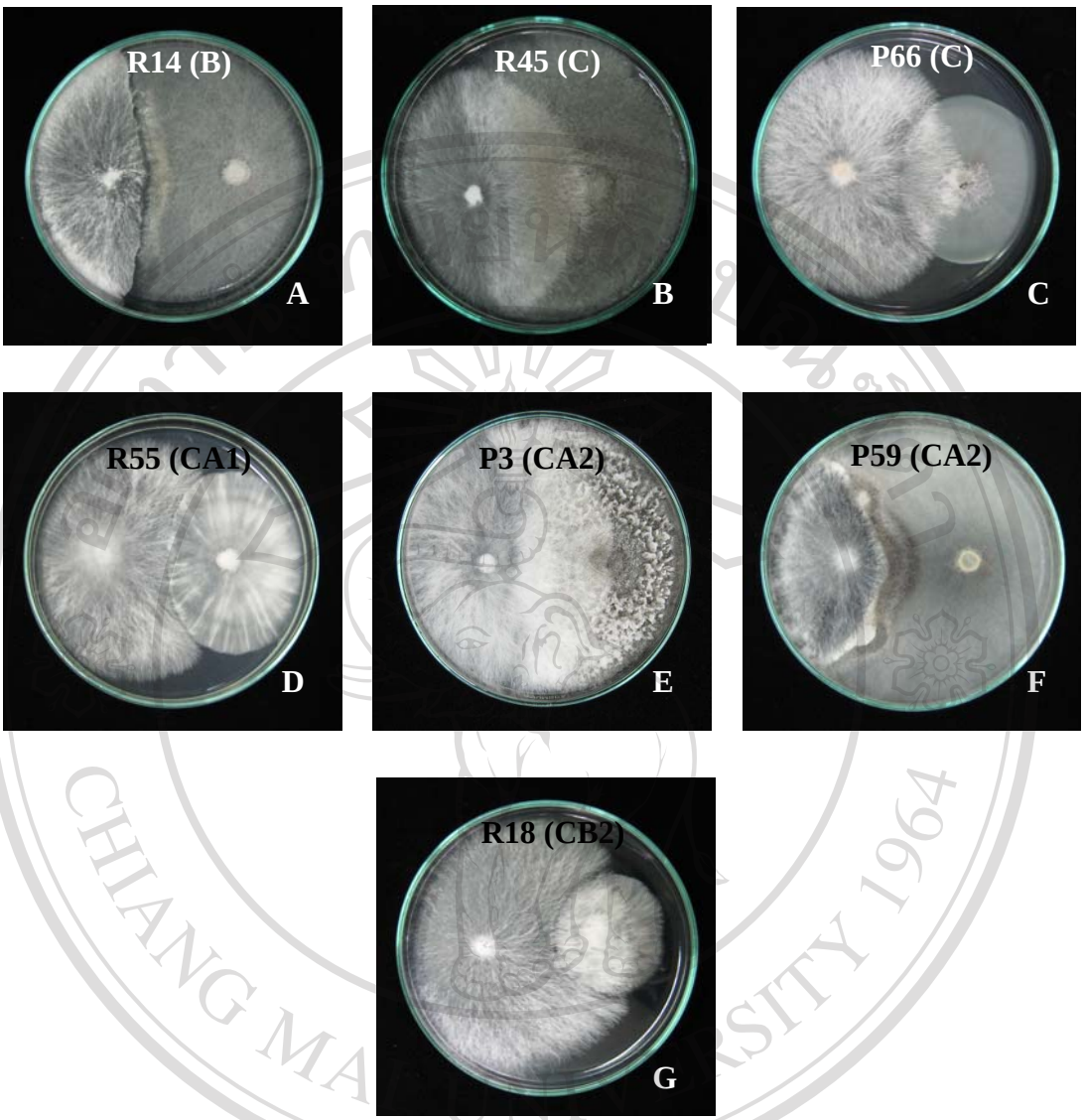

Figure 51 Interactions between *Sclerotium* sp. (left) and endophytic fungi (right) using dual culture technique on PDA; A: Deadlock at a distance, B and F: replacement of *Sclerotium* sp. by endophytic fungi and C, D, E,F and G; replacement of endophytic by *Sclerotium* sp

Interactions between endophytic fungi and *Phytophthora* sp. were observed in two type of competitive interaction were observed (Table 9); deadlock with mycelium contact (Figure 52A) and with distance (Figure 52B) and replacement, consisting in the inhibition of one organism, followed by complete over growth (Figure 52C-E). The frequency of deadlock was considerably more frequent (95.29) than replacement (3.67+1.04) (Table 9). There were two type of replacement; replacement of pathogen by endophytic fungi and replacement of endophytic by pathogen which frequency were 3.67% and 1.04%, respectively.

Table 9 Frequency of type and sub-type of interactions between mycelium of endophytic fungi and *Phytophthora* sp. using dual culture technique on PDA.

Deadlock		Replacement of pathogen by endophytic fungi		Replacement of endophytic by pathogen	
Sub-type	%	Sub-type	%	Sub-type	%
A	90.05	C	0	C	1.04
B	5.24	CA1	0	CA1	0
		CA2	2.09	CA2	0
		CB1	1.58	CB1	0
		CB2		CB2	
Total	95.29		3.67		1.04

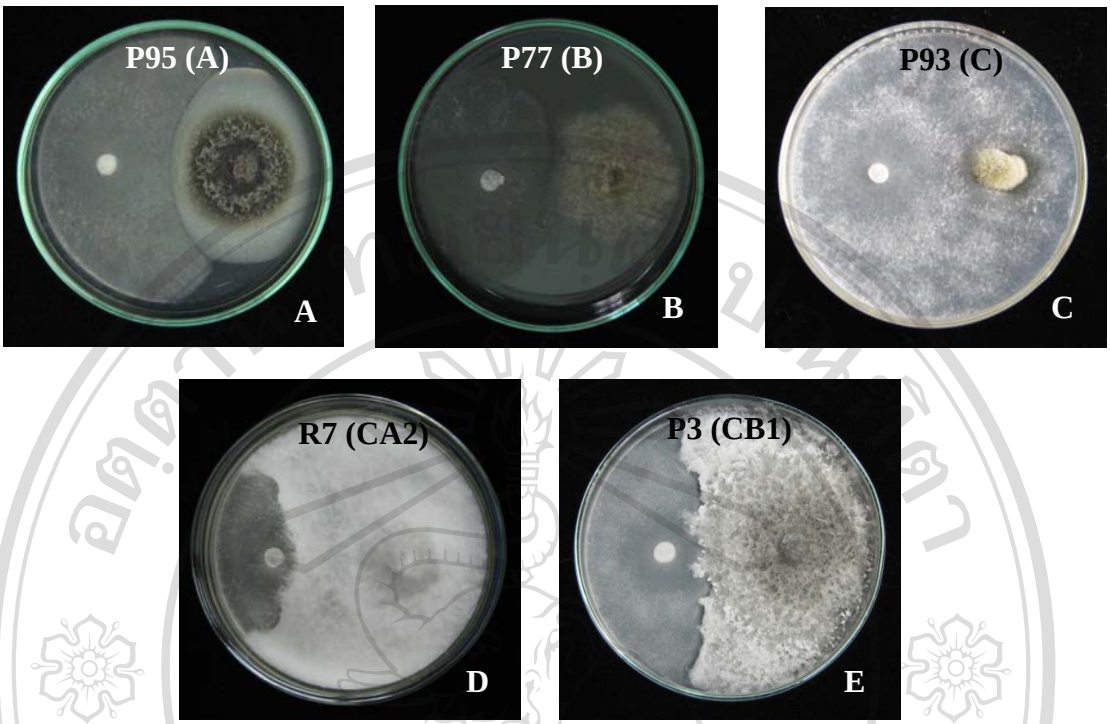

Figure 52 Interactions between *Phytophthora* sp. (left) and endophytic fungi (right) using dual culture technique on PDA; A,B: Deadlock, D, E: replacement of *Phytophthora* sp. by endophytic fungi and C: replacement of endophytic fungi by *Phytophthora* sp.

Interactions between endophytic fungi and *Curvularia senegalensis* showed in two type of competitive interaction were observed (Table 11); deadlock with mycelium contact (Figure 53A) and with distance (Figure 53B) and replacement, consisting in the inhibition of one organism, followed by partial growth (Figure 53D,F) and complete over growth (Figure 53E, G). Deadlock was considerably more frequent (77.47) than replacement (22.53). Moreover, only replacement of *Curvularia senegalensis* by endophytic fungi (Table 11).

Table 10 Frequency of type and sub-type of interactions between mycelium of endophytic fungi and *Curvularia senegalensis* using dual culture technique on PDA.

Dead lock		Replacement of pathogen by endophytic fungi		Replacement of endophytic by pathogen	
Sub-type	%	Sub-type	%	Sub-type	%
A	71.72	C	4.18	C	0
B	5.75	CA1	4.72	CA1	0
		CA2	8.92	CA2	0
		CB1	4.19	CB1	0
		CB2	0.52	CB2	0
Total	77.47		22.53		0

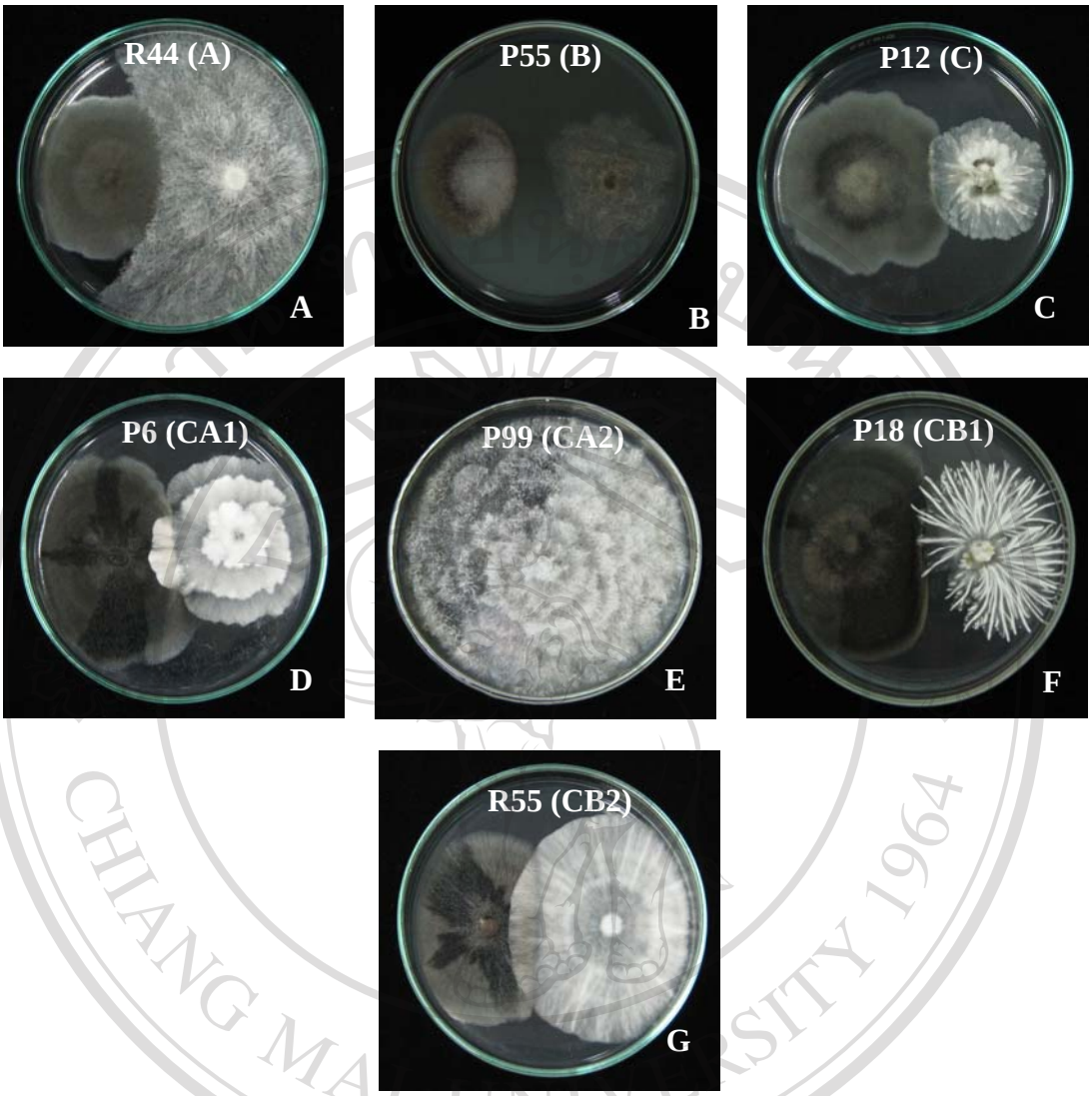

Figure 53 Interactions between *Curvularia senegalensis* (left) and endophytic fungi (right) using dual culture technique on PDA; A, B: Deadlock and C-G: replacement of pathogen by *C. senegalensis*.

According to the result from level of potential and interaction type, P59, R12 and R14 showed a very high potential to control all tested pathogen, moreover other isolates which high to very high potential and subtype CA2 and CB2 were also good candidate for further study. (Table 11).

Table 11 List of interest fungal endophyte for further study

	Sclerotium sp.	*Phythopthora* sp.	*Curvularia senegalensis*
P10	-	-	H (CA2)
P14	-	Eh (CA2)	Eh (CA2)
P37	-	-	H (CA2)
P59	Eh (CA2)	Eh (CA2)	Eh (CB1)
P82	H (CB2)	-	-
P99	-	-	H (CA2)
R2	-	-	H (CA2)
R7	-	Eh (CA2)	-
R8	H (CA2)	-	-
R11	-	-	H (CA2)
R12	Eh (B)	Eh (A)	Eh (CA2)
R14	Eh (B)	Eh (CA1)	Eh (A)
R18	-	-	H (CA2)
R22	-	Eh (CA2)	-
R30	-	-	H(CA2)
R44	H (CA2)	-	-
R55	-	-	H (CB2)
R81	-	-	H (CA2)
R86	H (CA2)	-	-