CHAPTER 8

ENDOPHYTIC AND EPIPHYTIC FUNGI OF THAI DWARF FISHTAIL PALM

8.1. Introduction

The phyllosphere is a term used in microbiology to refer to leaf surfaces or total above-ground surfaces of a plant (Carroll *et al.*, 1977) and is colonized by a variety of microorganisms (Andrews and Hirano, 1991). Phyllosphere fungi include endophytes and epiphytes that colonize the interior and surface of the phyllosphere, respectively, thereby occupying two distinct habitats of the plants (Petrini, 1991). Phyllosphere fungi occur not only on living parts but also on decomposing plant parts at the initial stages of decomposition, and their succession during decomposition has been demonstrated in several litter types (Petrini, 1991). The ecology of phyllosphere fungi on decomposition process should be studied to evaluate the role of phyllosphere fungi in energy flow and nutrient dynamics in ecosystem (Andrews, 1991).

In succession, biotic communities change over time, and fungal communities generally go through three stages (Dix and Webster, 1985; Gessner *et al*., 1993): *Pioneer community* which is dominated by fast growing, short-lived and capable of rapid and wide dispersal (Luczkovich and Knowles, 2000), mostly hyphomycetes; *mature community* which is dominated by ascomycetes and some hyphomycetes; and *impoverished community* which is dominated by very slow growing fungi such as basidiomycetes.

Classical method has been widely used in measuring fungal community on different ecosystems or habitats. This method includes direct sampling of fungal fruiting bodies, incubation of substrata in moist chambers, culturing of endophytes and particle plating (Schmit and Lodge, 2005). The advantages of a classical study are widely recognized due to it generates a list of species found during the study. Species list assembly enables researchers to compare data across sites and studies and among different taxonomic or ecological groups. By combining species lists from multiple studies, researchers can determine basic information about individual species, such as geographic range, host relationships and ecological distribution. The fungal communities of different areas can be compared to determine patterns of species diversity. Classical methods are also the only methods that can be used to demonstrate which fungi are reproducing in a particular environment or on a given substratum, as opposed to which fungi are present but cannot reproduce.

In the study of palmicolous fungi, direct sampling of fungal fruiting bodies from decaying substrata collected from the field (Fröhlich and Hyde, 2000; Yanna, 2001, Yanna *et al.*, 2001; Taylor and Hyde, 2003; Pinnoi *et al.,* 2004, 2006; Pinruan *et al.*, 2007) and culturing endophytes (Rodrigues, 1990, 1994; Rodrigues and Samuels, 1990; Rodrigues and Petrini, 1997; Taylor *et al.*, 2000; Fröhlich *et al.*, 2000) have been the most common methods used until present time. The first method revealed several taxa such as *Anthostomella*, *Arecomyces*, *Astrosphaeriella, Linocarpon* and *Oxydothis* as common fungi associated with terrestrial palms in various localities; and Xylariaceae, Hyponectriaceae and Lophiostomataceae at higher level (Fröhlich and Hyde, 2000; Taylor and Hyde, 2003). Furthermore, in non-terrestrial habitat, Pinruan *et al.* (2007) reported different taxa such as *Annulatascus*

velatisporus, *Microthyrium* sp., *Phaeoisaria clematidis*, *Massarina bipolaris*, *Phruensis brunneispora*, *Thailiomyces setulis* and *Solheimia costaspora* as common taxa associated with palms. These results indicated that host or biogeography affects the fungal community on tissue of plants. In second method of direct sampling, culturing of endophytes, xylariaceous fungi are the most commonly isolated endophytes in tropical and temperate regions (Rodrigues and Samuels, 1990; Rodrigues, 1994; Rodrigues and Petrini, 1997; Taylor *et al.*, 2000; Fröhlich *et al.*, 2000). This method is less favored than the direct sampling due to most of the fungi grow on the isolation media are mycelia sterilia.

Another classical method, incubation of substrata in moist chambers, has also been widely used to examined the fungal community on the plants particularly phyllosphere (Tokumasu, 1996; Osono *et al.*, 2004; Osono, 2007). This method has most often used for fungi growing on leaves or small woody debris, such as ascomycetes (Polishook *et al.*, 1996; Rambelli *et al.*, 2004) and slime molds (Snittler and Stephenson, 2000) and fungi growing on dung (Richardson, 2001). The substrata are usually placed on moist paper towels in an inflated plastic bag or in a container with a lid. The samples are then examined for the presence of fruit bodies periodically for 2 to 6 weeks. This method is also commonly used in the study of fungal succession (Tokumasu, 1998a, 1998b). In contrast to the previous classical methods, incubation of substrata in moist chambers has not widely employed to analyze the fungal community on palms yet. Therefore, it is necessary to examine this alternative method in analyzing the fungal community occurs on palms.

It has been hypothesized that the fungal community patterns of phyllosphere fungi at early stage of decomposition is dominated by fast growing taxa (mostly

hyphomycetes) (Gessner *et al*., 1993). Thus, in this chapter, the fungal community pattern at the early stage of decomposition resulted from incubation of substrata method in moist chambers is presented.

8.2. Materials and methods

Sampling

Samples were collected at Huay Kog Ma, Doi Suthep-Pui National Park, Chiang Mai, Thailand. Ten fronds from ten trees of the palm were sampled on November 2007. The samples were randomly selected, without regard to their age, their size and sites. From each tree, healthy and necrotic fronds (living but with a dead portion of tissue), were collected. On returning to the laboratory, the samples were processed within 24 h and the rest were stored at 4ºC.

Fungal Isolation and Identification

From each tree, five primary rachis pieces (0.5 cm diam., 0.5–1 cm long), five pinnae discs (1 cm diam.) and five secondary rachis segments (0.2 cm diam., 0.5–1 cm long) were randomly obtained from both necrotic and healthy branches and processed by two different methods:

(1) The first method includes growing mycelia on petri dishes containing sterilized wet filter paper. Surface disinfection was performed by dipping in ethanol 70% (v/v) before soaking in 2% (w/v) sodium hypochlorite solution (5 min for leaf samples and 10 min for twig and bark samples) and washed three times in sterile distilled water, with the subsequent plating of the surface-disinfected fragments on petri dishes containing sterilized wet paper. The plates were then sealed with parafilm

and incubated at room temperature (24 ± 2°C) in diffused daylight for three months. Cultures were identified according to morphological characteristics.

(2) The second method consists of finding fruit bodies on plant tissues (primary rachis, secondary rachis, and pinnae) after incubating them on petri dishes containing sterilized wet filter paper at room temperature (24 ± 2°C) in diffused daylight for three months. The samples used in this method were not surface-disinfected in order to find endophytes and fungal epiphytes. Cultures were also identified according to morphological characteristics.

Statistical Data Analyses

The total number of species and the number of fungi per sample were recorded and calculated. Isolation rate analysis was employed in order to measure fungal richness in a given treatment/plant/tissue (Fröhlich *et al*., 2000).

$$\text{Isolation rate} = \frac{\text{Total number of isolates yielded by a given sample}}{\text{Total number of leaf discs/rachis segments in}}$$

One-way ANOVA analysis was performed to compare the differences between isolation rates using surface disinfection and non-surface disinfection methods.

Percentages abundance and frequency of occurrence of each species were employed in order to compare the dominance of fungi among different collections (Cai *et al.*, 2006) as follow:

$$\% \text{ abundance of a taxon } X_a = \frac{\Sigma \text{ records of taxon } X_a}{\Sigma \text{ records of all taxa}_a} \times 100$$

$$\% \text{ occurrence of a taxon } X_a = \frac{\Sigma \text{ records of taxon } X_a}{\text{Number of plant parts investigated}} \times 100$$

The effect of experimental design (surface disinfection and non-surface disinfection) was evaluated by one-way analysis of variance (ANOVA) using isolation rates as dependent variable. The data was examined using descriptive table. The homogeneity of dataset was tested using Levene statistic test. To compare the similarity of the species composition between different microhabitats, Sørensen' index of similarity (*S'*) was applied and expressed with values between 0 (no similarity) and 1 (absolute similarity) (Magurran, 1988).

Hierarchical Cluster Analysis (HCA) was performed to classify species recorded on the palms fronds. Species with frequency of occurrence > 10% were selected as cases in the analysis. Ward method was used as a clustering method, Squared Euclidean Distance was selected as an interval measurement and Z-scores was used to standardize the transform value of variable group. In order to constructs a configuration/map of the fungal community on specimens collected, Multi Dimensional Scaling (MDS) ALSCAL analysis using Euclidian model distance was performed based on dissimilarity matrices and down-weighting of rare taxa (with frequency of occurrence < 5%). The relationship between assemblage of the fungal community (with frequency of occurrence > 10%) and different type of palm tissues

was also analysed using a simple correspondence analysis. Two dimensional plots were generated from the MDS and Correspondence Analyses (CA).

All analyses were performed using SPSS version 16.0 (Anonymous, 2007) and XLSTAT-Pro version 7.5 (Anonymous, 2004).

8.3. Results

A total 47 species and 387 numbers of isolates (abundance) were yield from 150 total specimens examined (50 specimens from each tissue) (appendix 10). The taxa consist of 7 Ascomycetes (representing 14.9% of all taxa), 6 Coelomycetes (12.8%), 32 Hyphomycetes (68%) and 2 Zygomycetes (4.3%) (appendix 10). Several taxa that commonly found as endophytes in the previous studies (Rodrigues, 1990, 1994; Rodrigues and Samuels, 1990; Rodrigues and Petrini, 1997; Taylor *et al.*, 2000; Fröhlich *et al.*, 2000) also appeared as the most frequent taxa encountered in this study, namely, *Gliocladium penicillioides* (frequency of occurrence 39.3%), *Colletotrichum gloeosporioides* (23.3%), *Acremonium alternatum* (20.7%), *Arthrinium phaeospermum* (16%), *Fusarium* sp. (12.7%), *Pestalotiopsis guepinii* (12%) and *Cladosporium cladosporioides* (11.3%), respectively (appendix 10). The list of taxa with their Σ records, total frequency of occurrence and total % abundances are presented in appendix 10. A more detail information of Σ records, total frequency of occurrence and % abundances of all taxa on different tissue types is given in appendix 11.

Isolation rate analysis that measure of fungal richness in a given site/plant/tissue indicated the higher species richness fungal community resulted from non-surface disinfection technique than surface disinfection technique on each tissue

type (pinna, primary rachis and secondary rachis) (table 8.1). When the datasets of non-surface disinfection and surface disinfection were combined, the highest fungal richness was found on pinna, followed by primary rachis and secondary rachis with 2.9, 2.5, and 2.4 isolation rate values, respectively (table 8.1). A similar proportion of fungal group isolation rate values were also yield when abundance dataset was calculated (fig. 8.1).

Table 8.1 Isolation rate of fungal community on *Wallichia siamensis* at five different site based on tissue types and combination of tissue types-experimental design.

Microhabitats	**Methods**	**Sites**					**Average**
		1	**2**	**3**	**4**	**5**	
Pinna	Surface disinfection	1.8	1.2	1.6	1.4	4.2	2
	Non-surface disinfection	3	6.8	3.2	1.4	4	3.7
Secondary rachis	Surface disinfection	1.4	0.6	1.6	1.6	2.6	1.6
	Non-surface disinfection	2.2	5.8	3.2	2	3	3.2
Primary rachis	Surface disinfection	2	2	2.2	1.8	2.8	2.2
	Non-surface disinfection	2.4	3.2	2.8	1.8	3.8	2.8
Pinna		2.4	4	2.4	1.4	4.1	2.7
Secondary rachis		1.8	3.2	2.4	1.8	2.8	2.4
Primary rachis		2.2	2.6	2.5	1.8	3.3	2.5

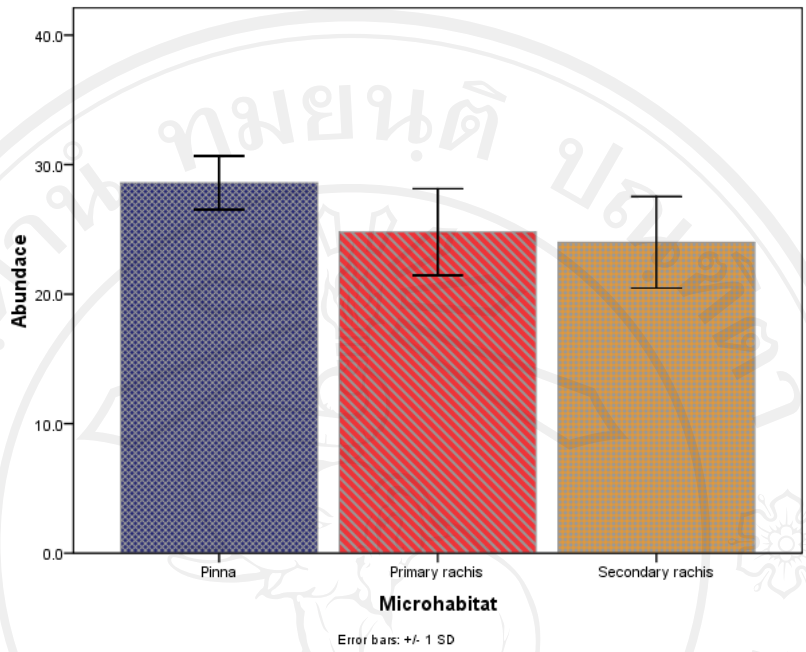

Fig. 8.1 Bar errors showing mean of total fungal individual records based on tissue types as microhabitat.

Based on total number of records (abundance) dataset of taxa generated from total samples examined, several common anamorphic taxa such as *Gliocladium penicillioides*, *Colletotrichum gloeosporioides*, *Acremonium alternatum*, *Arthrinium phaeospermum*, *Fusarium* sp. and *Cladosporium cladosporioides* appeared as the most frequent taxa recorded with number of records and % abundances 59 (15.3%), 35 (9%), 31 (8%), 24 (6.2%), 19 (4.9%) and 17 (4.4%), respectively (fig. 8.2; appendix 10).

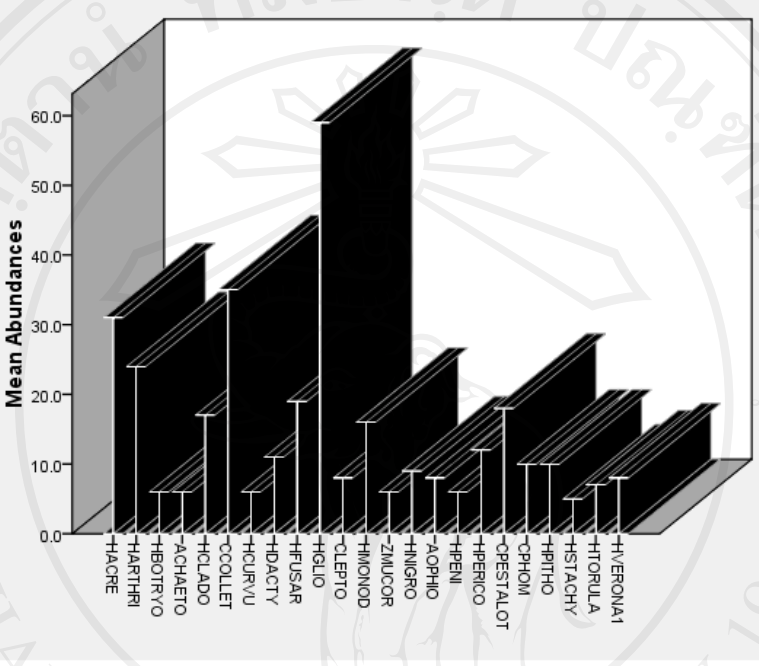

Fig. 8.2 Distribution of all taxa on *Wallichia siamensis* based on abundances data (Σ records > 5).

Box plots illustration showed that *Gliocladium penicillioides*, *Colletotrichum gloeosporioides*, *Acremonium alternatum* and *Fusarium* sp. occurred more frequently on primary rachis, but less on secondary rachis and pinna (fig. 8.3). *Cladosporium cladosporioides* which is well known as an endophyte and phytopathogenic fungus occurred frequently on pinna as well as on secondary rachis (fig. 8.3). On the other hand, *Arthrinium phaeospermum* occurred more frequently on pinna and primary rachis (fig. 8.3).

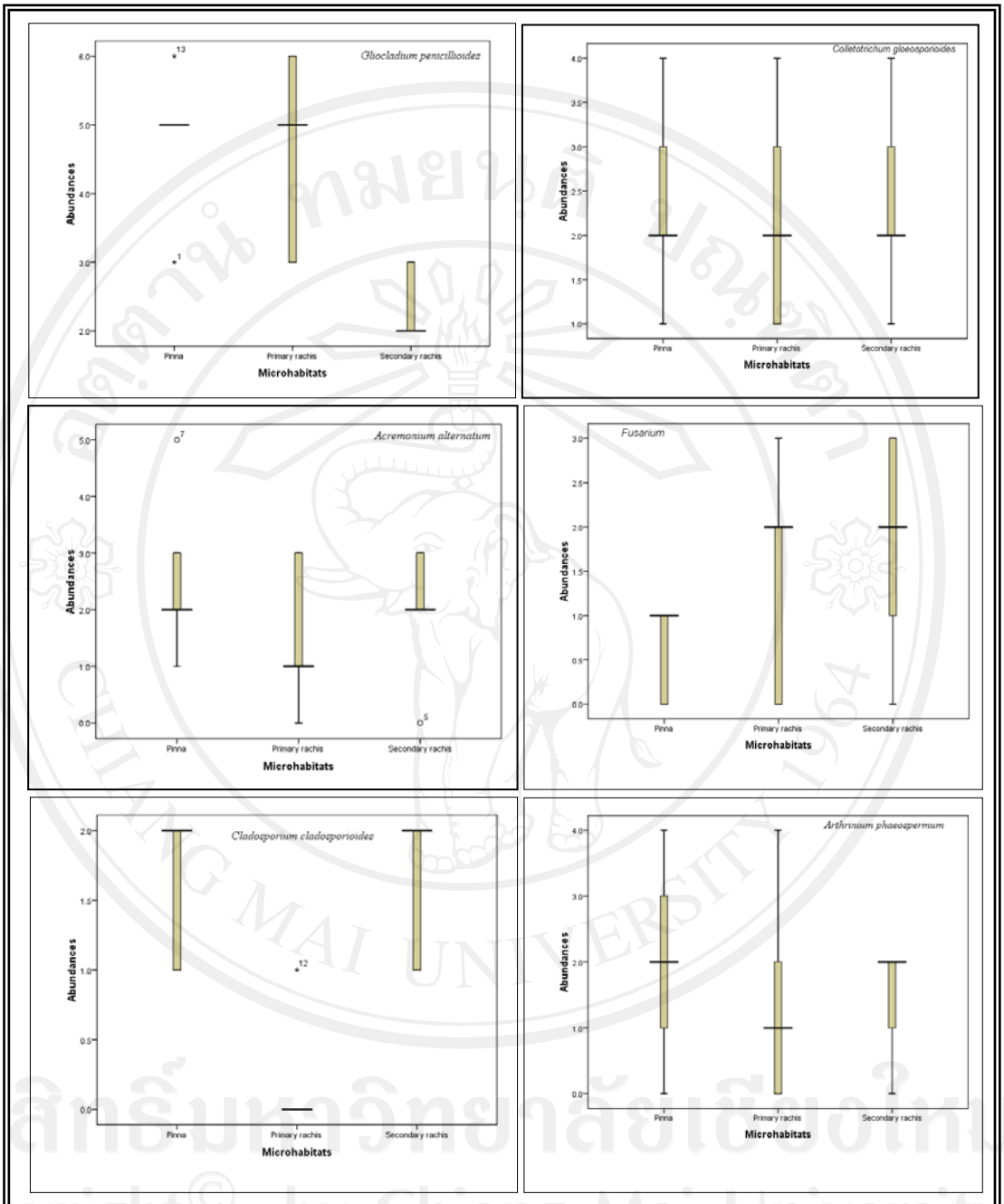

Fig. 8.3 Box plots showing the distribution of the most dominant species on *Wallichia siamensis* based on tissue types as microhabitat. The lower and upper boundaries of each box enclose 25-75% of the data. The line within the box shows the median values, the bar lines above and below the boxes indicate minimum and maximum values, and ° indicates outliers.

In order to analyze the difference between treatments in the experimental design (surface disinfection and non-surface disinfection), and to examine the difference among fungal communities abundance at various tissue types, bar errors diagram based on abundance datasets was performed (figs. 8.4 and 8.5). The bar errors apparently showed that total fungal abundance was highest when the specimens were treated by using non-surface disinfection method before incubation period (fig. 8.4). On the other hand, the abundance of fungal community on pinna was the highest at each collection site than fungal community on primary and secondary rachis (this data generated from combination of surface disinfection and non-surface disinfection datasets (fig. 8.5).

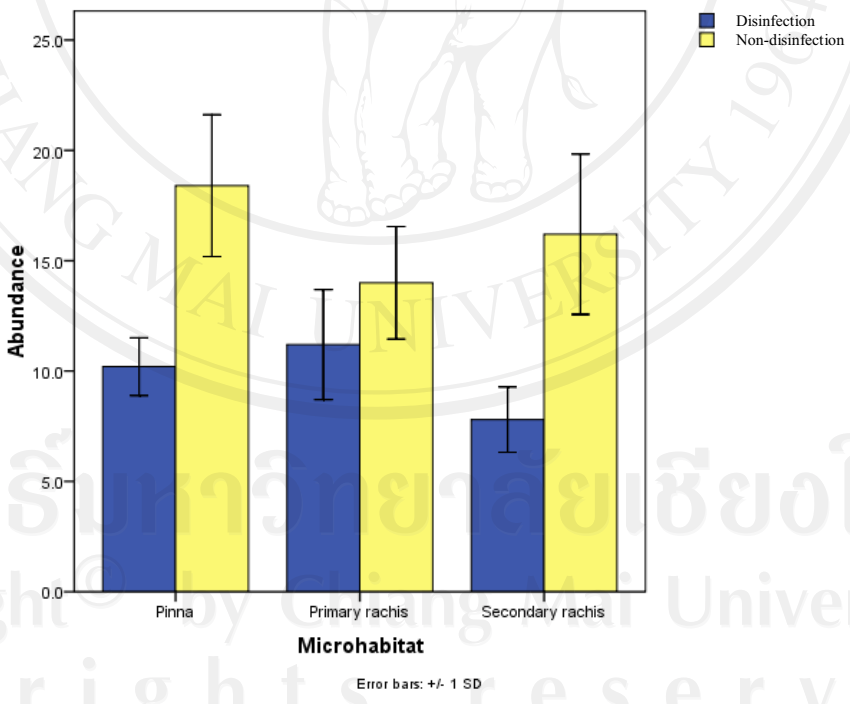

Fig. 8.4 Bar errors showing mean of total fungal individual records based on tissue types and experimental design (surface disinfection and non-surface disinfection).

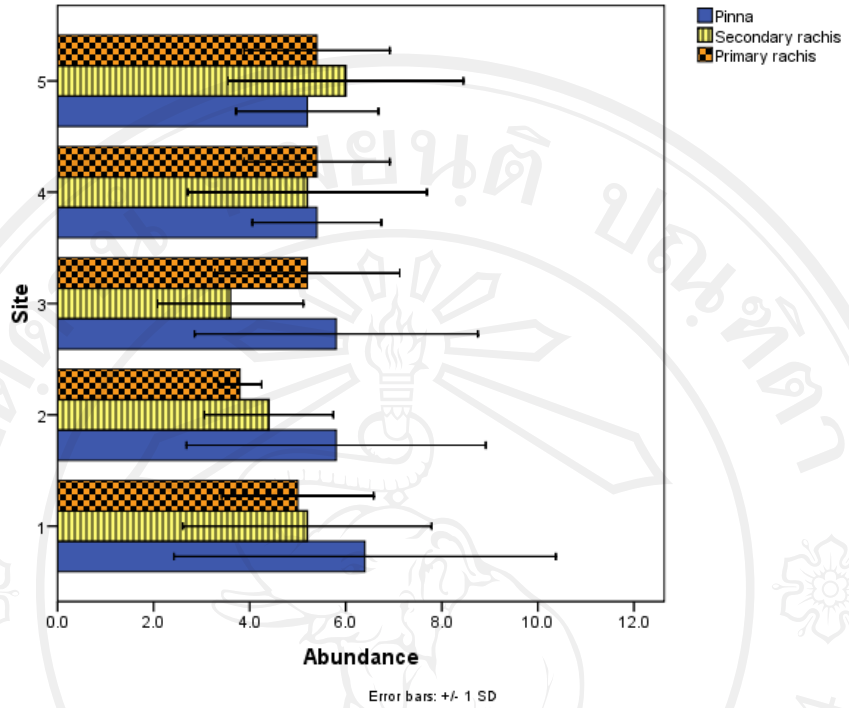

Fig. 8.5 Bar errors showing mean of total fungal individual records on different tissue types and sites.

The dataset from non-surface disinfected tissues also showed that hyphomycetous taxa occurred at very high frequencies (figs. 8.6; 8.7). Of the total 36 species, 26 were hyphomycetes (72%). In addition, hyphomycetous fungi also dominated total number of records with 83% abundance. Two common endophytic species (also reported as plant pathogens), namely, *Cladosporium cladosporioides* and *Fusarium* sp. were also dominant on *W. siamensis* using non-surface disinfection method (appendix 10).

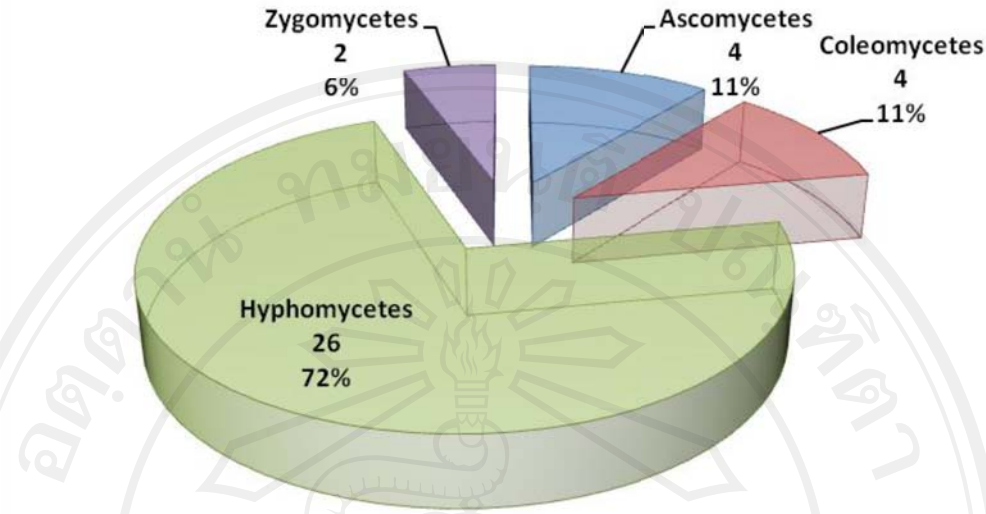

Fig. 8.6 Graphic showing distribution of fungal community on *Wallichia siamensis* generated from non-surface disinfection method based on species richness dataset. All taxa grouped according to their pseudo-systematic classification.

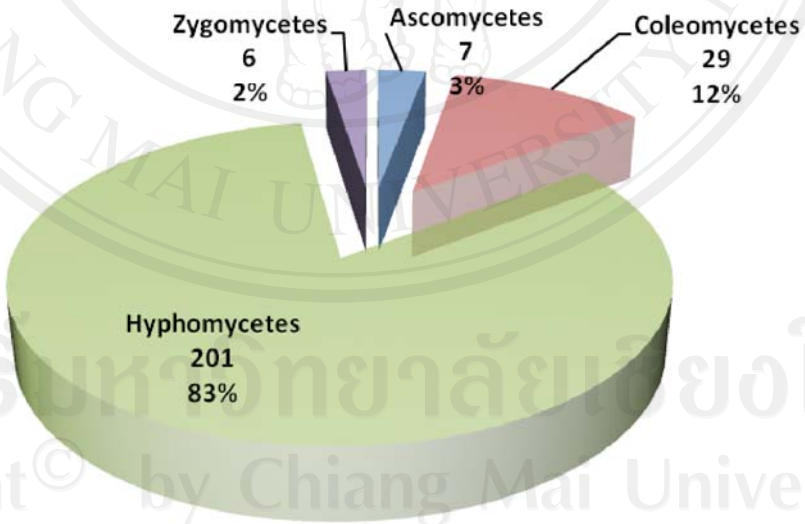

Fig. 8.7 Graphic showing distribution of fungal community on *Wallichia siamensis* generated from non-surface disinfection method based on species abundances dataset. All taxa grouped according to their pseudo-systematic classification.

Fungal endophytes on *W. siamensis* isolated from surface-disinfected samples were also high in diversity as they consist of four major pseudo-systematic taxa include Ascomycetes, Coelomycetes, Hyphomycetes and Zygomycetes. The members of Hyphomycetes also occurred at highesr frequencies based on this experimental design (65% of total species recorded; 56% of total abundances) (figs. 8.8; 8.9).

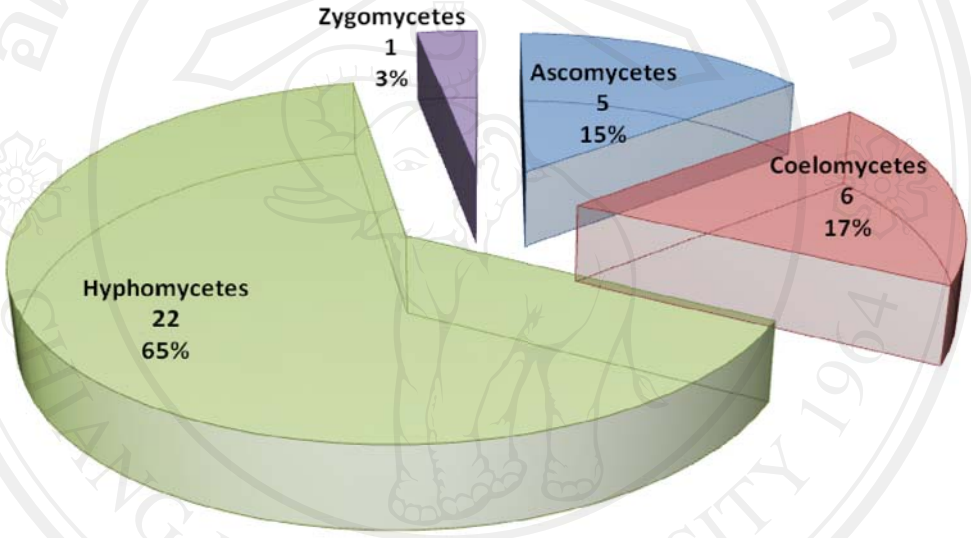

Fig. 8.8 Graphic showing distribution of endophytic fungal community on *Wallichia siamensis* based on species richness dataset. All taxa grouped according to their pseudo-systematic classification.

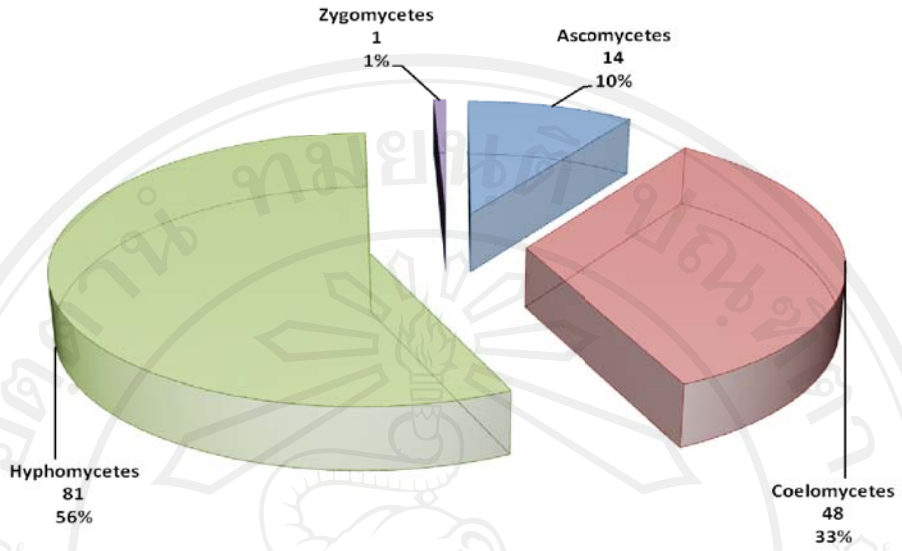

Fig. 8.9 Graphic showing distribution of endophytic fungi community on *Wallichia siamensis* based on abundances dataset. All taxa grouped according to their pseudo-systematic classification.

The Sørensen index of similarity (S') in table 8.2 indicated the highest similarity between fungal communities on pinna and secondary rachis (S' = 0.7), followed by the fungal communities on secondary-primary rachis (S' = 0.6) and the fungal communities on pinna-primary rachis (S' = 0.6). When combining the two datasets (tissue types and experimental design), it was clear that the highest similarity occurred between fungal community on UP-USR (S' = 0.7), followed by between the fungal communities on UP-UPR (S' = 0.6) and USR-UPR (S' = 0.6) (table 8.4). The lowest similarity occurred between fungal communities on SP-UPR (S' = 0.3), followed by between the fungal communities on SSR-UPR (S' = 0.4) and SPR-UP (S' = 0.4) (table 8.2).

Table 8.2 Table showing Sørensen index of similarity among different type of microhabitat. SP: Surface disinfection-Pinna, SSR: Surface disinfection-Secondary Rachis, SPR: Surface disinfection -Primary Rachis, UP: Non-surface disinfection-Pinna, USR: Non-surface disinfection-Secondary Rachis, UPR: Non-surface disinfection-Primary Rachis.

Microhabitats	S'
SP-SSR	0.6
SP-SPR	0.5
UP-USR	0.7
UP-UPR	0.6
SSR-SPR	0.5
USR-UPR	0.6
SP-UP	0.5
SP-USR	0.5
SP-UPR	0.3
SSR-UP	0.5
SSR-USR	0.6
SSR-UPR	0.4
SPR-UP	0.4
SPR-USR	0.5
SPR-UPR	0.5
PINNA-SECONDARY RACHIS	0.7
PINNA-PRIMARY RACHIS	0.6
SECONDARY RACHIS-PRIMARY RACHIS	0.6

Hierarchical cluster analysis (HCA) clearly separated palmicolous fungal community on *W. siamensis* into two major groups/clusters (fig. 8.10). The first cluster contains taxa with frequency of occurrence more than 20%, namely, *Gliocladium penicillioides*, *Colletotrichum gloeosporioides* and *Acremonium alternatum* with frequency of occurrence of 39.3%, 23.3% and 20.7%, respectively (fig. 8.10; appendix 10). The second cluster consisted of taxa with frequency of occurrence less than 20% (fig. 8.10; appendix 10), and this cluster could be separated into two subclusters. The first subcluster consisting of eight taxa with frequency of occurrence less than 10%, namely, *Leptodothiorella* sp. (5.3%), *Veronaea botryosa* (5.3%), *Ophioceras tenuisporum* (5.3%), *Dactylaria* sp. (7.3%), *Periconia byssoides* (8%), *Phoma* sp. (6.7%), *Pithomyces sacchari* (6.7%) and *Nigrospora oryzae* (6%). The second subcluster consisting of five taxa with frequency of occurrence between 10-20%, namely, *Cladosporium cladosporioides* (11.3%), *Monodictys putredinis* (10.7%), *Pestalotiopsis guepinii* (12%), *Fusarium* sp. (12.7%) and *Arthrinium phaeospermum* (16%).

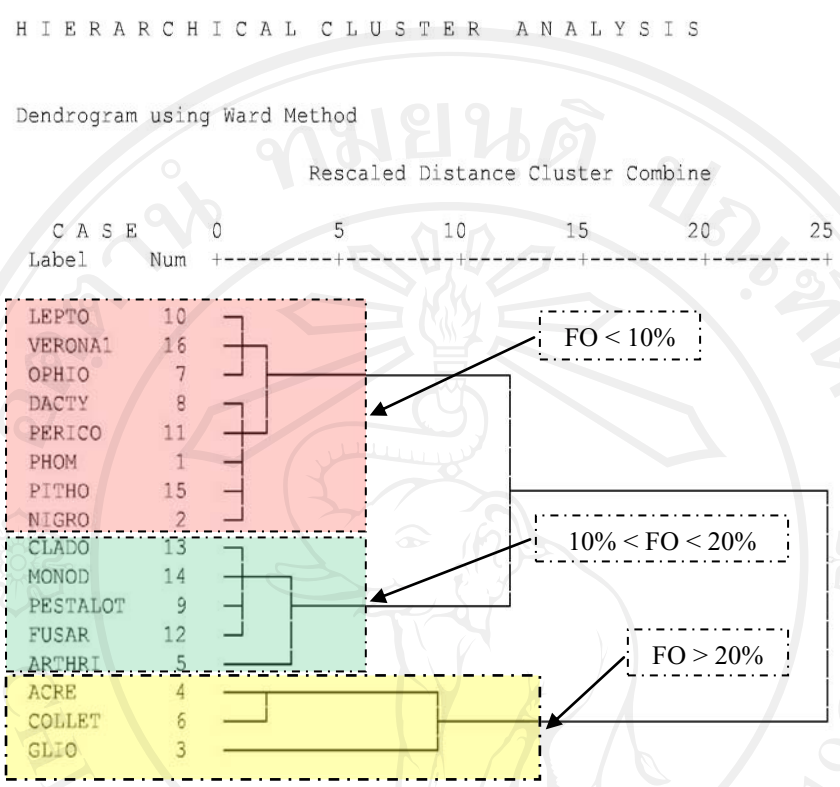

Fig. 8.10 Dendogram for hierarchical clustering (using Ward method) of all taxa with frequency of occurrence > 5% from total samples collected based on rescaled distance cluster combine matrix.

The two dimension of symmetric plot generated from simple correspondence analysis (CA) distinctly separated dominant taxa into surface disinfection and non-surface disinfection groups (showed by vertical axis/Dim1) (fig. 8.11). Several common hyphomycetous taxa found in this study, namely, *Monodictys putredinis*, *Fusarium* sp. and *Cladosporium cladosporioides* commonly occurred on non-surface disinfected samples (fig. 8.11). On the other hand, *Acremonium alternatum*, *Arthrinium phaeospermum*, *Colletotrichum gloeosporioides* and *Pestalotiopsis guepinii* occurred more frequent on surface disinfected samples (fig. 8.11). In addition,

a horizontal axis (Dim2) separated fungal community on primary rachis from pinna and secondary rachis (fig. 8.11). Based on this category, several anamorphic taxa such as *Monodictys putredinis*, *Pestalotiopsis guepinii*, *Fusarium* sp. and *Gliocladium penicillioides* showed a close association with primary rachis tissue as their microhabitat (fig. 8.11). On the other hand, *Colletotrichum gloeosporioides*, *Arthrinium phaeospermum*, *Acremonium alternatum* and *Cladosporium cladosporioides* were more common on pinna and secondary rachis (fig. 8.11).

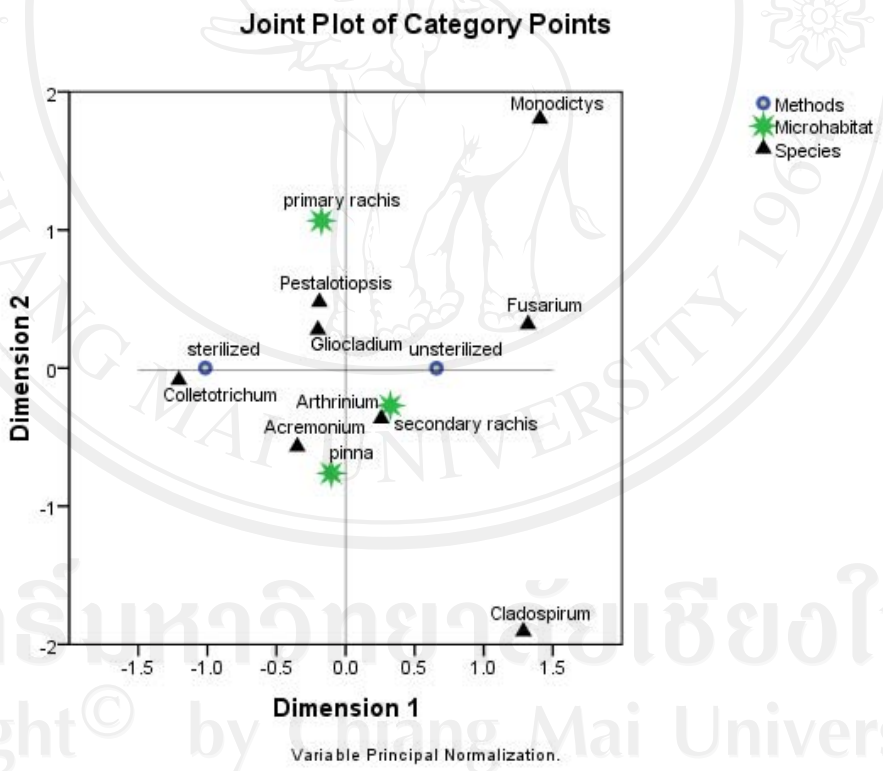

Fig. 8.11 Two dimensional of symmetric plot generated from multiple correspondence analysis showing the relationship among seven dominant taxa (represented by genus name), tissue types and experimental treatment.

8.4. Discussion

Composition of Endophyte and Non-Endophyte Fungal Communities

The experimental design which separated samples into surface disinfected and non-surface disinfected categories was purposed to examine the occurrence of fungal endophyte and epiphyte communities. Hyphomycetous fungi were very dominant in diversity and abundance compares to other pseudo-systematic fungal groups, not only on surface-disinfected samples but also on non-surface disinfected samples.

The pattern of which the fungal endophyte community on palm tissue was dominated by hyphomycetous taxa is common in investigation of fungal diversity/species richness at laboratory scale, and probably is unique to endophytic studies. The similar compositions were also found in other previous studies of fungal endophyte on palms (Rodrigues, 1994; Rodrigues and Petrini, 1997; Taylor *et al*., 1999; Fröhlich *et al*., 2000). Although the overall fungal endophytes composition of *W. siamensis* was distinct from other similar studies on palms (Rodrigues, 1994; Rodrigues and Petrini, 1997; Taylor *et al*., 1999; Fröhlich *et al*., 2000; Osono *et al*., 2004; Osono, 2007), but the most dominant endophytic taxa found were similar as several common endophytes such as *Colletotrichum gloeosporioides*, *Pestalotiopsis guepinii* and *Acremonium alternatum* were also dominant on *W. siamensis*.

This finding was consistent with a hypothesis that surface disinfection treatment will diminish the chance of air borne fungi and other epiphytes fungi to inhabit the samples during incubation period. It was proved by the appearance of several common saprobic species such as *Torula herbarum*, *Stachybotrys kampalensis* and *Stlbella* sp. only on non-surface disinfected samples (table 8.1). On the other hand,

xylariaceous taxa such as *Nodulisporium acervatum* and *Anthostomella* sp. were only isolated from surface disinfected samples along with coelomycetous fungi such as *Phomopsis caryotae-urentis* and *Diplodia* sp., which were frequently reported as endophytes on other hosts (Rodrigues, 1994; Sridhar and Raviraja, 1995; Lodge *et al*., 1996; Rodrigues and Petrini, 1997; Fröhlich *et al*., 2000; Taylor and Hyde, 2003).

Higher isolation rate of fungal community resulted from non-surface disinfection than surface disinfection method was supported statistically using one-way ANOVA analysis (table 8.3). The descriptive table showed the isolation rate of using non-surface disinfected method was higher 1.3 point than surface disinfected method (table 8.3). The Levene statistic of the analysis rejects the hypothesis of the homogeneity of dataset used in ANOVA analysis ($P < 0.05$), thus the dataset was sufficient for the analysis. The ANOVA table rejected the hypothesis that isolation rate of fungal community of *W. siamensis* fronds using surface disinfection and non-surface disinfection methods were equal ($P < 0.05$), therefore, surface disinfection and non-surface disinfection techniques were significantly contributed to the difference in fungal communities yield in this study.

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	15	1.9200	.83083	.21452	1.4599	2.3801	.60	4.20
2	15	3.2400	1.44460	.37299	2.4400	4.0400	1.40	6.80
Total	30	2.5800	1.33840	.24436	2.0802	3.0798	.60	6.80

Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
1.938	1	28	.175

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13.068	1	13.068	9.411	.005
Within Groups	38.880	28	1.389		
Total	51.948	29			

Table 8.3 Descriptive, test of homogeneity of variances, and ANOVA tables generated from One-Way ANOVA analysis showing the statistical test of isolation rate of disinfected and non-surface disinfected samples. 1 = Surface disinfected, 2 = Non-surface disinfected.

Xylariaceous taxa, unexpectedly, make up only a small proportion of the fungal community on *W. siamensis*. Only few results were reported similar findings such as fungal endophytes on *Trachycarpus fortunei* (Arecaeae) (Taylor *et al*., 1999), *Quercus ilex* (Fagaceae) (Fisher *et al*., 1994) and *Eucalyptus* (Fisher *et al*., 1993). In general, this result is in contradicting with the most endophytes studies using culturing method that suggested xylariaceous fungi as the most commonly isolated

endophytes in tropical and temperate regions (Rodrigues and Samuels, 1990; Rodrigues, 1994; Rodrigues and Petrini, 1997; Taylor *et al.*, 1999; Fröhlich *et al.*, 2000). The difference of fungal composition in this study with other previous studies was probably affected by two factors. Firstly, the method which did not use culturing of endophytes on artificial medium, but using moist chambers on natural substrates combined with sterilized filter paper as isolation medium. Secondly, the endophytes on such host are specific. The specificity of some endophytes was also previously suggested (Rodrigues and Samuels, 1990; Rodrigues, 1994; Rodrigues and Petrini, 1997), and these specific fungal communities along with plant pathogenic fungi are generally thought to have co-evolution with their hosts (Pirozynski, 1988).

Incubation of substrata in moist chambers has widely been used to examine the fungal community on the plants in particular phyllosphere (Polishook *et al.*, 1996; Tokumasu, 1996; Osono *et al.*, 2004; Rambelli *et al.*, 2004; Osono, 2007) and fungal succession (Tokumasu, 1998a, 1998b), but have never been used on palms. Thus, this study has demonstrated that a particular method combined with surface disinfection/non-surface disinfection techniques and a given substratum affected extremely to the fungal community resulted in the fungal diversity studies. Due to the significant different in the fungal community patterns resulted using the combination methods of moist chamber and surface disinfection/non-surface disinfection to the samples, therefore, it is necessary to employ this alternative method more frequent in analyzing the fungal community on palms.

The difference in isolation rate values among fungal endophytes and epiphytes communities on different tissue types suggested that not only method affected fungal community structures yield in the study, but also tissue anatomy (internal structure of

living things) and morphology (structure and configuration of an organism, includes aspects of the outward appearance such as shape, structure, color, size and pattern) as well as dispersal behavior of fungal spores. Chapela *et al.* (1990) and Rodrigues *et al*. (1993) noted that some endophytic fungi of tropical plants appear to be mainly transmitted when airborne propagules land and germinated on the leaf surface. Some endophytes in tropical and temperate regions are suggested to enter petioles and rachis of palms or twig of other plants via leaflets (palm)/leaves (other plants) (Fröhlich *et al*., 2000; Fisher *et al*., 1994). On the other hand, Petrini *et al*. (1992) insisted that different plant tissues and organs may, in particular, resemble distinct microhabitats. Endophytes may occur in certain tissues and if they become saprobes after the death of their hosts, the saprobes may be restricted to the tissues in which they have been endophytes.