DIVERSITY OF *FUSARIUM* SPECIES IN PEAT SOILS

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DIVERSITY OF *FUSARIUM* SPECIES IN PEAT SOILS

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

µl	Microliter
AFLP	Amplified Fragment Length Polymorphism
bp	Base pair
CFU	Colony forming unit
cm	Centimeter
CM	Complete medium
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
EtBr	Ethidium bromide
f. sp.	Formae speciales
Fe(NH4)2(SO4)2.6H2O	Ferrous ammonium sulfate hexahydrate
g	Gram
gcm^{-3}	Gram per cubic centimeter
h	Hour
IGS	Intergenic spacer
ITS	Internal transcribed spacer
kb	Kilobase
$K_2Cr_2O_7$	Pottasium dichromate
KCL	Potassium chloride
kg	Kilogram
KH_2PO_4	Potassium hydrogen phosphate
L	Liter
m	Meter
MAT	Mating type

mcf	Moisture correction factor
$MgSO_4.7H_2O$	Magnesium sulphate
ml	Milliliter
min	Minutes
$NaNO_2$	Sodium nitrite
NH_3	Ammonia
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PPA	Peptone pentachloronitrobenzene agar
p.s.i	Per. Square inch
RAPD	Random amplified polymorphic DNA
rDNA	Ribosomal deoxyribonucleic acid
RFLP	Restriction Fragment Length Polymorphism
rpm	Revolutions per minute
s	Second
SIS	Single image stereograms
SSU rDNA	Small subunit ribosomal ribosomal deoxyribonucleic acid
TBE	Tris-Boric acid-EDTA
TEF-1α	Translation elongation factor 1-α
UPGMA	Unweighted pair group method with arithmetical mean
USDA	United States Department of Agriculture
UV	Ultraviolet light
var.	Variety
VCG	Vegetative compatibility group
WA	Water agar

LIST OF SYMBOLS

%	Percentage
°C	Degree of Celsius
®	Registered
±	Plus minus
™	Trade mark

KEPELBAGAIAN SPESIES *FUSARIUM* DALAM TANAH GAMBUT

ABSTRAK

Kehadiran dan kepelbagaian spesies *Fusarium* telah dikaji daripada 23 jenis tanah paya gambut yang diperolehi daripada hutan paya gambut, paya gambut berair dan tanah gambut daripada ladang kelapa sawit. Daripada analisis tanah, kebanyakkan tanah paya gambut adalah berpasir dan lom berpasir, berasid (pH 3-4) serta mengandungi kandungan nitrogen, karbon dan kelembapan tanah yang rendah. Berdasarkan ciri-ciri morfologi makrokonidia, mikrokonidia, sel konidiogenus, rupa bentuk koloni dan pigmentasi, lima spesies *Fusarium* telah dikenalpasti iaitu *F. oxysporum* (60%), *F. solani* (23%), *F. proliferatum* (14%), *F. semitectum* (1%) dan *F. verticillioides* (1%). Spesies-spesies tersebut tersebar secara meluas dalam tanah di seluruh dunia dan merupakan penghuni tanah yang bertindak sebagai saprofit dan pengurai. Identiti setiap spesies telah disahkan melalui penjujukan DNA gen faktor pemanjangan translasi 1-α (TEF-1α). Bagi spesies dalam spesies kompleks *Gibberella fujikuroi* iaitu; *F. verticillioides* dan *F. proliferatum*, kajian pengawanan telah dijalankan. Keputusan kajian pengawanan menunjukkan sembilan pencilan *F. proliferatum* dan dua pencilan *F. verticillioides* membawa alel *MAT 2*. Ujian persilangan kesuburan menunjukkan sembilan pencilan *F. proliferatum* dikenalpasti secara morfologi telah disahkan sebagai *F. proliferatum* setelah dikacukkan dengan populasi mengawan D (*Gibberella intermedia*) dan hanya satu pencilan yang telah disahkan sebagai *F. verticillioides* setelah dikacukkan dengan populasi mengawan A (*Gibberella moniliformis*). Analisis filogenetik menggunakan jujukan gen TEF-1α dan β-tubulin berdasarkan set data individu dan set data gabungan menggunakan kaedah hubungan jiran (NJ) dan kebolehjadian maksimum (ML), menunjukkan pencilan daripada spesies yang sama dikelompokkan dalam klad yang sama. Variasi

intraspesies juga diperhatikan melalui analisis filogenetik. Oleh itu, kehadiran spesies *Fusarium* dalam tanah gambut yang berbeza menunjukkan tanah gambut boleh menjadi takungan spesies patogenik dan ini memberikan pengetahuan tentang kemandirian dan evolusi spesies-spesies tersebut.

DIVERSITY OF *FUSARIUM* SPECIES IN PEAT SOILS

ABSTRACT

The occurrence and diversity of *Fusarium* species were determined from 23 peat soil samples collected from peat swamp forest, water-logged peat and peat soils from oil palm plantations. From soil analysis, the peat soils were mostly sandy and loamy sand, acidic (pH 3-4) with low nitrogen and carbon content and low moisture content. Based on the morphological characteristics of macroconidia, microconidia, conidiogenous cells, colony appearance and pigmentation, five *Fusarium* species were identified namely, *F. oxysporum* (60%), *F. solani* (23%), *F. proliferatum* (14%), *F. semitectum* (1%) and *F. verticillioides* (1%). These species are widely distributed worldwide and are common soil inhabitants which act as saprophyte and decomposer. Species identity was confirmed through DNA sequencing of translation elongation factor (TEF-1α). For species from *Gibberella fujikuroi* species complex, *F. verticillioides* and *F. proliferatum*, mating study was conducted. Mating study results showed that nine isolates of *F. proliferatum* and two isolates of *F. verticillioides* carried *MAT 2* allele. Cross fertility test indicated that nine morphologically identified *F. proliferatum* were confirmed as *F. proliferatum* after cross-fertile with mating population D (*Gibberella intermedia*) and only one isolate was confirmed as *F. verticillioides* (*Gibberella moniliforme*) after cross-fertile with mating population A. From phylogenetic analysis using TEF-1α and β-tubulin genes based on individual dataset and combined dataset using neigbour-joining (NJ) and maximum likelihood (ML) methods, showed that the isolates from the same species were clustered in the same clade. Intraspecific variations were observed through the phylogenetic analysis. Thus, from this study, the occurrence of *Fusarium* species in peat soils suggested that different types of peat soils could be a reservoir of

pathogenic species and will provide knowledge on the survival and evolution of the species.

CHAPTER ONE

INTRODUCTION

Tropical peat lands comprised about 10 - 15% of global land (Immirzi and Maltby, 1992; Lappalainen, 1996). A total of 60% of the world' tropical peat is located in South East Asia which is the largest area of peat lands, estimated about 20 - 30 million ha (Regional Physical Planning Programme for Transmigration, 1990; Rieley *et al.*, 1996). Peat lands are located in the low altitude in coastal lowlands in Borneo, Papua New Guinea, Sumatra, Sarawak and the Malay Peninsular. Small peat land areas can also be found in Vietnam and the Philippines. Pahang Forestry Department (2005), estimated that 1.45 million ha peat swamp forest were found in Malaysia and there are about 200,000 ha remained in Peninsular Malaysia, which mostly are found in Pahang.

Peat soils can be obtained from peat land forest, peat land under crops and water-logged peat swamp. Peat land forest and peat land under crops provides dry area while water-logged peat swamp provides wet area. These three types of peat soils shared similar characteristics in which the environment is acidic (pH 2-4), high moisture content, high carbon content and low nitrogen content. Most of the tropical peat lands in Malaysia are geologically recent where most of the area formed over the past 5,000 years (Yule and Gomez, 2009).

Peat lands were build by poor drainage, permanent water logging and slow decomposition of the organic matter by acidic environment due to the rainfall and the topography. Most of the lowland peat contained partially decomposed trunks, branches and roots of the trees (Rieley *et al.*, 1996) which resulted in low nutrients in the peat soils due to litter build up as peat (MacKinnon *et al*., 1996). While, peat soil in peat swamp were formed by the accumulation of partially decaying plant debris in waterlogged conditions, high level of acidity (2.5 – 4.7) and lack of oxygen. These conditions prevent microorganisms from rapidly decomposing the plant debris (Pinruan *et al*., 2007). Recently, peat swamp forests are rapidly vanishing due to peat land conversion into agricultural land, logging, drainage and fire (Yule and Gomez, 2009).

Generally peat soil conditions are regarded as an extreme environment by the acidic and anaerobic conditions for microbial growth. Thus, the inhibition of the microbial activities resulted in slow rate of decomposition of leaf litter especially in water-logged condition (Gorham, 1991; Whitten *et al*., 2000; Pahang Forestry Department, 2005). Among the microbe found in peat soil is *Fusarium* species in which the fungus has been reported by Thorman and Rice (2007) and Latiffah *et al*. (2010). It was not surprising as previously the *Fusarium* species were also been found in other type of extreme environment such as in the desert, Arctic and mangrove (Gordon, 1954; Gordon, 1960; Kommedahl *et al*., 1975).

The genus *Fusarium* is one of the most important groups of Ascomycetes fungi and well-distributed in the soils as soil inhabitants. Majority of the species survived in plant residues and lived close to the soil surface (Nwanma and Nelson, 1993). *Fusarium* species have special characters which are chlamydospores and resistant conidia that can help in their survival in the soils or plant debris and as the ability to be parasites or saprophytes.

The first step to identify and characterize *Fusarium* isolates is by using morphological characteristics such as the features of macroconidia, microconidia, pigmentation and types of conidiogenous cells. Morphological characteristics are mainly used to sort the isolates into groups or sections (Leslie and Summerell, 2006). Previously, most researchers identify *Fusarium* species morphologically by separated them into sections through similar morphological characters. Morphological characteristics are based on the similarity of observable morphological characters, such as spore size and shape (Leslie and Summerell, 2006). To distinguish the species, macroconidia is the main characters observed. However, some species produced very similar macroconidia characteristics such as species in the section Liseola. Therefore, other methods of identification such as molecular characterization and mating studies are applied.

Mating study refers to the sexual fertility crossses in which the teleomorph of *Fusarium* species are used to identify the mating populations which represent different biological species (Leslie and Summerell, 2006). In this approach, sexually fertile members of the same mating populations will cross or mate and produced perithecia with eight ascospores which indicate fertile progeny (Leslie, 1993).

Molecular characterization using DNA sequencing has been used widely for identification and characterization of *Fusarium* species. DNA sequencing data can also be used to determine the genetic variations within and between species, and to provide information on phylogenetic relationships among closely related species. Among protein-coding gene, translation elongation factor 1-alpha (TEF-1α) is the most widely used for identification as it is highly informative among closely related taxa, exist as simple copy and non-orthologous (Geiser *et al*., 2004).

For phylogenetic inference, commonly more than one gene is applied. Another protein coding gene, β-tubulin gene has been used to determine the relationships among fungi including the studies of species complex in *Fusarium* (O' Donnell *et al.*, 1998b). β-tubulin gene can give additional information on the phylogenetic relationship among the *Fusarium* species as the gene is one of the more commonly used genes for phylogenetic inferences. A study by O'Donnell *et al*. (1998b), showed that β-tubulin provides 3.5 times more phylogenetic information than mitochondrial SSU rRNA genes and proposed that β-tubulin as a useful marker for studying closely related *Fusarium* species. β-tubulin gene has been also been widely used for molecular phylogenetic resolution in Ascomycetes including *Calonectria* sp. (Schoch *et al*., 2001), *Epichloe* sp. (Craven *et al*., 2001), *Aspergillus* sp. (Geiser *et al*., 1998; Peterson, 2001) and *Penicillium* sp. (Samson *et al*., 2004).

The information on the occurrence of *Fusarium* species in peat soil is important as many *Fusarium* species is soil-borne pathogen, and some of the plant pathogenetic *Fusarium* species are cosmopolitan. Since *Fusarium* can occur and survive in extreme environments, peat ecosystem could be a reservoir for pathogenic species. Moreover, many peatland areas are converted to agricultural land which might provide suitable conditions for growth for many species of *Fusarium*.

Therefore, the objectives of the present study were:

1) To isolate *Fusarium* spp. from peat soils and identify using morphological characteristics and molecular approaches.

2) To determine the phylogenetic relationship among *Fusarium* spp. from peat soils using TEF-1α and β- tubulin sequences.

3) To determine the mating population of *Fusarium* spp. from section Liseola.

CHAPTER TWO

LITERATURE REVIEW

2.1 Distribution of peat land

The largest tropical peat is in the areas of South China Sea and in Papua-New Guniea which together forming up to 68% of all known tropical peat land (Immirzi *et al*., 1992). In temperate region, peat lands can be found in the US with total acreage of 30 million ha and in Canada and Russia with 170 and 150 million ha, respectively (Hartlen and Wolski, 1996).

Southeast Asia and western Pasific Islands are the epicenter of tropical peat land (Hirano *et al.,* 2007), in which two third (about 30 million ha) of the total world coverage of tropical peats are in Southeast Asia (Huat *et al*., 2005; Murdiyaso *et al*., 2009). The Regional Physical Planning Programme for Transmigration (RePPProT, 1990) recorded that the total area of peat swamps forest in Southeast Asia is estimated to be about 33 million ha, where approximately 82% are located in Indonesia, 8.8% in Papua New Guinea and 8.3% in Malaysia, with smaller areas in the Philippines (240 000 ha), Vietnam (183 000 ha) and Thailand (68 000 ha). In Indonesia, 26 million ha of the country land areas are peat lands, with almost half of the peat land total areas are located in Kalimantan (Huat *et al*., 2005).

In Malaysia, Wetland International (2010) reported that peat land encompass 2,457,730 ha (7.45%) of Malaysia’s total land area (32,975,800 ha). Sarawak supports the largest area of peat land in Malaysia with 1,697,847 ha or 69.08 % of the total peat land area in Malaysia, followed by Peninsular Malaysia, 642,918 ha (26.16%) and Sabah, 116,965 ha (4.76 %) (Table 2.1).

Table 2.1: The area (ha) of peat land in Peninsular Malaysia, Sarawak and Sabah

Region	Hectares	Percentages
Sarawak	1,697,847	69.08
Peninsular Malaysia	642,918	26.16
Sabah	116,965	4.76
Total	**2,457,730**	

Source: Wetland International Malaysia, 2010

Pahang has the highest distribution of peat land areas which supports 57% of peat swamp forest with more than 70% canopy cover remaining in Peninsular Malaysia (Table 2.2).

Table 2.2: Estimated extent of peat swamp cover in Malaysia

State	Hectares
Johor	13,000
Pahang	200,000
Selangor	76,000
Terengganu	13,000
Sabah	120,000
Sarawak	1,120,000

Source: United Nations Development Program, 2006

Most of this area comprises the South East Pahang Peat Swamp Forest, within the Pekan, Kedondong, Nenasi and Resak Forest Reserves. The distributions of peat land in Malaysia are shown in Figure 2.1.

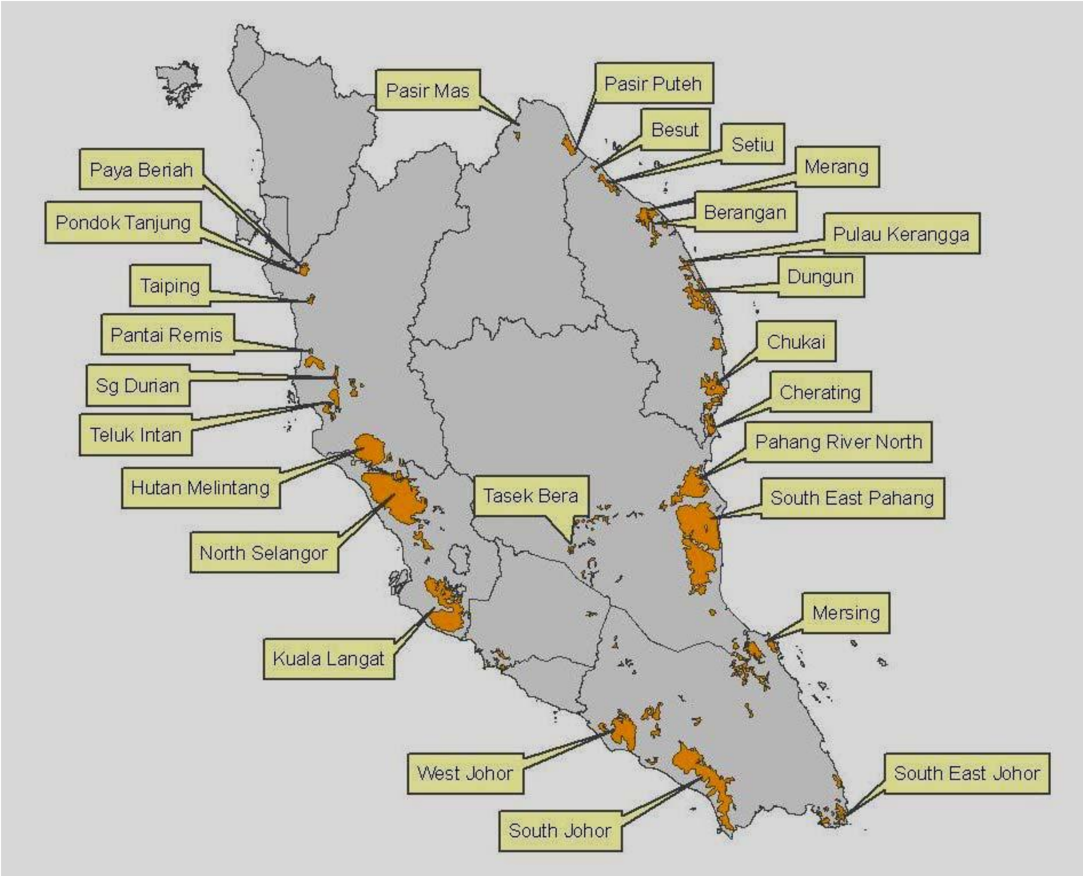

Figure 2.1: Major peat swamp areas in Malaysia
(Source: Wetland International Malaysia, 2010)

Lately in Malaysia, the tropical peat swamp forests are rapidly decreasing because of agricultural conversion, which is mainly to oil palms, logging, drainage and fire. Only a few peat lands can be found along the east and west coasts and very little pristine forest remains (Yule and Gomez, 2009).

2.2 Peat land vegetation

The natural vegetation of peat lands in Malaysia is generally peat swamp forest. There are a few peat land areas with a natural vegetation of sedges, grasses and shrubs, especially where peat areas are found around water areas. Species compositions in peat swamp forest flora are related to the peat depth and hydrology which in turn affecting water table depth and nutrient content. Decreasing fertility, increased incidence of periods of water stress, and problems with uptake of water with very high concentrations of leached plant defensive compounds can affect the vegetation in the peat land area (Wetland International Malaysia, 2010).

Most of Malaysia's peat lands have a dome-like structure especially in Sarawak, thus different vegetations are found. In Sarawak and Brunei, Anderson (1963) described six communities of plants from the edge to the centre of the dome. The communities includes mixed peat swamp forest (mixed vegetation with height 40-45 m), Alan batu forest (mixed and uneven vegetation usually *Shorea albida* were found), Alan bunga forest (vegetation dominated by a single species with 50-60 m height), Padang Alan forest (vegetation with even canopy, mostly with 35-40 m height), Padang paya (dense vegetation with 15-20 m height) and Padang kerutum (Herbaceous flora consists of *Nepenthes*, sedges and *Sphagnum*). The timber trees are commonly found in peat swamp forest in Sarawak which includes *Shorea albida, Shorea macrantha, Dryobalanops* sp. (Dipterocarpaceae) and *Elaeocarpus beccari* (Tiliaceae). Dipterocarpaceae and the other timber tree have special rooting system (stilt roots) that helps in better mechanical support (Anderson, 1964).

In Sabah, three vegetation communities have been described in peat areas which include timber, ramin and dipterocarps. Both *Dactylocladus stenostachys* (timber) and *Gonystylus bancanus* (ramin) are found mainly in southwest Sabah. The dipterocarps, *Shorea platycarpa, S. scabrida* and *S. teysmanniana* can also be found in this forest type. The dipterocarp *Shorea albida* is not found in Sabah because the peat swamp is not dome shaped structure (Wetland International Malaysia, 2010).

In Peninsular Malaysia, there is no specific classification of the peat land vegetation. However, it seems that most plant communities are a mixed of peat swamp forest type (Wetland International Malaysia, 2010). Most species of trees found in peat swamp forest are from Myrtaceae and Dipterocarpaceae (Mansor, 1999). There are differences in vegetation grow between the shallower and sandy peats of the east coast and the deeper peats over clay of the west coast (Appanah, 1999).

In Pahang peat swamp forest, the common non-timber families have been recorded including Araceae, Zingerberaceae, Pandanaceae and Orchidaceae. In other peat swamp forest, such as in Pondok Tanjung, Perak, Palmae can be found and plays an important part in biodiversity of the peat swamp forest. This group of plant can survive and adapted to acidic environment and water logged conditions (Mansor, 1999).

Murdiyaso *et al*. (2009) reported that in Kalimantan, the most common vegetation found is dipterocarp species such as *Shorea* spp. The peat swamp habitat includes ramin (*Gonystylus bancanus*), ulin (*Eusideroxylon zwageri*) and jelutong (*Dyeracostulata*). In the disturbed areas, gelam (*Melaleuca cajuputi*), Pandan and the sealingwax palm (*Cyrtostachys lakka*) are also found.

2.3 Properties of peat soils

2.3.1 Physical properties

Physical properties of soil include soil development, color, texture, structure and bulk density. Peat soils were mostly formed 5,000 years ago and began to develop when rate of accumulation of organic material exceeds the rate of decomposition. The waterlogged conditions in peat swamp soils began to develop when rivers drain to the area (Firdaus *et al*., 2010). The depth of the peat soils usually ranging from 1 to 8 m, but can be as deep as 24 m in some areas (Giesen, 2004). Some conditions including poor drainage, waterlogged condition and high rainfall can lead to faster accumulation of the plant residues (Brady, 1997).

In Malaysia, most of the peat land especially in Sarawak, has a dome-like shaped, where there is an extensive flat bog in the centre which can achieved 10 m higher than the river level. In Marudi, Sarawak, the depth of the dome centre may reach 12 m (Anderson and Muller, 1975). In tropical peat swamp domes, water is stored above the peat surface between hummocks that surround tree trunks and between spreading buttress roots. The hummocks and other surface elements looked like a V-notch barriers that regulate water availability. Buttressed trees help in water regulation and retaining the water (Dommain *et al*., 2010).

Soil texture is described as the mixture of different sizes of soil particles such as sand, silt and clay (Table 2.3). These particle size distributions will affect the physical and chemical properties of the soil structures (Rowell, 1994). Soils that are high in clay content tend to have slower permeability while soils that are high in sand content tend to have faster permeability. Water will move almost straight down through sandy soil, whereas it will have more lateral movement in a heavier (clay) soils (Rowell, 1994). Generally peat soils can be fine or course depends on the soils characteristics. In Air Hitam Laut, Sumatra, Indonesia, the peat soils consist of light to heavy clays (Wosten *et al*., 2006).

Table 2.3: Particle size fraction

Particle class	Particle subclass	Particle size (mm)
Stones		> 2
Sand	Coarse	2-0.2
	Fine	0.2-0.06
Silt		0.06-0.002
Clay		<0.002

Source: Rowell (1994)

In peat swamps, the peat deposits can extend from 50 cm to 20 m. The thickness of the organic matter and humus make the soils black in color due to the high levels of polyphenols, tannins and other degradation products of organic matter, with low silt levels (Maltby and Proctor, 1996). Dark brown color of peat soils are due to tannins and heavily shaded by forest canopy (Yule and Gomez, 2008). Thus, the colors of the peat soils are commonly black, dark or dark brown.

Bulk density is a reflection of the amount of pore space in the soil. In tropical peat soils, the bulk density is low ranging from 0.1 to 0.32 gcm^{-3} and decreases with depth (Brady, 1977). Bulk density of the upper 30 cm layer varies between 0.12 and 0.17 gcm^{-3} in undisturbed area (Sajarwan *et al.*, 2002), while in cultivated areas, it ranges from 0.17 to 0.31 gcm^{-3} (Kurnain *et al.*, 2001). Bulk density increases during land reclamation where peat begins to decompose and compacted (Martini *et al*., 2006). Generally, peat soils have lower bulk density (Rowell, 1994).

2.3.2 Chemical properties

Some of the chemical properties of peat soils are pH, organic matter and carbon to nitrogen ratio. Peat soils are acidic with pH levels as low as 3.5, have low nutrient content and high carbon/nitrogen ratio. In peat soils in Kalimantan, Indonesia, the average pH is around pH 3.1 (Page *et al*., 1999). Peat soils in peat swamps are acidic and nutrient deficient because these areas are not drained by flooding (Wetland International Malaysia, 2010).

Lowland tropical peat consists of partially decomposed trunks, branches and roots of trees (Rieley *et al*., 1996). Although peat soils composed of large number of vegetations and microorganisms, the rate of decomposition is low. This is because of the high organic materials due to low decomposition rate and decreased the microorganism activities. Low decomposition rate are affected by the carbon and nitrogen content and particle size of the surface areas that exposed to surrounding areas for microbial attacks (Thomas *et al*., 1998).

Peat soils have low nutrients content since leaf litter decomposed slowly and build up as peat, rather than cycling rapidly and thus, there are no new nutrient inputs (MacKinnon *et al*., 1996). Page *et al*. (1999) reported that in peat swamps in Kalimantan, Indonesia, nutrients such as calcium, potassium and phosphorus decrease with increasing depth of the soils. In peat land that has dome structure, the inorganic nutrients decrease from the margin to the centre of the peat domes, with the centre has fewer phosphorus and potassium content (Dreissen, 1977).

Peat soils contain high carbon content and low nitrogen content. The organic carbon content of tropical peat usually exceeds 50% dry weight, with total nitrogen content up to about 2% (Wust *et al*., 2003). The C: N ratio of peat soils have a wide range and generally increase with increasing depth. For example in Kalimantan and Malaysia, the maximum C: N ratio exceeds 50% (Martini *et al*., 2006).

2.4 Microbial diversity of peat soils

2.4.1 Bacteria and fungi

Various types of microorganisms and their physiological activities have been discovered in peat soils (Thormann and Rice, 2007). In early 1932, Waksman and Purvis reported that bacteria and actinomycetes as well as fungi have been found at all depth of minerotrophic (ground water nourished) peat soils and in all layers of ombrotrophic (air nourished) peatlands (Wheatly *et al*., 1976). Bacterial genera that have been recorded in peat soils include *Bacillus, Pseudomonas, Achromobacter, Cytophaga*, *Micrococcus*, *Streptomyces*, and *Actinomyces* (Given and Dickinson, 1975). Since 1920s, researchers proposed that peat soil depth affected the occurrence of bacteria whereby the numbers decreased in deeper soils. When peat soil depth increased, the number of aerobic bacteria is lower while the anaerobes bacteria are higher as most of the bacteria in soils have resistant structures (Wheatly *et al*., 1976).

Yeast and filamentous fungi can also survive in peat soils which have limited oxygen supply and high salinity (Kurakov *et al*., 2008). Fungi are assumed to be more dominant compared to bacteria (William and Crawford, 1983; Kurakov *et al*., 2008). The density and the survival mechanisms of fungi are much affected by the high salinity content of the soil (Grishkan *et al*., 2003) and the extreme condition of the peat soils itself. The balanced population composition in soil ecosystem can be achieved once the fungi can adapt to this stressful environment (Migahed, 2003). Fungi survived in peat lands as saprobes which produced extracellular enzymes that can decomposed organic matter and degrade simple and complex structural plant materials such as cellulose and lignin (Thormann *et al*., 2001, 2002).

Fungal genera found in peat soils including *Penicillium, Cladosporium, Trichoderma* and *Mucor* (Given and Dickinson, 1975). Thorman and Rice (2007) reported that 601 species of fungi have been identified globally from different peat lands and one of the fungus is from the genus *Fusarium*. They also reported that Ascomycetes and Basidiomycetes were the most common fungal groups found in peat land with 519 species. *Penicillium* spp. were the highest number of species recovered from the Ascomycete groups while *Galerina* spp. were the highest number of species from the Basidiomycetes group.

2.4.2 Functions of microorganism

The soil microorganisms play vital roles in sustaining peat soil health and fertility (Capogna *et al*., 2009). Bacteria can be found in the upper and lower part of the soils and play major role at the early phases of decomposition in which the bacteria decomposed the organic materials, especially when moisture contents are high. Later, fungi dominated the decomposition process (Gupta and Roget, 2004). The organic materials contain among others simple sugars and simple carbon compounds, such as root exudates and fresh plant litter. Besides involved in decomposition process, other groups of bacteria such as nitrogen-fixing bacteria

(Rhizobia) formed mutualistic associations with plants (Hoorman, 2011). These bacteria live in root nodules on legumes to fix nitrogen in the soils. The nitrogen gas from the air were extracted and changed into nitrogen forms that plants can use (Gupta and Roget, 2004).

Fungi can act as a decomposer in acidic environment such as in the peat soils (William and Crawford, 1983) as fungi are equipped with hyphal network which has rapid growth rate, and the hyphae are used to translocate nutrients in the peat land ecosystem (Thormann and Rice, 2006). Fungi also produce extracellular enzymes that can degrade simple leachates and complex structural plant polymers, including cellulose, lignin, and their derivatives (Thormann *et al*., 2002) which assist in decomposition process. Fungi in the peat soils interact with plants in exchange for organic and inorganic compound that help in the carbon cycle (Kamal and Varma, 2008).

2.5 Significance of peat land

2.5.1 Carbon storage

Peat lands have a vital function on ecosystem carbon storage and can stored approximately up to one-third of all soil carbon as well as act as sinks of carbon in atmosphere (Moore, 2002; Smith *et al.*, 2004). The soils managed to store large amount of carbon because the soils have high root-shoot ratios and contain high organic matter (Komiyama *et al.*, 2008). Bragazza *et al.* (2006), reported that peat lands are able to store carbon in long term by accumulation of partially decomposed organic matter in the form of leaf litter and peat layer. A part of carbon taken by the vegetations is released back to the atmosphere but the remaining carbon is stored in living and organic matter and also in peat layers for prolonged periods. The decomposition of the organic matter can produced large amount of gases as carbon dioxide and nitrous oxide into the atmosphere (Chimner and Ewel, 2005). The submerge environment of tropical peat swamp is very suitable for conservation of regional ecosystems and reduction of carbon dioxide gas release to the atmosphere (Wosten *et al.*, 2006).

In recent years, a lot of lowland peat lands are cleared and converted to plant agricultural crops such as oil palm and economically important timbers are logged (Curran *et al.*, 2004). When the peat lands are cleared, large quantities of carbon are released to the atmosphere (Murdiyarso and Adiningsih 2007). Thus, the degradation of tropical peat lands have an impact on the emission of greenhouse gasses (Neuzil, 1995) as the carbon accumulated in the organic matter in peat soils is released, which in large quantities can affect climatic changes.

2.5.2 Hydrological function

Peat land has the ability to retain and store large amount of water (Ingram, 1978) as the high organic content has high water holding capacity. Peat soil has higher infiltration capacity, drainable pore space and hydraulic conductivity, but lower capillary rise, bulk density and plant-available water compared to the other types of soils (Ritzema, 2006).

Peat land helps to reduce flood and drought conditions, as it can act as sponges which stored and released water according to the amount of water around the area, thus maintaining water flows in rivers (Ritzema & Wösten, 2002). Some peat lands have a stable hydrology which can maintain specific amount of water. During dry periods, water is lost to dry areas as well as through evaporation and transpiration. While, during wet periods, peat soils have the capacity to store more water than it usually contain (Demissie and Khan, 1993).

The floodplains of the peat lands can help in controlling floods. Major rivers downstream from their headwaters will create peat land system of floodplains. This floodplains will act as a natural storage reservoirs by flowing the excess water to other wide area that can decrease its depth and speed. Peat lands close to the headwaters of the streams and rivers can slow down rainwater runoff thus prevent sudden flooding into the nearby area and damaging the ecosystem (Page *et al*., 1999; Wosten *et al*., 2006; Wetland International, 2010).

2.6 *Fusarium* species

Fusarium is a hyphomycete fungus from the class Sordariomycetes. The classification of *Fusarium* is based on classification in the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi? id=5506):

Superkingdom : Eukaryota

Kingdom : Fungi

Subkingdom : Dikarya

Phylum : Ascomycota

Subphylum : Pezizomycotina

Class : Sordariomycetes

Subclass : Hypocreomycetidae

Order : Hypocreales

Genus : *Fusarium*

Fusarium species are widely distributed in the environment and have been isolated in temperate, tropical, desert, Arcticand Alpine regions. *Fusarium* species are commonly found in plant residues in cultivated soils and close to the soil surface (Nwanma and Nelson, 1993). This fungus is well known as a saprobe and ubiquitous in soil and act as a decomposer in decaying plant materials (Nelson *et al*., 1983).

Many *Fusarium* species are cosmopolitan soil fungi, associated with plant roots and are able to survive in soil under unfavaourable conditions (Kurakov *et al*, 2008). Thus, *Fusarium* is among well known plant pathogenic fungi and responsible for causing diseases like wilts, blights, root rots, and cankers in many economically important crops (Girish and Goyal, 1986). Among important plant diseases caused by *Fusarium* are Fusarium wilt of banana and oil palms, kernel rot of maize, crown and root rot of tomato and fruit rot on tropical and temperate fruits (Agrios and Beckerman, 2011).

Among *Fusarium* species causing economically important diseases are *Fusarium oxysporum* f. sp. *cubense* (FOC) causal agent of banana wilt, *F. verticillioides*, ear rot of maize, (Oren *et al*., 2003), *F. oxysporum,* crown and root rot of the tomato by (Muslim *et al*., 2003), and *F. semitectum*, crown-rot disease of banana (Knight *et al*., 2008). Three *Fusarium* species, *F. oxysporum, F. proliferatum* and *F. solani* were reported to be the causal agent of stem and root arot of orchid (Latiffah *et al*., 2008).

Fusarium species are also associated with serious human diseases. For example, *F. solani* caused keratitis, onychomycosis, endophthalmitis, and skin and musculoskeletal infections (Mansoory *et al*., 2003). The infection are commonly occurs in patients with acute leukemia and prolonged neutropenia. Skin lesions usually occurred at the trunk and face of the patients (Guarro and Gene, 1995).

Some *Fusarium* species are endophyte. Endophytic fungi live inside healthy plant tissues without causing any damage to the host and form mutual beneficial relationships with the host plant (Petrini *et al*., 1992; Lodge *et al*., 1996). However, some endophytic fungi can harm the host when suitable conditions arise. For example, *F. verticillioides* can caused symptomless endophytic colonization of maize without any visual signs by contaminating the maize kernel before and after kernels development and become pathogenic and causes systemic infections of maize kernels (Bacon and Hinton, 1996).

Fusarium is also toxigenic fungi, produced several types of mycotoxins. Among the mycotoxins produced by *Fusarium* species are fumonisins, moniliformin, trichothecenes and zearalenone which are harmful to human and animal health (Marasas *et al*., 1984; Miller *et al*., 1991). These mycotoxins enter the food chain through contaminated food and feed (Pittet, 1998; Pitt, 2000).

2.6.1 Taxonomy of *Fusarium* species

The genus of *Fusarium* was first introduced by Link in 1809 and the species described was *F. roseum* based on canoe or banana shaped conidia. After Link, there were numerous *Fusarium* species described but many were poorly defined and the type of specimen was no longer available (Booth, 1971; Leslie and Summerell, 2006).

The classification of *Fusarium* was intensively studied by Wollenweber and Reinking (1935). In their classification system approximately 1,000 species were described. The species which showed common similarities were put in the same sections based on primary characteristics such as the shapes of macroconidia, presence and shape of microconidia and the presence of chlamydospores. Sixteen sections were developed namely Arachnites, Arthrosporiella, Discolor, Elegans, Eupionnotes, Gibbosum, Lateritium, Liseola, Macroconi, Martiella, Pseudomicrocera, Roseum, Spicarioides, Sporotrichiella, Submicrocera and Ventricosum in which 65 species 55 varieties and 22 forms were described. The main weaknesses of Wollenweber and Reinking classification system were species identification and descriptions was based on cultural variations, incubation period was not standardized and the culture was not originated from single conidia (Leslie and Summerell, 2006).

Later, Snyder and Hansen (1940, 1941, 1945) also carried out a comprehensive study on the classification of *Fusarium* species. They introduced the used of single spores method for species identification and focused on the morphological similarities of macroconidia to differentiate species. Synder and Hansen reduced the number of species to only nine species namely *F. episphaeria, F. lateritium, F. moniliforme, F. nivale, F. oxysporum, F. rigidiuscula, F. roseum, F. solani* and *F. tricinctum*. Part of Synder and Hansen descriptions of *F. oxysporum* and *F. solani* are widely accepted until today. The lumping of several sections mainly Arthrosporiella, Discolor, Gibbosum and Roseum in *F. roseum* (Synder and Hansen, 1945) was not accepted by many taxonomists.

In taxonomic descriptions by Gordon (in 1930s and 1960s), *Fusarium* species from cereal seeds, various types of plants and soils from temperate and tropical regions were used for species descriptions. Gordon's taxonomic system combined the work of Wollenweber and Reinking as well as Snyder and Hansen. Gordon modified certain section such as Lateritium, Liseola, Elegans and Martiella and 26 species were described (Nelson, 1991).

In 1950, a taxonomic system by Raillo was published. The taxonomic system was based on the characteristics of macroconidia, microconidia and chlamydospores. Single spore technique was used to culture the isolates for species descriptions. In Raillo' taxonomic system, the shape of apical cell was the main character and other characters used were pigmentation and mode of spore formation (Nelson *et al*., 1983).

Booth (1971) published a monograph, The Genus *Fusarium*. The descriptions of *Fusarum* species were based on the characteristics of macroconidia and the morphology of conidiogenous cell, the cells that produced microconidia. Booth also included the information on the teleomorph stage of *Fusarium* and identification keys to differentiate the isolates into sections and species.

Gerlach and Nirenberg (1982) applied the species concept and descriptions of Wollenweber and Reinking in their classification system. They described 78 species that were arranged in sections and provide photographs and drawings which originally from Wollenber and Reinking. Gerlach and Nirenberg emphasized on the morphological differences of the isolates which were cultured on eight different media, but the cultures originate from single spore method (Nelson, 1991; Leslie and Summerell, 2006).

Nelson *et al*. (1983) published a manual on *Fusarium* species identification which was based on a combination of several classification systems by other researchers and their own work. For species identification, the cultures are grown on standardized media as described in the manual and uniform morphological characters of macroconidia, microconidia, conidiosphores and chlamydosporesare are among the important characters observed.

Leslie and Summerell (2006) published a manual which is a compilation of species descriptions of several researchers. The manual provides descriptions of 70 *Fusarium* species. Media preparations, techniques for isolations and maintaining *Fusarium* isolates as well as morphological, biological and phylogenetic species concept are also included.

2.7 Species concept for identification of *Fusarium* species

The number of species reported very much depends on the species concept applied. Therefore, to accurately identify and characterize species in the genus *Fusarium,* three species concept are widely used, namely morphological, biological and molecular species concept.

2.7.1 Morphological species concept

Morphological species concept is based on the morphological variations shown by an individual but represent variations within the whole species. This concept is widely used because of their important in early classification of biodiversity. The main strength of this concept is that it has been applied broadly to many fungal taxa and had been used more than hundred years for identification of fungal species (Taylor *et al*., 2000).

Morphological species concepts are widely used by many researchers since 1930s and earlier publications of *Fusarium* taxonomic systems were based on morphological characteristics such as Wollenweber and Reinking (1935), Synder and Hansen (1954), Booth (1971), Gerlach and Nirenberg (1982) and Nelson *et al*. (1983). Morphological species concepts for *Fusarium* are mainly based on primary and secondary characters. The primary characters are macroconidia, microconidia, chlamydospores and conidiosphores, while the secondary characteristics are the pigmentation, colony appearance and growth rate (Leslie and Summerell, 2006).

Macroconidia is the most important characters for *Fusarium* species identification in which the size, number of septation and the shapes of apical and