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Effect of Soil Physicochemical Factors on the Efficacy of Potential Antagonists Against Pathogenic *Ganoderma* of Oil Palm

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ABSTRACT

The studies on biocontrol agent against *Ganoderma* causal pathogen of oil palm basal stem rot have been carried out progressively in Malaysia and many other countries. Several non pathogenic microbes have been reported to inhibit the growth of *Ganoderma* sp. effectively through plate culture analysis in laboratory condition but, when these antagonists were applied in the field condition, often it does not give satisfactory results. Natural environmental conditions may have contributed to the occurrence of this problem especially in the soil. The objectives of this study was to analyze the soil properties in the oil palm plantations such as moisture content, soil microorganisms, soil pH and soil nutrients and to investigate the effect of pH, nutrients, and soil microorganisms on the efficacy of potential antagonists against *Ganoderma* sp. Three potential antagonists, *Penicillium citrinum*, *P. pinophilum* and *Burkholderia* sp. were used in this study. The analysis of soil from non-infected and *Ganoderma* infected oil palm plantations in Sarawak was carried out in order to determine the moisture content, pH, nutrients, and microorganisms. Results obtained in this study indicated that the soil moisture content and pH was highly correlated with the soil depth in both non-infected (r^2= 0.97) and *Ganoderma* infected oil palm areas (r^2= 0.99). No specific pattern of correlation was recorded between soil moisture content, pH, nutrients and microbes with the occurance of *Ganoderma* infection in oil palm plantations. Different pH levels and nutrients sources

were tested in order to observe the ability of mixed antagonists against *Ganoderma* sp. It was discovered that the antagonists performed best in controlling the *Ganoderma* sp. on media with pH 5.0. Extreme acidic and basic medium disrupted the cellular activity of the antagonists leading to cell lysis or death. Macroelements such as nitrogen, phosphate, potassium, and magnesium have significant effects on the efficacy of the antagonists against the *Ganoderma* sp. The antagonists perfomed optimally on media containing sucrose, glycine, potassium chloride, and all sources of magnesium and phosphate which resulted with higher inhibiton percentage of radial growth of the *Ganoderma* sp. 71.74%, 69.44%, 54.76%, 53% and 47% respectively. The studies on the effect of soil extract agar (SEA) on the antagonistic ability of the antagonists against the *Ganoderma* sp. were also carried out which indicated that SEA of P10 from Sempadi plantation was the best media compared to other SEA with reduction radial growth of *Ganoderma* 68.83%. Different methods of antagonists inoculation were tested on *Ganoderma* sp. growth which were simultaneously, after 2 and 5 days inoculation of *Ganoderma* sp. It was found that the simultaneous inoculation of antagonists and the *Ganoderma* sp. was the best method to control the pathogen growth effectively with highest reduction radial growth of *Ganoderma* sp. of 68.83% . The effect of volatile compound of soil microbes on the efficacy of mixed antagonists against *Ganoderma* sp. were also tested. High reduction radial growth of *Ganoderma* sp. (48.33%) were recorded on medium without the presence of volatile compounds. In medium with the presence of volatile compounds, the radial reduction of *Ganoderma* sp. ranged from 18.78% - 32.32%. It is suggested that the major factors contributing to the inefficacy of the antagonists in the field might be due to the presence of volatile compounds produced by soil microorganisms which rapidly enhance the growth of the *Ganoderma* sp. The action of the antagonists alone might be insufficient to control the pathogen. Further studies should be carried out on the volatile compound

produced by soil microorganisms especially in non-infected and *Ganoderma* sp. infected oil palm plantation areas in order to detect specific compound which could trigger the growth of the pathogen.

Kesan Faktor Fisikokimia Tanah ke atas Keberkesanan Antagonis Berpotensi Mengawal Patogen *Ganoderma* pada Kelapa Sawit

Hasma Binti Mat Nor

ABSTRAK

Kajian ke atas agen kawalan biologi untuk mengawal *Ganoderma* patogen penyebab reput pangkal batang kelapa sawit telah giat dijalankan di Malaysia dan negara-negara lain. Beberapa mikrob yang tidak patogenik telah dilaporkan menghalang pertumbuhan *Ganoderma* sp. secara efektif melalui piring kultur analisis di dalam makmal tetapi, apabila antagonis tersebut digunakan di lapangan, ia tidak menunjukkan keputusan yang memuaskan. Keadaan persekitaran semulajadi mungkin menyebabkan masalah ini berlaku terutama di dalam tanah. Tujuan kajian ini adalah untuk menganalisa keadaan tanah dalam ladang kelapa sawit seperti kandungan kelembapan, microorganisma, pH dan nutrien dan untuk mengkaji kesan pH, nutrien, dan mikroorganisma dalam tanah ke atas keberkesanan antagonis yang berpotensi mengawal *Ganoderma* sp. Tiga antagonis, *Penicillium citrinum*, *P. Pinophilum* dan *Burkholderia* sp. telah digunakan dalam kajian ini. Analisis tanah dari ladang kelapa sawit yang tidak dijangkiti dan dijangkiti *Ganoderma* di Sarawak telah dijalankan untuk menentukan kandungan kelembapan tanah, pH, nutrien, dan mikroorganisma. Keputusan diperoleh dalam kajian ini menentukan bahawa kandungan kelembapan tanah mempunyai hubungkait yang tinggi dengan kedalaman tanah di kedua-dua kawasan kelapa sawit yang tidak dijangkiti (r^2= 0.97) dan dijangkiti *Ganoderma* (r^2= 0.99). Tiada corak hubungkait yang spesifik dicatatkan antara kandungan kelembapan tanah, pH, nutrien, dan mikrob dengan kejadian jangkitan *Ganoderma* dalam ladang kelapa

sawit. Tahap pH dan sumber nutrien yang berbeza telah dikaji untuk melihat kemampuan campuran antagonis mengawal *Ganoderma* sp. Didapati bahawa antagonis bertindak dengan baik dalam mengawal *Ganoderma* sp. pada media pH 5. Media yang terlampau berasid dan beralkali boleh menganggu aktiviti sel antagonis mengakibatkan sel mengecut dan mati. Makroelemen seperti nitrogen, fosfat, potasium dan magnesium juga memberi kesan ke atas keberkesanan antagonis melawan *Ganoderma* sp. Antagonis bertindak dengan optimum pada media yang mengandungi sukrose, glycine, potasium klorida dan semua sumber magnesium dan fosfat yang menyebabkan peratus pembantukan radial pertumbuhan patogen meningkat masing-masing 71.74%, 69.44%, 54.76%, 53% dan 47%. Kajian ke atas kesan agar ekstrak tanah (SEA) terhadap keupayaan antagonis melawan *Ganoderma* sp. juga telah dilakukan yang mana menunjukkan bahawa SEA dari P10 dari ladang Sempadi adalah media yang terbaik berbanding SEA lain dengan peratus pengurangan radial koloni *Ganoderma* sp. 68.83%. Kaedah inokulasi antagonis yang berlainan telah di kaji pada pertumbuhan *Ganoderma* sp. iaitu secara serentak, selepas 2 dan 5 hari *Ganoderma* sp diinokulasi. Didapati bahawa inokulasi antagonis dan *Ganoderma* sp. secara serentak adalah kaedah yang terbaik untuk mengawal pertumbuhan pathogen secara berkesan dengan peratusan pengurangan radial pertumbuhan *Ganoderma* sp. 68.83%. Kesan komponen meruap dari microorganisma dalam tanah ke atas keberkesanan campuran antagonis untuk mengawal *Ganoderma* sp. juga telah dikaji. Peratus pengurangan radial pertumbuhan *Ganoderma* sp. telah direkodkan tinggi (48.33%) pada media tanpa kehadiran komponen meruap. Dalam media yang mempunyai komponen meruap, peratus pengurangan radial *Ganoderma* sp. adalah dari 18.78% - 32.32%. Telah dicadangkan bahawa faktor utama terlibat dengan ketidakberkesanan antagonis di lapangan mungkin disebabkan oleh kehadiran komponen meruap yang dihasilkan oleh microorganisma dalam tanah yang menggalakkan pertumbuhan *Ganoderma* sp. lebih

singkat. Tindakan antagonis sahaja mungkin tidak mencukupi untuk melawan patogen. Kajian lanjut perlu dilakukan ke atas komponen meruap yang dihasilkan oleh mikroorganisma dalam tanah terutamanya di ladang kelapa sawit yang tidak dijangkiti dan dijangkiti *Ganoderma* sp. untuk mengesan komponen spesifik yang mempercepatkan pertumbuhan patogen.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
Al	Aluminium
$NH_4H_2PO_4$	Ammonium Dihydrogen Phosphate
BSR	Basal Stem Rot
Ca	Calcium
CFU	Colony Forming Unit
Cu	Copper
CMC	Carboxymethyl Cellulose
Fe	Ferum
HCl	Hydrochloric Acid
HNO_3	Nitric Acid
K	Potassium
KH_2PO_4	Potassium Dihydrogen Orthophosphate,
K_2HPO_4	Dipotassium Phosphate
KNO_3	Potassium Nitrate
K_2SO_4	Potassium Sulphate
Mg	Magnesium
MgCl	Magnesium Chloride
MgO	Magnesium Oxide
$MgSO_4$	Magnesium Sulphate
$MgSO_4.7H_2O$	Magnesium Sulphate Heptahydrate
Na	Sodium

NaOH	Sodium Hydroxide
$NaNO_3$	Sodium Nitrate
Pb	lead
PDA	Potato Dextrose Agar
NaH_2PO_4	Sodium Dihydrogen Phosphate
PDB	Potato Dextrose Broth
SEA	Soil Extract Agar

CHAPTER 1

INTRODUCTION

1.1 Research Background

Malaysia is known as the world's second largest producer and exporter of palm oil after Indonesia (USDA, 2012) and it currently accounts for 39% of world palm oil production and 44% of world exports (MPOB, 2014). Basiron (2007) reported that 4.05 million hectares of areas in Malaysia were planted with oil palm. Until now, the expansion areas of oil palm plantation continue to increase rapidly since the demands of palm oil keep arising throughout the years.

The achievement of Malaysian palm oil industry in global market contributes towards the country's economy. Various efforts continuously adopted in order to maximize the palm oil production. Starting as an ordinary ornamental plant, oil palm now develops into industrial crop. The oil palm was initially introduced in Malaysia as an ornamental plant in 1871 (Basiron & Chan, 2004). It was then commercially planted as an oil crop in 1971 (Sundram *et al*., 2003). The investment in the oil palm industry is very profitable compared to other oil crops because of their highest oil production. The average of palm oil production is approximately 4-5 tonnes per hectare annually which is ten times the yield of soybean oil.

Malaysian oil palm industry has always placed strong emphasis on research and development of the various aspects of oil palm cultivation and management, from planting techniques, waste management technologies, by product utilization to palm oil product utilization to palm oil product development (Singh *et al*., 1999). This development has resulted with various productions of palm oil products, especially for foods, medications, cosmetics, and alternative biodiesel.

Basal Stem Rot (BSR) disease caused by pathogenic *Ganoderma* sp. is known as the major problem in Malaysian oil palm plantation that leads yield reduction. *Ganoderma* sp. produce enzymes that degrade the oil palm tissues and affect the xylum, thus causing serious problems to the distribution of water and other nutrients to the top of the tree (Su'ud *et al*., 2007). With no known cure at present, it is the major disease of oil palm and, therefore of great economic importance to the Malaysian oil palm industry (Sapak *et al.*, 2008). Previously, *Ganoderma* sp. was only found in older plants, but in recent years it has been found in younger plants where symptoms appear earlier and are more severe, leading to greater replanting (Idris *et al*., 2004). According to Turner (1981), BSR can kill more than 80% of stands by the time they are half-way through normal economic life.

In order to prevent greater losses of oil palms yields, various studies on control methods related to BSR disease have been carried out progressively. Cultural, biological, chemical and land clearing practice are the basic strategies that were implemented in oil palm plantation but unfortunately, these control measures are less effective (Flood *et al*., 2000). Azadeh *et al.* (2010) reported that the current available control measures are only aimed at

minimizing the incidence of BSR in replanting, prolonging the productive life of infected palms, and delaying the progress of *Ganoderma* sp. infection and the results in field application are still unsatisfactorily. The characteristics of *Ganoderma* sp. with many forms of resting stages including resistant mycelium, basidiospores, chlamydosphores, and pseudosclerotia (Susanto *et al*., 2005) may have contribute to the failure in controlling the disease.

The exploration on biocontrol agent against *Ganoderma* sp. is very crucial in order to inhibit their early development stage in a short time without affecting the growth of the oil palm, yields, and also the environment. Studies using different microbes as antagonists are done in several research institutions including MPOB, UPM and UNIMAS. Several promising microbes have been proven to have antagonistic effect on pathogenic *Ganoderma* in laboratory condition and controlled environment such as *Trichoderma harzianum, Gliocladium viride* (Susanto *et al*., 2005), *Burkholderia cepacia* and *Pseudomonas aeruginosa* (Sapak *et al*., 2008). The mechanisms involved in plant pathogen suppression by these potential antagonists were categorized based on nutrient competition, amensalism, microbial antagonism, parasitism and systemic induced resistance (Garbeva *et al*., 2004; Chen *et al*., 2012).

1.2 Problem Statement

Despite various findings on the effectiveness of potential antagonists against pathogenic *Ganoderma* sp. through culture plate analysis and controlled environment, it is often still does not provide satisfactory results in preventing the occurrence of BSR disease incidence when potential antagonists are applied directly in the field. The lack of appropriate screening methods for microorganisms which have the potential to be used for disease control in diverse soil environment is believed to be one of the most important factors that influence biocontrol failure (Merriman and Russel, 1990). Futhermore, Sarim (2013) reported that very little attention is given to the biology of the soil environment in oil palm management. As a result, the interactions between biotic and abiotic factors of the soil environment are unintentionally neglected.

The natural environment in the field plays significant roles in promoting the effectiveness of antagonists against pathogenic *Ganoderma*. Gadd *et al*. (2001) reported that in nature, fungi are rarely encounter conditions that allow their optimal growth due to the nutritional and other environmental constraints. Previous researches reported that the environmental conditions, soil type, host plant cultivar, variation in pathogen response to inoculated antagonists and inoculation strategy significantly influence the efficacy of antagonists (Deacon, 1991; Dik *et al*., 1998).

Furthermore, the interactions between microorganisms and plants in soil environments are very complex and with a few exceptions such as rhizobia and to a much lesser extent

mycorrhizal fungi, have proven difficult to manage which caused variable responses to inoculants (Richardson and Simpson, 2011). As a consequence, details information on the association of soil microbial communities with environmental factors and ecosystem function has often proven difficult to be accessed accurately (Barns *et al*., 1998).

Rousk *et al.* (2009) reported that soil pH is one of the most influential factors that affect microbial soil community which is strongly influences abiotic factors such as carbon availability, nutrient availability, and the solubility of metals. Since biotic and abiotic factors are correlated with each other, the biomass composition of fungi and bacteria are also changed according to pH in the soil (Fierer and Jackson, 2006).

Another vital factor that influences the successful colonization of biocontrol agent in rhizosphere is nutrient competition. Although it is very difficult to observe directly, a lot of indirect evidence suggests that competition between pathogen and non-pathogens for nutrient resources is important for limiting disease incidence and severity (Pal and Gardener, 2006).

The variable interactions between both biotic and abiotic factors in the soil and plant host pathogen warrant more efforts on details assessment and management of soil microbial community structure and environment in order to achieve successful suppression of plant pathogens (Chen *et al*., 2012). Detail information on the performance of biological control agent in biotic and abiotic factors which independently or in combination, influence the efficacy in suppressing BSR disease of oil palm is prerequisite to implement in large-scale

areas (Ryan *et al*., 2004). Hence, knowledge of the ecological interactions taking place in the soil and root environment is required to predict the conditions under which the biocontrol agents can function (Deacon, 1994) and finally the effective biocontrol formulation would be achieved in suppressing pathogenic *Ganoderma* infection in the oil palm.

1.3 Objectives

In order to achieve the efficacy of biocontrol agent against BSR of oil palm, this study was carried out:

1) To study and analyze the soil properties of several oil palm plantations including soil moisture content, soil microorganisms, soil pH, and soil heavy metals.

2) To investigate the effect of soil pH, nutrients and microorganisms on the efficacy of potential antagonists against *Ganoderma* sp.

CHAPTER 2

LITERATURE REVIEW

2.1 Oil Palm (*Elaeis guineensis* Jacq.)

Oil palm (*Elaeis guineensis* Jacq) is a tropical tree crop belonging to the Palmae family. It is one of the major sources of the world supply of oils and fats after soybean (Wahid *et al*., 2005). The oil palm is widely planted in large scale, aiming for the industrial production of its vegetative oil (Verheye, 2010). The oil product obtained from the oil palm fruits are from two types which are palm oil from the flesh or mesocarp and also palm kernel oil from the seed or kernel inside the hard shelled mesocarp (Chong, 2010). Palm oil is used mainly for food, while palm kernel oil goes mainly into the oleochemical industry for making soaps, detergents and toiletry products (Basiron, 2007). Currently, most of the world's production of palm oil comes from South-East Asia, in particular Malaysia and Indonesia

2.1.1 Origin and distribution

Oil palm is native to the tropical rainforest region of West Africa (Poku, 2002). The centre of origin of oil palm is the West and Central African coastal belt between Guinea and

northern Angola (Verheye, 2010) which was based on the discovery of deposited fossil *Elaeis* pollen grains deep in the Niger River Delta in West Africa (Bergert, 2000). The oil palm was then introduced to South America with the advent of the slave trade in the early seventeenth century and abundant groves are found in Brazil (Ng, 1972). The primary record shows that four palms, two from Amsterdam and two from Reunion or Mauritius were planted in Bogor Botanical Garden, Indonesia in 1848. The progenies of this palm were brought to Deli in Sumatra, from seeds of the Deli palms which was the first commercial plantation started by Hallet in 1911. At that time, Deli palms were also established in Selangor, Malaysia and the seeds of these palms were used to plant oil palm in the first estate known as Tennamaram in Selangor (Ng, 1972). The development and extension of oil palm in Malaysia and Indonesia has promoted both countries as the main production areas of palm oil and palm kernel production (Verheye, 2010).

2.1.2 Botanical description

Elaies guineensis Jacq is a large, pinnate-leaved palm and typically reaching up to 20 m tall (Corley and Tinker, 2003). The trunk is stout, erect, and fairly uniform column matured palm and is covered by persistent leaf bases. When the leaf bases slough off, scars are found to occupy a large portion of the stem surface (Ng, 1972).

The leaves can reach between 2-5 m in length and one palm can ideally carry 45-50 opened leaves in various stages of development (Bergert, 2000). The stalk is hard and fibrous. The leaflet which attached laterally and supported by a stiff midrib is known as rachis. The

petiole is shorter and thicker than the rachis and bears short spines instead of leaflets (Bergert, 2000). The life of a mature leaf after unfurling is about 2 years and the leaf area of a mature palm is around 400 m^2 (Verheye, 2010).

The flowers are produced in dense clusters and each individual flower is small, with three sepals and three petals. The separate upper and lower ranks of leaflets on the rachis give the palm a characteristic of untidy appearance (Corley and Tinker, 2003). An inflorescence primordium is produced in the axil of each lead at the time of leaf initiation. The inflorescence reaches the cental spear stage in two years and a further 9-10 months is required to flowering and anthesis. Each flower primordium is a potential producer of male and female inflorescence (Verheye, 2010). Male and female inflorescences form separately on the same palm which is called monoecious (Ng, 1972). The female bunch bears about 2500-3000 fruits borne on 100-120 spikelets attached to a peduncle from the axil of a frond (Basiron and Chan, 2004).

Oil palm fruit is sessile drupe varying in shape from nearly spherical to ovoid or elongated (Ng, 1972). The fruit takes five to six months to mature from pollination to maturity. It comprises an oily, fleshy outer layer (pericarp), with a single seed (kernel) which also rich in oil. Unlike its relative, the coconut palm, the oil palm does not produce off shoots. The propagation is by sowing the seeds (Corley and Tinker, 2003).

2.1.3 Ecology and growing conditions

Oil palm is normally grown in the rainy tropical lowland areas (Verheye, 2010). It requires suitable climate conditions to grow optimally such as a relatively stable high temperature and continuous moisture throughout the year. The suitable temperature required by the trees to achieve maximum yields has been recommended with mean temperature ranging from 29-33^{o}C and a mean of minimum temperature from 22 - 24^{o}C (Ndon, 2006; Obahiagbon, 2012). Cold temperature below minimum the range affected oil palm growth, bunch development and also reduce the yields production (Adzimah and Seckley, 2009). The crop grows mostly in tropical lowlands below 400 m altitudes (Verheye, 2010). Paterson *et al*. (2013) reported that oil palm grows less well at higher altitudes (above 500-600 m) and latitudes above 10^{o}. Hartley (1988) also reported that at high altitude, 500 m and at low temperature the palms bearing fruits at least a year later than palms grown at usual altitude.

A total 2000 mm rainfall or more per year is required for the oil palm to maintain a continuous supply of soil moisture content (Obahiagbon, 2012). Verheye (2010) reported that dry periods of more than 2-3 months do not specifically damage vegetation growths, but it seriously affects the production and quality of the fruit bunches. Caliman and Southworth (1998), concluded in their findings that water deficit of 100 mm in a particular year resulted in fresh fruit bunches (FFB) yield reduction of 8-10% in the first following year and by 3-4% in the second year. Besides that, oil palms are only able to establish in forest climate either on river banks or where an opening is created to allow sufficient

sunlight. In forested areas, oil palm cannot grow well in the shade of the larger trees and forest disturbance must occur to open the canopy for germination of oil palm seedlings (Bergert, 2000).

Soil physical and chemical properties are also very crucial for the growth of oil palm. The soil which permits extensive root development, firm anchorage and also stores plentiful of water and plant nutrients is required for oil palm growth. Ng (1972) stated that the oil palm grows well on a wide range of tropical soils ranging from loamy sand to heavy clays and deep peats. Chemically, most soils planted with oil palm are found in acidic condition ranging from pH 4-7 (Ng, 1972). Within a certain set of climatic conditions, soil variation can cause appreciable differences in yield due mainly to impeded drainage leading to disease, shallow rooting and moisture stress because of shallow soil profile (Hew and Ng, 1968; Ng, 1972). Ndon (2006) reported that soil physical properties are more important than soil chemical properties because the shortage of nutrients can be amended by addition of mineral fertilizers to improve soil chemical properties but, soil physical limitations is very difficult to rectify. Hence, physical soil properties such as the depth, texture and structure of the soil contribute to the suitability of successful oil palm cultivation (Ng, 1972).

2.1.4 Economic importance

Oil palm is very important as a commercial oil crop. The fruits contain highest content of vegetative oil compared to other oil seed crops like soybean, rapeseed, and sunflower

(Shanmuganthan and Narayanan, 2014). Among the other competing oil seed crop, the oil extracted from palm seed kernel still holds the largest share which is 27% of the world's oil and fat consumption (Shanmuganthan and Narayanan, 2014). Oil palm is the most efficient oil crop in the world. It produces the highest yield per hectare which is almost 10 times higher than soya bean, 6.9 times than sunflower seed and 6.3 times than that of rapeseed (MPOB, 2014).

Generally, various products can be made using different parts of oil palm. It is classified into three categories which are the oil derived from the fruits and seeds of the oil palm, the drinks from the 'sap' of the palm and products that utilize the leaf stem and foliage of the plant such as basket and household item (Bergert, 2000). However, the oil derived from oil palm fruit and seeds are the most important product in term of economic value (Bergert, 2000).

Palm oil can be used for making edible products and non-edible products. Some of the edible products are cooking oil, margerines, baked goods and sweets while non-edible products are biodiesel, detergent and cosmetics (UNEP and UNESCO, 2007; UNEP, 2011). Instead of palm oil, the biomass raw materials from crude palm oil, empty fruit bunches, palm mesocarp fibre and palm kernel shells also can be processed into value added products such as fertilizers, fuel pellets and briquettes, pellets, composite fibre boards, paper pulp, and paper products (Hoong, 2011).

2.2 Malaysian oil palm industry

In Malaysia, the oil palm was commercially exploited as an oil crop starting from 1911 since the first oil palm estate was established (Basiron and Chan, 2004). Began with a slow performance, the oil palm plantation areas was impressively expanded throughout the years. In 2012, Malaysian has over 5 million hectares of oil palm areas (USDA, 2012) compared to only 54,000 hectares in 1960 (Basiron, 2007).

Palm oil is widely consumed around the world in term of thousands of different products including processed foods, pharmaceutical and detergent products. The high palm oil consumption has leading to the great production of world palm oil with around 45.3 million tonnes per year (Dalingeer, 2011). As in 2012, Malaysia is in the second rank of the world major producer and exporter of palm oil after Indonesia with total production of 19.4 million tones and total exports of 17.6 million tonnes (MPOB, 2014). MPOB (2014) also reported that Malaysian palm oil is exported to more than 170 countries worldwide and the export earnings in 2013 are RM 61.29 billion.

The success of Malaysian palm oil industry was accompanied by the implementation of initiatives in the marketing of palm oil products, research and development and a conducive regulatory framework by three main institutions known as Palm Oil Registration and Licencing Authority (PORLA), Malaysian Palm Oil Board (MPOB) and Malaysian Palm oil Promotion Council (MPOPC) (Simeh and Tengku Ahmad, 2001).

2.3 Pests and diseases of oil palm

Although oil palm industry has been shown to have remarkable achievements in Malaysian economies, its cultivation processes and management has been surrounded by various problems and limitations which cause severe yield losses. Like other crops, oil palm is also prone to attack by a number of pests and diseases (Ariffin *et al*., 2000). In poorly controlled infected plantations, the yield losses can be as high as 50% or more as compared to the potential yields (Verheye, 2010)

Pests of oil palm can be classified into insects, diseases and vertebrates (Ariffin and Basri, 2000). The main insect pests in oil palm consist of leaf defoliators such as bagworms, nettle caterpillars and crown attacker such as Rhinocerus beetle and bunch moth (Ariffin and Basri, 2000). Among important diseases of oil palm were Freckle (Cercospora leaf spot) and blast which were firstly recorded in West Africa, Corticum leaf rot in Republic of Congo, Vascular wilt, Armillaria root and trunk rot in Zaire, Marasmius bunch rot in Malaya and Sabah, sudden wilt in Colombia, Peru, Brazil and Equador, spear rot in Africa, Malaysia, and South America, and basal stem rot in South East Asia especially in West Malaysia and North Sumatra (Aderungboye, 1977). However, among all of oil palm pests and diseases, only basal stem rot was reported as the most destructive disease in Malaysian oil palm plantation which requiring urgent solution (Idris and Ariffin, 2005) .

2.4 Basal stem rot disease

Basal Stem Rot (BSR) disease is the most serious oil palm disease in Southeast Asia especially in Malaysia and Indonesia which destroy thousands of hectares of oil palm plantings (Anderson and May, 1979; Azahar *et al*., 2011). Lower incidence was recorded in Africa, Papua New Guinea and Thailand (Idris *et al*., 2004).

2.4.1 Causal agent of BSR disease

Basal stem rot disease is caused by species of *Ganoderma* (Idris and Ariffin, 2005). The genus *Ganoderma* belongs to the family Ganodermataceae, which causes white rot of hardwoods in many woody plants by decomposing lignin as well as cellulose and related polysaccharides (Hepting, 1971). In Malaysia, Thompson (1931) initially identified the pathogen as *G. lucidum*. Turner (1981) listed fifteen species of *Ganoderma*, which have been reported as likely pathogens from different locations worldwide to be associated with BSR disease and among them, seven species, *G. applanatum* (Pers.) Pat., *G. boninense*, *G. chalceum* (Cooke) Steyaert, *G. lucidum* (W. curt. Et. Fr.) Karst, *G. miniatocinctum* Steyaert, *G. pseudoferreum* (wakef.) Overh. And Steinmann, and *G. tornatum* (Pers) Bres. were reported from Peninsular Malaysia (Idris and Ariffin, 2005). Ho and Nawawi (1985) concluded that all *Ganoderma* sp. isolates from diseased oil palm from various locations in Peninsular Malaysia were all the same species *G. boninense* (Ariffin *et al*. 2000). Idris *et al.* (2001) considered that two other species, *G. miniatocinctum* and *G. zonatum* were also

pathogenic while *G. tornatum* not pathogenic and only found on dead palms which presumed to be saprophytic (Corley and Tinker, 2003).

2.4.2 The occurrence of BSR disease

The occurrence of BSR disease in the oil palm was first reported by Thompson (1931) and was initially thought to be economically unimportant as their occurrence were only discovered on oil palms over 25 years old (Ho and Nawawi, 1985; Latiffah and Ho, 2005). However, continuous rotation of oil palm plantation in the same field has promoted the disease to infect the oil palm as young as 1 to 2 years old (Singh, 1991; Latifah and Ho, 2005). The disease is well developed in the coastal area compared to inland areas. In Malaysian coastal areas, Lim *et al.* (1992) reported that the average of 50% yields losses from 80% of 13-year-old plantings. This situation was attributed by planting in previous coconut stands (Turner, 1981; Latiffah and Ho, 2005) and also the nature and water table of the soil (Turner, 1981; Khairudin, 1990; Latiffah and Ho, 2005). Although high disease incidence was recorded in coastal area, typical levels of disease incidence of 30% on 13-year-old palms in both inland and peat soils were also discovered (Rao *et al*., 2003; Cooper *et al*., 2011).

2.4.3 Symptom of BSR disease

The main symptoms of BSR are decay of the bottom of the stem from where basidiocarps emerge and often involve the decay of the roots (Hushiarian *et al*., 2013). Decay leads to a restriction of water and nutrient supply to the aerial parts of tree, causing symptoms resembling those of water stress and nutrient imbalance (Turner and Gillbanks, 1974). At the early stage of infection, the disease symptoms progress is very slow and difficult to be detected because the infection only occurs inside palm stem and no physical symptoms appeared externally (Naher *et al*., 2013). Turner (1981) reported that the earliest external symptoms of BSR only occur in the foliage which generally after at least half of the cross-sectional area of the stem base has been killed by the pathogen.

Generally, the first foliar symptoms of BSR are the presence of excessive number of spear leaves compare to healthy palm which normally has two to three spears per month and much paler green in colour (Turner 1981). The older leaves become one-sided yellowing or mottling followed by necrosis and finally dry and die (Singh, 1990). The dried leaves later snap at the petioles and hang down encircling the upper part of the stem and eventually will drop off giving a condition of palms devoid of older leaves (Turner, 1981). The basidiocarp of *Ganoderma* begins to develop at the lower end of the trunk, close to the soil level. Singh (1991) reported that occasionally, the death of the infected palm occur within six to twelve months after the foliage symptoms appeared.

2.5 BSR control and management strategies

Great efforts have been made up on research and management strategies in controlling *Ganoderma* infection in oil palm plantation. However, there is still no effective method that is able to control the disease from spreading (Sariah *et al*, 2003). The available methods to control *Ganoderma* diseases of perennial crops are only aimed at either delaying the progress of infection, or prolonging the productive life of the oil palm trees (Sankaran *et al*., 2005). Soepena *et al*. (2000) reported that variety of methods is required for an integrated approach to control the disease. Three main methods that were applied in controlling the disease include cultural, chemical, and biological control.

Some of the previous treatments that claim to be effective in managing *G. boninense* are preventive treatments such as soil drenching, clean clearing, crop rotation, fallow period, burning and windrowing (Susanto *et al*., 2005) and ameliorative treatments by using different types of fungicide and application methods, surgery, biological control and attempt to develop resistance genotypes (Sariah and Zakaria, 2000).

2.5.1 Cultural control

A proper cultural practice play significant roles in controlling the disease from spreading especially before the tree replanting was carried out. Several methods that have been used in controlling BSR disease are clean-clearing techniques (Idris *et al*., 2004), sanitation

(Ariffin and Idris, 2002), surgery and soil mounding (Ariffin and Idris, 2002), digging trench (Wakefield, 1920) and also collecting basidiomata of *Ganoderma* from diseased palms (Ariffin and Idris, 2002).

Clean clearing procedure which emphasis on the removal of all stumps as well as stems of oil palm prior to field transplanting appears to be the most practical methods in reducing the disease incidence (Aderungboye, 1977). This attempt involves complete destruction of previous crop which is carried out by excavation and burning, followed by uprooting to bring the surface of any rotting tissue in the stumps due to *Ganoderma* infestation, which can be infection foci for the new planting (Turner and Gillbanks, 2003).

In existing plantings with a high disease incidence of *Ganoderma*, regular inspection is necessary in order to ensure possible treatment at an early stage of infection (Flood and Hasan, 2005). Surgery was carried out through manual excision of large and discrete lesions of infected oil palm. However, the survival of the treated palm is highly depends on the early stage of *Ganoderma* infection and small size of the lesion (Chung, 2011). In most cases, surgery is unsuccessful when it was applied on the oil palm that severely damaged with large disease lesion and lesion extended below ground including infected root masses (Chung, 2011). Turner (1976) also reported that surgery has been found unsatisfactory due to physical collapse of treated palms.

2.5.2 Chemical control

Chemical methods in combination with soil amendments form short-term solutions for managing the disease and improving productivity (Sankaran *et al*., 2005). Several chemical treatments that were applied in BSR infected oil palm include drenching of fungicides, trunk injection fungicides (Chung, 1990) and pressurized trunk injection (Idris *et al*., 2002). A number of systemic fungicides have been applied by various workers such as triadimenol (Chung, 1990), tridemorph and dazomet (Ariffin, *et al*., 2000), and hexaconazole (Idris *et al*., 2010) but the results are inconclusive. Majority of systemic fungicides were only managed to prolong the economic life of BSR infected palms, but failed to completely cure the disease (Chung, 2011). Moreover, the application of chemical control has raised problems such as environmental pollution and resistance of disease causative organisms to fungicides (Suryanto *et al*., 2012).

2.5.3 Biological control

Biological control approach against BSR has attracted special attention of many researchers especially from palm oil producing country. The interest on biological control study was increased due to the fact that they can reduce the reliance on chemicals and form a sustainable long term solution to disease management with long term benefits to both the oil palm industry as well as the effect on the environment (Chung and Sharma, 1999).

2.6 Biocontrol agents

Natural soil ecosystems, even in disturbed agriculture ecosystems, contain a certain spectrum of biodiversity which is considered important in protecting plant from stress factors including disease infection (Vilich and Sikora, 1998). This factor has attributed researchers to find effective biocontrol agents among the soil microorganisms including mychorhiza. Microbes such as *Aspergillus*, *Fusarium*, *Penicillium*, *Gliocladium*, *Trichoderma, Actinomycetes and Bacillus* (Soepena *et al*., 2000) have been reported as potential agents to control BSR disease. Ariffin *et al*. (2000) reported that the most promising antagonists against *Ganoderma* were *Trichoderma, Aspergillus* and *Penicillium*. Some of endophytic microorganisms such as *Serratia, Burkholderia, Pseudomonas,* and *Fusarium* also have been found to induce systemic resistance in plants and shown biological traits like antibiotic activity and lysis (Kloepper *et al*., 1992).

2.6.1 Characteristics of biocontrol agents

Generally, biocontrol agent can impair the development of soil borne plant pathogen and consequently inhibit or reduce disease severity (Idris and Ariffin, 2005). The selection of biocontrol agent are mostly depends on several characteristics or modes of action against plant pathogen like competition, antibiosis, parasitism, induce resistance, and plant growth promotion along with highly specialized mechanisms such as hypovirulence (Whipps and McQuilken, 2009).

2.6.2 *In vitro* test of biocontrol agents

The successful application of several potential biocontrol agents in controlling *Ganoderma* sp. through *in vitro* studies have been reported by many researchers including Dennis and Webster (1971), Sapak *et al*. (2008), and Sundram *et al*. (2011). Sundram *et al*. (2011) suggested that endophytic bacteria have the potential to be used in combination with Arbuscular Mychorrhizal Fungi (AMF) as biological control agents against *Ganoderma* sp. The association between both endophytic bacteria and AMF were significantly promoted AMF spore germination and hyphal length while the endophytic bacteria caused damage to *Ganoderma* sp. (Sundram *et al*., 2011).

However, the major problem of *in vitro* test is that antagonism successfully shown in laboratory condition does not consistently reflect the antagonism in the field or greenhouse conditions (Tronsmo and Hjeljord, 1998). This is because the soil in natural environment allows the development of extensive chemical-physical gradients that are difficult to stimulate in *in vitro* studies of biological interactions (Vilich and Sikora, 1998). It is important to provide the *in vitro* culture condition that mimic natural conditions in order to prevent overestimate of potential antagonists (Tronsmo and Hjeljord, 1998).

2.6.3 *In vivo* application of biocontrol agents

Introduction of biological control agents to control plant diseases has been practiced in agriculture since in 1927 (Millard and Taylor, 1927). Generally, most of the potential biological control agents failed to survive the fluctuations in the physical environment and the action of the indigenous and competitive microbiota (Whipps and Robert, 2001). As a consequence, there are still no effective biocontrol agents have been found to control *Ganoderma* sp. in the field. Yang and Rio (2002) reported that majority of potentially good biocontrol agents cannot be moved from the experimental phase to a commercialization phase due to impractical dosage recommendations, limited or inconsistent control efficacy, inadequate delivery systems, and incompatibility with current production methods. According to Powell *et al.* (2000), only 5% of the potential biocontrol agents chosen from *in vitro* screening tests have achieved the aim to control the disease.

2.7 Biotic constraints on the efficacy of biocontrol agents

The field or greenhouse condition is very complex and there is very limited understanding on the mechanisms of microbial interaction that occur under different environmental and cultural conditions (Schroth and Becker, 1990). Multiple aspects of microbiota interactions and survival need to be considered before established the potential antagonists in the field. Tronsmo and Hjeljord (1998) reported that competition between antagonists and indigenous microflora on the plant surface may make establishment of an introduced antagonist difficult. Moreover, the efficacy of biological control also depends on the

population density of the antagonistic microorganisms (Alabouvette *et al*., 1996). Several potential antagonists especially fungi often failed to survive in the field due to the presence of fungal-feeding microarthropods, nematodes, and amoebae (McGonigle and Hyakumachi, 2001). Knudsen and Dandurand (2014) found that fungivorous nematode significantly reduced hyphal growth and total population of potential antagonists of *Trichoderma harzianum* under some environmental conditions in non-sterile field soil. Old and Patrick (1976) stated that there are many examples where the amoebae feed on the conidia, chlamydospores, sporangia, teleutospores, basidiospores, and hyphae of various fungi (Nigam and Mukerji, 1988).

2.8 Abiotic constraints on the efficacy of biocontrol agents

Abiotic factors fluctuation especially water availability, temperature, length of dew periods, microclimate, canopy type and rainfall events, have an important impact on the performace of biocontrol agents in controlling diseases (Magan, 1997). Among all the abiotic factors, soil moisture is the main factor affecting microbial and microfaunal community structure and activity in forest floor or soil (Wagener and Schimel, 1998). Excessive soil moisture content could affect soil microorganisms' performance and soil physicochemical properties including pH and nutrients. Nutrients deficiencies in soil especially copper and calcium could reduce lignification of plant which is very important for plant defense mechanism (Vance *et al*., 1980). Plants with low lignin content would be more susceptible to disease infection (Marschner, 1995) and could reduce the efficacy of biocontrol agents in soil. Alabouvette (1990) indicated that the general mechanisms of

competition between soil microorganisms are very important in establishment and maintenance of microbial balance in the soil.

CHAPTER 3

MATERIALS AND METHODS

3.1 Sources of Microbes

Cultures of *G. boninense*, pathogen of basal stem rot of oil palm and three potential biocontrol agents, *Penicillium citrinum*, *Penicillium pinophilum, and Burkholderia* sp. were obtained from Mycology Laboratory, Faculty of Resource Science and Technology, UNIMAS. Preliminary study on the isolates has shown that the antagonists can inhibit the growth of *Ganoderma* sp. (Wan Nuur Fatiha, 2013). Microorganisms from freshly collected soils from different locations of oil palm plantations were also isolated.

3.2 Study area

Soil samples were collected from five different locations of oil palm plantation areas which were Sg. Mata, Bangka Semong, Sg. Noren, Sg. Staman and Sempadi, Sarawak. The oil palm trees in all the locations are about 15-20 years old except at Sg. Mata and Bangka Semong which are about two years old. Initially, only the top soils from two plots of each location were collected in order to determine the organisms in different location. Samplings in Sempadi area were done more often as a selected area for detail observation

for the microclimate of the oil palm plantation. Samplings were done in areas which were identified by FELDA plantation management as the BSR infected area and the disease free area. The samples were collected monthly starting from February 2014 until July 2014. The samples were collected in three different plots (P1, P2, and P3) from the infected area and the other three plots (P4, P5, and P6) were from non-infected area. In every plot, the samples were collected in three different depths which were 0 - 15 cm, 15 - 30 cm, and 45 - 60 cm. Each soil sample from each location was collected in three replicates.

All fresh samples were brought back to Mycology laboratory at FRST, UNIMAS. The fresh soil samples were used for the isolation of soil microorganisms and determination of soil moisture content while the dry samples were used for soil analysis to determine the soil pH, nutrients, and preparation of soil extract.

3.3 Preservation of soil samples

The soil samples were preserved as much as possible in its original condition in order to maintain their properties (Tan, 2005). The soil samples were dried in the oven at 50^{o}C for three to four days. When the sample was completely dried, it was ground with a mortar and pastel to separate the soil particles and passed through the 0.2 mm sieve before stored in a clean container.

3.4 Isolation of soil microorganisms

The isolation of soil microorganisms was followed Apinis (1963) through serial dilution agar plating. 10 g of soil sample was mixed in 100 mL sterilized distilled water by shaking in an orbital shaker at 100 rpm for 10 minutes. Serial dilution of the soil sample was conducted until 10^{-3} dilution and 1 mL of the final (10^{-3}) diluted soil was pipetted into a petri dish. 15 mL of Potato Dextrose Agar (PDA) medium was poured onto the diluted soil, gently shaken and left to cool. Three replicates were prepared and all the plates were incubated at 27 ± 1^{o}C. After five to eight days, the colonies of microbes in the plate were observed and each of the colonies present was isolated onto a new PDA in order to obtain the sub culture. The fungi obtained were observed under light microscope and then were identified based on morphological characteristics.

Basically, it is supposed to use PDA for isolating fungi, nutrient agar for bacteria and actinomycete isolation agar for actinomycetes. But in this study, only PDA was used as the purpose of this study was to test the mix-culture bacteria and fungi as potential antagonists against *Ganoderma* sp. by using only one type of medium with different pHs and nutrient sources. Hence, the basis of using PDA was to isolate all PDA favourable microbes and some of them were selected for further mix-culture test of soil fungi, bacteria and actinomycetes with the antagonists against *Ganoderma* sp. in section 3.10.

3.5 Determination of soil moisture content

The moisture content of all soil samples was measured. Known weight of crucible with lid was cleaned, dried and added with 30 g of moist soil. The weight of the crucible with the moist soil was recorded before it was dried in an oven (105^{o}C and 110^{o}C) to a constant weight. After drying, the crucible was removed from the oven and allowed to cool. The weight of the dried crucible with the contents was measured again. Three replicates were prepared for each sample. The percentage of soil moisture content was calculated based on the following formula:

$$MC\ \% = \frac{(W_2 - W_3)\ X\ 100}{(W_3 - W_1)}$$

Where :

W_1 – Weight of crucible with lid (g)

W_2 – Weight of moist soil + crucible with lid (g)

W_3 – Weight of dried soil + crucible with lid (g)

3.6 Determination of soil pH

The method to measure soil pH was according to Tan (2005) method. 15.0 g of soil was weighed in a clean 100 mL centrifuge tube. 30 mL of distilled water was added and swirled frequently for five minutes. The mixture was centrifuged at 2500 rpm for 15

minutes, and the supernatant solution was filtered into a 150 mL beaker. The pH of the solution was determined by using a pH meter. Three replicates were prepared.

3.7 Determination of soil nutrient content

Determination of soil nutrient contents was carried out using Aqua Regia Digestion Method which according to a manual of soil analysis from UPM (2010) with minor modification. Aqua regia solution was prepared by mixing Hidrochloric acid (HCl) with Nitric acid (HNO_3) in a ratio of 3:1. 4 g of soil was placed in a 100 mL beaker followed by 40 mL of aqua regia solution. The sample was heated for 30 minutes until clear solution was obtained. The sample was filtered through ashless filter (Whatman filter paper No. 2) into 100 mL volumetric flask and the sample was diluted to a volume 100 mL with distilled water. The concentration of copper (Cu), sodium (Na), aluminium (Al), carbon (Ca), zinc (Zn), magnesium (Mg), ferum (Fe), potassium (K) and lead (Pb) in the sample was measured and recorded using Atomic Absorption Spectroscopy (ICE^{TM} 3000 AAS).

3.8 Effect of pH on the growth of *Ganoderma* and Antagonists

Flask culture experiment was performed using Potato Dextrose Broth (BD Difco^{TM}) with eight different pH which were pH 2.0, 3.0, 4.0, 5.0, 5.6 (control), 6.0, 7.0, 8.0. The medium was prepared by diluting 12 g of PDB in 500 ml of distilled water. The mixture was heated until it dissolved completely before sterilized using an autoclave (TOMY SX 300).

Before pouring into sterile 100 mL conical flasks, the medium was adjusted to eight different pH by adding HCl or Sodium Hydroxide (NaOH). The non-adjusted pH of PDB with pH 5.6 served as control. A total of eight treatments were prepared and each treatment was done in three replicates.

An agar containing mycelia of *Ganoderma* from 5-days-old culture was inoculated into each conical flask containing 30 mL culture medium of tested pH. Then, the flasks were incubated at 27 ± 1^{o}C under stagnant condition. After eight days, the mycelium was harvested using a pre-weighted filter paper and dried in an oven overnight to a constant weight at 60^{o}C. The mycelia biomass was determined and expressed as mycelia dry weight (g) per 30 mL of culture medium.

3.9 *In vitro* test of potential antagonists against *Ganoderma* on different media conditions

The antagonistic ability of the mixtures of *P. citrinum*, *P. pinophilum*, and *Burkholderia* sp. on *Ganoderma* sp. were tested using different media conditions including different pH of growth media, different soil extracts agar media, and media with different sources of nutrient.

3.9.1 Preparation of inoculum suspension (Antagonists)

The preparation of mixture inoculum suspension of antagonists, *P. citrinum*, *P. pinophilum*, and *Burkholderia* sp. was according to Wan Nuur Fatiha (2013) with some modifications.

3.9.1.1 Conidial suspension of *P. citrinum* and *P. pinophilum*

P. citrinum and *P. pinophilum* were grown individually on PDA. The medium was incubated at room temperature, 27 ± 1˚C for five days. After five days, the plates were flooded with 10 mL of sterile distilled water and a drop of tween-80 as wetting agent. The conidia were gently scraped with a sterile inoculation needle. The inoculum suspension was stirred for 10 minutes and hyphal debris was removed by filtration through 0.45 µm filter. The inoculum concentration was determined using a haemocytometer. 200 mL each of the inoculum suspension of *P. citrinum* and *P. pinophilum* with concentration of 8 x 10^7 cfu/mL was prepared.

3.9.1.2 Cell suspension of *Burkholderia* sp.

Burkholderia sp. was grown on PDA in petri dish at room temperature, 27 ± 1^oC for 48 hours. A loopful of the culture was transferred into 250 mL conical flask containing 50 mL

PDB. The flask was incubated on a rotary shaker at 150 rpm for 48 hours at room temperature, 27 ± 1^{o}C. One mL of the culture was mixed with 9 mL sterilized distilled water to make 10^{-1} dilution. The dilution was done in three times to make 10^{-3} dilution. The inoculums concentration was determined using haemocytometer by counting the cells. The inoculums suspension was made up to 200 mL with the inoculums concentration 8 x 10^{7} cfu/mL.

3.9.1.3 Preparation of mixed inocula suspension

200 ml each of the *P.citrinum*, *P. pinophilum* and *Burkholderia* sp. suspension with concentration 8 x 10^{7} cfu/mL was mixed in a sterilized bottle. The final concentration of the mixture was 2.5 x 10^{7} cfu/mL.

3.9.2 Effect of pH on antagonistic ability of the potential antagonists against *Ganoderma*

The ability of potential antagonists against *Ganoderma* sp. was tested on PDA media with different pH. 7.8 g of ready-made PDA was mixed with 200 ml of distilled water and the mixture was heated to dissolve it completely. Eight bottles of 200 ml of dissolved PDA were prepared. The pH of the medium in each bottle was adjusted to pH 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 by adding HCl or NaOH. The non-adjusted pH served as control. The PDA was autoclaved at 121^{o}C for about 15 minutes. Fifteen ml of PDA was poured into a petri

dish and was left to solidify. Mycelial plug 0.5 cm diameter of *G. boninense* was inoculated on PDA at 2.0 cm away from the centre of the petri dish. 20 µl of mixed inoculum suspension was inoculated on the plate at 2.0 cm from the centre of the petri dish which was opposite with the culture of *G.boninense* (Figure 1). The cultures were prepared in three replicates for each treatment and incubated at room temperature, 27 ± 1^{o}C. The radial growth of *Ganoderma* was observed and measured after 8 days. The observation was made based on the inhibition of mycelia growth of the pathogen by the antagonists using the formula by Fokkema (1973):

$$I = \frac{r_1 - r_2}{r_1} \text{ X } 100\%$$

Where I = percentage of inhibition (%)

r_1 = radius of the pathogen in the control plate

r_2 = radius of the pathogen in dual culture plates

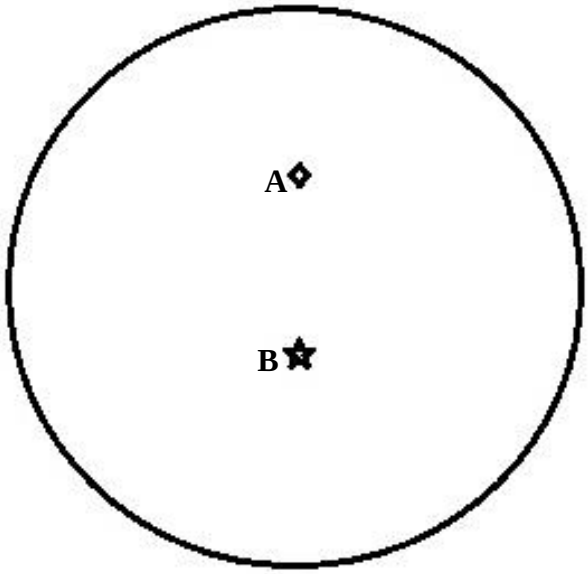

Figure 1: Antagonistic test between the pathogenic *G. boninense* and the antagonists, **A**; inoculums of the *G. boninense*, **B**; Inoculum of mix antagonists

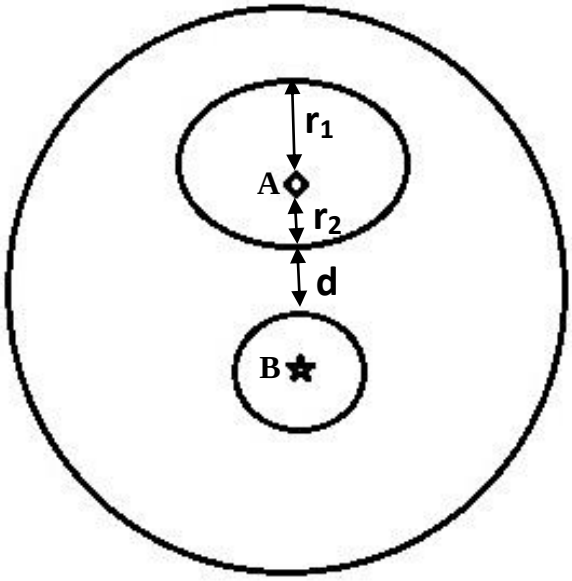

Figure 2: Colony measurement of *G. boninense*. **A**; inoculum of *Ganoderma* sp. **B**; inoculum of the antagonists. Radius of the pathogen ($\mathbf{r_1}$ and $\mathbf{r_2}$) and the zone of inhibition **(d)** were also noted.

3.9.3 Effect of Soil Extracts on antagonistic ability of potential antagonists against *Ganoderma*

The top soil from all the sampling locations of Sg. Mata, Bangka Semong, Sg. Noren, Sg. Staman and Sempadi were used in preparation of the Soil Extracts Agar (SEA) medium. The SEA was prepared by mixing 50 g of soil with 500 mL of sterilized distilled water. The mixture was shaking in an orbital shaker at 100 rpm for 10 minutes. 30 g of agar were mixed with 500 mL of sterilized distilled water in a clean bottle and was heated until the agar completely dissolved. The agar solution was autoclaved at 121^{o}C for 15 minutes. Both the soil extracts and agar solution were mixed in a petri dish with ratio 1:1 and left to solidify. Before the medium solidified, the pH of each SEA were measured and recorded. PDA without soil extraction was used as control.

3.9.3.1 Method of inoculation

Three methods of inoculation of antagonists were choose in this study in order to determine the suitable time for application of antagonists or treatments in the oil palm plantations to achieve more effective result in controlling the disease.

The antagonists mixture were inoculated simultaneously, after two days, and after five days inoculation of the *Ganoderma* on SEA medium in the same petri dishes. Three replicates were prepared for each SEA plate.

For inoculation, the inoculum of the antagonists mixture and *Ganoderma* were inoculated simultaneously on SEA in the same petri dish. Mycelial plug (0.5 cm diameter) of *Ganoderma* was inoculated on SEA at 2.0 cm away from the centre of petri dish. Then, 20 µl of the mixture of the antagonists suspension was inoculated on SEA at 2.0 cm from the centre of the petri dish which was opposite with the *Ganoderma* inoculation site. The inoculated plates were incubated at room temperature, 27 ± 1^{o}C. The radial growth of *Ganoderma* was observed and measured after eight days (Figure 2). The observation was made based on the percentage inhibition of mycelia growth of the pathogen by the antagonists using the formula by Fokkema (1973).

3.9.4 Effect of nutrients sources on the efficacy of potential antagonists against *Ganoderma* sp.

The effects of different sources of carbon, nitrogen, potassium, magnesium, and phosphate on the efficacy of the potential antagonists against the *Ganoderma* sp. were tested.

The basic complete media contained of glucose (40 g/L), Magnesium sulphate ($MgSO_{4)}$ (1.25 g/L), potassium dihydrogen orthophosphate (KH_2PO_4) (2.5 g/L), potassium nitrate (KNO_3) (5 g/L), agar (20 g) and distilled water (1 L) was used. The method of inoculation was carried out as in Figure 1 and the radial growth of *Ganoderma* sp. was observed and measured after eight days (Figure 2).

3.9.4.1 Carbon

The effect of different carbon sources on the efficacy of potential antagonists against the *Ganoderma* sp. was studied by replacing glucose in the basic complete media with fructose (40 g/L), lactose (40 g/L), sucrose (40 g/L), soluble starch (40 g/L) and carboxymethyl cellulose (CMC) (40 g/L) or without carbon sources.

3.9.4.2 Nitrogen

The effect of different nitrogen sources on the efficacy of potential antagonists against *Ganoderma* were studied by replacing nitrogen source (KNO_3) in the basic complete media with yeast (5 g/L), peptone (5 g/L), glycine (5 g/L), sodium nitrate (5 g/L), or without nitrogen.

3.9.4.3 Potassium

The effect of different potassium sources on the efficacy of potential antagonists against *Ganoderma* sp. were studied by replacing potassium source (KH_2PO_4, KNO_3) in basic complete media with potassium chloride (KCl) (5 g/L), Potassium sulphate (K_2SO_4) (5 g/L), Dipotassium phosphate (K_2HPO_4) (5 g/L), or without potassium. The source of

phosphate and nitrogen in KH_2PO_4 and KNO_3 in the basic medium was replaced by sodium nitrate ($NaNO_3$) (5 g/L).

3.9.4.4 Magnesium

The effect of different magnesium sources on the efficacy of potential antagonists against *Ganoderma* sp. were studied by replacing magnesium source ($MgSO_4$) in the basic complete media with magnesium chloride (MgCl) (1.25 g/L), magnesium oxide (MgO) (1.25 g/L), magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) or without magnesium. The source of sulphate in $MgSO_4$ in basic medium was replaced by potassium sulphate (K_2SO_4) (2.5 g/L).

3.9.4.5 Phosphate

The effect of different phosphate sources on the efficacy of potential antagonists against *Ganoderma* sp. were studied by replacing phosphate source (KH_2PO_4) in the basic complete media with di-potassium hydrogen phosphate (K_2HPO_4) (5 g/L), ammonium dihydrogen phosphate ($NH_4H_2PO_4$) (5 g/L), sodium dihydrogen phosphate (NaH_2PO_4) (5 g/L), or without phosphate.

3.10 Interaction of soil microbes and potential antagonists on growth of *Ganoderma*

Fifteen isolates of soil microorganisms that were successfully isolated from top soils of each location in Sempadi, Sg. Mata, Bangka Semong, Sg. Staman and Sg. Noren were randomly selected and formulated into a mixture of inoculum suspension. The mixture of the soil microbes and the antagonists were used to test the interaction effect against *Ganoderma* growth. Table 1 shows the number of isolates of the soil microbial suspension from each location used for the interaction study.

Table 1: Number of soil microbes from different locations of oil palm plantation areas used for the interaction study

Soil microbial suspension	No. of isolates used			
	Fungi	Bacteria	Actinomycete	Total
Mixture A (Sempadi)	7	5	3	15
Mixture B (Sg. Mata)	9	3	3	15
Mixture C (Bangka Semong)	7	5	3	15
Mixture D (Sg. Noren)	7	5	3	15
Mixture E (Sg. Staman)	5	7	3	15

The mixture of soil microbes from each location were mixed with the mixture of antagonists and the suspension concentration were determined using a haemocytometer by counting the conidia number of fungi or cell number of bacteria.

3.10.1 Preparation of inoculums suspension

3.10.1.1 Fungi

Thirty five of selected fungi as in Table 1 were grown individually on PDA media. The media were incubated at room temperature, 27 ± 1˚C for five days. After five days, the plates were flooded with 10 ml of sterile distilled water and a drop of tween-80 as wetting agent. The conidia were gently scraped with a sterile inoculation needle. The inoculum suspension was stirred for 10 minutes and hyphal debris was removed by filtration through 0.45 µm filter. The inoculum concentration was determined using haemocytometer. Each inoculum suspension of *P. citrinum* and *P. pinophilum* were made up to 50 mL with the inoculums concentration 8 x 10^7 cfu/mL by adding sterilized distilled water.

3.10.1.2 Bacteria

Fifteen selected bacteria were grown on PDA in petri dish at room temperature, 26±2 ^oC for 48 hours. A loop of each culture was transferred into 250 ml conical flask containing 50 ml potato dextrose broth. The flask was incubated on a rotary shaker at 150 rpm for 48 hours at room temperature, 27 ± 1^oC. One ml of culture was mixed with 9 ml sterilized distilled water to make 10^{-1} dilution. The dilution was done in three times to make 10^{-3} dilution. The inoculums concentration was determined using haemocytometer. The

inoculums suspension was made up to 50 ml with the inoculums concentration 8 x 10^7 cfu/mL by adding sterilized distilled water.

3.10.1.3 Preparation of mixed inocula suspension

Twenty mL each of the 15 selected soil microbes and antagonists suspensions with concentration 8 x 10^7 cfu/ml were mixed in a sterilized bottle. The final concentration of the mixture was 2.5 x 10^7 cfu/mL.

3.10.2 Effect of soil microbes on the efficacy of potential antagonists against *Ganoderma*

PDA plates were prepared using 9 cm diameter petri dish. A mycelial plug (0.5 cm diameter) of *Ganoderma* and 20 µl suspension of inoculum prepared in 3.10.1.3 were inoculated onto the media (Figure 1). The PDA plate inoculated with only antagonists' suspension and *Ganoderma* pathogen were served as a control. The radial growth of *Ganoderma* pathogen was observed and measured after eight days (Figure 2)

3.10.3 Effect of volatile compounds of soil microbes on the ability of antagonists against *Ganoderma* sp.

The method that was used to test volatile compounds of soil microbes on radial growth of antagonists was according to Dennis and Webster (1971) with some modifications. Two lower portions of the petri dishes containing PDA were used which inoculated with the *Ganoderma* and antagonists mixture at the opposite site which are 2 cm away from the center of the petri dish. Another lower portion of the PDA plate was streaked with 20 µl mixture of unsterilized soil suspension using L-shaped glass rod. Both inoculated lower plates were placed facing each other and sealed with parafilm. A PDA plate inoculated with only *Ganoderma* and antagonists served as control. Radial growth of *Ganoderma* was observed and measured after 8 days of incubation at 27 ± 1^{o}C (Figure 2). All cultures were prepared in three replicates. The observation was made based on the inhibition of mycelia growth of the pathogen by the antagonists using the formula by Fokkema (1973).

3.11 Statistical analysis

The experiments were arranged in completely randomized design arrangements. The data were analyzed using SPSS statistic software version 19.0. Statistical analysis of one-way ANOVA and Tukey's test were used. Figure 3 and 4 shows the general methodology for soil analysis and *in-vitro* test.

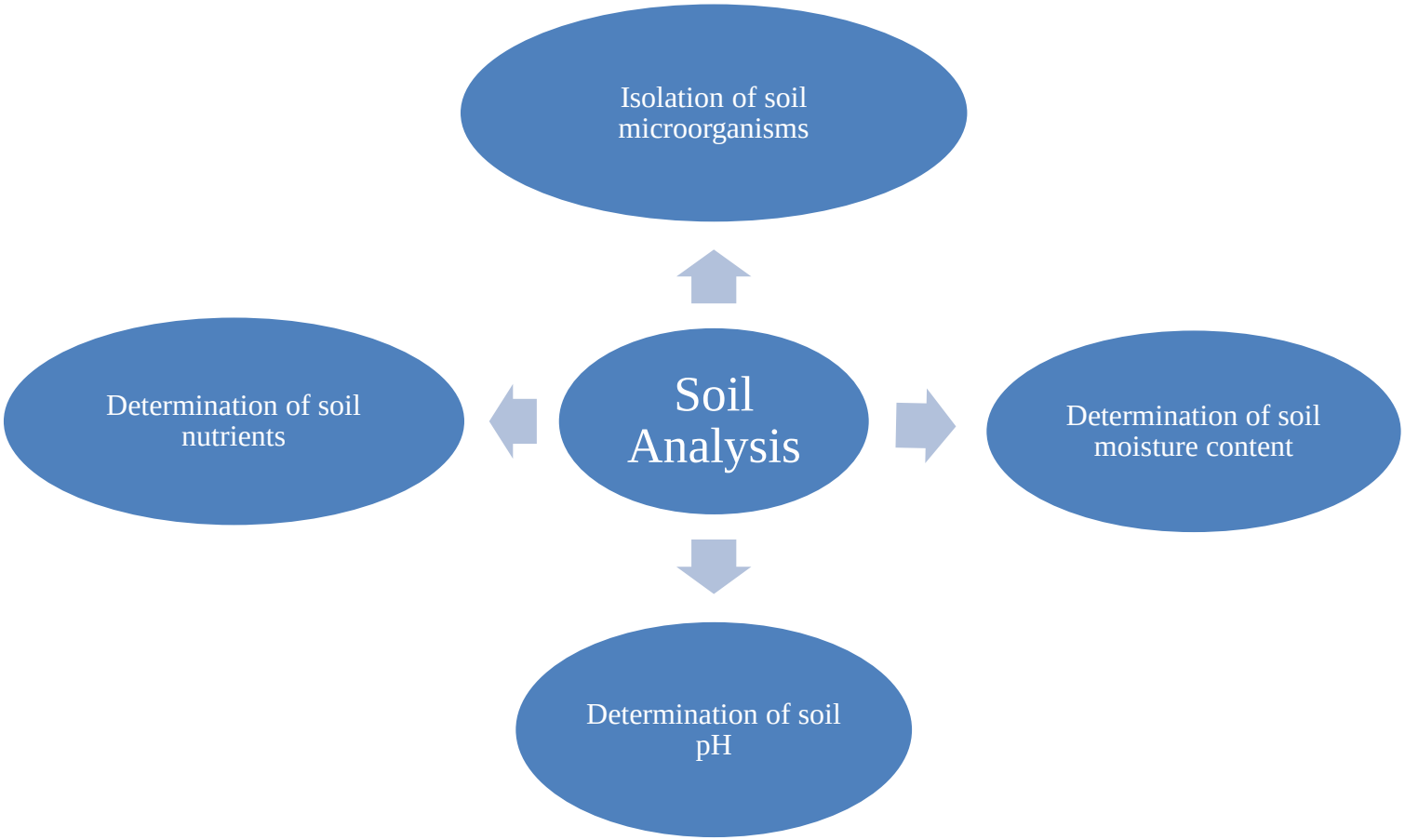

Figure 3: General methodology for soil analysis

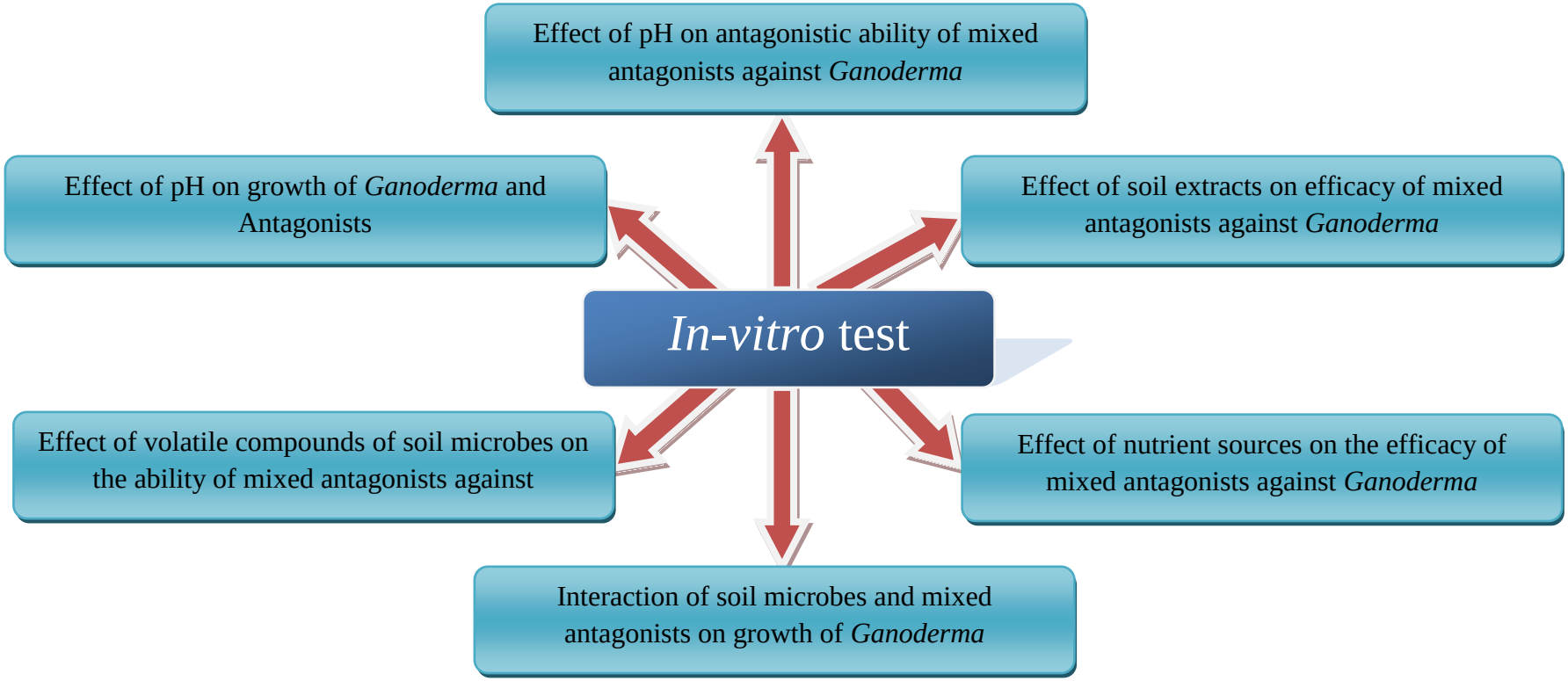

Figure 4: General methodology for *In-vitro* test on the ability of mixed antagonists against *Ganoderma*

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Soil microorganisms from different locations

Sub cultures of soil microorganisms from five different locations of oil palm plantation areas in Sarawak were successfully isolated using PDA media. In general, there were differences in the number of isolates of the microorganisms that were successfully isolated which were according to the locations.

In general, the isolates that were successfully obtained were mainly of fungi followed by bacteria and actinomycetes. A total of 639 isolates of the microorganisms were isolated from the top soil from four different locations comprised 447 of fungal strains, 143 of bacterial strains and 49 of actinomycete strains. Table 2 shows the total number of microorganisms that were successfully obtained from different oil palm plantation areas.

Table 2: Total number of microorganisms successfully isolated from top soil of different oil palm plantations

Location	Plot	No. of soil isolates			Total isolates
		Fungi	Bacteria	Actinomycete	
Sg. Mata, K. Samarahan					
(N 01^{o}28.085', E 110^{o}25.833')	P1	44	8	3	55
(N 01^{o}26.877', E 110^{o}32.084')	P2	44	12	3	59
Bangka Semong, K. Samarahan					
(N 01^{o}24.140', E 110^{o}32.602')	P3	36	12	3	51
(N 01^{o}24.136', E 110^{o}32.612')	P4	38	9	6	53
Sg. Noren, Bau					
(N 01^{o}21.992', E 110^{o}05.376')	P5	24	6	4	34
(N 01^{o}21.985', E 110^{o}05.374')	P6	22	8	3	33
Sg. Staman, Bau					
(N 01^{o}20.239', E 110^{o}01.177')	P7	33	13	4	50
(N 01^{o}21.985', E 110^{o}05.374')	P8	36	15	4	55
Sempadi					
	P9	30	13	3	46
	P10	32	10	4	46
	P11	29	9	3	41
	P12	25	12	2	39
	P13	29	7	4	40
	P14	25	9	3	37
Total		447	143	49	639

Of these sub cultures, the highest amounts of successfully isolated fungal strain were from Sg. Mata at P1 and P2 and bacteria from Sg. Staman at P8. The lowest number of isolated fungal strains was from Sg. Noren at P6 while bacteria was from Sg. Noren at P5. The highest amount of actinomycete was obtained in soil of Bangka Semong oil palm plantation at P4 while the lowest was in soil of Sempadi at P12.

The isolated fungi that were identified morphologically were the species from the genera *Trichoderma*, *Penicillium*, *Gliocladium*, *Botryodiplodia*, *Fusarium*, *Aspergillus*, *Colletotrichum*, *Pestalotiopsis*, *Nigrospora*, *Rhizopus*, *Chaetomium*, *Phomopsis* and *Alternaria* (Table 3). Among the genera, *Penicillium* was the highest number of isolates successfully isolated from the soil from all locations of the oil palm plantation areas except in Sg. Noren. The genus *Aspergillus* was the highest number of isolates obtained from Sg. Noren.

Table 3: Total number of soil fungi according the genus isolated from different locations of oil palm plantation areas

Fungi	Total number of fungi isolates in different location														Total
	Sg. Mata		Bangka Semong		Sg. Noren		Sg Staman		Sempadi						
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	
Penicillium	9	7	5	6	3	4	8	7	8	9	11	6	8	5	96
Trichoderma	5	6	6	4	1	-	4	5	4	2	3	-	2	-	42
Gliocladium	2	3	3	-	-	-	2	1	-	-	-	2	3	1	17
Botryodiplodia	2	1	-	-	4	2	-	-	3	2	1	-	-	-	15
Fusarium	5	6	2	3	1	2	-	2	3	3	2	3	-	3	35
Aspergillus	6	4	3	5	4	6	-	-	5	2	-	4	5	-	44
Colletotrichum	1	-	-	1	-	-	2	1	-	3	1	-	-	-	9
Pestalotiopsis	4	5	2	3	3	1	1	3	-	2	3	2	4	2	35
Nigrospore	-	2	6	5	-	-	5	3	-	-	-	3	4	1	29
Rhizopus	3	5	-	-	2	-	2	3	5	4	3	2	-	5	34
Chaetomium	-	-	-	-	1	2	-	-	-	-	-	-	1	2	6
Phomopsis	-	-	3	3	1	1	-	-	-	-	-	1	-	1	10
Alternaria	-	-	-	-	2	1	4	4	1	1	2	-	-	-	15
Others	7	5	6	7	2	3	5	7	1	4	4	2	2	5	60
total	44	44	36	38	24	22	33	36	30	32	29	25	29	25	447

There are several limitations of isolating soil fungi using standard protocols. Bakken (1997) reported that only a small percentage of soil microbes are culturable on artificial media. This may be due to the complex interdependencies between microorganisms in the soil that cannot be replicated in the laboratory and the presence of bacteria in dormant or viable but non-culturable states. Generally, artificial media used for microbial isolation only favored the growth of several species due to inability of culture media to mimic soil habitats (Bridge and Spooner, 2001). Dix and Webster (1995) reported that artificial media are selective and favors fast growth microorganism and also fungi which produce large number of spores. Furthermore, the competition for nutrients and space among soil microbes might inhibit or hinder the slow-growth microbes. Hence, the total number of isolates obtained from this study does not necessarily indicate the dominancy of *Penicillium* sp. and *Aspergilus* sp. in the selected location of oil palm plantation areas.

Microbiologists have consistently proved that most of the microbes successfully isolated from soil on artificial media using standard protocols are normally fast growing and sporing fungi such as *Trichoderma*, *Fusarium*, *Penicillium*, *Alternaria*, *Rhizopus*, and *Mucor* (Gilliam, 1978). The slow growing fungi are inhibited at an early stage by the competition of faster growing fungi and they are unable to form visible colonies (Garret, 2013). Azaz and Hasenekoglu (1997) reported that the richest genera in terms of the number of species in soil of Harran Plain in Turkey were *Penicillium* sp., *Aspergillus* sp., *Acremonium* sp. and *Fusarium* sp. Gaddeyya *et al*. (2012) proved that *Aspergillus* sp. and *Penicillium sp*. were dominant among the isolates obtained from different crop fields at Salur Mandal, India. Besides that, several researchers also reported that *Penicillium* sp., *Trichoderma* sp. and *Aspergillus* sp. were dominat in the forest soil (Moubasher and El-

Dohlob, 1970; Sarawanakumar and Kaviyarasan, 2010). Similarly, this study also showed that majority of isolates obtained from oil palm plantation areas belonging to *Penicillium* sp., *Aspergillus* sp. and *Trichoderma* sp.

Each microbes especially bacteria have a very specific nutritional requirements (Garland, 1995). Eventhough the rich agar media such as Potato Dextrose Agar (PDA) are the most widely used for fungal isolation, it may only promote the sporulation of anamorphic fungi while other microbes are rather difficult to grow (Watanabe, 2010). In order to avoid biases in isolating the soil microbes, it is suggested to use variety of methods, culture media and isolation strategies covering soil microbial characteristics from the diverse taxonomic and physiological groups in the soil ecosystems (Pfenning and Abreu, 2008).

4.1.1 Microorganisms in soil of different depths

The highest amount of microbial strains was obtained from the top soil of Sempadi oil palm plantation and the number decreased in the deeper soils. A total of 118 isolates of soil microbes were successfully isolated from the top soil (0 - 15 cm depth) comprise of 92 fungal strains, 26 bacterial strains and eight actinomycete strains. Ninety nine soil microbial strains were isolated from soil of 15-30 cm depth comprise 83 fungal strains, 16 bacterial strains and seven actinomycete. Only 65 microbes were isolated from soil of 45-60 cm depth including 52 fungal strains, 13 bacterial strains, and three actinomycete strains. Figure 5 shows the total number of microbial strains obtained from different depths of soils collected at different plots in Sempadi oil palm plantation.

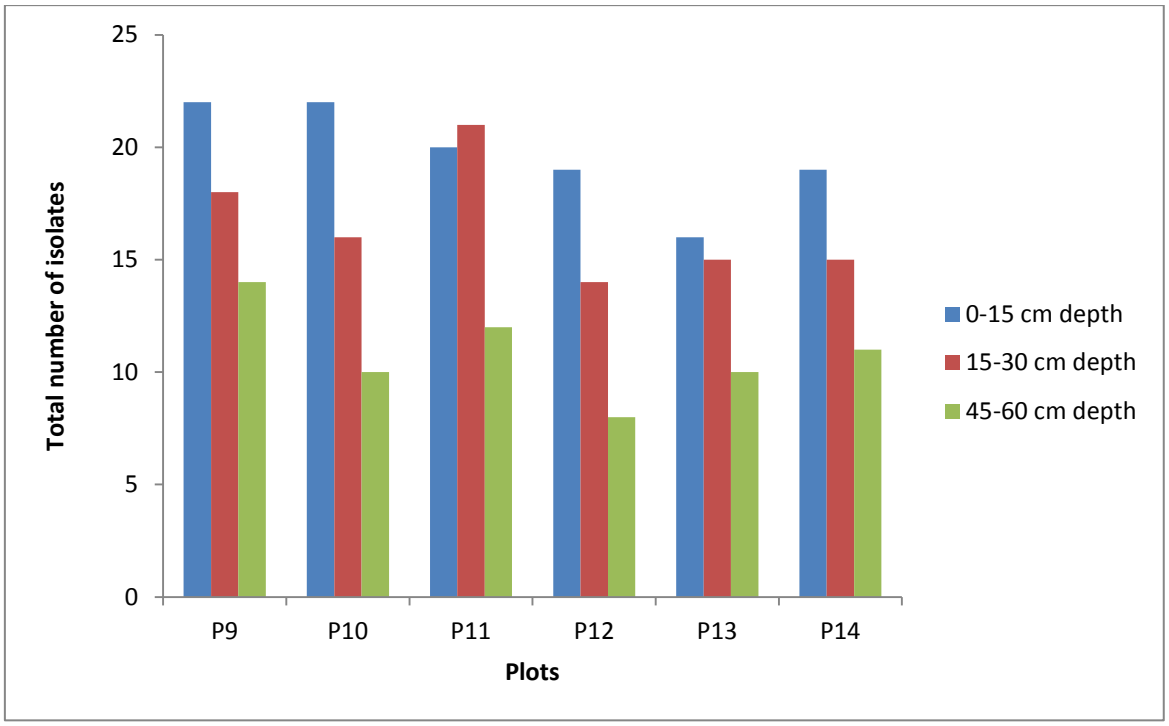

Figure 5: Total number of microbes obtained from different soil depths at different plots in Sempadi oil palm plantation

In this study, more microbial isolates were isolated from the surface soil compared to deeper soils. This might be due to the microbial community enrichment that occurs on the surface of the soil. The microbial enrichment in the soil surface occur due to high production of organic matter content in that areas through root distribution, fertilizer input and litter return (Griffith *et al.*, 2011; Cui and Holden, 2014). The organic matter content in soils is very important since it provides energy and nutrient for microbial growth and reproduction. Ekelund *et al.* (2001) reported that the biomass of the organisms generally tends to decrease with increasing depth of soils. Research on arbuscular mycorrhizal fungi (AMF) distribution at different soil depth which was carried out by Becerra *et al.* (2014) also indicated that the highest number of species and diversity of AMF was near the surface of the soil (0-20 cm depth) and decrease significantly in the deepest layer of the

soil. According to Dkhar (1983), fungi grow slowly with increasing depths due to shortage of mineral nutrients and compaction of soil.

4.1.2 Cultures of microorganisms from soils of *Ganoderma* infected and non-infected oil palms

More cultures of soil microbial strains that were successfully isolated from *Ganoderma* infected oil palm areas compared to non-infected areas either for fungi, bacteria or actinomycete. Figure 6 shows the total number of cultures of different microorganisms that were successfully isolated from *Ganoderma* infected and non-infected oil palm areas in Sempadi.

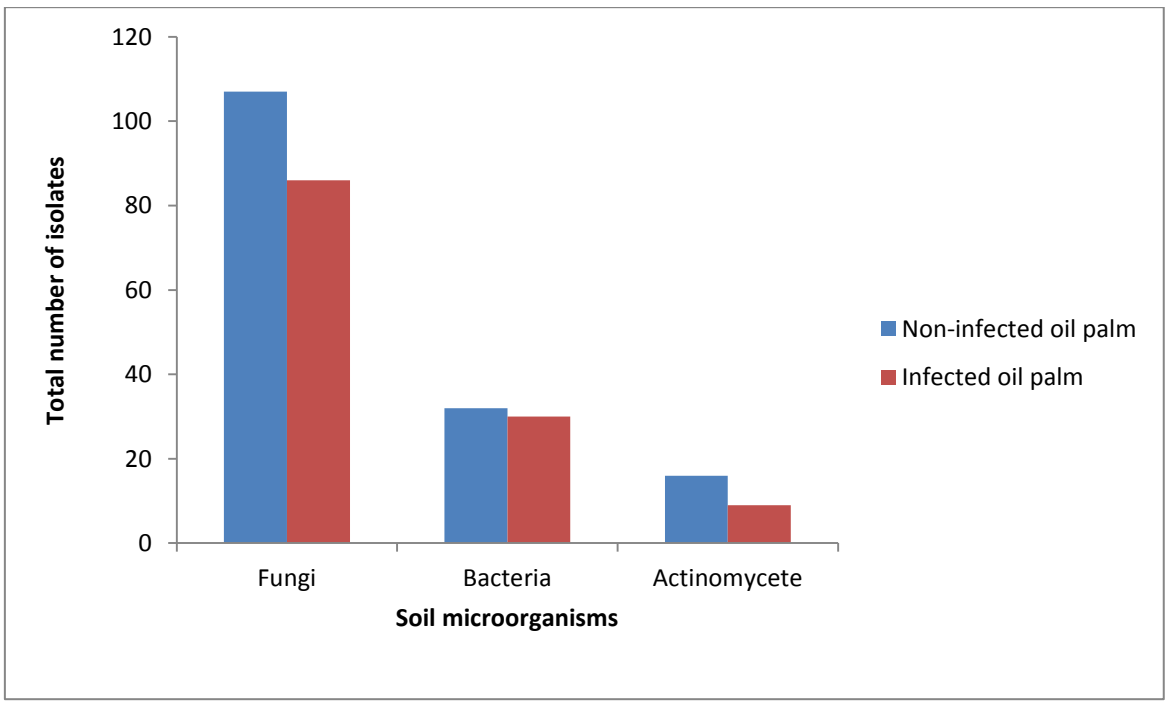

Figure 6: Number of cultures of the microorganisms successfully isolated from infected and non-infected oil palm plots in Sempadi oil palm plantation

One hundred and seven fungal isolates were successfully isolated from three different plots of non-infected oil palm soils in Sempadi plantation area. Among the most common fungi obtained from soil of non-infected oil palm plots were from the genera *Penicillium*, *Trichoderma*, *Botryodiplodia*, *Fusarium*, *Aspergillus*, *Colletotrichum*, *Pestalotiopsis*, and *Rhizopus* (Table 4). In *Ganoderma* infected oil palm plots, 86 fungal isolates were successfully isolated, mostly species from the genera *Penicillium*, *Trichoderma*, *Gliocladium*, *Fusarium*, *Aspergillus*, *Pestalotiopsis*, *Nigrospora*, *Rhizopus*, *Chaetomium* and *Phomopsis*.

A total of 32 bacterial isolates were successfully isolated from non-infected oil palm soils while in soil of *Ganoderma* infected oil palm plots, only 30 isolates of bacteria were isolated. Sixteen isolates of actinomycete were obtained from free disease oil palm plots while from infected oil palm plots, only nine isolates of actinomycete were obtained.

Table 4: Total number of soil fungi according to the genus from non-infected soil and *Ganoderma* infected oil palm plantation areas

Fungi	Total number of fungi isolates in different location					
	Non-infected oil palm areas			Infected oil palm areas		
	P9	P10	P11	P12	P13	P14
Penicillium	13	9	10	7	9	7
Trichoderma	4	1	4	-	2	1
Gliocladium	-	-	-	4	3	2
Botryodiplodia	4	3	3	-	-	-
Fusarium	3	3	3	3	-	2
Aspergillus	3	2	-	5	4	-
Colletotrichum	-	3	1	-	-	-
Pestalotiopsis	-	1	4	2	3	3
Nigrospore	-	-	-	1	5	2
Rhizopus	7	5	4	2	-	5
Chaetomium	-	-	-	-	2	3
Phomopsis	-	-	-	2	-	1
Alternaria	2	2	5	-	-	-
Others	2	3	2	1	2	3
Total	39	32	36	27	30	29

In this study, certain genera of fungi which have been reported to have antagonistic effect on pathogenic *Ganoderma* in non-infected soil and *Ganoderma* infected oil palm soils were successfully isolated. The genera were *Trichoderma*, *Penicillium*, and *Gliocladium*. Dharmaputra and Tjitrosomo (1990) reported that through *in-vitro* test the antagonistic ability of *Penicillium* sp. and *Trichoderma* sp. against pathogenic *Ganoderma*. Susanto *et*

al. (2005) reported that *Trichoderma* sp. and *Gliocladium* sp. have very high capacity in controlling *Ganoderma* infection in glasshouse and nursery trials.

Results obtained in this study indicated the presence of potential antagonists in both non-infected and infected plots. In the infected plots, it is possible that the colony density of potential antagonists is insufficient to control *Ganoderma* infection. Vergas Gil *et al*. (2009) proposed that in order to determine the capacity of the soil to control the disease caused by soilborne fungi, the colony density of the potential antagonists should be sufficient. Hence, further studies on quantification of potential biocontrol agents in soil are required.

Through field observation, there were a few healthy oil palm trees in the *Ganoderma* infected plots. This might be due to the existence of high resistant traits which enable them to prevent the disease infection. The high resistant tree will detect the infection and trigger suitable defence system which prevent the establishment of the pathogen (Jones and Dangl, 2006; Dangl *et al.*, 2013). According to Ennos (2014), there are genetic variation in natural tree populations which some trees produce genotype (R+) which able to detect pathogen and some trees produce genotype (R-) which unable to detect the pathogen. These factors could also explain the occurrence of BSR disease in oil palm plantation and thus, continuous studies are still required in order to fully understand the soil microbial interactions and genetic variations in accordance to prevent *Ganoderma* infection in oil palm plantation.

4.2 Soil moisture content

4.2.1 Moisture content of top soil at different location

Data analysis of one way ANOVA indicated that there were significant differences at $P<0.05$ of moisture content between the top soils in the various locations of oil palm plantation (Table 5). The moisture content in these areas was in a range between 27.56% - 236.31%. The highest moisture content was soils in Sg. Mata followed by soils in Bangka Semong plantation. The top soils moisture contents of these two areas were between 122.57% - 236.31%. The top soils moisture contents in the other three plantations were significantly lower at $P<0.05$ than of the soils in Sg. Mata and Bangka Semong. The top soils moisture content in these three plantations were between 27.56% - 50.43%. In general, the moisture content of different plot in the same location showed no significant differences except in P1 and P2 in Sg. Mata.

Table 5: Average top soil moisture content at five location of oil palm plantation taken on **a.** January 2012: Sg. Mata, Bangka Semong, Sg. Noren and Sg. Staman; **b.** February 2014: Sempadi

Location	Plot	Average top soil moisture content (%)
Sg Mata, K. Samarahan	P1	165.7 ± 10.98d
	P2	236.31 ± 14.70e
Bangka Semong, K. Samarahan	P3	122.57 ± 15.43c
	P4	135.40 ± 13.77c
Sg. Noren, Bau	P5	50.43 ± 2.74b
	P6	38.83 ± 3.96ab
Sg. Staman, Bau	P7	32.41 ± 1.31ab
	P8	44.04 ± 8.56ab
Sempadi, Lundu	P9	27.56 ± 2.28ab
	P10	46.65 ± 1.34ab
	P11	23.50 ± 0.77a
	P12	34.38 ± 3.23ab
	P13	34.24 ± 3.42ab
	P14	39.15 ± 2.59ab

*Mean ± standard deviation. Different letters in the column indicate significantly different mean ($P<0.05$, Tukeys test)

Soil type of Sg Mata, Bangka Semong, Sg. Noren and Sg. Staman oil palm plantation areas are peat soil while Sempadi are mineral soil. It is well known that peat soil has high water holding ability that makes them highly compressible, low in shear resistance and low stability (Kolay *et al.*, 2011).

The soils of Sg. Mata and Bangka Semong oil palm plantation areas have very high moisture content compared to other locations probably also due to the low stability of the soil since the oil palm were planted only two years while the soils in Bau and Lundu are much stable as the oil palm have been planted for almost 15 – 20 years.

Different types of soil in different locations have different water holding capacity which is depending on their structure, texture and other properties (Piedallu *et al*., 2011). Data from Jabatan Pertanian Kuching, Sarawak indicated that the soil of oil palm plantation in Sg. Mata, Bangka Semong and Sg. Noren are from Bijat series while Sg.Staman are of Seduau series and Sempadi from Nyalau and Merit series (Table 6).

The soils of Sg. Mata and Bangka Semong which is known as Bijat series are clayey and the soils terrain in those areas are flat to undulating floodplains or valleys (Jabatan Pertanian Kuching, 2014). According to Mohidin *et al*. (2009), Bijat series is characterized by clayey texture, poor drain and has high water holding capacity. The wetness and inundation problems of Bijat series have been reported as moderate limitation for agricultural activities (Mohidin *et al*., 2009). The soils of Sg. Staman or Seduau series are from group of alluvial soil. It is clayey texture, well drain and permeable with good water holding capacity, deep soil profile and also have limitation of wetness and inundation problems (Shaliha *et al*., 2012).

Nyalau series and Merit series in Sempadi oil palm plantation areas are both from red-yellow podzolic soil group. The Merit series are clayey texture, well drained and slow

water runoff while the Nyalau series are coarse loamy texture and have lower moisture holding and nutrient retention capacities compared to the Merit series (Sabang, 1996).

Table 6: Soil series description in Sg. Mata, Bangka Semong, Sg. Noren, Sg. Staman, and Sempadi

Soil Series	Bijat series	Seduau series	Nyalau series	Merit series
Location	Sg. Mata, Bangka Semong, Sg. Noren	Sg. Staman	Sempadi (P9, P10, and P11)	Sempadi (P12, P13, and P14)
Group	Gleysols	Alluvial soils	Red-yellow podzolic	Red-yellow podzolic
Main characteristic	Clayey; alluvial; non-sulphidic; white to grey	Clayey; non-calcareous sedimentary rocks; yellow	Coarse loamy; residual; non-calcareous	Fine clayey; residual; non-calcareous; yellow; high CEC
Terrain	Flat to undulating floodplains or valleys	Flat to undulating floodplains and levees	Moderately steep to steep hills	Moderately to very steeply dissected hills
Capability	Class 3	Class 2 to 4	Class 2 to 5	Class 3 to 5
Limitations	Wetness, inundation	Wetness, inundation	Fertility, slope, erosion hazard	Slope, erosion hazard

Source: Pejabat tanah, Jabatan Pertanian Kuching Sarawak (2014)

4.2.2 Soils moisture content in Sempadi oil palm plantation

In general, the soil moisture contents in Sempadi plantation which were recorded from February 2014 until July 2014 varied according to the plots, depths, and the sampling periods. Data analysis of one-way ANOVA showed that the moisture content in all six plots were significantly different at $P<0.05$ at different depths and sampling periods.

4.2.2.1 Moisture content of the top soil according to month

Moisture contents of the top soils in the plantation which were collected in February to July 2014 at Sempadi oil palm plantation were inconsistent over the study period. Statictical analysis of one-way ANOVA indicate that there were significant differences at $P<0.05$ between the moisture contents of top soil of the six plots (P9-P14) within the plantation. Overall, the highest soil moisture contents were in February except at P9 and P11 plots. The moisture content reduced in the following months and the lowest moisture content was recorded between May and June 2014 before increased again in July (Table 7).

The monthly total rainfall in Sempadi oil palm plantation during this study period were unable to be accessed by Sarawak Meteorological Department due to technical problems that occurred at Sempadi nearest meteorology station which located in Lundu. However, previous research showed that the climate variations in different months affect the moisture

content of the soil. Roxy *et al*. (2010) reported that variation of rainfall in different seasons causes the variation in the soil moisture.

Almost all plots in the plantation had the highest soil moisture content in February which related with heavy rainfall that regularly occurred in Sarawak from November to March due to Northeast Monsoon Season or locally known as 'Landas' season (Chemsain Consultant Sdn. Bhd., 2011). Ruji (2007) reported that the monthly rainfall during 'Landas' season was much higher than the average month rainfall. The Landas season has an average monthly rainfall from 400 and 500 mm (Mah *et al*., 2010).

The lowest moisture content of soil was recorded in May and June. This is due to Southwest Monsoon season or known as dry season that occurs from May to September (Chemsain Consultant Sdn. Bhd., 2011). In drier season, the average monthly rainfall is normally range from 200 to 300 mm (Mah *et al*., 2010).

Table 7: Monthly average moisture content of the topsoil at different plots in Sempadi oil palm plantation from February to July 2014

Plot	February	March	April	May	June	July
P9	27.56 ± 2.28c	23.44 ± 0.61b	45.80 ± 1.19d	7.86 ± 2.32a	8.67 ± 0.25a	24.81 ± 0.69bc
P10	46.65 ± 1.34e	24.37 ± 0.13b	25.50 ± 0.12b	7.59 ± 1.86a	32.31 ± 1.14c	35.45 ± 0.13d
P11	23.50 ± 0.77b	23.96 ± 0.47b	29.56 ± 2.21c	43.08 ± 1.23d	8.05 ± 3.91a	26.14 ± 0.60bc
P12	34.38 ± 3.23d	19.87 ± 1.71c	18.26 ± 0.81bc	15.60 ± 0.23b	5.73 ± 0.08a	21.54 ± 0.15c
P13	34.24 ± 3.42 d	14.33 ± 0.55b	18.48 ± 0.27c	7.61 ± 0.13a	17.06 ± 0.18bc	14.96 ± 0.17bc
P14	39.15 ± 2.59e	11.67 ± 0.41b	20.50 ± 0.03d	17.96 ± 0.09cd	4.34 ± 0.09a	15.20 ± 0.03c

*Mean ± standard deviation. Different letters in the row indicate significantly different mean (P<0.05, Tukeys test)

4.2.2.2 Soil moisture content at different depths

Data analysis of one-way ANOVA shows that there were significant differences at $p<0.05$ between the soils moisture contents at different depths. In general, the variations in soil moisture at different depths of the six plots showed almost similar pattern. The highest soils moisture contents in almost all the plots was of the top soil and reduced in deeper soils except at P11 in February, P14 in March, P10 and P13 in May, and P12, P13, and P14 in June (Appendix 1). The highest moisture content of the soils at all depths was in February and lowest in May and June. Simple regression linear analysis also indicate that the soil moisture content was negatively high correlated with the soil depth in free disease ($r^2 = 0.9664$) and *Ganoderma* infected area ($r^2 = 0.9995$). Figure 7 and 8 showed the moisture content according to depth in non-infected and *Ganoderma* infected oil palm areas.

Variations of soil moisture content are mostly influenced by several topography factors including relative elevation and hillslope position (Qiu *et al*., 2001), soil properties and organic material (Rozenswerg and Hillel, 1998). The soil moisture content in Sempadi plantation could also be influenced by weather conditions since the highest moisture content was during wet or Landas season while the lowest was during the dry season.

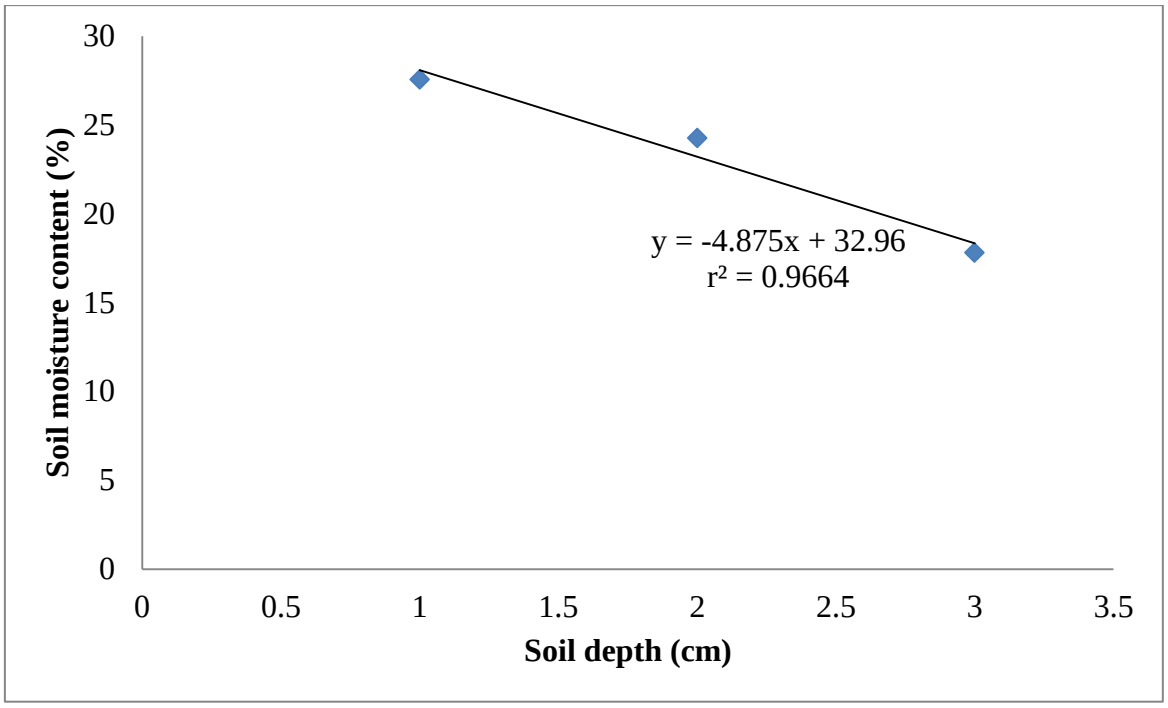

Figure 7: Soil moisture content according to depth in non-infected oil palm area. x-axis: **1**= 0-15 cm depth, **2**= 15-30 cm depth, **3**= 45-60 cm depth

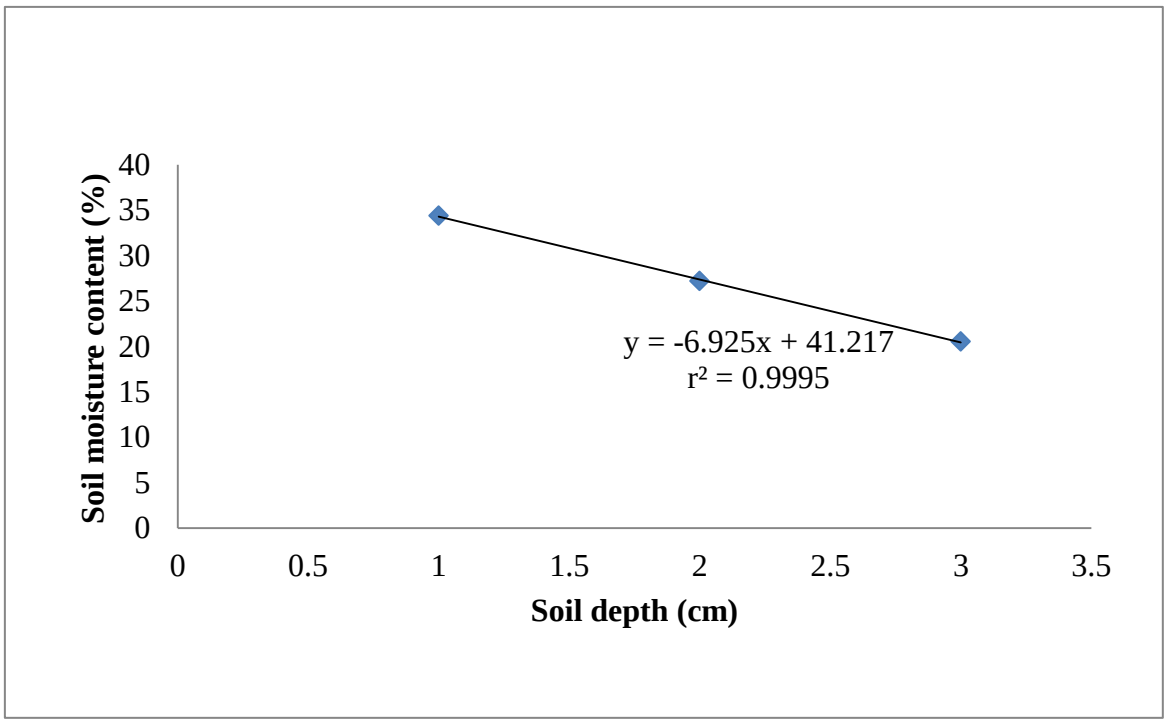

Figure 8: Soil moisture content according to depth in *Ganoderma* infected oil palm area. x-axis: **1**= 0-15 cm depth, **2**= 15-30 cm depth, **3**= 45-60 cm depth

4.2.2.3 Soil moisture content in *Ganoderma* infected and non-infected plots

Statistical analysis of one-way ANOVA revealed that there were significant differences at $P<0.05$ of top soils moisture content in plots of *Ganoderma* infected and non-infected plots. The moisture content of top soil in the non-infected plots for the six months sampling period were between 7.59% - 46.65% while in the disease infected plots were between 4.34% - 34.38% (Table 8).

The soils of non-infected plots are Nyalau series which have coarse loamy texture. The topsoil colour is yellow brown while the subsoil is brownish yellow. The top soil was sandy and the amounts of sand decreasing with depth while the amount of clay increasing with the depth. The soil of *Ganoderma* infected plots is of Merit series which have fine clayey texture. The topsoil is yellowish brown while the subsoil is brownish yellow.

Results obtained from this study indicated that the moisture content of coarse loamy soil in non-infected plots was higher than the fine clayey soil in *Ganoderma* infected plots. According to Zotarelli *et al*. (2010), the coarse loamy soil stores relatively small amount of soil moisture but has high infiltration rates while the clay soil stores more moisture but has slow infiltration rates. The contradict moisture content obtained might be due to the accumulation of organic materials from the oil palm empty fruit bunch (EFB) and pruned oil palm fronds on the coarse loamy soil which increased water holding capacity. Hudson (1994) reported that increasing of organic matter content in soil could promote water infiltration and reduces water runoff from particular soil. Hence, it is suggested that the soil

texture and soil organic matter content plays important role as it can affect water storage capacity and water infiltration rates of soil in plantation.

Table 8: Monthly average moisture content of top soils in non-infected and *Ganoderma* infected oil palm plots in Sempadi from February – July 2014

Areas	Plot	Average moisture content of top soil in different month (%)					
		February	March	April	May	June	July
Non-infected area	P9	27.56 ± 2.28b	23.44 ± 0.61a	45.80 ± 1.19c	7.86 ± 2.32a	8.67 ± 0.25a	24.81 ± 0.69a
	P10	46.65 ± 1.34c	24.37 ± 0.13a	25.50 ± 0.12a	7.59 ± 1.86a	32.31 ± 1.14b	35.45 ± 0.13c
	P11	23.50 ± 0.77a	23.96 ± 0.47a	29.56 ± 2.21b	43.08 ± 1.23b	8.05 ± 3.91a	26.14 ± 0.60b
Ganoderma infected area	P12	34.38 ± 3.23a	20.53 ± 0.98c	18.26 ± 0.81a	15.60 ± 0.23b	5.73 ± 0.08ab	21.54 ± 0.15b
	P13	34.24 ± 3.42 a	14.33 ± 0.55b	18.48 ± 0.27a	7.61 ± 0.13a	17.06 ± 0.18c	14.96 ± 0.17a
	P14	39.15 ± 2.59a	11.67 ± 0.41a	20.50 ± 0.03b	17.96 ± 0.09c	4.34 ± 0.09a	15.20 ± 0.03a

*Mean ± standard deviation. Different letters in the column indicate significantly different mean ($P<0.05$, Tukeys test)

4.3 Soil pH

In general, the soil pH in different selected oil palm plantations varied according to the locations, depths and sampling periods of which the pH were ranging from pH 4 to pH 7.6. The results indicated that there were no specific patterns of soil conditions that influence the soil pH value.

4.3.1 Top soil pH at different locations

Statistical analysis of one-way ANOVA reveals that there were significant differences at $P<0.05$ of top soil pH in different locations of the oil palm plantation. pH of the top soil in different plots within the same location also showed significant differences except in Bangka Semong and Sg. Noren plantation. The most acidic soil was recorded at P9 in Sempadi which showed pH 4.34 while the least acidic soil was recorded at P7 in Sg. Staman which was at pH 6.77 (Table 9).

Table 9: Top soil pH at different locations of oil palm plantation areas taken in **a.** January 2012 (Sg. Mata, Bangka Semong, Sg. Noren and Sg. Staman) and February 2014 (Sempadi)

Location	Plot	Average of pH
Sg. Mata	P1	4.97 ± 0.06c
	P2	4.63 ± 0.06b
Bangka Semong	P3	5.47 ± 0.06d
	P4	5.33 ± 0.06d
Sg. Noren	P5	5.73 ± 0.06e
	P6	5.90 ± 0.10e
Sg. Staman	P7	6.77 ± 0.06g
	P8	6.50 ± 0.10f
Sempadi	P9	4.34 ± 0.05a
	P10	4.97 ± 0.06c
	P11	4.73 ± 0.02b
	P12	5.08 ± 0.05c
	P13	4.59 ± 0.04b
	P14	5.59 ± 0.03d

*Mean ± standard deviation. Different letters in the column indicate significantly different mean ($P<0.05$, Tukeys test)

The soil pH in all selected locations of the oil palm plantation was slightly acidic which are very suitable for agricultural practices. According to Akbar *et al.* (2010), most plants grow best in the soil with slightly acidic conditions where it promotes optimal yield production.

The variation of soil pH according to the locations could be due to the soil physical properties and the amount of rainfall received. The soils in Sg. Staman which is of alluvial

soils group or Seduau series showed less acidic (pH 6.77) while the soil in Sempadi which is of red-yellow podzolic soil group or Nyalau series showed the most acidic (pH4.34). According to Sylvia *et al.* (2005), the consuming or releasing H^+ through redox reaction, fermentation, and also rainfall that leaches the bases in the soil could reduce the soil pH. The soil texture plays important roles in water and nutrient holding capacity. The amount of pH reduction of soil through water leaching largely depends on the soil texture, since it is the determinant of water holding capacity, nutrient availability and aeration (Knudsen, 2006).

Besides rainfall and soil physical properties, the accumulation of organic matter from oil palm residues could also affect soil pH. Moradi *et al*. (2012) reported that there were changes of soil pH in oil palm plantation areas by the accumulation of empty fruit bunches (EFB), and pruned oil palm fronds in the area. The accumulation of those organic matters in soil might reduce the pH of the soil. Hudson (1994) suggested that low soil pH indicated that the soil contain high amount of organic matter.

4.3.2 pH of soils in Sempadi oil palm plantation

4.3.2.1 pH of top soils according to month

Data analysis of one-way ANOVA indicated that the pH of top soil of the six plots in Sempadi plantation that were measured monthly from February 2014 until July 2014 were

significantly different at P <0.05. Comparatively, the most acidic soil was at P9 in March 2014 with pH 4.33 while the most basic soil was at P14 in June 2014 with pH 7.67.

The soil pH in all plots in Sempadi plantation in every month of sampling periods was not consistent. However, the pH of the top soils at every plot was the lowest in March 2015 and the highest in July 2014 except at P12 and P14 (Table 10). The soil pH changes regularly with slight fluctuations as it is affected by rainfall patterns, decomposing organic matter, and bacterial activity in the soil ground. Studies in South Dakota soils by Woodard and Bly (2010) suggested that there were small and inconsistent fluctuations of soil pH even after four years of liming application

This present study showed that the soil pH during Landas season which is in February and March 2014 were lower compared to dry season which is in May, June and July. This might be due to heavy rainfall which promotes decomposition of organic matter in the soil and thus reduce the pH of the soil (Shaliha *et al*., 2012).

Table 10: Monthly average pH of the top soil at different plots in Sempadi oil palm plantation from February - July 2014

Plot	Average pH of the top soil every month					
	February	March	April	May	June	July
P9	5.33 ± 0.06b	4.33 ± 0.06a	5.67 ± 0.06c	7.30 ± 0.00e	6.67 ± 0.06d	6.70 ± 0.00d
P10	5.20 ± 0.10b	4.97 ± 0.06a	6.53 ± 0.06d	6.50 ± 0.00d	6.17 ± 0.06c	6.70 ± 0.00e
P11	5.03 ± 0.06b	4.73 ± 0.06a	5.77 ± 0.06c	5.97 ± 0.06d	5.90 ± 0.00cd	6.87 ± 0.06e
P12	4.60 ± 0.00a	5.10 ± 0.10b	5.60 ± 0.00c	5.97 ± 0.06d	6.53 ± 0.06f	6.27 ± 0.06e
P13	4.90 ± 0.00b	4.60 ± 0.00a	4.83 ± 0.23ab	6.37 ± 0.06d	5.30 ± 0.00c	6.70 ± 0.00e
P14	6.57 ± 0.06c	5.60 ± 0.00b	5.07 ± 0.06a	5.10 ± 0.00a	7.67 ± 0.06e	7.10 ± 0.00d

*Mean ± standard deviation. Different letters in the row indicate significantly different mean ($P<0.05$, Tukeys test)

4.3.2.2 pH of soil at different depths

Data analysis of one-way ANOVA showed that there were significant differences at $P<0.05$ of soil pH at different depths in all plots in the Sempadi plantation.There were consistent patterns of soil pH according to the depths. Within the six month of sampling period, the pH values of the soil at different depths in the plantation varied ranging from pH 3.53 – pH 7.67(Appendix 2). Further analysis through simple linear regression indicates that the soil pH was negatively high correlated with the soil depth at free disease (r^2 = 0.9597) and *Ganoderma* infected area (r^2 = 0.9868) means that the soil pH was reduced in deeper soils. Figure 9 and 10 shows the correlation between soil pH and depth of free disease and *Ganoderma* infected areas.

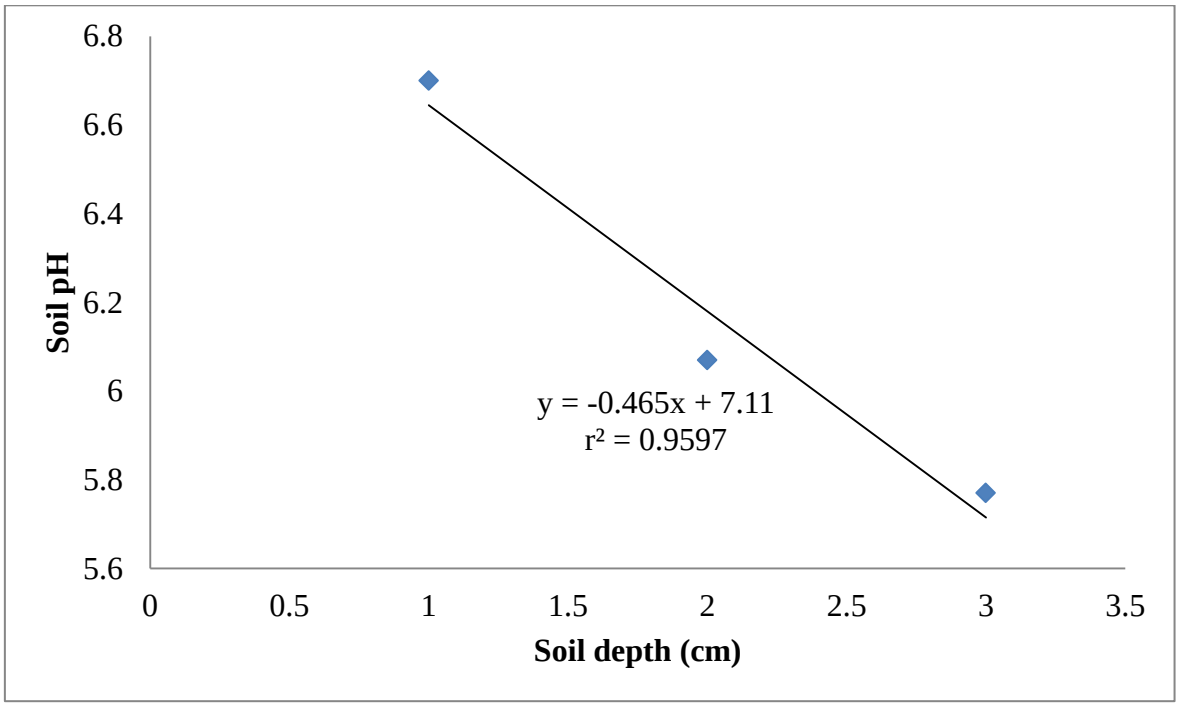

Figure 9: Soil pH according to depth in non-infected oil palm area. x-axis: **1**= 0-15 cm depth, **2**= 15-30 cm depth, **3**= 45-60 cm depth

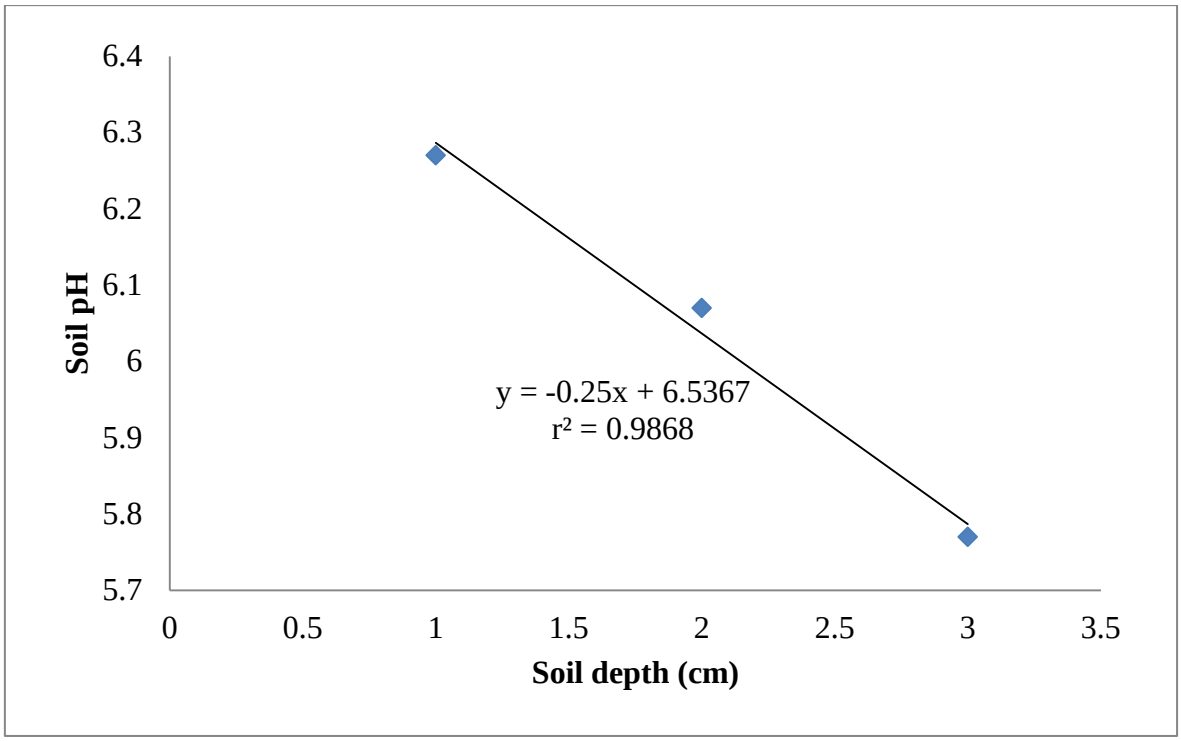

Figure 10: Soil pH according to depth in *Ganoderma* infected oil palm area. x-axis: **1**= 0-15 cm depth, **2**= 15-30 cm depth, **3**= 45-60 cm depth

According to Barik *et al*. (2011), the main factors that cause fluctuation of soil pH at different depth are mostly due to the decomposition of soil organic matter and nutrient content, soil physical structures and also rainfall patterns.

During Landas season in February 2014, most plots in the Sempadi plantation showed an increment of pH with depth except P9 and P10 plots. However in other months, most plots showed decrease of pH with increasing depth while in a few plots the soil pH increased. The increase of pH with increasing depth might be related to high contents of Fe and Al oxides while the decrease of pH might be related to high organic matter (Sanchez, 1976; Zaidey *et al*., 2010).

4.3.2.3 Top soil pH at non-infected and *Ganoderma* infected oil palm plots

Statistical analysis of one-way ANOVA showed that there were significant differences at $P<0.05$ of soil pH in the non-infected and *Ganoderma* infected palms plots. Overall, the pH of top soil in the different plots fluctuated throughout the sampling periods which were from February 2014 until July 2014 (Appendix 3). As stated before, the organic matter and nutrients content in the soil could affect the soil pH. Coarse loamy soil which has low filtration rate could leach away the organic matter and nutrients in the soil in a shorter periods compared to fine clayey soil which has higher filtration rate (Barik *et al*., 2011). This situation causes faster fluctuation of pH in coarse loamy soil compared to pH of fine clayey soil.

Simple linear regression analysis indicates very low positive relationship between the soil moisture content and pH at non-infected oil palm plots (r^2 = 0.4603) but high at *Ganoderma* infected oil palm plots (r^2= 0.7810). Figure 11 and 12 shows the correlation between the moisture content and pH of soil at non-infected oil palm plots and *Ganoderma* infected oil palm plots.

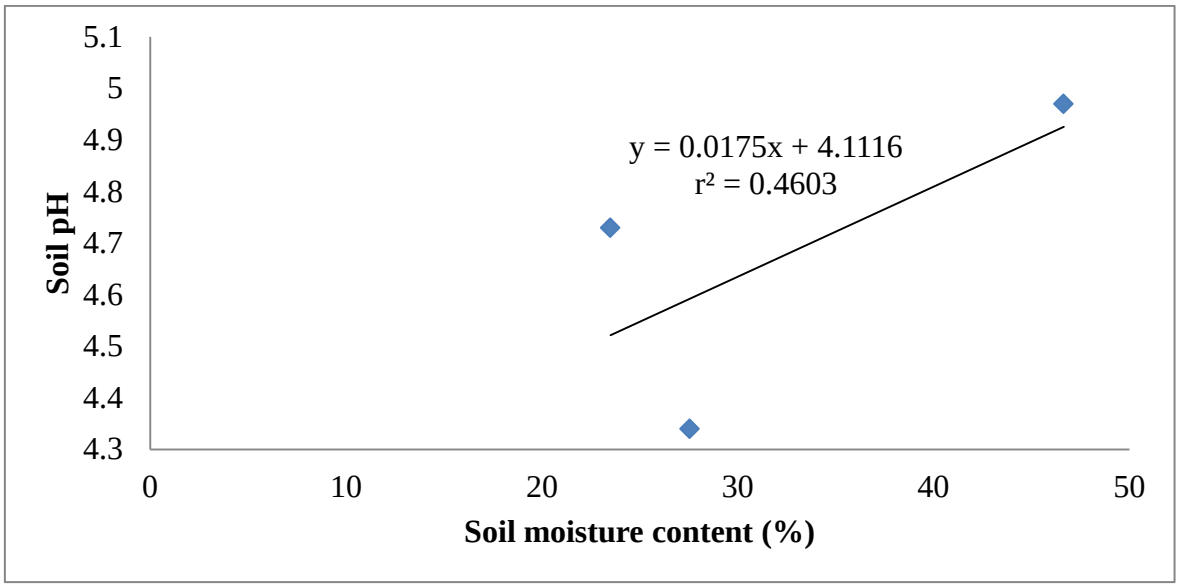

Figure 11: Soil pH according to different moisture content in non-infected oil palm area

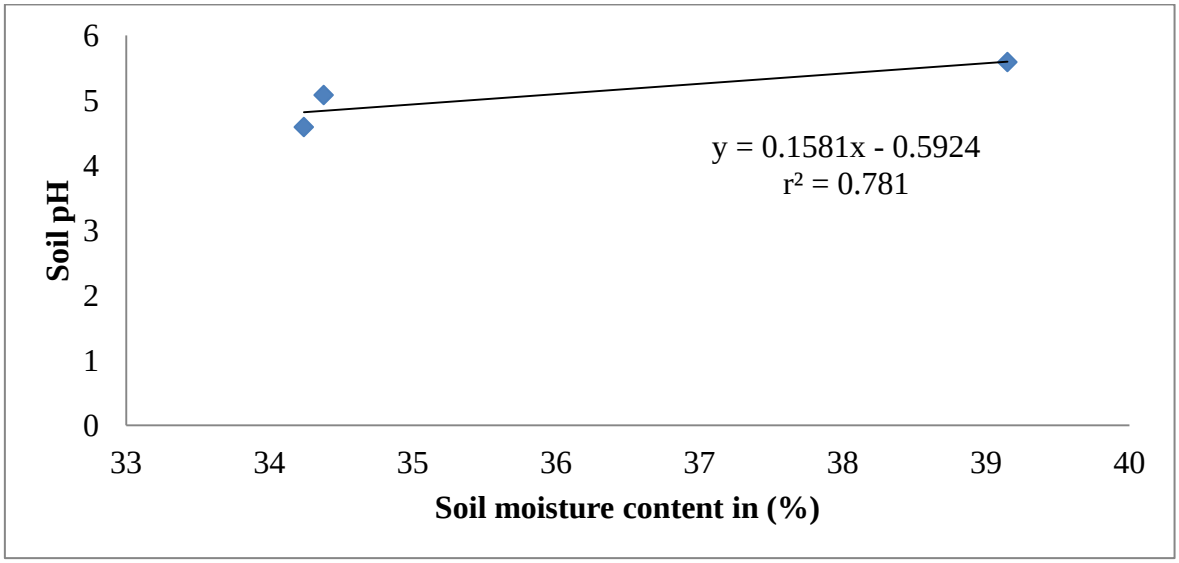

Figure 12: Soil pH according to different moisture content in *Ganoderma* infected oil palm area

4.4 Soil heavy metals in the selected oil palm plantation plots

Statistical analysis of one way anova indicated that the average concentrations of heavy metals in soils at different plots of the oil palm plantations were significantly different. Higher concentrations of Al and Fe were present in the soils compared to other heavy metals. The highest concentration of Al was at P10 of Sempadi plantation while Fe was at P8 of Sg. Staman with concentrations of 1075.75 mg/kg and 1282.60 mg/kg respectively (Table 11). Zn, Cu, Na and Pb were detected at lowest concentration in all selected locations of sampling plots which were less than 2.18 mg/kg.

Table 11: Average concentration of heavy metals (mg/kg) in five locations of oil palm plantation taken in February 2012

Location	Plot	Average concentration of heavy metals (mg/kg)								
		Zn	Mg	Fe	K	Ca	Al	Cu	Na	Pb
Sg. Mata	P1	1.47 ± 0.06e	161.77 ± 3.75e	342.65 ± 13.84b	74.88 ± 5.58d	1.01 ± 0.04a	258.66 ± 23.47ab	0.21 ± 0.01c	0.47 ± 0.06b	0.06 ± 0.00ab
	P2	0.75 ± 0.10d	88.74 ± 6.75d	431.77 ± 47.57b	1.70 ± 0.11a	397.27 ± 17.36c	92.28 ± 20.16a	0.12 ± 0.01b	1.89 ± 0.28d	0.09 ± 0.01b
Bangka Semong	P3	0.28 ± 0.01bc	177.01 ± 8.01e	250.22 ± 10.60ab	55.17 ± 5.31b	33.14 ± 1.72a	597.83 ± 9.10b	0.16 ± 0.00bc	0.43 ± 0.05b	0.15 ± 0.03b
	P4	0.21 ± 0.01b	208.70 ± 11.83f	176.16 ± 18.63ab	2.45 ± 0.32a	25.01 ± 2.13a	362.51 ± 30.25b	0.12 ± 0.01b	0.58 ± 0.06b	0.10 ± 0.01b
Sg. Noren	P5	0.62 ± 0.01d	1.48 ± 0.05a	971.77 ± 118.20c	63.59 ± 1.17c	115.07 ± 1.60ab	204.41 ± 10.37ab	0.27 ± 0.01c	0.72 ± 0.02b	0.48 ± 0.00e
	P6	0.38 ± 0.04c	1.24 ± 0.10a	477.01 ± 35.36b	1.94 ± 0.03a	8.77 ± 0.70a	217.75 ± 12.88ab	0.52 ± 0.06e	0.32 ± 0.04ab	0.43 ± 0.04e
Sg. Staman	P7	0.41 ± 0.03c	38.03 ± 0.82b	503.93 ± 70.49b	47.98 ± 5.99b	188.63 ± 29.97b	1075.75 ± 21.62d	0.38 ± 0.01d	0.50 ± 0.14b	0.22 ± 0.03c
	P8	2.18 ± 0.11f	65.93 ± 12.20c	1282.60 ± 53.51	70.96 ± 0.45cd	58.03 ± 2.98ab	361.74 ± 20.66b	1.33 ± 0.06f	1.47 ± 0.07c	0.29 ± 0.03d
Sempadi	P9	0.06 ± 0.01a	0.72 ± 0.08a	30.59 ± 0.27a	0.30 ± 0.03a	0.61 ± 0.15a	6.58 ± 0.89a	0.00 ± 0.00a	0.17 ± 0.02ab	0.04 ± 0.01ab
	P10	0.09 ± 0.01ab	0.42 ±0.01a	906.63 ± 149.31c	0.39 ±0.02a	0.15 ± 0.08a	1505.80 ± 46.17d	0.00 ± 0.00a	0.05 ± 0.03a	0.02 ± 0.01a
	P11	0.08 ± 0.01ab	0.45 ±0.03a	209.40 ± 8.09ab	2.02 ± 0.06a	11.06 ± 13.67a	994.43 ± 205.12c	0.01 ± 0.00a	0.09 ± 0.06ab	0.02 ± 0.01a
	P12	0.03 ± 0.00a	0.96 ± 0.02a	2.14 ± 0.03a	1.17 ± 0.12a	1.71 ± 0.05a	2.04 ± 2.61a	0.01 ± 0.01a	0.09 ± 0.06ab	0.03 ± 0.01a
	P13	0.04 ± 0.00a	1.14 ± 0.07a	1.42 ± 0.17a	0.31 ± 0.02a	2.19 ± 2.30a	5.70 ± 4.34a	0.01 ± 0.01a	0.09 ± 0.06ab	0.03 ± 0.02a
	P14	0.10 ± 0.00ab	1.43 ± 0.02a	0.53 ± 0.02a	0.05 ± 0.10a	1.65 ± 0.12a	3.66 ± 4.42a	0.01 ± 0.01a	0.09 ± 0.06ab	0.04 ± 0.02ab

*Mean ± standard deviation. Different letters in the column indicate significantly different mean ($P<0.05$, Tukeys test)

4.4.1 Zinc (Zn)

In general, the concentration of zinc was very low in all sampling plots of oil palm plantation which were below 2.20 mg/kg. Statistical analysis of one way ANOVA indicated that there were significant different at $p<0.05$ between the zinc concentration in different locations of the oil palm plantations. The highest concentration of zinc was recorded at P8 of Sg. Staman plantation which was 2.18 mg/kg while the lowest concentration was recorded at P12 of Sempadi plantation which was only 0.03 mg/kg (Table 11). Based on the result obtained, the zinc concentration in different plots of the same plantations varied except in Sempadi and Bangka Semong plantations.

Most of the zinc in soils exists in unavailable forms (Schulte, 2004). In this study, the concentrations of zinc in all plantation plots were below the normal range. Lindsay (1979) reported that normal range of Zinc in the soil is between 10-300 mg/kg. The high exchangeable zinc may be toxic to agronomic crops (Shulte, 2004) and soil microbes (Salminen *et al*., 2001).

4.4.2 Magnesium (Mg)

In general, statistical analysis of one way ANOVA shows that there were significant different at $P<0.05$ between the magnesium concentrations in soil of the different locations in the oil palm plantation. The magnesium concentrations in soil of all locations ranged

from 0.42 mg/kg to 208.70 mg/kg. The result also showed that the magnesium concentrations in different plots of the same locations except in Sempadi and Sg. Noren plantation were varied (Table 11).

According to Lipinski (2005), the concentration of magnesium depends on soil texture, soil type, pH, and humus content. In this study, the lowest concentration of magnesium content was recorded in coarse loamy soil in Sempadi and clayey soil in Sg. Noren plantations. Staugaitis and Rutkauskiene (2010) reported that the concentration of magnesium was the lowest in sandy loam soils and the highest content was in clay loam. Excessive magnesium concentration lead to poor plant growth and poor soil physical conditions (Mikkelsen, 2010)

4.4.3 Ferum (Fe)

Statistical analysis of one way ANOVA indicated that there were significant different at $P<0.05$ between ferum concentrations in five different locations of oil palm plantation. The highest concentration of ferum was recorded in soil at P8 of Sg. Staman plantation which was 1282.60 mg/kg while the lowest was recorded at P14 of Sempadi plantation which was 0.53 mg/kg. The ferum concentrations in soil of the different plots at the same location varied except in Sg. Noren and Sg. Staman (Table 11).

In this study, the ferum concentration measured from the selected soil of oil palm plantation areas are below the normal range in the soil. Lindsay (1979) reported that the normal ranges of soil ferum ranging from 7000 - 550,000 mg/kg. The excessive concentration of ferum in soil may have toxic effect on plants and soil microbes (Jankiewicz *et al*., 2002).

4.4.4 Potassium (K)

Statistical analysis of one way ANOVA indicated that potassium concentration in soils at the different locations of the oil palm plantation were significantly different at $P<0.05$. The potassium concentrations in the studied areas ranged between 0.05 mg/kg to 74.88 mg/kg (Table 11). The results also showed that there were large differences between the potassium concentrations in different plots of the same location except at Sempadi plantation. This indicated that there was no specific pattern of potassium concentration in soils regarding oil palm plantation areas even though at different plots in the same location except at Sempadi plantation. Potassium content in the soils of all plots at the oil palm plantation in Sempadi plantation was very low, ranged from 0.05 – 2.02 mg/kg.

The results indicated that available potassium in the soil of the five oil palm plantation was insufficient because it was below critical value. According to Al-Zubaidi and Pagel (1979), the critical value of potassium concentration in the soil is 160 mg/kg. The low value of potassium in the soil was most probably due to leaching by rainfall. Johnston and Goulding (1992) reported that approximately 1kg potassium ha-1 was lost for every 100 mm of

rainwater leached throughout the soil in the field. Besides that, the palm uptake and retention exceeding potassium applications also contribute to the losses of potassium level in soil (Ogeh and Osiomwan, 2012).

4.4.5 Calcium (Ca)

Statistical analysis of one way ANOVA revealed that there was significant difference at $P<0.05$ between calcium concentration in the soils at different plots of oil palm plantation. The highest calcium concentration was recorded at P2 of Sg. Mata plantation which was 397.37 mg/kg while the lowest (0.15 mg/kg) was at P10 of Sempadi plantation (Table 11). The results also indicate that there was no significant difference between the calcium content in soil of the different plots of the same location except of Sg. Mata plantation.

Calcium is an essential nutrient for plant growth that is used in the formation of wood and in the maintenance of cell walls (Marschner, 1995). According to Mc Laughlin and Wimmer (1999), Ca deficiency is rare in nature, but may occur on soils with low base saturation and high levels of acidic deposition. Ca deficiency could affect the lignification of oil palm trees and cause the trees susceptible to *Ganoderma* infection (Marschner, 1995).

Generally, the concentration of calcium was sufficient in both *Ganoderma* infected and non-infected oil palm plantations. The results obtained in this study indicated that the

calcium concentration in the soil at different oil palm plantation plots varied according to the sampling plot. Shallari *et al*. (1998) reported that natural existence of calcium in soil is related to several factors including geographic location, type of rock, pH, nature of drainage water, clay content, cation exchange capacity, weathering and climatic conditions. In agricultural soil, the existence of calcium content is mostly from fertilizer application in order to increase salinity of soil. Philips and Chiy (2002) reported the increment of calcium concentrations in leachate after applied with sodium fertilizer. The application of compost in tropical agricultural soil could also promote the increment of calcium concentration (Slattery *et al*., 2002).

4.4.6 Aluminium (Al)

Statistical analysis of one way ANOVA revealed that the aluminium concentration in different plots of the oil palm plantation were significantly different at $P<0.05$. The aluminium concentrations varied from 2.04 mg/kg to 1075.75 mg/kg (Table 11). Within the same plantation, there was no significant difference of aluminium concentration in soils at different plots except in Sg. Staman and Sempadi plantations.

In this study, the aluminium concentration in the soil was very low which were below the normal range of Al in soil. According to Lindsay (1979), the normal range of aluminium concentration in soil is 10,000 - 300,000 mg/kg. The excessive amount of aluminium content in soil caused toxicity. According to Belliveau *et al*. (1987), the aluminium ion is non-essential elements for plants and lacking biological function. Thus, the presence of

aluminium has been recognized as a serious pollution problem related to acidification of soil and water because it can solubilizes aluminium to toxic levels (Pina and Cervantes, 1996). Thus, the results of this study indicated that the aluminium level in these plantations was safe and not harmful to the oil palms health.

4.4.7 Copper (Cu)

Statistical analysis of one way ANOVA showed that there were significant differences at $P<0.05$ between copper concentration in the soils from different oil palm plantations. The concentrations of copper varied from 0.00 mg/kg to 1.33 mg/kg. The copper concentrations in different plots from the same location differed except in Bangka Semong and Sempadi plantations (Table 11). In P9 and P10 of Sempadi plantation, copper was not detected.

Copper is an essential microelement which involved as an enzyme activator and is thought to be involved in chlorophyll formation, protein synthesis (Tucker, 1999), oxidative stress reduction, cell expansion and most importantly for cell wall lignification which acts as defense mechanisms from pathogen infection (Marschner, 1995).

The results from this study indicated that copper concentrations in soil from different locations are below normal range. According to Lindsay (1979), the copper concentration in soil normally ranges from 2 - 100 mg/kg which means that the soils in all selected plots of the oil palm plantations having copper deficiency. Hence, the copper deficiency in all

plantation plots probably reduces the lignification process in oil palm trees and thus, increases the susceptibility of *Ganoderma* infection. The excessive amount of copper in soil however leads to copper toxicity problems which affect plants physiological process and microbes in the soil (Schulte and Kelling, 2004).

4.4.8 Sodium (Na)

Statistical analysis of one way ANOVA showed that there were significant differences at $P<0.05$ between sodium concentrations in five oil palm plantations. The highest sodium concentration in soil was recorded at P2 of Sg. Mata plantation which was 1.89 mg/kg while the lowest was at P10 of Sempadi plantation which was 0.05 mg/kg (Table 11). The results also indicated that the concentrations of sodium in the soils at different plots of the same oil palm plantation were not significantly different except at Sg. Mata and Sg. Staman plantations.

Sodium is one of the alkali metals and it is non-essential element for several plants (Shah and Shah, 2011). High sodium content in the soil would lead to high salinity or pH of soil (Ahmad and Khan, 1988). Based on the results of soils pH (Part 4.3.1), the soils in all different sampling plots were acidic (Table 9). The low soil pH could be related to low sodium content in the soil. Excessive content of sodium in the soil could lead to soil salinity and sodicity which adversely affect plant physiological processes and microbial growth due to osmotic stress, ion toxicity or reduced absoption of essential nutrients

(Launchli and Epstein, 1990). Hence, the results indicated that the sodium level in these plots were acceptable for the oil palm health and growth.

4.4.9 Lead (Pb)

Statistical analysis of one way ANOVA indicated that lead concentrations in the soil from five oil palm plantations were significantly different at $P<0.05$. The lead concentrations were from 0.02 mg/kg to 0.48 mg/kg (Table 11). The result also showed that the concentration of lead within the same oil palm plantation was not significantly different except in Sg. Staman plantation.

The concentration of lead in soils of all the sampling plots was very low which was below 0.48 mg/kg and this lead concentration values are below the normal range. According to Lindsay (1979), lead naturally exists in soil at levels of 2 - 200 mg/kg. Excess lead concentration could causes toxicity in plants such as stunted plant growth, chlorosis and blackening of root system (Sharma and Dubey, 2005). Atuanya and Oseghe (2006) also reported that higher lead concentrations in soil might reduce soil microbial density due to their toxicity.

4.5 Growth of *Ganoderma* and antagonists at different pHs

Statistical analysis of one way ANOVA showed that there were significant differences at $P<0.05$ of dry mycelial weight of *Ganoderma* at different pHs of Potato Dextrose Broth (PDB). The highest dry mycelial weight was recorded at pH 5 while the lowest was at pH 2, pH 7, and pH 8 (Table 12).

In this study, abundant mycelia growth of *Ganoderma* was formed in PDB with pH ranged from pH 4 - 5.6. The growth was very poor at pH 3 and pH 6 and it was negligible at pH 2, pH 7 and pH 8. Nawawi and Ho (1990) reported that negligible growth of *Ganoderma* was at pH 6.9-7.5. This indicated that the *Ganoderma* grows optimally at pH 4 to pH 5.6 and cannot tolerate strong acidic condition and basic conditions. According to Rietsz and Haynes (2003), high basic media cause osmotic stress to the microbial community and thus limit the microbial growth and activity. The high acidic conditions cause toxicities to the microbes which also inhibit their growth (Rietsz and Haynes, 2003). Generally, the optimum pH required for fungal growth is pH 5 (Pardo *et al*., 2006). Deshmukh *et al*. (2012) suggested that fungi grow at pH 3 – pH 8 with maximum production of dry mycelial weight and sporulation at pH 5.5 and pH 6.5 respectively in liquid media.

Table 12: Average dry mass weight (g) of the *Ganoderma* sp. in PDB at different pH

pH of PDB	Dry mass weight of *Ganoderma* sp.(g)
2.0	0.03 ± 0.00a
3.0	0.15 ± 0.03b
4.0	0.47 ± 0.04c
5.0	0.62 ± 0.08d
5.6 (-ve control)	0.60 ± 0.03d
6.0	0.23 ± 0.06b
7.0	0.04 ± 0.05a
8.0	0.04 ± 0.01a

*Mean ± standard deviation. Different letters in the column indicate significantly different mean ($P<0.05$, Tukeys test)
PDB: Potato Dextrose Broth

4.5.2 Growth of antagonists at different pHs

Data analysis of one way ANOVA showed that there were significant differences at $P<0.05$ of dry mass weight of the three potential antagonists, *P. pinophilum*, *P. citrinum* and *Burkholderia* in PDB at different pH values (Table 13). The highest dry mass weight of *P. pinophilum* and *P. citrinum* were at pH 4.0 -6.0 which suggested that *P. pinophilum* and *P. citrinum* grow best in acidic conditions. According to Deacon (2006), *Penicillium* spp. show high tolerance to acidic media and they grow optimally on media of pH 5.5 - 6.0. The present results also showed that the lowest dry mass weight of *Penicillium* spp. were in PDB of pH 2.0 and 8.0. According to Li *et al*. (2010), the growth of *Penicillium* spp. was minimal and spore germination was inhibited at pH 2.0 and 8.0.

It has been reported that the optimal pH for *Burkholderia* sp ranged from pH 5.0 to pH 8.0 with rapid bacterial inactivation below pH 4.5 (Tong *et al*., 1996). High dry mass of *Burkholderia* sp. was recorded in PDB of pH 6.0 -7.0. In PDB of pH 2.0 and 3.0, the dry mass weight of *Burkholderia* sp. was not detected. This might be due to unfavorable acidic condition which disrupts the cellular activity of *Burkhoderia* sp. Rice and Bayles (2008) also has proved that extreme pH condition cause autolysis of the bacteria.

Table 13: Dry mass weight (g) of the potential *Ganoderma* sp. antagonists in different pH of PDB

pH of PDB	Dry mass weight (g)		
	P.pinophilum	*P.citrinum*	*Burkholderia*
2.0	0.01 ± 0.02a	0.01 ± 0.01a	0.00 ± 0.00a
3.0	0.07 ± 0.02b	0.05 ± 0.03b	0.00 ± 0.00a
4.0	0.32 ± 0.01d	0.12 ± 0.01c	0.05 ± 0.01b
5.0	0.45 ± 0.0e	0.37 ± 0.01d	0.10 ± 0.01c
5.6 (-ve control)	0.47 ± 0.0e	0.46 ± 0.01e	0.11 ± 0.01c
6.0	0.30 ± 0.02d	0.48 ± 0.04e	0.15 ± 0.01d
7.0	0.11 ± 0.01c	0.09 ± 0.03b	0.15 ± 0.01d
8.0	0.02 ± 0.02a	0.01 ± 0.01a	0.06 ± 0.00b

*Mean ± standard deviation. Different letters in the column indicate significantly different mean ($P<0.05$, Tukeys test)

PDB: Potato Dextrose Broth

4.5.3 Antagonistic Ability of mixed antagonists against *Ganoderma* at different pH

Growths of the pathogen and the mix antagonist were good at pH 5.0 to pH 6.0 and were poorer as the pH increased or decreased. There was no growth of both the mixture of antagonists and *Ganoderma* sp. on PDA at pH 2.0.

Further observation showed that there was a formation of inhibition zone between the colony of *Ganoderma* sp. and the mixed antagonists. The margin of the *Ganoderma* sp. was thinner towards the antagonist. This inhibition zone was clearly seen on PDA at pH 5.0 and pH 6.0. The mycelia thickness of the *Ganoderma* sp. and antagonist colony was also influenced by the pH of the medium. The mycelia were thinner on PDA at pH 3.0, 4.0, 7.0 and 8.0 compared to other pH. Amrita and Richa (2014) also found that the nutrient in the media and pH were major factors that influence the growth and sporulation of fungi. Thus, it can lead to suggestion that to get the optimum antagonist activity against *Ganoderma* sp., the medium should be at pH 5.0.

Statistical analysis of one-way ANOVA reveals that there were significant differences at $P<0.05$ of mycelia reduction percentage of the *Ganoderma* sp. on PDA of different pH value when it was simultaneously inoculated with the mixed antagonists on the media of the same plate. The highest percentage of radial growth inhibition of *Ganoderma* sp. colony growth on PDA was at pH 5.0 (41.80%) (Table 14).

Table 14: Reduction of radial growth of *Ganoderma* sp. after simultaneously pairing with mixed antagonists on PDA of different pH after 8 days incubated

pH	Average percentage reduction of *Ganoderma* radial growth (%)
2.0	0.00 ± 0.00a
3.0	24.57 ± 0.93b
4.0	25.70 ± 1.31b
5.0	41.80 ± 1.61d
5.6 (-ve control)	30.47 ± 0.70c
6.0	33.53 ± 1.96c
7.0	32.17 ± 2.11c
8.0	25.13 ± 1.78b

*Mean ± standard deviation. Different letters in the column indicate significantly different mean ($P<0.05$, Tukeys test)

4.6 Antagonistic ability of potential antagonists against *Ganoderma* on different soil extracts agar

Data analysis of one-way ANOVA showed that there were significant differences at $P<0.05$ of percentage reduction of radial growth of the *Ganoderma* sp. with mixed of potential antagonists on different soil extracts agar (SEA) from different locations. The average percentage reduction ranged from 30.20% to 68.83% (Table 15). Among all the media, the highest percentage reduction of the *Ganoderma* sp. radial growth colony was on SEA of P10 while the lowest was on PDA without soil extract. This might indicated that SEA of P10 contained the highest antibiosis compound against the *Ganoderma* sp.

Since the soil extract used in this study was non-sterile, abundant of soil microbes appeared on the media. This might promote better interactions between the soil microbes and antagonists in controlling the *Ganoderma* sp. Chaube and Singh (2000) reported that common types of interaction between soil microbes, antagonists and *Ganoderma* were mutual intermingling, partial mutual intermingling, inhibition at contact point and formation of inhibition zone. These mechanisms occur due to nutrient competition (Chaube and Singh, 2000), antibiosis (Marone *et al*., 1988), fast growth of microbial isolates (Velusamy and Gnanamanickam, 2008), ability of microbial isolates to adhere and parasitize the pathogen hyphae, and production of lytic enzyme (Nielson *et al*., 1998).

Table 15: Average percentage reduction of radial growth of *Ganoderma* after treated with mixture of potential antagonists on different location of SEA media

Growth media	Average percentage reduction of radial growth (%)
P1	62.14 ± 1.40cd
P2	56.20 ± 3.45c
P3	49.28 ± 1.25bc
P4	47.35 ± 2.79bc
P5	45.38 ± 3.87 b
P6	47.89 ± 2.09bc
P7	43.61 ± 5.29b
P8	51.96 ± 2.34bc
P9	48.83 ± 5.50bc
P10	68.83 ± 5.50d
P11	51.45 ± 1.25bc
P12	48.15 ± 3.21bc
P13	56.55 ± 6.27c
P14	62.93 ± 1.79cd
PDA	30.20 ± 2.03a

*Mean ± standard deviation. Different letters in the column indicate significantly different mean ($P<0.05$, Tukeys test)

4.6.1 Interactions of antagonists and soil organisms against *Ganoderma* at different inoculation times

In general, time of inoculation the antagonist mixture applied into the media affected the interaction patterns against the *Ganoderma* sp. The highest percentage reduction of radial growth of the *Ganoderma* sp. was when the antagonist was inoculated simultaneously with

the *Ganoderma* sp. on SEA media. The lowest percentage of radial reduction of the *Ganoderma* was when the treatment was paired after 5 days of inoculation of the *Ganoderma* sp (Table 16).

Clear zone of inhibition produced in *in-vitro* experiment is an indicative of antibiosis by biocontrol agent against fungal pathogens (Killani *et al*., 2011). The antibiosis production by the antagonists was suggested to be their primary mode of action. Dharmaputra and Tjitrosomo (1990) reported that antibiotic from antagonists diffused into the medium. The present study showed the highest inhibition zone during simultaneous inoculation of pathogen and antagonists. The pairings of antagonists after 5 days of inoculation of the pathogen however showed no inhibition zone. This might be due to the dominancy and stability of the pathogen over the time onto the media which increased their resistant towards antibiotic substances produced by the antagonists. This might indicated that timing of the pairing or inoculation and treatment is crucial to achieve the effectiveness of the antagonists against the pathogen. Linderman (2000) also reported that simultaneous inoculation of antagonists and pathogen was the most effective.

Hence, it is suggested that the simultaneous inoculation of antagonists and *Ganoderma* sp. was the best method to control the pathogen growth. The results also showed that the times of application on SEA media has a huge impact on the efficacy of potential antagonists against pathogenic *Ganoderma*.

Table 16: The average percentage reduction of *Ganoderma* radial growth after inoculated with potential antagonists with different inoculation period of *Ganoderma* on different types of SEA media

Soil extract	Average percentage reduction of radial growth (%)		
	simultaneous	after 2 days	after 5 days
P1	62.14 ± 1.40b	60.79 ± 2.18b	11.11 ± 19.24a
P2	56.20 ± 3.45b	37.82 ± 4.21ab	22.02 ± 19.59a
P3	49.28 ± 1.25b	32.44 ± 4.58a	33.97 ± 5.74a
P4	47.35 ± 2.79b	42.20 ± 1.96b	11.11 ± 19.24a
P5	45.38 ± 3.87 b	38.89 ± 12.73b	0.00 ± 0.00a
P6	47.89 ± 2.09b	39.22 ± 4.61a	33.33 ± 0.00a
P7	43.61 ± 5.29a	55.87 ± 8.97a	38.69 ± 10.76a
P8	51.96 ± 2.34b	49.60 ± 7.74b	17.78 ± 16.78a
P9	48.83 ± 5.50b	52.78 ± 4.81b	17.86 ± 15.57a
P10	68.83 ± 5.50c	48.18 ± 7.44b	0.00 ± 0.00a
P11	51.45 ± 1.25b	44.36 ± 3.05ab	35.12 ± 9.16a
P12	48.15 ± 3.21b	41.67 ± 8.34b	8.33 ± 14.43a
P13	56.55 ± 6.27b	46.11 ± 4.19b	17.86 ± 15.57a
P14	62.93 ± 1.79c	28.89 ± 11.35b	0.00 ± 0.00a
PDA	30.20 ± 2.03a	31.07 ± 4.26a	28.92 ± 2.57a

*Mean ± standard deviation. Different letters in the column indicate significantly different mean ($P<0.05$, Tukeys test)

4.6.2 Effect of nutrient sources on the efficacy of the antagonists against the *Ganoderma*

4.6.2.1 Carbon

In general, the mixed of antagonists and *Ganoderma* sp. were able to grow on various carbon sources. Data analysis of one way ANOVA showed that there were significant differences $P<0.05$ of percentages reduction of the *Ganoderma* sp. radial growth on different carbon sources. The highest percentage reduction of the radial growth was on medium containing sucrose while the least percentage reduction was on media containing lactose (Table 17). Thus, the results indicated that sucrose is the best carbon source for growth of the antagonists in controlling the *Ganoderma* sp. Lily and Barnett (1951) reported that some microbes were not able to grow on sucrose medium because of their inability to hydrolyze disaccharide due to the of invertase enzyme (Leite *et al*., 2003). Hence, it is suggested that antagonists is more effective to inhibit the growth of the pathogen when sucrose was added to the medium.

Table 17: The average percentage reduction of *Ganoderma* radial growth on media with different carbon sources

Sources of Carbon	Average percentage reduction of *Ganoderma* radial growth (%)
Fructose	60.00 ± 0.00c
lactose	34.72 ± 2.41a
sucrose	71.74 ± 2.17d
starch	57.69 ± 0.00c
CMC	50.00 ± 2.38b
glucose (control)	47.78 ± 1.92b
without carbon	38.89 ± 5.56a

*Mean ± standard deviation. Different letters in the column indicate significantly different mean ($P<0.05$, Tukeys test)

4.6.2.2 Nitrogen

The findings in this study indicated that the percentages radial reduction of *Ganoderma* sp. were significantly different at $P<0.05$ on medium containing different nitrogen sources. The highest percentage reduction of the radial growth was on the medium containing glycine (Table 18). High reduction percentage indicated that the efficacy of antagonistswas higher when glycine was used. Madan and Thind (2000) reported that glycine was a good source of nitrogen for most microbes. The lowest percentage of mycelial reduction of *Ganoderma* sp. was on medium without nitrogen sources which indicated that nitrogen is essential for growth of antagonists against *Ganoderma sp.* Papadianni (2004) also reported that omission of nitrogen in the medium greatly affects fungal growth and metabolites.

Table 18: Average percentage reduction of the *Ganoderma* radial growth on media with different nitrogen sources

Nitrogen sources	Average percentage reduction of *Ganoderma* radial growth (%)
Yeast	50.70 ± 1.21c
Peptone	60.87 ± 0.00d
Glycine	69.44 ± 2.40e
Sodium nitrate	26.47 ± 2.94b
Without nitrogen	18.18 ± 0.00a
Potassium nitrate (control)	47.78 ± 1.92c

*Mean ± standard deviation. Different letters in the column indicate significantly different mean ($P<0.05$, Tukeys test)

4.6.2.3 Potassium

Data analysis of one way ANOVA showed that there was no significant difference at $P<0.05$ between the average percentage reduction of radial growth of the *Ganoderma* sp. on the media containing different potassium sources (Table 19). Ingold and Hudson (1993) stated that potassium was essential for fungal nutrition. The lowest reduction of radial colony of *Ganoderma* sp. in the present study was on the medium without potassium. This might be due to loss of potassium from inhibition of various metabolic processes including glycolisis and respiration (Garraway and Evans, 1984). Potassium is required for both of these two processes and plays a role as cofactor in some enzyme systems (Chang and Miles, 1987). Higher percentage reduction was recorded when KCl was used. This indicated that the efficacy of the antagonists would be more effective to control *Ganoderma* sp. when KCl is used.

Table 19: Average percentage reduction of radial growth of *Ganoderma* on media with different potassium sources

Potassium sources	Average percentage reduction of *Ganoderma* radial growth (%)
KCl	54.76 ± 4.12c
K_2SO_4	46.88 ± 3.13bc
K_2HPO_4	41.67 ± 3.61b
Without potassium	16.19 ± 3.30a
KH_2PO_4, KNO_3 (control)	47.78 ± 1.92bc

*Mean ± standard deviation. Different letters in the column indicate significantly different mean ($P<0.05$, Tukeys test).

4.6.2.4 Magnesium

The results of this study indicated that there was no significant difference between the average percentages reduction of radial growth of the *Ganoderma* sp. on media of different magnesium sources (Table 20). The growth of antagonists and the *Ganoderma* sp. were very slow without magnesium. Morphologically, the mycelia on the media were thin and no spores were observed. This may indicated that the antagonists require magnesium elements in order to grow well and against growth of *Ganoderma* sp. Hawker (1950) reported that magnesium had a great effect on fungal sporulation. High percentage reduction of radial growth of the pathogen were recorded when MgCl was used which determined the effectivity of antagonists against the *Ganoderma* sp. It is suggested that the usage of MgCl is better in promoting the antagonists against growth of *Ganoderma* sp.

Table 20: The average percentage reduction of radial growth of *Ganoderma* on media with different magnesium sources

Magnesium sources	Average percentage reduction of *Ganoderma* radial growth (%)
MgCl	53.92 ± 4.49c
MgO	40.35 ± 3.04b
$MgSO_4.7H_2O$	46.88 ± 3.13bc
Without mg	12.53 ± 3.98a
$MgSO_4$ (control)	47.78 ± 1.92bc

*Mean ± standard deviation. Different letters in the column indicate significantly different mean ($P<0.05$, Tukeys test)

4.6.2.5 Phosphate

Statistical analysis of one way ANOVA showed that there were significant differences at $P<0.05$ between the average percentages reduction of radial growth of the *Ganoderma* sp. on media containing phosphate from different sources. The antagonists grew well on media containing different sources phosphate against *Ganoderma*. The highest reduction percentage of the *Ganoderma* sp. radial was on the media containing $NH_4H_2PO_4$, Na_2HPO_4, or KH_2PO_4 while the lowest was on media without phosphate (Table 21). This indicated that the antagonists require phosphate in order to grow optimally against the *Ganoderma* sp. Boddy *et al*. (1989) reported that phosphorus has a primary role in the storage and transfer of energy in most microbes. Hence, the absent of phosphate element might inhibit the growth of the antagonists in the media due to phosphorylation where the carbon compound from glucose could not be synthesized.

Table 21: The average of *Ganoderma* radial reduction percentage (%) on basic media with different phosphate sources

Phosphate sources	Average percentage reduction of *Ganoderma* radial growth (%)
K_2HPO_4	35.90 ± 4.44b
$NH_4H_2PO_4$	46.49 ± 1.52c
Na_2HPO_4	47.13 ± 3.98c
Without PO_4	14.29 ± 2.39a
KH_2PO_4 (Control)	47.78 ± 1.92c

*Mean ± standard deviation. Different letters in the column indicate significantly different mean ($P<0.05$, Tukeys test)

4.7 Interaction of soil microbes and antagonists on growth of *Ganoderma*

Data analysis of one way ANOVA indicated that there were significant differences at $P<0.05$ of interactions between the antagonists and selected soil microbes isolated from different oil palm plantations against the *Ganoderma* sp. The average percentages reduction of colony radial growth of the *Ganoderma* sp. ranged from 20% - 48% (Table 22). The highest percentage reduction of the *Ganoderma* sp. radial growth were recorded in the interaction between the antagonists alone (48.33%) while the lowest percentage reduction was observed from mixture D, from Sg. Noren plantation (20.20%).

Physical observation indicated that the diameter of the *Ganoderma* sp. was longer when treated with mixture of soil microbes and antagonists compared to the treatment with the antagonists alone. This might be due to the diffusion of metabolic compounds from the soil microbes in the media which could enhance the pathogen growth. Klett *et al*. (2011) also

reported that some bacterial metabolites are able to stimulate hyphal growth of certain fungi by promoting the extension of the fungal mycelium. Hence, it is suggested that the presence of the soil microbial mixture in the media could reduce the efficacy of the antagonists against the *Ganoderma* sp.

Table 22: Average percentages (%) reduction of colony radial growth of the *Ganoderma* sp. after treated with the potential antagonists and selected microbes isolated from different sampling plots

Soil microbes	Average percentage reduction of *Ganoderma* radial growth (%)
Mixture A (Sempadi) + Antagonists	33.50 ± 5.81ab
Mixture B (Sg Mata) + Antagonists	24.24 ± 3.03ab
Mixture C (Bangka Semong) + Antagonists	41.41 ± 5.26b
Mixture D (Sg Noren) + Antagonists	20.20 ± 6.31a
Mixture E (Sg Staman) + Antagonists	38.38 ± 7.63b
Antagonists alone	48.33 ± 0.70c

*Mean ± standard deviation. Different letters in the column indicate significantly different mean ($P<0.05$, Tukeys test)

4.8 Effect of volatile compound of soil microbes on the efficacy of the antagonists against the *Ganoderma* sp.

The results showed that all mixture of the microbes isolated from the soil of five oil palm plantations produced volatile compounds. There was no significant difference at $P<0.05$ on the effect of the volatile compound produced by selected soil microbes on the efficacy of the antagonists against the *Ganoderma* sp. The average percentage reduction of colony

radial growth of the *Ganoderma* sp. in the presence of volatile compounds ranged from 19% - 32% (Table 22).

Table 23: The percentage (%) reduction of colony radial growth of the *Ganoderma* sp. after treated with volatile compounds of soil microbes from different oil palm location

Volatile compound	Average percentage reduction of *Ganoderma* radial growth (%)
Sempadi	28.43 ± 5.85a
Sg Mata	18.78 ± 5.08a
Bangka Semong	20.47 ± 8.91a
Sg Noren	32.32 ± 3.88a
Sg Staman	20.48 ± 6.39a
without volatile compound	48.33 ± 0.70b

*Mean ± standard deviation. Different letters in the column indicate significantly different mean ($P<0.05$, Tukeys test)

Comparison on the colony of the *Ganoderma* sp. in the mixed culturesshowed that the pathogen growth was faster compared to the antagonists alone as well as on untreated *Ganoderma* sp. (Figure 13 and 14). According to Alstrom (2001), the fungi and bacteria synthesize and emit many volatile compounds. Vespermann *et al*. (2007) reported that the volatiles compound produces by soil bacteria can act as antibiotics and growth promoting features on the organisms. Thus, the result in this study indicated that rapid growth of the *Ganoderma* sp. might be due to the presence of volatile compounds produced by soil microbes.

When the mix cultures were exposed to volatile compound, it was shown that the percentage reduction of the *Ganoderma* radial growth decreased except when exposed to volatile compound produced by microbes in soils of Sg. Noren plantation. This indicated that the efficacy of the potential antagonists to inhibit growth of *Ganoderma* sp. was reduced in the presence of volatile compounds.

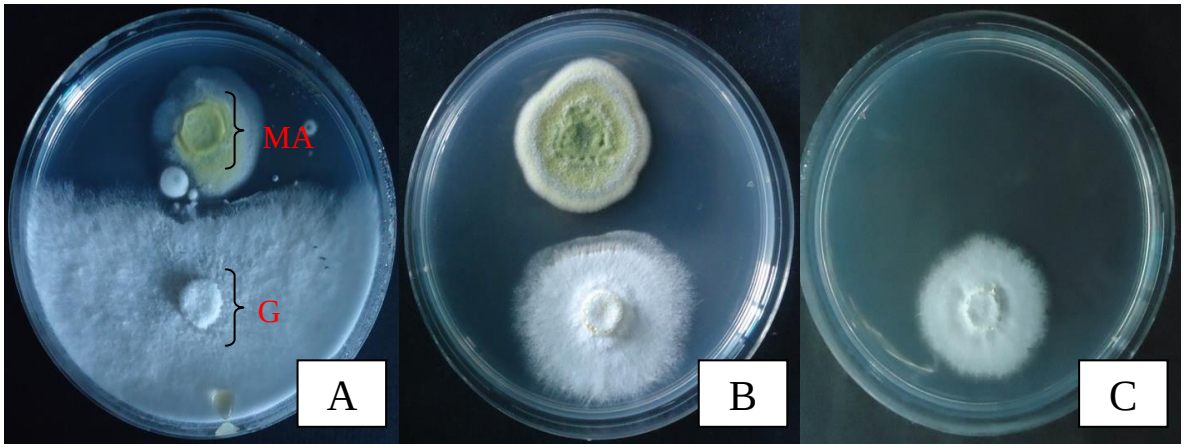

Figure 13: Culture appearance of the *Ganoderma* sp. on PDA after 8 days incubated at room temperature. A. Inoculated together with mixture of antagoists and exposed to volatile compounds of soil microorganisms from Sempadi plantation. B. Inoculated together with mixture of the antagonist only. C. Without volatile compound and mixture of antagonists

G. *Ganoderma* sp. **MA**. Mixture of antagonists

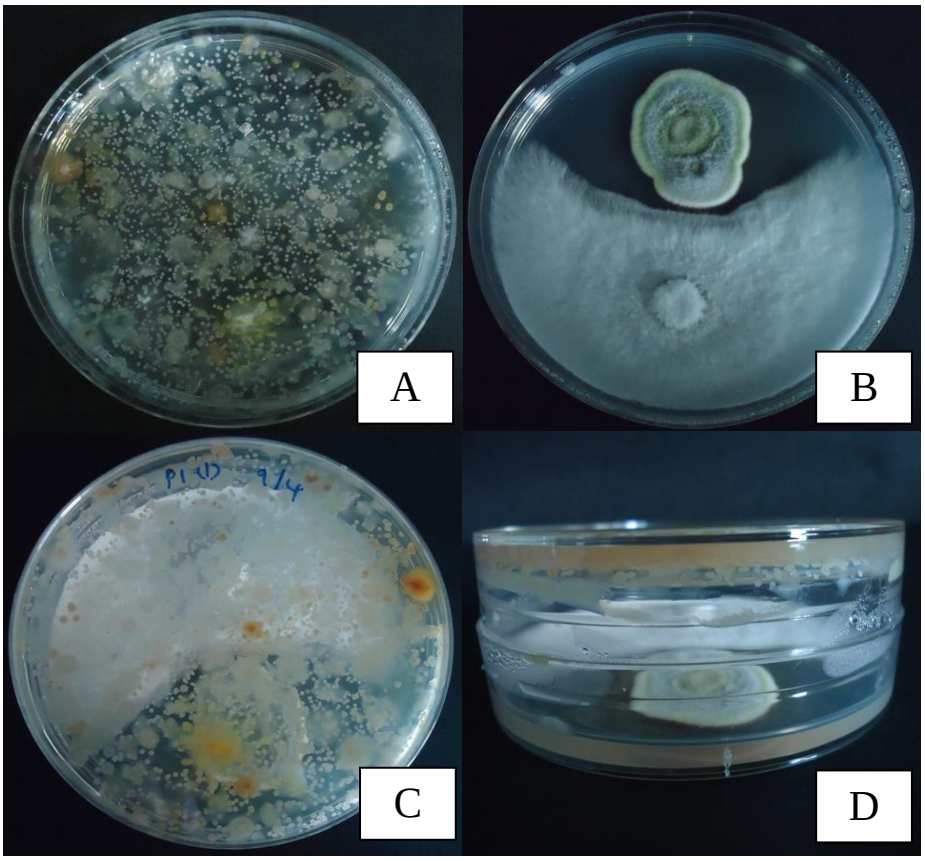

Figure 14: Volatile test of soil microbes on the efficacy of mixed antagonist against *Ganoderma* sp. A. Soil microorganisms from Bangka Semong plantation B. *Ganoderma* sp. and mixed antagonists C. Top view of culture D. Side view of culture

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

Results from this study proved that the efficacy of antagonists against the *Ganoderma* sp. were affected by environmental conditions especially soil physicochemical properties and microorganisms in the field.

In this study, the association of soil microorganisms, moisture content, pH, and nutrients concentration with non-infected and *Ganoderma* sp. infected oil palm were successfully investigated. Although both location of free disease and *Ganoderma* sp infected areas showed the presence of several potential antagonists, their colony density in the infected area might be insufficient to combat *Ganoderma* sp infection. Hence, it warrants further studies on the quantification of potential biocontrol agents in soil in order to determine the capacity of the soil to control the disease caused by soilborne fungi.

It was discovered that the soil moisture content and pH was highly correlated with the soil depth in both non-infected and *Ganoderma* infected oil palm areas. The variations of moisture content and pH were probably influenced by weather condition, topography factors, soil properties and organic materials. However, no specific pattern of correlation was recorded between soil moisture content, pH, nutrients and microbes with the occurrence of *Ganoderma* infection in oil palm plantations.

macroelements such as nitrogen, phosphate, potassium and magnesium had significant effects on the efficacy of the antagonists against *Ganoderma* sp. The antagonists perfomed optimally on media containing sucrose, glycine, potassium chloride, and all sources of magnesium and phosphate resulted with higher inhibiton percentage of radial growth of *Ganoderma* sp.

This study suggested that the major factors contribute to the inefficacy of antagonists in the field might be due to the presence of volatile compounds produced by soil microorganisms which trigger rapid growth of *Ganoderma* sp. and finally the action of natural antagonists alone might be insufficient to control the pathogen. It is suggested that further studies should be carried out on the volatile and non-volatile compounds produced by soil microorganisms in non-infected and *Ganoderma* sp. infected oil palm plantations in order to detect specific compound which could triggers the growth of *Ganoderma* sp.

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Appendix 1

Monthly Average moisture content of soil of different plots in Sempadi oil palm plantation at different depth from February – July 2014

Month	Plot	Average soil moisture content in different depth (%)		
		0-15 cm	15-30 cm	45-60 cm
February	P9	27.56 ± 2.28b	24.26 ± 2.21b	17.81 ± 1.81a
	P10	46.65 ± 1.34c	27.87 ± 1.24b	17.82 ± 0.64a
	P11	23.5 ± 0.77a	24.45 ± 1.86a	38.59 ± 3.12b
	P12	34.38 ± 3.23c	27.19 ± 1.62b	20.53 ± 1.43a
	P13	34.24 ± 3.42b	25.26 ± 2.68a	22.53 ± 1.03a
	P14	39.15 ± 2.59b	26.34 ± 1.86a	26.19 ± 2.40a
March	P9	23.44 ± 0.61c	21.81 ± 0.02b	20.00 ± 0.36a
	P10	24.37 ± 0.13b	24.31 ± 0.31b	20.45 ± 0.28a
	P11	23.96 ± 0.47c	22.70 ± 0.38b	18.70 ± 0.13a
	P12	20.53 ± 0.98b	10.43 ± 0.38a	9.67 ± 0.26a
	P13	14.33 ± 0.55a	14.06 ± 0.32a	14.55 ± 0.46a
	P14	11.67 ± 0.41a	19.38 ± 0.24c	17.56 ± 0.17b
April	P9	45.80 ± 1.19c	31.65 ± 0.66b	25.72 ± 0.11a
	P10	25.50 ± 0.12c	22.23 ± 1.33b	19.91 ± 0.20a
	P11	29.56 ± 2.21b	24.49 ± 0.54a	21.65 ± 0.36a
	P12	18.26 ± 0.81b	15.20 ± 0.60a	15.13 ± 0.22a
	P13	18.48 ± 0.27c	16.73 ± 0.22a	17.66 ± 0.44b
	P14	20.50 ± 0.03b	19.99 ± 0.56b	18.45 ± 0.13a
May	P9	7.86 ± 2.32a	7.67 ± 1.63a	8.47 ± 1.76a
	P10	7.59 ± 1.86a	12.00 ± 0.41b	19.38 ± 0.07c
	P11	43.08 ± 1.23c	22.71 ± 1.85b	17.65 ± 0.24a
	P12	15.60 ± 0.23c	14.13 ± 0.11b	13.04 ± 0.08a
	P13	7.61 ± 0.13a	9.27 ± 0.25b	10.62 ± 0.45c
	P14	17.96 ± 0.09b	18.83 ± 0.07c	17.45 ± 0.20a
June	P9	8.67 ± 0.25b	7.64 ± 0.03a	8.24 ± 0.30b
	P10	32.31 ± 1.14c	20.60 ± 0.50b	10.72 ± 3.74a
	P11	8.05 ± 3.91a	7.65 ± 3.57a	12.28 ± 1.43a
	P12	5.73 ± 0.08a	7.10 ± 0.15b	9.78 ± 0.22c
	P13	17.06 ± 0.18a	17.63 ± 0.12b	18.56 ± 0.31c
	P14	4.34 ± 0.09a	6.10 ± 0.14b	7.57 ± 0.11c
July	P9	24.81 ± 0.69c	19.28 ± 0.46b	17.30 ± 0.52a

	P10	35.45 ± 0.13c	21.82 ± 1.66b	18.18 ± 0.61a
	P11	26.14 ± 0.60c	23.59 ± 0.61b	20.46 ± 0.25a
	P12	21.54 ± 0.15c	21.07 ± 0.04b	19.05 ± 0.07a
	P13	14.96 ± 0.17b	14.53 ± 0.28b	12.44 ± 0.11a
	P14	15.20 ± 0.03c	14.25 ± 0.05b	12.80 0.29a

*Mean ± standard deviation. Different letters in the row indicate significantly different mean ($P<0.05$, Tukeys test)

Appendix 2

Monthly average pH of soil of different plots in Sempadi oil palm plantation at different depth from February – July 2014

Month	Plot	Average pH of soil in different depth		
		0-15 cm	15-30 cm	45-60 cm
February	P9	5.33 ± 0.06b	4.77 ± 0.06a	4.87 ± 0.06a
	P10	5.20 ± 0.10c	4.87 ± 0.06b	4.60 ± 0.00a
	P11	5.03 ± 0.06c	4.77 ± 0.06a	4.90 ± 0.00b
	P12	4.60 ± 0.00a	5.13 ± 0.07b	5.87 ± 0.06c
	P13	4.90 ± 0.00a	5.33 ± 0.06b	5.17 ± 0.12b
	P14	6.57 ± 0.06a	6.83 ± 0.12b	6.57 ± 0.06a
March	P9	4.33 ± 0.06a	5.00 ± 0.00c	4.60 ± 0.00b
	P10	4.97 ± 0.06c	4.50 ± 0.00b	4.23 ± 0.06a
	P11	4.73 ± 0.06b	4.40 ± 0.00a	4.30 ± 0.10a
	P12	5.10 ± 0.10a	5.23 ± 0.06ab	5.37 ± 0.06b
	P13	4.60 ± 0.00c	3.90 ± 0.10b	3.53 ± 0.06a
	P14	5.60 ± 0.00b	4.33 ± 0.06a	5.70 ± 0.00c
April	P9	5.67 ± 0.06c	5.50 ± 0.00b	5.27 ± 0.06a
	P10	6.53 ± 0.06c	5.60 ± 0.00b	5.03 ± 0.06a
	P11	5.77 ± 0.06c	5.23 ± 0.06b	4.77 ± 0.06a
	P12	5.60 ± 0.00b	5.43 ± 0.06b	4.90 ± 0.10a
	P13	4.83 ± 0.23a	4.63 ± 0.12a	4.87 ± 0.12a
	P14	5.07 ± 0.06a	5.10 ± 0.10a	5.60 ± 0.00b
May	P9	7.30 ± 0.00b	6.97 ± 0.06a	6.90 ± 0.10a
	P10	6.50 ± 0.00c	5.87 ± 0.06b	5.60 ± 0.00a
	P11	5.97 ± 0.06c	5.80 ± 0.00b	5.47 ± 0.06a
	P12	5.97 ± 0.06b	5.90 ± 0.00b	5.67 ± 0.06a
	P13	6.37 ± 0.06a	6.43 ± 0.06a	6.60 ± 0.00b
	P14	5.10 ± 0.00a	5.27 ± 0.06b	5.23 ± 0.06b

June	P9	6.67 ± 0.06a	6.80 ± 0.00b	6.63 ± 0.06a
	P10	6.17 ± 0.06c	5.97 ± 0.06b	5.67 ± 0.06a
	P11	5.90 ± 0.00c	5.57 ± 0.06b	5.07 ± 0.06a
	P12	6.53 ± 0.06b	6.60 ± 0.00b	6.37 ± 0.06a
	P13	5.30 ± 0.00b	5.43 ± 0.06c	5.20 ± 0.00a
	P14	7.67 ± 0.06b	7.50 ± 0.00a	7.67 ± 0.06b
July	P9	6.70 ± 0.00c	6.07 ± 0.06b	5.77 ± 0.06a
	P10	6.70 ± 0.00a	7.00 ± 0.00b	7.07 ± 0.06b
	P11	6.87 ± 0.06c	6.00 ± 0.10b	5.20 ± 0.00a
	P12	6.27 ± 0.06c	6.07 ± 0.06b	5.77 ± 0.06a
	P13	6.70 ± 0.00a	6.77 ± 0.06a	6.90 ± 0.00b
	P14	7.10 ± 0.00a	7.27 ± 0.06b	7.33 ± 0.06b

*Mean ± standard deviation. Different letters in the row indicate significantly different mean ($P<0.05$, Tukeys test).

Appendix 3

Monthly average pH of top soil in free disease and *Ganoderma* infected oil palm areas from February – July 2014

Areas	Plot	Average pH of top soil in different month					
		February	March	April	May	June	July
Non-infected area	P9	5.33 ± 0.06c	4.33 ± 0.06a	5.67 ± 0.06b	7.30 ± 0.00e	6.67 ± 0.06e	6.70 ± 0.00b
	P10	5.20 ± 0.10c	4.97 ± 0.06c	6.53 ± 0.06c	6.50 ± 0.00d	6.17 ± 0.06c	6.70 ± 0.00b
	P11	5.03 ± 0.06b	4.73 ± 0.06b	5.77 ± 0.06b	5.97 ± 0.06b	5.90 ± 0.00b	6.87 ± 0.06c
Ganoderma infected area	P12	4.60 ± 0.00a	5.10 ± 0.10c	5.60 ± 0.00b	5.97 ± 0.06b	6.53 ± 0.06d	6.27 ± 0.06a
	P13	4.90 ± 0.00b	4.60 ± 0.00b	4.83 ± 0.23a	6.37 ± 0.06c	5.30 ± 0.00a	6.70 ± 0.00b
	P14	6.57 ± 0.06d	5.60 ± 0.00d	5.07 ± 0.06a	5.10 ± 0.00a	7.67 ± 0.06f	7.10 ± 0.00d

*Mean ± standard deviation. Different letters in the column indicate significantly different mean ($P<0.05$, Tukeys test)