PRELIMINARY SCREENING FOR POTENTIAL ANTAGONIST ORGANISMS AGAINST *Poria hypolateritia* OF RED ROOT DISEASE

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DECLARATION

I admitted that this dissertation is my own effort except for some reference information which each one had been quoted its source.

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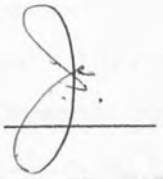

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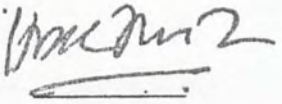

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ABSTRAK

Poria hypolateritia dikategorikan sebagai patogen penyakit akar pokok teh yang boleh menyebabkan kematian. Objektif utama kajian ini adalah untuk membuat pengesanan asas organisma antagonis ke atas pertumbuhan *Poria*. Kaedah dual kultur dipraktikkan untuk memerhatikan interaksi antara *Poria* dan isolat tanah. Kaedah ini diubahsuai dengan menempatkan *Poria* di tengah dan isolat tanah diletakkan 2 cm dari sisi piring Petri; bertentangan antara satu sama lain. Isolat-isolat tanah yang diperolehi dilabel sebagai NF 1, NF 2, NF 3, NF 4, NF 5, NF 6, NF 7, NF 8, NF 9, NF 10, NF 11 dan NF 12. Selepas interaksi selama 12 hari, hanya 2 isolat iaitu NF 1 dan NF 5 boleh disifatkan sebagai organisma antagonis yang berpotensi dengan nilai Peratus Perencatan Pertumbuhan Jejari (PIRG) masing-masing pada 86.67 peratus dan 85.33 peratus. Namun, sebenarnya kedua-dua isolat tersebut adalah dalam genus yang sama. Melalui pemeriksaan mikroskopik, NF 1 berkemungkinan adalah *Cunninghamella*. Kulat-kulat lain yang berjaya diasingkan dari tanah adalah *Trichoderma, Stylopage, Verticillium, Blastomyces, Ovulariopsis, Periconia* dan *Ustilago*. Kulat yang lain menunjukkan sama ada interaksi agonisma atau mutualisma ke atas *Poria*. Kajian lanjut perlu dijalankan dengan menitikberatkan interaksi yang mungkin berlaku dalam persekitaran yang sebenar sebagai salah satu kriteria yang utama. Malah, kajian ke atas kemampuan organisma-organisma agonis juga perlu dijalankan kerana kemungkinan mereka juga mampu bertindak sebagai perencat yang berpotensi. Impak organisma agonis ini ke atas tumbesaran pokok teh memerlukan penilaian yang teliti dan bukti yang kukuh sebelum dikomersilkan.

ABSTRACT

Poria hypolateritia is categorized under root disease pathogen for tea plants which can bring fatality to tea bushes. The objective of this study was mainly on preliminary screening for potential antagonist organisms against *Poria*. Dual culture method was practiced in order to see the interaction between *Poria* and soil isolates. This method was slightly modified by allocating *Poria* in the middle and the soil isolate was inoculated 2 cm from the periphery of the plate; opposite to each other. The soil isolates was labeled as NF 1, NF 2, NF 3, NF 4, NF 5, NF 6, NF 7, NF 8, NF 9, NF 10, NF 11 and NF 12. After 12 days of interaction, solely two isolates; NF 1 and NF 5 were pointed as potential antagonist organisms with the percentage of inhibition in radius growth (PIRG) of 86.67 percent and 85.33 percent respectively. But, both isolates were virtually in the same genus. Through microscopic examination, NF 1 was suspected to be *Cunninghamella*. Other successfully isolated fungi are *Trichoderma, Stylopage, Verticillium, Blastomyces, Ovulariopsis, Periconia* and *Ustilago*. The rest of the fungi showed either agonism or mutualism interaction against *Poria*. Further study need to be implemented considering the interaction that might be practiced in the natural environment as one the vital criteria. Besides, research on the capability of agonist organisms need to be done as they also can be pointed as potential inhibitors. The impact of agonist organisms on tea's growth needs detail evaluation and strong evidence prior to commercializing them.

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°C	celsius
µm	micrometer
psi	pressure
min	minute
cm	centimeter

CHAPTER 1

INTRODUCTION

1.1 Introduction

The science of plant pathology was born in Ireland in the 1840s (Schumann 1999). This field was born out of necessity. The pests and diseases that robbed the people's food had plagued humanity for millennia and reached a climax during the suffering of the Irish during the potato famine. The desperate calls for help finally brought the most eminent scientists to search for the answer. The answer was provided by Anton deBary, long considered as the father of plant pathology (Sequeira, 2000).

Diseases are not self-perpetuating. Only the pathogen is. On that account diseases cannot be triggered, kindled or incited. In fact, if the pathogen that causes a disease is removed, the disease stops progressing (Chandniwala, 1995). Pathogen development may facilitate by various factors and may also be varnished through various methods.

Pytopathologists have neglected for a long time the role of the microbial population of the soil, considering only the parasite and the disease. Sanford (1926)

and Millard and Taylor (1927) began the study of the relations existing between soil microorganisms and the pathogen host complex (Chandniwala, 1995). However, the seminal work that crystallized biocontrol research into coherent discipline was 'Ecology of Soil-Borne Plant Pathogens: Prelude to Biological Control', based on the meeting in 1963 at Berkeley, California (Paulitz, 2000).

The distribution of antagonist organisms varied widely according to the different samples of the soil. Among the many antagonistic bacteria were *Bacillus subtilis*, and the most frequently occurring antagonistic fungi were species of *Penicillium*, *Aspergillus*, *Trichoderma* and *Trichothecium* (Chandiwala, 1995). During the past decade biological control of soil borne plant pathogens was demonstrated with the organic soil amendments containing *Trichoderma* spp. (Bell *et al*., 1982).

One of the species in *Trichoderma*; *Trichoderma pseudokoningii* found to be able to impede the spread over of *Poria hypolateritia*; the causal agent of red root disease in tea plantation. *Aspergillus* also can hamper this pathogen but merely as pre-pathogen (Cooray & Balasuriya, 2002). The infection of *Poria* on tea bushes not only means a gradual loss of yield and income during the period of infection, but also a loss of capital because of death of the entire bush (Fuchs, 1989).

Recently, Sabah Tea Plantation has been found to be an area infected by this disease. Countless methods have been applied including planting Guatemala grass, uprooting, drenching and sanitation by burning the infected area. All the mentioned methods did not reach the target to fully eradicate the disease severity.

In fact, methyl bromide had been sprayed over to eradicate *Poria*. But, this merely jeopardizes Sabah Tea Plantation as one of the few organic tea producers. Therefore, application of methyl bromide has been discontinued. Thus, biological control may be the best alternative to overcome this problem.

1.2 Scope of Research

The research is focus on isolating *Poria* from infected areas and residual potential antagonist organisms against this causal agent from healthy areas of Sabah Tea Garden.

1.3 Research Objective

Preliminary screening on potential soil isolates to inhibit *Poria hypolateritia.*

1.4 Hypothesis

Null: There are no potential antagonist organisms which are able to inhibit *Poria hypolateritia.*

Alternative: There are potential antagonist organisms which are able to inhibit *Poria hypolateritia.*

CHAPTER 2

LITERATURE REVIEW

2.1 Tea

Tea or *Camellia sinensis* belongs to the family Theaceae or Cameliaceae. It is a common beverage made by processing the leaves or buds of the tea bush. The habitat is native to Southwest Asia, China and India, where it still grows wild. It is widely farmed in the above mentioned countries as well as in Brazil and in tropical Africa (Roger, 2001).

The shrub grows up to 10 m high when wild and 1 to 2 m when farmed. In a wide plantation area, the better type of bushes is roughly cone-shaped. The root distribution is distinctly variable. Some bushes developed deep laterals whereas some may produce horizontal roots which preferred very shallow penetration in soil. When almost mature, the roots are devoid of root hairs. Besides the rudimentary functions of nutrients and water absorption, the roots lay down starch granules in their cells, thus act as essential storage organ when they reached a diameter of between 1 and 2 mm. This stored carbohydrate has a critical function after pruning.

It is a perennial plant with dark green and waxy surface leaves. Young leaves and branches develop from buds in the axils of mature leaves. These leaves are evergreen, obovate, lanceolate in shape and acuminate. Normal mature leaves are serrated at the margin. The leaf length does not exceed 5 cm with short petiole. The buds and internodes are more profusely hairy.

Besides, it has a big and white aromatic flower. The globular flower buds are borne in the axils of scale leaves. The fully bloomed flower has a persistence calyx with 5 to 7 sepals. The petals, corresponding in the number to the sepals, are white in colour with a smooth appearance. The petals are obovate, emarginate and internally concave. The stamens are long with yellow twin-celled anthers and are free above 2 to 3 mm. The ovary is hairy with single style split into three to five arms.

The green fruit is three celled and thick-walled. The fruit dehisces by splitting from the apex into three valves. The brown seed is thin-celled with approximately 1 cm in diameter, either semi globose or rounded at the back and wedge-shaped in front. It consists of two large cotyledons; a distinct structure of radicle and plumule can be seen (Eden, 1976).

Tea leaves contain from 1% to 4% of caffeine (called theine to differentiate its origin), tannins approximately 15% to 20% and one essence. Its effects are very similar to those of coffee, though less intense as tea infusions are prepared less concentrated. Tea stimulates nervous system and increases the secretion of acid juices in the stomach (Kartasapoetra, 1992).

The legendary origin of tea drinking has been traced back to the Chinese Emperor Chen Nung of 2737BC, who was also a scholar and herbalist, who discovered this drink when he was sitting beneath a tree while his servant was boiling a pot of water. A few leaves from a tea plant dropped into the pot of water, gave an excellent aroma and he found it tasted as good when sipped (Sabah Tea, 2005).

2.1.1 Sabah Tea Plantation

Sabah Tea Plantation, nestled into the lush tropical wilderness of Malaysia's first ever World Heritage Site, Mount Kinabalu, sits on a 6,200-acre land at 2,272 feet above sea level. It is surrounded by the world's oldest rainforest of about 130 million years. Sabah Tea Plantation is the largest single commercial tea plantation in Borneo with approximated area of 1,000 acres.

Being certified by Control Union Certification or formerly known as SKAL International, Netherlands after fulfilling all the required criteria listed under Organic Exchange 100 Guidelines (OE 100 Guidelines) and EN 45011 (accreditation), Sabah Tea is one of the few tea plantations in the world to produce organic tea (Chong *et al.*, 2004).

The quality of the yield is well-known and famed besides widely exported to other countries such as Taiwan and Japan. But, the recent red root infection interrupted yield production and may cause serious lost to the country. At the moment, the fungus colonies are kept at bay. But, the spread of the disease to the whole area is possible as they proliferate rapidly (Chong *et al* 2004).

2.2 Diseases of Tea

There are a few of diseases discovered infecting tea bushes. Brown blight caused by *Glomerella cingulata* is a common disease infecting tea plantation. Grey blight brought by *Pestalotiopsis theae* is reported to be fairly common (Williams & Liu, 1976). Blister blight is economically the most important disease, but relatively simple to control (Fuchs 1989). Besides, red rust and grey leaf scabs are occasionally attacked tea plantation (Williams & Liu 1976). Nevertheless, the pathologists concerned more about root diseases as it results risky lost upon the country's economy.

2.2.1 Root Diseases of Tea

Root diseases are a subject of great importance to the tea industry. In North- East India, 11 root diseases are known, 6 of which are primary and may be the direct cause of death, while the others are secondary and only attack bushes weakened by some other cause, such as adverse soil conditions (Harler, 1966). Unlike most of the primary root diseases, the secondary ones may be hampered by adjusting the unfavourable conditions to creep up the yield.

The form of inoculum existing in soil that initiates infection of host tissue directly or indirectly is termed primary inoculum (Campbell & Benson, 1994). Primary root disease fungi on one bush may infect and then kill any or all of the surrounding bushes. Charcoal stump rot, is the commonest primary root disease in North-East India. Red root rot, *Poria hypolateritia*; brown root rot, *Fomes lamaoensis*;

black root rot, *Roseilinia arcuata*; tarry root rot, *Hypoxylon asarcodes* and purple root rot, *Helicobasidium compactum*, are the less common primary root diseases (Harler, 1966).

Secondary root disease, on the other hand, attack weak bushes and only the dead bush needs removal. The causal agents are including *Sphaerostilbe repens*, *Botryodiplodia theobromae*, *Rhizoctonia bataticola*, *Aglaospora* sp. and *Poria hypobrunnea*.

a. *Poria hypolateritia*

Poria hypolateritia belongs to the class of Basidiomycetes, the phylum of Basidiomycota. The fungi in this phylum is classified as a high level of fungi which produce mycelia and basidiocarp as their fruiting bodies. Spores are produced on the structure name basidium, which are usually quadruplet in number and called basidiospore(Chong *et al.*, 2004). Mycelium of the basidiomycetes, always presence a unique septum termed as dolipore as shown in Figure 2.1 ((Kwon-Chung & Popkin, 1976).

Poria is a common root disease in Sri Lanka and recently detected to be the main causal of yield reduction in Sabah Tea Plantation. The disease is said to have originated from the stumps of jungle trees, which were not completely destroyed when the tea was originally planted. The stumps of Dun trees, *Doona gardneri*, are said to have been hosts of this fungus (Arulpragasam, 1987).

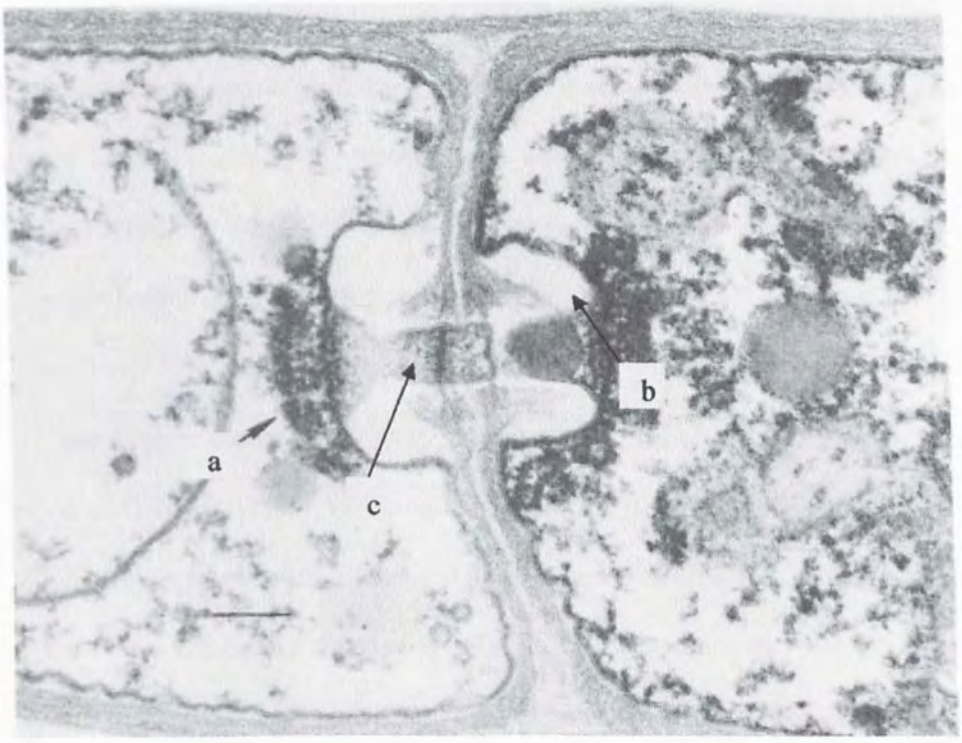

Figure 2.1 Unique structure of septum known as dolipore. The arrows show (a) pore cap, (b) rim and (c) pore. Bar, 0.5 µm (Kwon-Chung & Popkin, 1976).

2.3 Infection Process

P. hypolateritia can appear on solitary bushes or more usually in patches. It spreads by means of fungal threads or mycelial strands that grow from infected stumps or roots and infest adjoining healthy tea roots. Root contact is not essential for infection because the fungus is capable of growing via soil for some distance (Fuchs, 1989).

The disease can be easily recognized by the mycelial strands, which the fungus forms as a distinct surface of the infected root. Being at a full-grown stage, the mycelium on the roots becomes compacted into smooth, thin, red cords or sheets and gives the root a red colour mottled appearance. At this stage, the fungus thoroughly disintegrates the root tissues, leaving a formless moist pulp. The sudden death of a part or the whole of the tea bush follows with withered leaves remaining attached to the branches (Fuchs, 1989).

Optimal yield production at Sabah Tea is hampered by the presence of this particular disease (Chong *et al.*, 2004). Various methods have been applied to overcome the infection but yet the return still skews from the origin yield production.

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