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IDENTIFICATION OF CAUSAL PATHOGEN OF ROOT DISEASE AT SABAH TEA PLANTATION

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DECLARATION

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ABSTRACT

Sabah Tea Plantation is the largest organic tea plantation in the state of Sabah. It is situated in the elevation of 1200m as the highest and produces one of the finest tea grade around the world. The optimal yield production is hindered by the presence of root disease, with the most common one being the red root disease. The causal agent of this red root disease is believed to be *Poria hypolateritia* of the class Basidiomycetes. In identifying the causal pathogen of the root disease, three samples of roots were taken from each of five infected root tea bushes at Sabah Tea. The fifteen root samples were placed in sterilized blue capped bottles and were brought back to Plant Technology laboratory of UMS for culture and identification using two different methods. The methods include root tissues culturing which is of obtaining pure cultures and root sample segmentation method as direct visualization of microbial infection. The presence of characteristics such as the spores, fruiting bodies, hyphae attachments, the presence or absence of hyphal septa and clamp connections. The microscopic examination resulted the confirmed group of pathogen where there is clamp connections does show that *Poria hypolateritia* is the causal pathogen. Future research can be carried out as pathogenicity tests conducted on *Poría* spp. thus leave room for control or prevention methods.
ABSTRAK

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CHAPTER 1

INTRODUCTION

1.1 Tea Plant and Root Disease

Soil is a reservoir for innumerable plant pathogens. Root interacts with soil-borne pathogens exhibiting temporal and apatial variations. If the pathogen becomes dominant, disease is the result. Fungi dominates as the largest constituent as plant root pathogen in relation to plant health and productivity compared to soil-borne bacteria, nematodes and viruses (Alexander, 1961). Plant roots are beset with problems described as damping-off, seedling blight, root rots, crown rots, foot rots, club roots, pink roots, root browning, root parasites and wilts (Duggar, 2002). That brings us to the case of study where tea becomes exposed to this problem as well. Tea or the oldest known beverage is native of China in South East Asia. It was known to the Chinese as early as 2737 BC, but attained the status of a popular drink in England in 1664 AD.
The organic tea plantation in this area of research is at Sabah Tea Plantation, where it is the largest single commercial tea plantation in Borneo in approximated area of 10,000 acres (Sabah Tea Plantation Online, 2004). Glancing at whole, India is the largest producer, consumer and exporter in tea industry. Tea belongs to the genus *Camellia* and family Camelliaceae. The original species, which produces tea, were *C. assamica* (Assam jats), *C. sinensis* (China jats) and their natural hybrid, *C. assamica* subspecies lasiocalyx (Indo China or cambod type). In the sense of tea varieties, they are cultivated from the original tea plant *Thea sinensis* and *Thea assamica* (Grist, 1950). Organic cultivation of tea requires varieties (clones) with broad-scope resistances, and the ability to thrive under shade trees (upright, dark green leaves). Organically cultivated tea was first produced in 1986 in Sri Lanka. Since then, it has become widespread mostly in India and Sri Lanka and currently, about 5000 hectares of tea are being cultivated organically, in both the countries. Among other countries which produce tea include China, Japan, Seychelles, Tanzania, Kenya, Malawi, and Argentina (Glover et al., 1961).

When it comes to usage, tea is primarily drunk as black tea. Other sorts with less importance to the worlds market area green tea (East Asia, Arabian countries) and Oolong-tea (China, Taiwan). Recently, organic green tea is being manufactured in increasing quantities. Instant tea has also begun to be manufactured in increasing number as well. The majority of today's medical research involving tea is linked to green tea, but new studies are being conducted to include black tea. The Chinese have always believed that tea has strong medicinal uses. One Chinese proverb says, "The wisdom of 10,000 universes can be found in a cup of tea" Emperor Shen Nung presumably said, "Tea gives
you vigor of body, contentment of mind and determination of purpose" (Encyclopaedia Britannica Online, 2006). Through research, tea has been linked to the reduction of the risk of cancers, atherosclerosis, and heart disease because of its strong antioxidant properties. One study compared tea extracts to the antibiotics amoxicillin, cephradine, and eugenol because the extracts strongly inhibited *Escherichia coli*, *Streptococcus salivarius*, and *Streptococcus mutans* on dental caries (Rasheed and Haider, 1998).

Shifting towards the aspect of botany of the tea plant, it is an evergreen shrub, which in its natural state grows to a height of 15 to 30 ft., but the tea planter keeps it pruned down to a height of 3 to 5 ft. In its general appearance and the elliptic form of its two- to five-inch-long leaf, it resembles the myrtle. The five petaled blossoms are white, with yellow anthers; the fruit is a woody capsule containing three seeds. The plant grows well in the monsoon climate of the tropics, from sea level up to 6,000 ft., doing best in the higher altitudes; in more temperate zones, where there is danger of frost, it is grown at low elevations. The commercial tea belt of the world is largely confined to a ring of mountains around or near the equator, within 42 degrees N. and 33 degrees S (Encyclopaedia Britannica Online, 2006).

Specifying the scope where the pathogens involved in root rots in perennials, the phylum basidiomycetes dominates the pathogenic list. These fungi build up inoculums potential by means of rhizomorphs, mycelia strands, or mats around the root to be infected. Penetration occurs directly or through wounds. These fungi can attack actively growing, mature or weakened trees. Seedlings with primary tissues are rarely killed but
seedlings transplanted to former disease sites are often killed. Infections generally result in a slow host decline when lateral roots are infected first, and the infection slowly proceeds to the taproot. In some plants, a sudden wilt occurs, either as a result of stress, where the root system is destroyed to a point where it can no longer meet the water demands of the plant, or where the taproot is one of the first roots invaded and killed. The latter situation is important in plantations, where the inoculums density is high, and plants are crowded and have not yet developed an extensive root system (Holliday, 1980).

At Sabah Tea plantation, two varieties of tea are being cultivated. (Personal Communication) One is of Assamica (black tea) and the other China (green tea). Optimal yield production at Sabah tea is hampered by the presence of root disease. In about 8 hectares of cultivation, about 5% of its yield will be destroyed and thus affect the production. The severity of this root problem mainly lie in the lower land compared to highland of Sabah Tea area. The common symptoms of these root disease revealed include that of yellowing, thickening bark, wilting and so forth, and it is not obvious till the disease become severe where nothing else can be done except for uprooting the bush. Not only that, the two bushes nearby the infected tree will be uprooted as well as the disease spreads very fast. Due to the fact that Sabah Tea Plantation produces fine organic tea grade, pesticide-free, the common disease analyzed is the red root disease and the causal agent suspected is *Poria hypolateritia* a soil borne pathogen. Nevertheless, the presence of this pathogen is not detected; therefore this does leave room for other potential pathogen to be causal of the root disease. Presently, the management practice is
subsequent planting of Guatemala grass (*Tripsacum laxum*) that interrupts the life cycle of *Poria* and allows for replanting after six to nine months.

Thus, in identifying the pathogens being the causal agent of the root disease in this tea plant during this study, isolation and identification will be done and therefore also breed a significant step towards the betterment of management practices in Sabah Tea Plantation.

1.2 **Objective**

The major objective of this research according to its scope of study is as below:

i) To identify the pathogen being the causal agent of root disease in tea plant at Sabah Tea plantation
CHAPTER 2

LITERATURE REVIEW

2.1 About Tea Plant

2.1.1 History of Tea

The tea plant is rich in legend and a few of its prominent countries of production carry their own origin. According to Cramer (1967), on the origin and history of tea, the Japanese mythology records as tea being a tool for sleep banishing as a Buddhist monk was said to have his eyelids grown into tea leaves while he passed off in his sleep. Tea is composed chemically of caffeine which stimulates quality, tannin for the strength of the body and essential oil for its flavor and aroma. The Chinese started having their cup of tea as it began in the interior province of Szechwan in China and gradually extending from the Yangtze Valley to Seaboard provinces. England is known as the greatest coffee drinking country in the world when tea was first publicly sold at Garway’s Coffee House in London, 1657. It was formerly advertised as ‘That Excellent and By All Physicians approved China Drink’ and tea slowly gains popularity. Leather trade coins used in lieu of small changes and known as tea or coffee tokens, were generally accepted or currency
in London’s 2000 coffee houses. By mid-20th century, Englishmen were consuming about five times as many pounds of tea as coffee, while the Americans were consuming about 25 times as many pounds of coffee as tea. The people of Great Britain and Ireland being world’s greatest drinkers are currently, consuming about 10 pounds per person annually.

Tea is largely grown as a monoculture over large contiguous areas.

### 2.1.2 Status Quo of Tea

Tea in Asia is estimated to have sustained 8% loss in the crop due to pest infestation. Northeast India possibly loses about 29million kg of tea due to pests and these accounts for 13% of the production in that area (Glover et al., 1961). Greathead and Waages, (1983), reported that the tea plantation, on the whole, are highly suitable for biological control in view of the type climate, duration of crop, scale of economy and agronomic practices. India is the largest producer of black tea and supplies about one third of the total production in mid-1960s which also led to world exports. Almost 60% of India exports to United Kingdom, U.S.S.R, United States, and Canada. Other countries include China, Japan, Indonesia (mountainous regions) and several African countries. India was primarily introduced of tea when the East India Company took over its monopoly on the 1660s and then waddle its first step towards world trade of tea exports (Ananthakrisnan, 1995).
2.2 Common Pathogens of Tea Root Disease

2.2.1 *Poria hypolaterititia*

*Poria hypolaterititia* a soil-borne pathogen suspected being the causal agent of the red root disease affecting the tea plant. *Poria*, which is of the phylum of basidiomycota, belonging to the class of basidiomycetes where it also produces the sporophore or mycelial body as the fruiting body (Chong, K. p. *et al.*, 2004). A portion of sporophore is differentiated into a close layer, the hymenium, arises in a palisade manner, clavate or cylindrical basidia. Each basidum produces four (occasionally two, six, or eight) unicellular basidiospores (Photo 2.1) each on a relatively short sterigmatum (Saronins, 1973). The red root disease caused by it reflected the mycelium of the fungus strands being white and later attains a bright red color (Photo 2.2). The mycelia strands fuse with one another to form a sheet in advanced stage of infection and the mycelia shoot become dark, almost black in color.

2.2.2 *Verticillium* spp.

Nevertheless, in the experiment conducted, it was also mentioned *Verticillium* spp. being found as one of the specimens in the infected roots examined. It matches the fruiting body of this fungi belonging to the class of deuteromycetes where the presence of conidia and conidiophore was observed (Saronins, 1973). It has been concluded that there can be more than one group of causal pathogen of root disease at Sabah Tea (Chong K. P *et al.*, 2004).
Photo 2.1 Basidium and its basidiospores observed under electron microscope (Source: Saronins, 1973)

Photo 2.2 Red and whitish mycelia around infected root – Red root disease (Source: Saronins, 1973)
2.3 Mode of Infection of Root Disease in Tea Bushes

2.3.1 Root pathogenic fungi

In investigating the common root problems affecting the tea plantation around the world, Thai (1998) in his study the microorganism taken into case is fungi where among which include Armillaria mellea, Ganoderma philippi, Hypoxylon asarodes, Phellinus lamaensis, Phellinus noxium, Poria spp., Rosellinia arcuata, Rosellinia necatrix, Sphaerostilbe repens and Ustullina spp. The Armillaria, Ganoderma, Phellinus, and Poria belong to a broad range of taxonomic groups which is the phylum Basidiomycotina. The Hypoxylon, Rosellina, Ustullina belong to the phylum Ascomycotina and Sphaerostilbe is of phylum Deuteromycotina. Fungi usually are present in patches, but also must be taken note of that when the pathogen is present they often kill the entire tea bushes.

2.3.2 Infection mechanism and symptoms

The symptoms over time on tea bushes show similar characteristics as when it is affected by root diseases (Nguyen Phong Thai, 1998). The root disease all share the similar trait where the disease is never detected till several neighboring tea bushes are seriously affected. The growth of bushes slow down, leaves turn yellow, and during the flowering season, the tea bushes often produce excessive numbers of flowers. The bark of the tree may become much thicker as the bush tries to resist the fungus. As the disease worsen,
leaves wilt and die (though usually remain attached to the tea bush), and the bush die. Slowly, the trunk of the bush split and crack. Underneath the bark of the trunk, or on the surface of the roots, the fungus often forms whitish marks or patches, shaped like hands with many fingers or like leaves of the ferns. Threads or strings like components can be observed which are reddish black in color. In addition, fungus sometimes forms velvety ‘pillows’ or mushrooms on the surface of the roots or trunk. The disease cycle involved in causing the severity of root diseases in tea plants involve as follows. Usually, fungi develop in stumps or roots of dead trees that were left behind when clearing the land. The fungi spread to tea roots when the roots of the tea bush grow long enough to touch the roots of the dead tree. Also, the fungi can grow through the soil as threads or strings until they reach tea roots. Then, the fungus grows on the surface of the tea roots and inside the tea roots. The fungus can spread by growing as threads or strings from an infected tea bush to neighboring bushes (Nguyen Phong Thai, 1998).

The conditions that make the disease worse usually involve area where the soil is wet or poorly drained. It also happens in soil which is of shallow ‘hard-pan’, a hard layer that is difficult for roots to penetrate (William and Joseph, 1976). The presence of stumps or roots of dead trees also contribute to this phenomenon of root problems. Trees like Albizia and Tephrosia, which are used to provide shade in tea plantation, are susceptible to root rotting. Of course, the plants are safe when is planted in clean soil. But, if there are roots from forest trees left in the soil, these shade trees become infected. And because, the shade trees have large root systems, this helps build up a large population of root rot fungi that can later attack the tea plant. Another interesting point to be taken note of and deep
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http://www.tropentag.de/2004/proceedings/#foot9409


