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VARIATIONS IN *TRICHODERMA* FROM OIL PALM RHIZOSPHERE AND ITS BIOLOGICAL ACTIVITIES AGAINST *GANODERMA BONINENSE*

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By

HENDRY JOSEPH

Thesis Submitted in Fulfilment of the Requirements for the Degree of Master of Agricultural Science in the Faculty of Agriculture Universiti Putra Malaysia

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Agricultural Science.

VARIATIONS IN *TRICHODERMA* FROM OIL PALM RHIZOSPHERE AND ITS BIOLOGICAL ACTIVITIES AGAINST *GANODERMA BONINENSE*

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May 2000

Chairperson : Professor Dr. Sariah Meon

Faculty : Agriculture

A study on the distribution of Basal Stem Rot (BSR), frequency of isolation of *Trichoderma* spp. from oil palm rhizosphere, its biological activities and variations was attempted. The incidence of BSR was found to be correlated to age of palm. The percentage of disease incidence (PDI) in mature palm (OP74) was comparatively higher than the middle age palm (OP89) or young palm (OP94).

Frequency of isolation of *Trichoderma* spp. in the oil palm rhizosphere was found to be correlated to age of palm and disease incidence. Frequency of isolation (cfu/g soil) of *Trichoderma* spp. was higher in OP74 than OP89 or OP94.

Four main species aggregates identified from oil palm rhizosphere were *Trichoderma harzianum, Trichoderma virens, Trichoderma koningii* and *Trichoderma longibrachiatum. T. harzianum* was the highest in distribution in all the areas sampled with *T. longibrachiatum* being the lowest in its population dynamic.

In-vitro studies showed there were no variations in antagonistic activities between *Trichoderma* species aggregates. Meanwhile, a significant difference was observed within species aggregate as tested by dual culture and colony degradation tests, and production of volatile and non-volatile substances. Isolates TH1 of *T. harzianum* and TV1 of *T. virens* were observed to be consistent in their antagonistic and parasitic activities.

Variations between and within species aggregate were studied using intracellular isozyme and RAPD-PCR. Peroxidase activity was able to identify variations between species aggregates, however less sensitive to detect variations within intraspecific groups. RAPD was useful and able to detect variations within intraspecific groups, however was not able to identify variations between species aggregates *T. virens* could hold the possibility to be develop as a potential biopesticide based on its diverse genetic nature and biological activities.

Abstrak tesis yand dikemukakan kepada Senat Universiti Putra Malaysia sebagi memenuhi keperluan untuk Ijazah Master Sains Pertanian.

KEPELBAGAIAN KULAT *TRICHODERMA* PENCILAN DARIPADA RIZOSFERA KELAPA SAWIT DAN AKTIVITI BIOLOGI KE ATAS KULAT *GANODERMA BONINENSE*

Oleh

HENDRY JOSEPH

Mei 2000

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Satu kajian ke atas taburan Penyakit Reput Pangkal (BSR), frekuensi pencilan kulat *Trichoderma* spp. daripada rizosfera ladang kelapa sawit, aktiviti-aktiviti biologi serta kepelbagaian kulat tersebut telah dilakukan. Kejadian BSR di dapati berkorelasi dengan umur pokok kelapa sawit. Peratus kejadian penyakit daripada ladang kelapa sawit matang (OP74) adalah lebih tinggi berbanding dengan ladang kelapa sawit pertengahan (OP89) atau ladang kelapa sawit muda (OP94).

Frekuensi pencilan kulat *Trichoderma* spp. daripada rizosfera ladang kelapa sawit di dapati berkorelasi dengan umur pokok kelapa sawit dan kejadian penyakit. Frekuensi pencilan (cfu/g tanah) kulat *Trichoderma* daripada ladang OP74 adalah lebih tinggi berbanding dengan ladang OP89 atau ladang 94.

Empat kumpulan spesis agregat utama yang telah dikenalpasti daripada rizosfera ladang kelapa sawit, ialah *Trichoderma harzianum, Trichoderma virens, Trichoderma koningii* dan *Trichoderma longibrachiatum. T. harzianum* memberi taburan populasi yang tertinggi berbanding dengan spesis lain daripada semua kawasan yang dikaji, manakala *T. longibrachiatum* memberikan taburan populasi yang terendah.

Kajian *in-vitro* menunjukkan tidak terdapat perbezaan yang bererti bagi aktiviti antagonistik di antara kumpulan agregat kulat *Trichoderma*, manakala perbezaan yang sangat bererti di dapati di kalangan kulat spesis yang sama hasil ujian dwi kultur dan degradasi koloni, pengeluaran bahan-bahan volatil dan tidak volatil. Pencilan TH1 daripada *T. harzianum* dan pencilan TV1 daripada *T. virens* di dapati konsisten dalam aktiviti antagonistik dan parasitiknya.

Perbezaan di antara dan di kalangan kumpulan spesis agregat kulat *Trichoderma* dikaji dengan analisis isozim dan RAPD-PCR. Aktiviti isozim peroksidase di dapati hanya mampu untuk membezakan kepelbagaian di antara kumpulan spesis agregat, tetapi kurang sensitif untuk mengesan kepelbagaian di kalangan kumpulan kulat dari spesis yang sama. Analisis RAPD di dapati berupaya untuk mengesan kepelbagaian di kalangan kumpulan kulat dari spesis yang sama, tetapi tidak dapat mengesan kepelbagaian di antara kumpulan sepsis agregat. Kulat *T. virens* di dapati berpotensi untuk dibangunkan sebagai racun kulat biologi berdasarkan kepada kepelbagaian genetik dan aktiviti biologi.

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CHAPTER 1

INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) was introduced as an ornamental plant when it was first brought to Malaysia from Africa in 1917. Within 20 years the industry had expanded rapidly and consequently Malaysia became the largest producer and exporter of palm oil replacing Nigeria as the chief producer since 1971 (Ariffin, 1998). Malaysia's exports of palm oil accounts for 62 per cent of the global oil palm output and 22 per cent of the international oils and fats trade.

As a fast growing industry, there was an increase in hectarage from 640 thousand hectares in 1975 to 2.8 million hectares in 1997 and is projected to reach 2.9 million hectares in the year 2000. Meanwhile palm oil production has been increasing from 92 thousand tones in 1960 to 9.062 million tones in 1997 (Ariffin, 1998). Pakistan with imports of 460 811 tones was the largest importer followed by China, 326 649 tones. The European Union imported 305 660 tones during the period of January to May 1996 (Amiruddin, 1996) and it was traded at the average price of RM1226.42 per tone (Abdullah, 1996). Previously, a local press (Daily Express, Sabah) reported, that crude palm oil (CPO) price has touched a historic high of RM2300 per tone on 6 January 1998. Various factors support an optimistic stand for CPO to stay above RM2000 per tone for the rest of the year.

One of the many challenges of the industry is to maintain the economic production period of mature palms, which can be affected either due to poor management practices or pest and disease problems. *Ganoderma* Basal Stem Rot (BSR) disease has gained major attraction from both government and private sectors. An action committee to address the problem was formed. It consists of active bodies including UPM, PORIM, Oil Palm Grower's Association as well as international body, the Commonwealth Agricultural Bureau International (CABI) of United Kingdom.

The incidence of *Ganoderma,* a soil-borne pathogen has been recognized in Malaysia since 1928 (Sharples, 1928) where the incidence resulted in what is known as BSR. BSR is the most serious disease of oil palm in Malaysia. Though the disease is serious in coastal plantations which formerly supported coconut, its presence is also found in peat soils and some inland soils (Ariffin *et al.*, 1989a,b; Benjamin, 1993). Normally BSR was associated with old palms of over 30 years old but it was not until 1957, that the disease was found attacking younger palms as young as 5 years old (Larter, 1956; Anon, 1958). The incidence of BSR is usually slow to begin with but increase to more than 50% by the time the palms are replanted.

Although much is known on the occurrence of the disease, fundamental studies on the pathogen, pathogenesis and control are rather limited. Control measures commonly adapted are cultural control, surgery, clean clearing, flood fallow and use of fungicides. All the control measures above are short-live and considerably less economic due to the nature of the disease infection and epidemiology. To be effective, any practice must be sufficiently inexpensive so that the grower may use it as an assurance against disease outbreak. Biological control becomes more important lately because it is more environment friendly and inexpensive. Moreover nowadays all efforts are directed to enhance the usage of biologically-based technologies for plant disease control.

The most known fungal biological control agent is *Trichoderma.* Of the nine aggregates revised by Rifai (1969), *T. harzianum* Rifai is the most commonly cited species, followed by *T. virens* Gidden and Foster. However, problem arises in terms of its ability to adapt to different soil groups and characters, environmental conditions and its competitiveness as soil rhizosphere competent microorganism. Different workers reported different degree of disease controlling ability. This was the result of using different strains in different places. It was reported that the ability to compete is species dependent. Although *Trichoderma* has been used as a biological control agent for decades, the taxonomy, genetics and population composition of these fungi are still poorly understood.

Limited knowledge of variability in this fungi and infrequent culturing of their sexual stages make delineation of narrowly defined species difficult (Rifai, 1969). In addition little is known about such genetic components as ploidy levels, frequency of heterokaryosis, or the prevalence of parasexual events (Staz *et al*., 1988). Methods are lacking to differentiate among strains for patent purposes, or to determine variability and abundance of strains in natural ecosystem. It is important to differentiate among strains because there are variability in terms of their ability to colonize the soil rhizosphere and their specificity in controlling plant diseases (Papavizas, 1985).

It is not always possible to get an accurate and reliable identification of fungi by using morphological characters. Even when it is, identification of intraspecific elements are at best difficult and more often impossible (Mills, 1994). Recently a number of techniques comprised of biochemical and molecular methods have been developed. These include intracellular isozyme, and DNA-base method: Restriction Fragment Length Polymorphism (RFLP) analysis, DNA fingerprinting, Polymerase Chain Reaction (PCR), and DNA sequence analysis. The most rapidly used DNA-based method is PCR, meanwhile Random amplified polymorphic DNA's (RAPD) is a method that incorporates PCR technique. It is a method based on incorporation of single arbitrary primers, and proved to be able to distinguish variations within species.

In this experiment, attempt was made to study the frequency of isolation, distribution and diversity of antagonistic isolates of *Trichoderma* in oil palm rhizosphere, to characterize variations between and within species aggregate based on intracellular isozyme and DNA polymorphism and to select the potential antagonistic strain against *G. boninense* based on the *in-vitro* screening tests.

Therefore the specific objectives of this study are:

(a) to investigate the frequency of isolation and distribution of antagonistic isolates of *Trichoderma* in oil palm rhizosphere with respect to BSR severity and age of palm,

(b) to evaluate the biological activity of representative isolates of *Trichoderma* against *G. boninense in-vitro,* and

(c) to characterize variations between and within species of *Trichoderma* as expressed by intracellular isozyme and DNA polymorphism.

CHAPTER 2

LITERATURE REVIEW

Development of the Oil Palm Industry

The history of the oil palm in Malaysia begins when oil palm first entered this country through the Botanical Garden, Singapore in 1870, but the first commercial planting was not initiated until 1917. Long before its introduction into Malaysia, oil palm was abundantly found in tropical Africa under natural conditions and its kernel and pericarp oil were widely used by the natives (Bunting *et al.*, 1966).

The first commercial planting in Malaysia began in 1917 at Tenamaram Estate in Kuala Selangor. The second was Elmina estate which is also in Kuala Selangor where the acreage planted was 1010 acres (Bunting *et al.*, 1966). The rate of planting was accelerated during the 1960's. By 1965 areas planted in Peninsular Malaysia were 100 000 hectares, and by 1973 over 400 000 hectares. In Sabah, oil palm has become an important crop within 10 years. The planted areas rose from 400 hectares in 1960 to 68 000 hectares in 1975 (Hartley, 1977). Presently, 2.8 million hectares were planted in 1997 and is projected to reach 2.9 million hectares in the year 2000 (Ariffin, 1998).