
**UNIVERSITI PUTRA MALAYSIA**

***THE EFFICACY OF THREE SPECIES OF TRICHODERMA FOR THE CONTROL OF BASAL STEM ROT IN OIL PALM SEEDLINGS***

**JAYANTHI NAGAPPAN**

**FPSK(M) 2005 17**

# THE EFFICACY OF THREE SPECIES OF *TRICHODERMA* FOR THE CONTROL OF BASAL STEM ROT IN OIL PALM SEEDLINGS

**By**

**JAYANTHI NAGAPPAN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Masters of Science**

**February 2005**

*To my beloved parents for their love and patience and my twin sister, Jayasree for her moral support throughout my studies*

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

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**February 2005**

**Chairman: Associate Professor Faridah Abdullah, PhD**

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This study evaluated the potential of three *Trichoderma* species, namely *T. harzianum* (isolate BIO T32), *T. longibrachiatum* (BIO T28) and *T. virens* (BIO T128) for the control of *Ganoderma boninense* (EGB 01), the causal pathogen of basal stem rot (BSR) of oil palms in nursery trials. Besides their spore production and antagonistic properties, this study also investigated the growth response of each of the species towards a wide range of temperature and pH conditions. All three species exhibited particular strengths in the growth parameters studied but BIO T32 exhibited consistent and relatively good antagonistic properties and was used as the main inoculant in nursery trials against *G. boninense*. The type and size of wood block were found to influence the success and consistency of the inocula in establishing disease during artificial infection of seedlings. Very low infectivity rates were achieved when inoculum blocks were half to a quarter of the standard 6 x 6 x 12 cm; this size was found to give consistent infection rates leading to approximately 85% mortality. In

nursery trials, seedlings treated with a single inoculum of *T. harzianum* (T1) gave the lowest and most significant disease severity index (DSI) of 28.34. The conidial drench was stopped at week 14 and the first sign of disease was only observed on week 20. The uninfected and untreated control seedlings gave a DSI of 0 where as, the infected, untreated controls gave a DSI of 86.87. Soils under treatment using a single (T1), two mixed (T2) and three mixed (T3) inocula showed an increase in spore count based on colony forming units (cfu) starting from two weeks after application. When the soil drench was terminated at week 14, the spore count was peak on the $18^{th}$, $14^{th}$ and $10^{th}$ week for T1, T2 and T3 treatments respectively. Spore counts of BIO T32 were not significantly different on the upper (5 cm) and deeper (15 cm) layer of the treated soils. This study found that when *T. harzianum* (BIO T32) was used as a single inoculum, it gave the most significant and effective performance as a biological control agent. This was only followed by a mixture of *T. harzianum* and *T. longibrachiatum*. Lastly, the use of a combination of three *Trichoderma* species were found to give the poorest disease control, giving a DSI that was not statistically different from the infected, untreated control experiment.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**KEUPAYAAN TIGA SPESIS *TRICHODERMA* BAGI PENGAWALAN PENYAKIT REPUT PANGKAL BATANG ANAK POKOK KELAPA SAWIT**

Oleh

**JAYANTHI NAGAPPAN**

**Februari 2005**

**Pengerusi: Profesor Madya Faridah Abdullah, PhD**

**Fakulti: Sains**

Kajian ini menilai potensi tiga spesis *Trichoderma*, terutamanya *T. harzianum* (isolat BIO T32), *T. longibrachiatum* (BIO T28) dan *T. virens* (BIO T128) sebagai kawalan terhadap *Ganoderma boninense* (EGB 01), patogen reput pangkal batang (BSR) pokok kelapa sawit dalam kajian nurseri. Selain penghasilan spora dan ciri-ciri relatif antagonis mereka, kajian ini juga menilai tindakbalas ketiga-tiga spesis ini terhadap julat suhu dan pH yang luas. Ketiga-tiga spesis tersebut mempamerkan ciri-ciri tertentu dalam parameter yang dikaji tetapi BIO T32 mempamerkan ciri relatif antagonis yang baik dan konsisten serta dipilih sebagai inokulan utama dalam kajian nurseri terhadap *G. boninense*. Jenis dan saiz blok kayu didapati mempengaruhi kejayaan dan konsistensi inokula dalam memperkukuhkan penyakit semasa jangkitan secara buatan terhadap anak pokok kelapa sawit. Kadar jangkitan yang amat rendah diperolehi dengan blok inokulum yang bersaiz kecil berbanding dengan saiz 6 x 6 x 12 cm; saiz ini didapati memberi kadar jangkitan yang konsisten sehingga 85% kematian. Dalam kajian

nurseri, anak pokok yang dirawat dengan sejenis aplikasi inokulum *T. harzianum* (BIO T32) memberikan tahap kemerosotan penyakit (TKP) yang teramat rendah dan signifikasi iaitu sebanyak 28.34. Penggunaan cecair konidia ditamatkan pada minggu ke-14 dan kesan jangkitan hanya diperhatikan pada minggu ke-20. Anak pokok kawalan yang tidak dijangkiti dan tidak dirawat memberi nilai TKP 0, tetapi kawalan yang dijangkiti dan tidak dirawat memberi nilai TKP sebanyak 86.87. Tanah yang dirawat dengan satu (T1), kombinasi dua (T2) dan kombinasi tiga (T3) jenis inokula menunjukkan kenaikan kiraan spora berdasarkan unit pembentukkan koloni (upk) yang bermula dari minggu ke-2 selepas aplikasinya. Apablia aplikasi larutan inokulum ditamatkan pada minggu ke-14, kiraan spora memuncak pada minggu ke 18, 14 dan 10 untuk rawatan T1, T2 dan T3 masing-masing. Kiraan spora BIO T32 tidak signifikan pada tahap atas (5 cm) dan dalam (15 cm) tanah yang dirawat. Kajian ini mendapati apabila sejenis inokulum *T. harzianum* (BIO T32) digunakan, ia sangat signifikan dan efektif sebagai agen kawalan biologi. Ini diikuti dengan kombinasi *T. harzianum* dan *T. longibrachiatum*. Akhirnya, kombinasi ketiga-tiga spesis *Trichoderma* didapati memberikan pengawalan penyakit yang tidak memuaskan dengan TKP yang tidak signifikan dari anak pokok kawalan yang dijangkiti dan tidak dirawat.

## ACKNOWLEDGEMENTS

I would like to express my deepest and sincere gratitude to Prof. Madya Dr. Faridah Abdullah who has guided, supervised and supported my academic work and also thank her for providing financial support during the study from her IRPA project.

I would like to express my heartful thanks to Assoc. Prof. Dr. Umi Kalsom Yusuf and Assoc. Prof. Dr. Zainal Abidin Mior Ahmad for their invaluable advice throughout the research and thesis preparation.

I would like to thank the staff members of the Department of Biology of Soil Science for the use of the greenhouse and Department of Forestry for the cutting of wood blocks.

Special thanks were also extended to lab-mates Dr. G. N. M. Ilias, Mr. Nelson and Mrs. Shamala Sundram for their kind assistance during this study.

Special thanks were reserved for my parents, sister and friends, namely Mrs. Jasmin and Miss Gunavathi for putting up with me during the course of this study.

I certify that an Examination Committee met on to conduct the final examination of Jayanthi Nagappan on her degree thesis entitled "The Efficacy Of Three Species Of *Trichoderma* For The Control Of Basal Stem Rot Of Oil Palm Seedlings" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination committee are as follows:

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## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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**JAYANTHI NAGAPPAN**

Date:

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| - G | without *Ganoderma boninense* |
| + G | with *Ganoderma boninense* |
| BIO T28 | *Trichoderma longibrachiatum* |
| BIO T32 | *Trichoderma harzianum* |
| BIO T128 | *Trichoderma virens* |
| BSR | Basal stem rot |
| C1 | Control 1 |
| C2 | Control 2 |
| $C_6H_8O_7.H_2O$ | Citric Acid |
| CFU | Colony Forming Unit |
| DSI | Disease Severity Index |
| EGB 01 | Isolate of *Ganoderma boninense* (*Elaeis guineensis*-Banting) |
| MC | Moisture Content |
| MEA | Malt Extract Agar |
| MPOB | Malaysian Palm Oil Board |
| MW | Molecular Weight |
| $Na_2HPO_4$ | Sodium Hydrogen Phosphate |
| NaOH | Sodium Hydroxide |
| OPMF | Oil Palm Mesocarp Fibre |
| PCNB | Pentachloronitrobenzene |
| PDA | Potato Dextrose Agar |

| | |
|---|---|
| PIRG | Percentage Inhibition Radial Growth |
| PORIM | Palm Oil Research Institute of Malaysia |
| PP 28 | Isolate of *Ganoderma boninense* from Palm Oil Research Institute of Malaysia (PORIM), now know as Malaysian Palm Oil Board (MPOB) |
| RBA | Rose Bengal Agar |
| rpm | Rotation per minute |
| T1 | Treatment 1, *T. harzianum* (BIO T32) |
| T2 | Treatment 2, *T. harzianum* + *T. longibrachiatum* (BIO T28) |
| T3 | Treatment 3, *T. harzianum* + *T. longibrachiatum* + *T. virens* (BIO T128) |
| UPM | Universiti Putra Malaysia |
| w.a.i. | Weeks after inoculation |
| WP | Wettable Powder |

# CHAPTER I

# INTRODUCTION

Several million hectares in the world today are planted with commercially important edible oil crops that represent a significant fraction of the resources of the countries concerned (Ariffin and Idris, 2002). Among these species is the oil palm (*Elaeis guineensis*) as an important crop in the topical regions because of its two main raw materials produced, the palm oil and palm kernel oil. Currently, Malaysia is the leading producer of palm oil, with a total production of about 12.5 million tonnes for the year 2004 (MPOB Statistic, 2004) and seeks to maintain dominance in this field.

In order to maintain the current production as well as to strive towards higher yields, every aspect of oil palm cultivation will need to be carefully managed; one of these is in disease management. From seed germination to field planting, the oil palm is prone to attack by various disease-causing organisms, the most common being fungi. Nevertheless, diseases affecting seeds and nursery seedlings are under control in most cases and do not pose a serious threat to the industry. It is diseases of field palms, particularly a basal stem rot (BSR) caused by *Ganoderma* spp. that threaten crop development and requires urgent solution.

*Ganoderma* has been known to attack oil palms since the early years when the crop was introduced into this country (Turner, 1981). The disease was recognized since late 1920's (Thompson, 1931; as cited in Ariffin *et al*., 1996) but was regarded as of

negligible importance since only palms of over 25 years in age were affected. It was not until 1957 that BSR incidence was reported to increase at an alarming rate when younger palms of 10 to 15 years in age were also infected (Turner and Bull, 1967). Gurmit (1991) reported that the disease could set in as early as 12 to 24 months but the effects were only noticeable when they were four to five years old.

Currently, the approaches used to control the disease are mainly by adoption of hygienic cultural practices and the use of chemical control (tridemorph, carboxin, triadimefon, triadimenol, flutriafol, propiconazole and difenoconazole) to a certain degree (Gurmit, 1991). Bayleton® is one chemical that has been used in laboratory studies and in field trials as trunk injection (PORIM, 1984). Other fungicides tested in field trials were Benlate® T-20, Calixin®, Bayfidan®, Thiram® and Dazomet® but results from these trials were inconclusive (Ariffin and Idris, 1991). Several research institutes have studied this disease and developed means of control but despite many investigations and some 80 years of research no satisfactory solutions in terms of effectiveness, ease of use and cost could be offered.

During the next decade biological control may become an important component of plant disease management practices. The demand for alternatives to chemical control of plant pathogens has become stronger owing to concerns about the safety and environmental impacts of chemicals. The possibility of control of *Ganoderma* should be approached through manipulation of biological agents. Investigations on the use of fungi such as *Trichoderma* (Wijesekera *et al*., 1996; Ilias and Abdullah,

1999), *Aspergillus* (Shukla and Uniyal, 1989) and *Penicillium* (Dharmaputra *et al*., 1989) as antagonists of *Ganoderma* in culture have been reported. Particular attention is focused on species of *Trichoderma* that may not as yet given any 'wonder drugs' such as penicillin but has the potential to produce enzymes and to attack or inhibit other fungi (Samuels, 1996; Ilias and Abdullah, 1998; Ilias and Abdullah, 1999). Weindling (1932) was the first to discover the antagonistic ability of *Trichoderma* on the plant pathogen *Rhizoctonia solani*. Two major discoveries were reported; the first was that *Trichoderma* killed the pathogen by physical strangulation and the second was by killing them a short distance away through the production of toxic compounds in the media. Ilias and Abdullah (1998) showed situations where the fungal mycelia coiled tightly around the host hyphae resulting in physical strangulation, as well as the formation of hook-like structures by *Trichoderma*, which puncturing the fungal host cells. *Trichoderma* spp. was also found to produce volatile and non-volatile antibiotics (Dennis and Webster, 1971a,b; Ilias and Abdullah, 1998).

The most studied species of *Trichoderma* acting against antagonists of plant pathogens reported were *Trichoderma harzianum* (Wells *et al*., 1972; Elad *et al*., 1980; Chamswarng, 1992; Ilias and Abdullah, 1998), *T. virens* (Papavizas and Lewis, 1989; Sariah and Chan, 1999) and *T. longibrachiatum* (Chamswarng *et al*., 1992; Sreevinasaprasad and Manibushanrao, 1993; Saravanan *et al*., 2003). An *in vitro* study by Ilias and Abdullah (1999) showed that growth of *Ganoderma boninense* was inhibited using culture filtrates of *T. harzianum* and *T. virens* respectively. Further *in vitro* studies by Abdullah and Jayanthi (1999) found that a

metabolite mixture of strains of *T. harzianum*, *T. virens* and *T. longibrachiatum* resulted in a better antagonistic performance against growth of *Ganoderma boninense* than when applied singly.

Trials on using disease-controlling agents are still under explored and play an important role in inducing disease control in oil palm seedlings. A major obstacle towards achieving this objective is the inability to reproduce artificial infection accurately and consistently. Studies by Khairudin (1991), was the most successful and practicable thus far and is the model upon which the present study was based. However, the success in establishing induced disease in oil palm seedlings is meaningful only if the data can be quantified. Many attempts have been made in the earlier years to establish Koch's Postulate, one of which was by Navaratnam and Chee (1965). Khairudin *et al*. (1991) found that the oil palm seedlings were infected by rubber wood inocula but not on oil palm mesocarp fibre (OPMF). It was thus concluded that the type of substrate inocula used determined the success of infection by *G. boninense*. Besides the size, type and age of inoculum may also play an important role in establishing infection by *Ganoderma* (Khairudin, 1994; Abdullah *et al*., 2001).

Based on *in vitro* experiments by Ilias and Abdullah (1998), a further step was taken to test the antagonist activity in greenhouse trials. An *in vivo* trial carried out by Ilias (2000) found a strain of *T. harzianum* used singly in the form of conidial soil drench gave better results in suppressing diseased in oil palm seedlings than *T. virens*.

Abdullah *et al*. (2003b), reported that *T. harzianum* gave a DSI value of 5 after 20 weeks of treatment. Studies using a combination of *Trichoderma* species with other fungal species or chemical adjuvants has not been tried and they could be much more effective than when applied alone. Research in this directions include the control of *R. solani*, causal pathogen of root rot of eggplant, with *T. harzianum* combined with PCNB in soil (Hadar *et al*., 1979), *Sclerotium rolfsii* Sacc., seedling blight of barley by using seed coating with a combination of *Trichoderma* spp. and *Bacillus* sp. (Chamswarng *et al*., 1992) and against *Phytophthora erythroseptica*, the causal pathogen of pink rot of potato and root and stem rot of tomato by using a combination of Trichodex™ and *T. virens* (Etebarian *et al*., 2000).

*Trichoderma* species have been used in commercial preparations for biological control of fungal induced plant diseases (Samuels, 1996). *T. harzianum* is the active ingredient in Trichodex™, which is used against post-harvest rot of apple. *T. harzianum* is combined with *T. polysporum* in the product Binab-T™, which is used in the control of would decay and wood rot (Ricard, 1981). Hence, further trials are needed to determine whether a combination of reagents performed better to arrest the *Ganoderma* lesion.

To assess the efficacy of *Trichoderma* as a biological agent, the disease establishment of the pathogen onto the experimental plants must approach 90%. The state of 'infection' assessed by Navaratnam and Chee (1965) was as 'foliar symptoms'. There was a limitation in this concept. Due to this fact, Khairudin

(1991) assessed the 'infection' of the seedlings by the production of *Ganoderma* sporophores. This study was to establish a good infectivity with more than 80% infection. To document a spectrum of visual signs, from the early to late stages of infection, a disease severity index (DSI) was developed as an aid to quantify the disease establishment. Apart from that, the population dynamics of *Trichoderma* in the soil of treated seedlings was also assessed.

The four main objectives of the study are summarized as follows:

a) To determine the biological characteristics of the three species-isolates of *Trichoderma* namely, *T. harzianum* (isolate BIO T32), *T. longibrachiatum* (BIO T28) and *T. virens* (BIO T128).

b) To determine the success of an infectivity with two types of wood blocks (rubber and oil palm) that were tested on their relative suitability as substrate inocula in establishing disease to oil palm seedlings. Apart from that, the effect of smaller sized inoculum blocks in establishing disease on oil palm seedlings was also investigated.

c) To assess the treatments in the control of basal stem rot of oil palm seedlings using single and mixed inocula of *T. harzianum* (BIO T32), *T. longibrachiatum* (BIO T28) and *T. virens* (BIO T128) in greenhouse trials. To monitor the colony forming units (cfu) of *Trichoderma* in the soils of treated seedlings throughout the experiment.

The hypothesis of this study was to find out whether *T. harzianum* (BIO T32) with an integration of other *Trichoderma* species (*T. longibrachiatum* and *T. virens*) was able to give better control towards *G. boninense*, causal pathogen of basal stem rot of oil palm.

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