SHORT COMMUNICATION

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# FIRST REPORT: ISOLATION OF ENDOPHYTIC *Trichoderma* FROM OIL PALM (*Elaeis guineensis* Jacq.) AND THEIR *in vitro* ANTAGONISTIC ASSESSMENT ON *Ganoderma boninense*

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**ABSTRACT**

*The isolation of endophytic* Trichoderma *from different tissues* viz. *leaf, rachis, stem and root of oil palm (*Elaeis guineensis *Jacq.) was investigated in this study. A total of six palms were sampled from two plantation plots and it was found that* Trichoderma *isolates were specifically colonising the stems and roots of oil palm and none were found in the leaves and rachis.* Trichoderma *selective media E (TME) was used for isolation. Identification of the isolates was further established using slide cultures. A total of 40 isolates were obtained with 10 from stem and 30 from root samples of oil palm. All isolates were subjected to dual culture assay; a preliminary assay to screen mycoparatisms potential through antagonistic activity against* Ganoderma boninense. *Subsequently, seven potential* Trichoderma *isolates were tested for their antibiosis properties through poison food agar assay. This is the first report of endophytic* Trichoderma *isolated from oil palm with biocontrol potential against* G. boninense.

**Keywords:** oil palm, endophytic, *Trichoderma, Ganoderma boninense,* antagonistic.

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## INTRODUCTION

*Trichoderma* species are usually considered soil fungi that colonise superficially on the plant root surfaces, sometimes forming a symbiotic relationship. The genus is typically considered soilborne and associated with the roots of plants and is commonly considered for their potential to control plant disease in what can be a close association with many aspects typical of endophytic association (Harman *et al.*, 2004). Recent studies have reported that *Trichoderma* species are also capable of colonising the internal tissues of plants and characterised as having an endophytic relationship. Although *Trichoderma* species are typically considered common soil saprophytes, they are capable of more intimate associations with plant root systems in what has been characterised as an opportunistic avirulent sym-biotic relationship (Harman *et al.*, 2004). The critical characteristic of this association is the penetration of the plant's root system by *Trichoderma* and the persistent survival of the fungus within living plant tissues. Previous studies have shown, principally with *Theobroma cacao*, that *Trichoderma* species can persist not only within the plant's root

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system but also within above ground tissues in endophytic associations (Evans *et al*., 2003; Bailey *et al*., 2006; 2008). Wide range of *Trichoderma* isolates as biocontrol agents have been investigated for oil palm but these were isolated as soilborne microbes and were studied extensively for their biocontrol ability (Sariah *et al.*, 2005; Izzati and Abdullah, 2008; Sundram *et al.*, 2008). However, attempts to isolate *Trichoderma* species as an endophyte and the nature of the isolated in terms of biocontrol potential have yet to be investigated.

This study was executed to isolate *Trichoderma* spp. from leaf, stem, rachis and root tissues of the palm. The isolates obtained were subjected to *in vitro* assays to assess their antagonistic activity against *G. boninense;* the causal fungus of basal stem rot disease of oil palm.

## MATERIALS AND METHODS

### Isolation of Endophytic *Trichoderma*

Two local oil palm plantations were identified as sampling sites for this study. Six healthy oil palms were identified with their leaf, rachis, stem and root sections sampled for isolation of endophytic *Trichoderma* isolates. The isolation was carried out based on Arnold *et al.* (2001) and Evans *et al.* (2003) with minor modifications. The tissue samples were surface-sterilised through sequential immersion in 2% sodium hypochlorite (chlorine bleach), 70% ethanol and sterilised water followed by blotting dry on a sterile filter paper. The tissues were placed onto *Trichoderma* selective media E (TME) prepared as described by Papavizas and Lewis (1981). The agar plates were incubated for seven days at 28°C in the dark and examined daily for fungal growth. Each distinct fungal colony was subcultured on potato dextrose agar (PDA) and incubated between seven to 14 days.

### Morphological Characterisation

Colonies emerging from the TME were sub-cultured on PDA and were subjected to macroscopic and microscopic identification before subjecting to further *in vitro* assays. Morphological observations of the colonies and conidium-bearing structures were based on isolates grown on PDA for three weeks at 28°C. Microscopic observations were made using the monograph prepared by Rifai (1969). Slides were observed under Olympus BX41 microscope and Infinity MPX 2 digital camera.

### Mycoparasite Screening - Dual Culture Assay

Preliminary screening of *Trichoderma* isolates against *G. boninense* was carried out based on dual culture assessment (Dennis and Webster, 1971). A mycelial plug (5 mm in diameter) of *G. boninense* was placed against the test isolates (*Trichoderma*). The antagonistic potential of *Trichoderma* isolates were assessed after eight days of co-incubation by measuring the radial growth of *G. boninense* in the direction towards the growth of *Trichoderma* isolates (R2). The results were later transformed into percentage inhibition of radial growth (PIRG) in relation to radial growth of the pathogen in the control plate (R1), using the following formula used by Jinantana and Sariah (1998) as follows:

$$\text{PIRG}\ (\%) = (R1 - R2)/R2 \times 100$$

### Antibiosis Properties - Poison Food Agar Assay

The conidia of potential *Trichoderma* isolates from the dual culture were harvested from seven days old cultures grown at 27°C on PDA by placing 5 ml of sterilised distilled water on the plate surface and slowly agitating the surface using a sterile glass rod. The concentrated spore suspensions were collected using a syringe and filtered through sterile glass wool for mycelium removal. The concentrated suspension was adjusted to $1x10^6$ conidia of the endophyte using a haemocytometer. One millilitre was inoculated into a conical flask containing 150 ml of Difco PDB in an orbital incubator at 28°C and 110 rpm for seven days. The mycelia were removed by filtration after seven days of growth and the filtrate was sterilised by passing through a 0.22 $\mu$m membrane disposable unit (Sartorius). The culture filtrate was incorporated into PDA at 20%, 40%, 60% and 80%. The agar plates were cultured with *G. boninense* disc and percentage inhibition of mycelial growth (PIMG) of the pathogen was recorded after the control plate (without culture filtrate) was fully covered by mycelia. Measurement of radial growth in treatment plates (R2) was calculated against the radial growth in control plate (R1) using the following formula:

$$\text{PIMG}\ (\%) = (R1 - R2)/R2 \times 100$$

## RESULTS

### Endophytic *Trichoderma* Isolates

All colonies emerging from TME were sub-cultured onto PDA for macro and microscopic assessments. Young *Trichoderma* cultures' plate morphological characteristics appeared as faint white hyphal growth followed by green conidiation initiating from the centre of the plate on the third to fourth day after plating. *Trichoderma* cultures were fast growing fungi with mycelia typically covering the culture plate (90 mm diameter) within

four days. These cultures were examined for micro morphological characteristics on with slide cultures for typical structure of *Trichoderma* namely; phialides, conidia and conidiophores under the light microscope. *Table 1* shows the number of *Trichoderma* isolates isolated from different tissues of oil palm. Forty isolates of *Trichoderma* were recovered from the stem and root, but none were isolated from the stem and rachis. The typical structures of *Trichoderma* in the slide cultures are shown in *Figure 1*. The patterns of conidiophores branching and aggregation were the distinct characteristics that were used to identify *Trichoderma* isolates up to the genus level. Species identification requires molecular approach.

**Mycoparasite Screening and Antibiosis Properties**

All 40 *Trichoderma* endophytes were subjected to dual culture assay. *Table 2* shows the number of *Trichoderma* isolates exhibiting inhibition towards *G. boninense* growth through the PIRG values. Seven potential isolates with PIRG between 70% - 90% were identified (*Table 3*). *Trichoderma* isolate 6b gave the highest PIRG with 80.22% followed by 2b, 8b and 26b isolates. Some of the *Trichoderma* isolates were aggressive mycoparasites which completely colonised the *G. boninense* to the stage whereby the pathogen could not be recovered (*Figure 2*). Poor mycoparasites were eliminated from further assessment. The seven aggressive *Trichoderma* isolates were then subjected to poison food agar assay for inhibitory antibiosis properties. The isolates varied in their abilities to produce culture filtrate compounds that inhibited *G. boninense* growth. Culture filtrates from 6b isolate completely inhibited *G. boninense* (PER 71) growth with 80% incorporation of culture filtrate into the media (*Figure 3*). The isolate also gave significantly higher inhibition ($P<0.05$) at all percentages of culture filtrate incorporation tested when compared with the remaining six isolates.

**TABLE 1. ENDOPHYTIC *Trichoderma* ISOLATES ISOLATED FROM VARIOUS TISSUES OF OIL PALM**

| Sampled tissue | *Trichoderma* species |
|---|---|
| Leaf | - |
| Rachis | - |
| Stem | 10 |
| Root | 30 |

**TABLE 2. PERCENTAGE INHIBITION OF RADIAL GROWTH (PIRG) OF *Ganoderma boninense* BY ENDOPHYTIC *Trichoderma* THROUGH DUAL CULTURE ASSAY**

| | Percentage inhibition of radial growth (% PIRG) | | | |
|---|---|---|---|---|
| | < 60 | < 70 | < 80 | < 90 |
| Number of *Trichoderma* species | 20 | 13 | 6 | 1 |

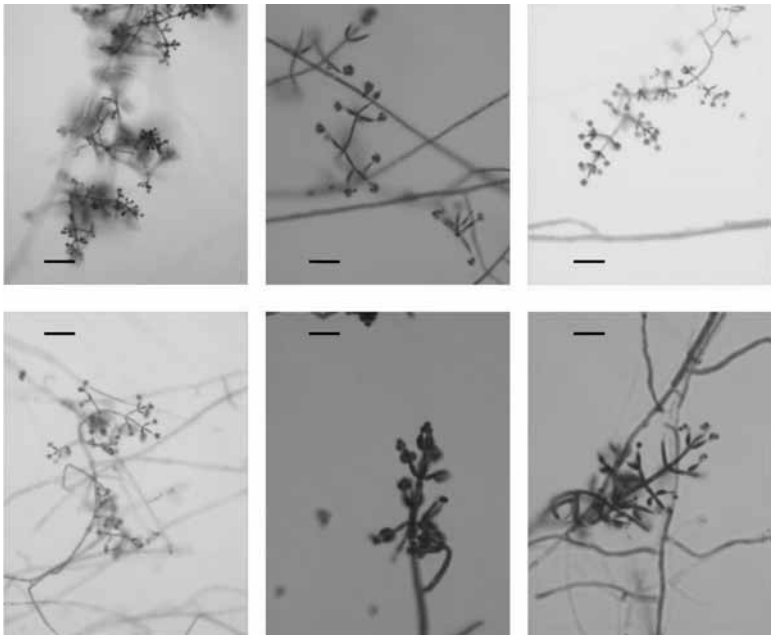

*Figure 1. The typical morphological structures of* Trichoderma *genus conidiophore branching and aggregation (scale bar represents 40 µm).*

**TABLE 3. ANTIBIOSIS PROPERTIES OF *Trichoderma* ISOLATES THROUGH POISON FOOD AGAR ASSAY**

| *Trichoderma* isolates | Percentage inhibition of radial growth (PIRG) | Percentage inhibition of mycelium growth (PIMG %) | | | |
|---|---|---|---|---|---|
| | | 20 | 40 | 60 | 80 |
| 2b | 75.56$^{ab}$ | 6.67$^{c}$ | 7.45$^{d}$ | 7.45$^{d}$ | 7.45$^{e}$ |
| 5b | 70.00$^{b}$ | 2.17$^{c}$ | 3.58$^{d}$ | 6.27$^{d}$ | 11.67$^{e}$ |
| 6b | 80.22$^{a}$ | 17.65$^{b}$ | 24.86$^{b}$ | 71.76$^{a}$ | 100.00$^{a}$ |
| 7b | 72.22$^{b}$ | 27.84$^{a}$ | 39.61$^{a}$ | 55.29$^{b}$ | 65.49$^{b}$ |
| 8b | 74.70$^{ab}$ | 1.18$^{c}$ | 38.04$^{a}$ | 59.61$^{b}$ | 71.82$^{b}$ |
| 23b | 70.00$^{b}$ | 20.00$^{c}$ | 19.22$^{c}$ | 27.06$^{c}$ | 28.63$^{c}$ |
| 26b | 74.22$^{ab}$ | 3.53$^{c}$ | 4.92$^{d}$ | 6.88$^{d}$ | 18.04$^{d}$ |

Note: Means within the same column followed by the superscript of same letters are not significantly different at P<0.05 with Tukey (n=3).

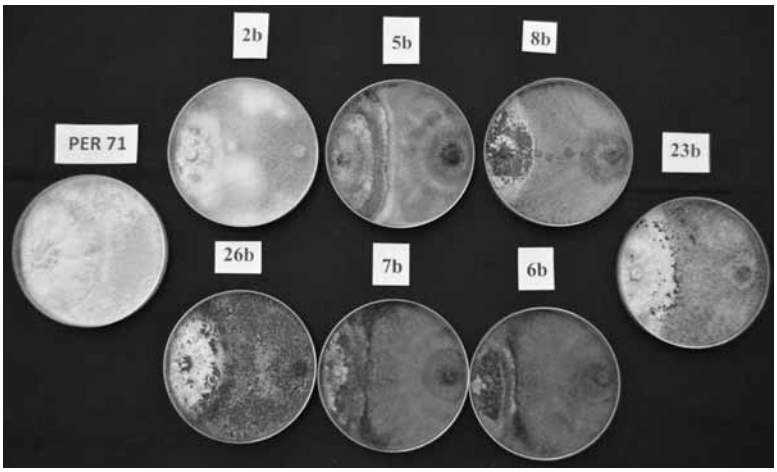


*Figure 2. Dual culture assay between several endophytic* Trichoderma *isolates and* Ganoderma boninense *(PER 71).*

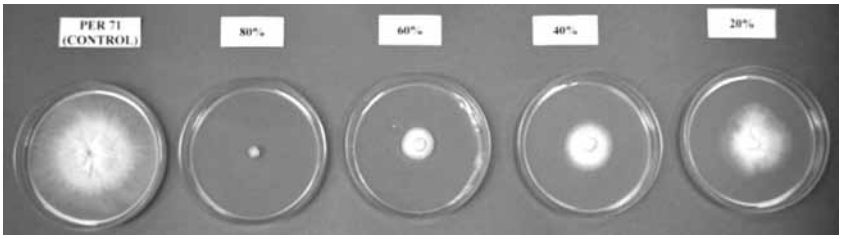


*Figure 3. Antibiosis properties via poison food agar assay for* Trichoderma *(6b) with culture filtrate incorporation into growth media at 20%, 40%, 60% and 80%.*

## DISCUSSION

This is the first known report on endophytic *Trichoderma* isolated from oil palm. The *Trichoderma* isolates exhibited antagonistic properties of mycoparasitism and antibiosis through the *in vitro* assays via dual culture and poison food agar assay respectively. Seven isolates of endophytic *Trichoderma* have the potential to inhibit *G. boninense* growth through *in vitro* assessment. Although *Trichoderma* has been extensively studied for its biocontrol potential, endophytic *Trichoderma* has only recently received its due attention (Harman *et al.*, 2004; Bailey *et al.*, 2008). Therefore, the results presented in this study are important as *Trichoderma* was found to colonise internal tissues of oil palm stem and root systems. Previous studies have shown that *Trichoderma* is a promising biocontrol agent against *G. boninense* both in the *in vitro* and *in*

*vivo* assessment. As for the endophytic *Trichoderma* isolated from this study, further assessment is required to evaluate *in vivo* assessment through seedling pathogenicity tests. The potential use of endophytic *Trichoderma* as biocontrol agent may be the answer for enhanced biocontrol strategy for basal stem rot disease. Endophytes may be a better and effective alternative as biocontrol agents, as they are buffered from environmental changes which are very important for rhizosphere competence of a biocontrol agent. However, more fundamental studies are required before a successful strategy can be implemented for control of oil palm basal stem rot disease. One of the key areas for research includes the extent of the endophytic *Trichoderma* colonisation within the oil palm root system.

Selected *Trichoderma* isolates will be identified using molecular tools for species identification. This will be carried out using PCR with Internal Transcribed Spacer (ITS) 1 and four primers. Oil palm being a perennial crop will require extensive research before the implementation of any potential biocontrol strategy. The selection of *Trichoderma* with good endophytic colonisation ability, in addition to other attributes such as mycoparasitism, antibiosis together with induced resistance could significantly improve the possibilities of developing an effective biocontrol against basal stem rot.

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