THE EFFECTS OF *Trichoderma* IN SURFACE MULCHES SUPPLEMENTED WITH CONIDIAL DRENCHES IN THE DISEASE DEVELOPMENT OF *Ganoderma* BASAL STEM ROT IN OIL PALM

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ABSTRACT

Biocontrol efficiency of Trichoderma *in surface mulches together with conidial drenches on disease suppression of basal stem rot (BSR) caused by* Ganoderma boninense *in oil palm seedlings was investigated. Surface mulches used were from palm press fibre (PPF). Two isolates of* Trichoderma (T. asperellum *T9 and* T. virens *T29) were selected based on the* in vitro *antagonistic assessment via dual culture followed by poison food agar test.* In vivo *results indicated disease development was lowest in plants treated with* Trichoderma *T29 and* Trichoderma *T9 compared to plants treated with mixed inocula of* Trichoderma *(T9 and T29), PPF alone and water. Disease development in the plants was assessed using disease severity (DS) and subjected to further analysis with area under the disease progression curve (AUDPC) and epidemic rate (R_L). Disease development was delayed by eight weeks in plants treated with* Trichoderma *T29 and* Trichoderma *T9 compared to control treatment. The study also suggests that the potential use of PPF, a biowaste product of palm oil mill as surface mulch, enriched with single* Trichoderma *species (T9/T29) effectively delayed disease onset in oil palm seedlings.*

Keywords: biocontrol, palm press fibre, *Ganoderma boninense*, basal stem rot, *Trichoderma asperellum.*

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INTRODUCTION

Oil palm, *Elaeis guineensis* Jacq. is an important perennial crop in Malaysia, which is currently one of the largest producers and exporters of palm oil in the world. Oil palm cultivation occupies approximately 14% or 4.85 million hectares of the land area in Malaysia. Malaysia contributes 47.9% of the world palm oil production with export revenue earnings from oil palm products being almost RM 60 billion in the year 2010 (Simeh and Fairuz, 2009; Abas *et al.*, 2011). However, the crop is very susceptible to a soil borne disease known as basal stem rot (BSR) caused by *Ganoderma boninense*, a white rot fungus. The fungus causes severe economic losses to oil palm in South-east Asia (Rees *et al.*, 2007). The infection occurs via the roots in close proximity to an infection source, *e.g.*, an infected stump of a felled tree (Sanderson, 2005). The disease has been found to infect oil palms as early as 12 to 24 months after planting, with increased incidence on oil palms that are four to five years old. The disease is most prevalent in mature oil palm areas in Malaysia and capable of killing 80% of the stand when the crop is halfway through its economic life-span (Turner and Gillbanks, 2003). Historically, the control of BSR involved cultural techniques via mechanical

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and chemical control with no demonstrable effects (Susanto *et al.,* 2005). Methods such as clean clearing and sanitation at the replanting stage with the complete removal of old diseased palm boles are being practiced by the oil palm growers with limited success (Idris, 1999). Basal stem rot management using chemical control such as hexaconazole, resulted in prolonging the economic life of the crop (Idris *et al.,* 2004). However, control methodologies involving long-term usage of chemicals can have a negative impact on the environment. Therefore, in addition to existing disease management tools, new and sustainable approaches are being explored to suppress diseases through natural and more eco-friendly means to reduce the use of synthetic fungicides. One of the eco-friendly approaches involves the use of naturally existing microbes as biological control agents against plant diseases. However, developing suitable technologies to utilise microbes in the field also requires the development of appropriate delivery technologies.

Mulching in plants has been commonly used and investigated for centuries using leaves, straw, sawdust, peat moss and compost (Jensen and Malter, 1995). Studies have indicated better growth and yield as mulching provides protection above the ground, usually to reduce evaporation, enrich the soil, prevent soil erosion, improve soil structures and prevent weeds (Crafts and Robin, 1962). Nature produces large quantities of mulch all the time with fallen leaves, needles, twigs, pieces of bark, spent flower blossoms, fallen fruit and other organic materials that provide protection or improvement of the area covered. Rice, oil palm, rubber, cocoa and vegetable crops are just a few examples of crops that generate considerable amounts of waste that can be utilised as mulching material. In the palm oil industry, palm press fibre (PPF), palm shell and empty fruit bunches (EFB) are produced as waste after crushing of palm fruitlets in the palm oil milling process (Ku and Cliffe, 1999). But now, the EFB are being convert to organic fertiliser to be recycled back to the field (Tohiruddin and Foster, 2013). Palm press fibre is usually burnt as fuel to provide energy for the mills which are self-sustaining (Choo and Yap, 1991).

Literature reports the importance of mycoparasitic fungi displaying lethal mechanisms against phytopathogenic fungi that could be of significance for plant disease biological control (Bélager *et al.,* 1995; Benhamou and Chet, 1996). Isolates of the genus *Trichoderma* have been the focal point of a number of studies concerning their ability to control plant pathogens. Reports of *Trichoderma* as a biocontrol agent for various other diseases in annual and perennial crops have been extensively investigated (Burgess and Hepworth, 1996; Etebarian *et al.,* 2000; Hanson, 2000; Howell, 2003; Shoresh *et al.,* 2005; Segarra *et al.,* 2007; Siddiqui *et al.,* 2008). However, reports of mulching with *Trichoderma* for disease suppression are relatively new. Jefferson *et al.* (2000) reported that the use of bio-enhanced mulches colonised with *Gliocladium virens* and *T. virens* as surface mulches proved to be the best combination in reducing the population of *Phytophthora cinnamoni* of avocado. It is well established that the right combination of compatible mixed cultures can exhibit a synergistic effect and significantly increase the efficacy of the end product. According to Etebarian *et al.* (2000), *T. harzianum* in combination with *T. virens* reduced disease severity in shoots and roots of potatoes after 10 weeks of inoculation of the pathogen, *Phytophthora erythroseptica* that causes root and stem rot in tomato. The biocontrol activity of microbial enriched mulches could therefore be enhanced with incorporation of suitable biocontrol inoculants into a planting mound or mulch (Chen *et al.,* 1988; Hardy and Sivasithamparam, 1991).

In view of the above, this study was carried out to determine the efficiency of *Trichoderma* spp. as single and mixed surface mulches in reducing and suppressing the disease development of *Ganoderma* basal stem rot in oil palm.

MATERIALS AND METHODS

Isolation of *Trichoderma* Species and *Ganoderma boninense*

Trichoderma species were isolated from soil samples obtained from three oil palm plantations located at Kluang, Bangi and Sepang in Peninsular Malaysia. *Trichoderma* spp. were isolated via the serial dilution method on *Trichoderma* Medium E (TME) (Papavizas and Lewis, 1981) while *Ganoderma boninense* (test pathogen) was isolated from a fruiting body of an infected oil palm, which was then identified as *G. boninense,* based on macroscopic, microscopic morphology and pathogenicity test (Idris *et al.,* 2000a, b; Sundram *et al.,* 2011). The pure cultures of *Trichoderma* spp. and *G. boninense* were maintained in potato dextrose agar (PDA) and stored at 4ºC until further study.

Screening of *Trichoderma* spp. against *G. boninense* via Dual Culture Assay

All the isolated *Trichoderma* spp. were subjected to screening via dual culture assay. Potential *Trichoderma* isolates were selected based on the dual culture assay described by Dennis and Webster (1971). Briefly, a mycelial plug (5 mm in diameter) obtained from the edge of a five-day old culture of *G. boninense* was placed 20 mm from the periphery of the 90 mm Petri plate. The culture plate was left to grow for four days. Each of the 5 mm diameter plug

obtained from the periphery of seven-day old culture of the test isolates (*Trichoderma*) was then placed on the opposite side of the same Petri plate. All the test antagonistic pairings were incubated at 28 ± 2°C and arranged in a completely randomised design (CRD) with seven replications. The antagonistic potential of *Trichoderma* isolates was assessed after eight days of co-incubation by measuring the radial growth of *G. boninense* in the direction towards the growth of *Trichoderma* isolates (R_2). 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 formula (Jinantana and Sariah, 1998) as shown below. Two isolates with PIRG of more than 75% were selected for poison food agar test.

$$PIRG\ (\%) = R_1 - R_2 / R1 \quad x \quad 100;$$

whereby R_1 is the radius of colony in control plates and R_2 the radius of *G. boninense* colony in a dual-culture plate.

Concurrently, the two selected *Trichoderma* isolates were sent to Centre for Agricultural and Bioscience International (CABI) for species identification. The isolates were subjected to genetic characterisation of Inter-Simple Sequence Repeat (ISSR), Variable Number Tandem Repeat (VNTR) and Random Amplification of Polymorphic DNA (RAPD-type) using a total of at least four separate primer reactions.

Poison Food Agar Test

Based on the results of dual-culture assay, two most potential *Trichoderma* isolates were further tested for the production of non-volatile metabolites in the culture filtrate as described by Dennis and Webster (1971) via poison food agar test. One hundred millilitres of potato dextrose broth (PDB) were dispensed into separate 250-Erlenmeyer flasks and inoculated with mycelium plugs (5 mm in diameter) from the edge of seven-day-old cultures of the *Trichoderma* spp. Each flask was inoculated with three plugs of the respective *Trichoderma* spp. and incubated on an orbital shaker at 150 rpm at 28 ± 2°C for seven days. Culture filtrates were harvested by filtration using double layer of Whatman No.1 filter papers and sterilised by membrane filter (Milipore, 0.22 μm). The sterilised filtrates were amended in Petri plates containing PDA to a concentration of 20%, 40%, 60% and 80%, respectively. The solidified PDA plates were inoculated with mycelia plugs of *G. boninense* (6 mm in diameter) at the centre followed by incubation at 27°C for eight days. The plates with the absence of culture filtrates served as control. Inhibition of mycelial growth of *G. boninense* was recorded as the difference between mean radial growth in the presence and absence of fungal filtrate. Percentage inhibition of mycelia growth (PIMG %) was calculated based on the formula described in Skidmore and Dickinson (1976).

Efficacy of *Trichoderma*-Palm Press Fibre (PPF) Applied as Surface Mulches on Disease Development

A total of 90 oil palm seedlings of four months old were used in this study. Seedlings were obtained from a local commercial seedling producer in Selangor, Malaysia. Seedlings were planted in polybags of 38 cm x 45 cm and arranged in an open nursery in a randomised complete block design (RCBD). Six treatments were evaluated, replicated three times with each replication accommodating five seedlings. Basal fertiliser was applied monthly according to standard nursery practice (Corley and Tinker, 2003).

Preparation of Conidial Suspension and *Trichoderma*-PPF Surface Mulch

Conidial suspension of *Trichoderma* was prepared according to Jinantana and Sariah (1998). Selected *Trichoderma* spp. were grown in PDA. After a period of seven days, the *Trichoderma* conidia were dislodged gently from the surface of the mycelial mat using an L-shaped glass rod by adding 20 ml of sterilised distilled water. A total of 40 Petri plates were prepared for a single conidial suspension preparation. The conidial suspension obtained was filtered through a double layer Whatman No.1 filter paper and was adjusted to 1 $x10^8$ conidia ml^{-1} using Neubauer hemocytometer. Each seedling received 150 ml of the conidial suspension.

The PPF which is a by-product of the oil palm fruit mesocarp was obtained from local oil palm mill in Banting, Selangor, Malaysia. The PPF was soaked in tap water overnight and dried at room temperature. The dried PPF was then packed into plastic bags at 500 g each and sterilised before being inoculated with a seven-day old *Trichoderma* culture at one plate per bag. The bags were aseptically sealed and incubated in the dark for a period of two weeks before use.

Artificial Infection by *G. boninense* on Oil Palm Seedlings and Application of *Trichoderma*-PPF Surface Mulch

Artificial infection of *G. boninense* on oil palm seedlings was carried out using rubber wood blocks of 6 x 6 x 12 cm. Method of preparing the inocula for artificial infection was performed as described by Ilias (2000). The wood blocks were packed in heat resistant plastic bags. Each bag received approximately 100 ml of malt extract agar (MEA) before sterilisation. The wood blocks were sterilised

at 120°C, 1.05 kg cm^{-2} for 55 min. Upon reaching the room temperature, each of the wood blocks was inoculated with macerated mycelium obtained from eight-day-old culture of *G. boninense* (1 plate/block). The wood blocks were incubated for eight weeks at room temperature in the dark before carrying out infection on the seedlings.

Treatment seedlings were subjected to *Ganoderma* infection with the exception on seedlings in positive control (T1) where no inoculation of either *Ganoderma* or *Trichoderma* was carried out. The remaining seedlings were subjected to the following treatments; water (T2), only PPF (T3), *Trichoderma* T9-PPF surface mulch (T4), *Trichoderma* T29-PPF surface mulch (T5) and mixed inocula *Trichoderma* (T9 and T29)- PPF surface mulch (T6).

Ganoderma infection on the seedlings involved removal of each seedling from the existing polybag to uncover the roots. The fully colonised woodblocks were then directly placed under the base of the seedlings before transplanting into a new polybags pre-filled one-third with soil. Once in place, more soil was added to cover the roots and inoculum. The soil surface was then covered with the respective treatments and applied with *Trichoderma* conidial suspension at 10^8 conidia ml^{-1} to each seedling (150 ml/seedling). Preliminary investigation found that a single conidial application of *Trichoderma* without any food substrate was insufficient for significant disease control; follow up studies found that more than one application was necessary to sustain the *Trichoderma* population dynamics in soil and delay the disease development. Hence, five more applications of conidial suspension at the rate of 10^8 conidia ml^{-1} were continued at fortnightly intervals at weeks 4, 6, 8, 10 and 14.

Disease Severity Assessment of Basal Stem Rot

Development of *Ganoderma* BSR in oil palm was recorded at intervals of four weeks using a disease rating scale of 0-4, where scale 0 indicates healthy seedling, scale 1 indicates appearance of 3 or more necrotic leaves, scale 2 indicates appearance of 3 or more necrotic leaves along with presence of white mycelial mass at the bole of seedling, scale 3 indicates appearance of 3 or more necrotic leaves and the presence of basidioma and scale 4 indicates formation of well-developed basidioma with more than 50% severe leaf necrosis or dead plant. Disease severity (DS) and disease severity index (DSI) were expressed according to the formula given by Tarig *et al.* (1998):

$$DS\ (\%) = \Sigma \frac{\text{(Number of seedlings in disease rating scale x disease rating scale)}}{\text{Total number of seedlings assessed x highest disease rating scale}} \times 100$$

A disease progression curve was produced based on the disease severity data for each treatment. The same data was subjected to express the disease reduction as a measurement of effectiveness of treatments in suppressing the disease. The area under the disease progressive curve (AUDPC) was assessed to determine the progression of disease. In addition to this, epidemic rate (unit/weeks) was also assessed to determine the rate of infection and extent of disease progression (Vanderplank, 1984). The slopes of curves were obtained by transforming the DS (%) data using monomolecular model (Monit) by Campbell and Madden (1990). Values for each treatment epidemic rate were generated from the slope of disease curve which paired weeks after *G. boninense* infection as a non-variable (x axis) and monit values as variable values (y axis). Monit (M) values were generated from the following formula M=log [1 / (1-Y)] where Y represented DS. This model was fitted into a non-linear regression analyses using the sigma plot software (Version 9.0).

Estimation of *Trichoderma* spp. Spore Counts

Soil samples of all the six treatments were sampled at two week intervals for assessment of population dynamic of *Trichoderma* isolates over time. The soil was sampled at 5 cm depth from soil surface and placed in a sterile plastic bottle prior to spore counts. The *Trichoderma* species were re-isolated via the serial dilution method as described earlier on TME (Papavizas and Lewis, 1981).

Leaf and Root Biomass

The plants were harvested at the end of the 24 weeks of assessments. They were separated into two parts; leaf and root. Each plant part was carefully washed and labeled before drying in the oven at 65°C for 48 hr or until a constant weight was achieved.

Statistical Analysis

Statistical analyses were performed using SAS statistical software (Version 8.2). All experimental data was subjected to analysis of variance (ANOVA) and treatment means were compared using Tukey's studentised range test (HSD) at $P \leq 0.05$. Data percentages were arcsine transformed before subjecting to ANOVA. To confirm the reproducibility of data, all experiments were repeated thrice.

RESULTS

Isolation and Screening of *Trichoderma* spp. against *Ganoderma boninense*

Preliminary screening of *Trichoderma* yielded more than 100 isolates of *Trichoderma* based on cultural and morphological characteristics (Rifai, 1969). A thorough screening for potential biocontrol

agent was carried out by subjecting the *Trichoderma* isolates in dual culture assay, where 15 isolates recorded PIRG (%) values more than 60% (*Figure 1*). The dual-culture assay revealed that, all 15 tested isolates were able to inhibit the mycelia radial growth of *G. boninense* with different inhibition levels. However two *Trichoderma* isolates namely, T9 and T29 recorded the highest PIRG values of 78.60% and 80.50% respectively. Therefore, T9 and T29 were further selected for *in vitro* assessment using the culture filtrate via poison food agar test followed by *in vivo* assessment. CABI identified both the *Trichoderma* isolates of T9 and T29 as *T. asperellum* and *T. virens*, respectively. Their unique reference number is IMI 398618 for T9 and IMI 398619 for T29.

Poison Food Agar Test

Four concentrations at 20%, 40%, 60% and 80% of culture filtrates derived from two *Trichoderma* spp. were tested against *G. boninense*. Both isolates varied in their abilities in producing anti-fungal compounds that inhibited *G. boninense*'s radial growth. Suppression was greater at higher concentration of culture filtrate. Cultures filtrates that were incorporated in the poison food agar test showed highest PIMG was significantly recorded by *T. virens* (T29) at 68%, while the lowest recorded by *T. asperellum* (T9) at 26.90% in 80% incorporation of culture filtrate into agar, respectively (*Figures 2* and *3*). Poison food agar test demonstrated that both *Trichoderma* cultures produced culture filtrates that were inhibitory to the pathogen's growth.

Efficacy of *Trichoderma*-Palm Press Fibre (PPF) Applied as Surface Mulches on Disease Development

The assessment of disease severity was based on the sequential progression of disease using index values of 0 to 4. Lower disease severity indicates the effectiveness of *Trichoderma*-PPF surface mulch in suppressing *Ganoderma* BSR in oil palm through *in vivo* assessment. DS was significantly higher ($P \leq 0.05$) in T2 (plants treated with water) with 95.0% with an index score of 4 and lowest in seedlings treated with *Trichoderma* T29–PPF surface mulch (T5) (46.67%) and in treatment using *Trichoderma* T9 – PPF surface mulch (T4) (58.33%) with an index score of 2. Treatment using only PPF (T3), mixed inocula of *Trichoderma* - PPF surface mulch (T6) recorded a disease severity of 96.67% and 83.33% with a similar disease index of 4 at the end of the 24 weeks (*Figure 4*).

Disease progression was further expressed as AUDPC and R_L. Based on the AUDPC values, treatment with *Trichoderma* T29-PPF surface mulch (T5) was significantly lower ($P \leq 0.05$) compared to the control treatment (T2) at 24 weeks after infection, as shown in *Table 1*. The development of the disease was delayed in plants treated with *Trichoderma* T9-PPF surface mulch (T4) and *Trichoderma* T29-PPF surface mulch (T5), whereby the symptoms were observed only at week 16, compared to the control treatment (T2), as early as week 8 (*Figure 4*).

The epidemic rates (slope) were assessed to determine the rate of infection and the extent of disease progression. The highest epidemic rates was recorded by PPF treatment (T3) ($R_L = 0.16$ unit/week) followed by control treatment (T2) ($R_L = 0.15$/week). The lowest epidemic rate was recorded by treatment using *Trichoderma* T9-PPF surface mulch (T4) and *Trichoderma* T29-PPF surface mulch (T5) as shown in *Table 1*. Treatment using mixed inocula of T9-T29 (T6) was not significantly different ($P \leq 0.05$) compared to the control treatment (T2). The disease was reduced in treatment using *Trichoderma* T29-PPF surface mulch (T5) and *Trichoderma* T9-PPF surface mulch (T4) with a disease reduction of 38.59% and 50.87% respectively, compared to the control treatment (T2) using water at the end of the experiment (*Table 1*).

Disease development in oil palm seedlings was delayed in the single species application of *Trichoderma*-PPF surface mulch. Plants receiving empty PPF (T3) developed disease symptoms as early as eighth week with significantly higher ($P \leq 0.05$) disease suppression parameters (DS%, AUDPC, epidemic rate, disease reduction and dry weight). The disease progressed rapidly in the non-*Trichoderma* treatments with severe necrosis of lower fronds and emergence of *Ganoderma* fruiting bodies on the plant base.

Estimation of *Trichoderma* spp. Spore Counts

The population counts (CFU g^{-1}) of T1, T2 and T3 marginally increased until reaching a peak at week 2, respectively and declined thereafter (*Figure 5*). On the contrary, the population dynamics recorded by T4, T5 and T6 was different, whereby the *Trichoderma* spp. populations were found to sustain over a period of 22 weeks of assessment. The population counts (CFU g^{-1}) were statistically higher in T6 (2.45×10^4) followed by T4 (2.32×10^4) and T5 (1.87×10^4) compared to the control (4.11×10^3), respectively at week 2. However, the population counts started to decline at week 4 and peaked again at week 6, 8, 12 and 16 in T4, T5, and T6, respectively. Applications of booster containing T9 and T29; singly or in combination at week 4, 6, 8, 10 and 14 weeks of assessment contributed in the increase of the population counts recorded by these three treatments The population counts were highest in these three treatments with the values for T6 at 7.44×10^3, T4 at 6.56×10^3 and T5 at 3.78×10^3 compared to the control T2 (4.4×10^2), in week 22.

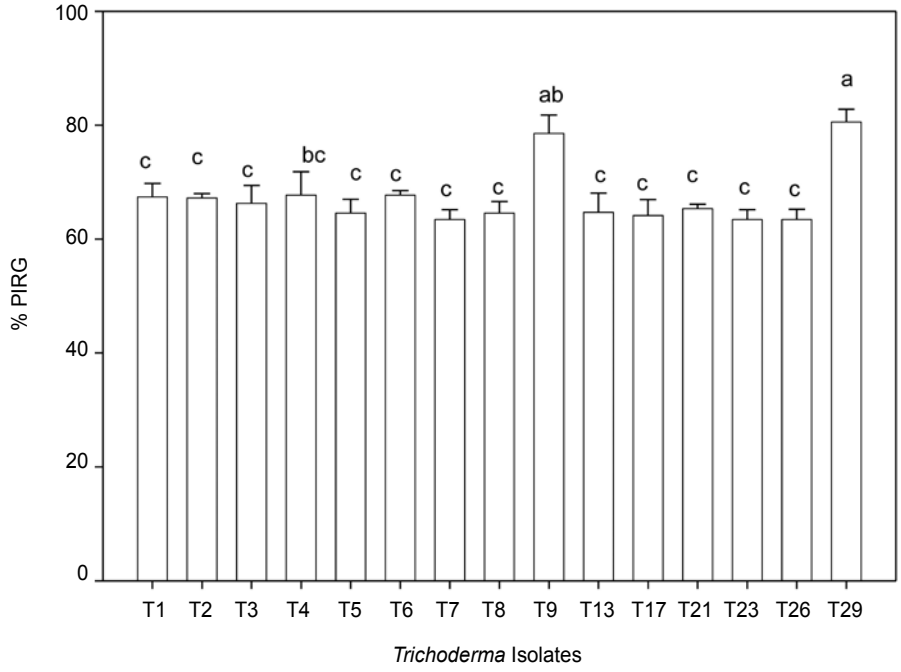

Figure 1. In vitro *antagonistic assessment with percentage inhibition of radial growth (PIRG) of* Ganoderma boninense *by selected* Trichoderma *isolates. Vertical bar with the different letter denotes significant difference (P ≤ 0.05) according to Tukey's test (HSD) test. Vertical error bars indicate standard error.*

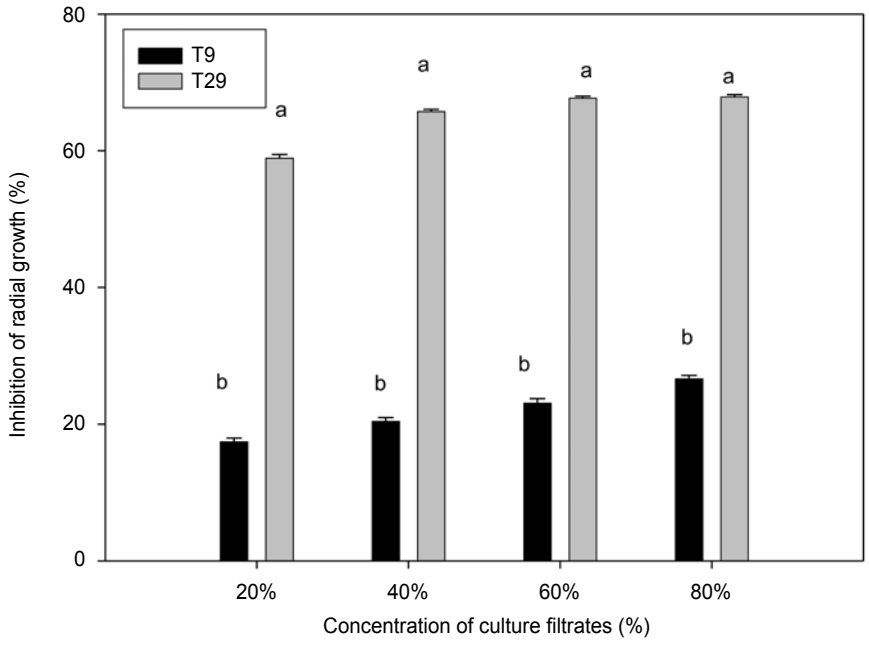

Figure 2. In vitro *inhibitory effect of T9 and T29 culture filtrates against* Ganoderma boninense*. Values with the different letter at each concentration are significantly different (P ≤ 0.05) according to Tukey's test (HSD) test on their transformed values. Vertical error bars indicate standard error.*

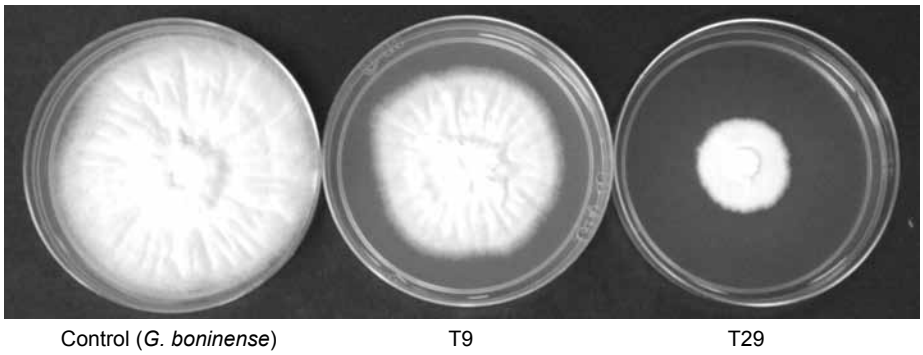

Figure 3. In vitro *inhibitory effect of culture filtrates of T9 and T29 against* Ganoderma boninense *at 80% concentration, respectively.*

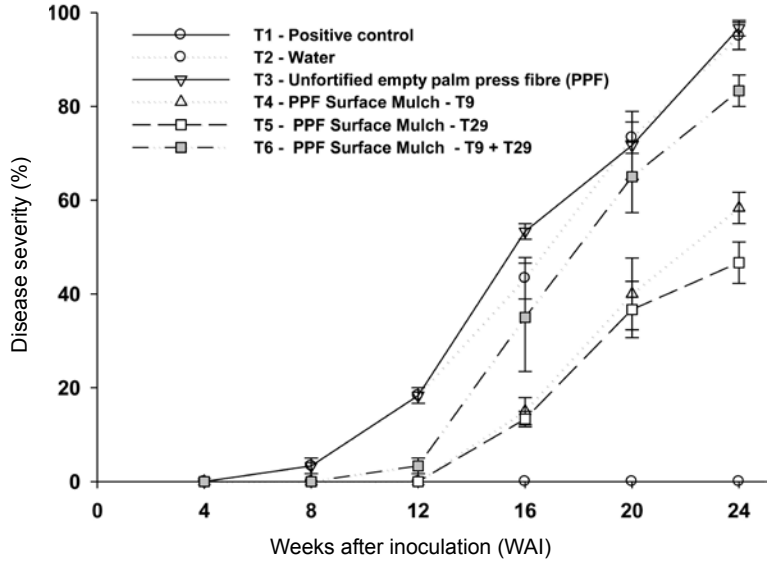

Figure 4. Disease development in oil palm treated with Trichoderma*-palm press fibre (PPF) at four weekly intervals (n=3). Vertical error bars indicate standard error.*

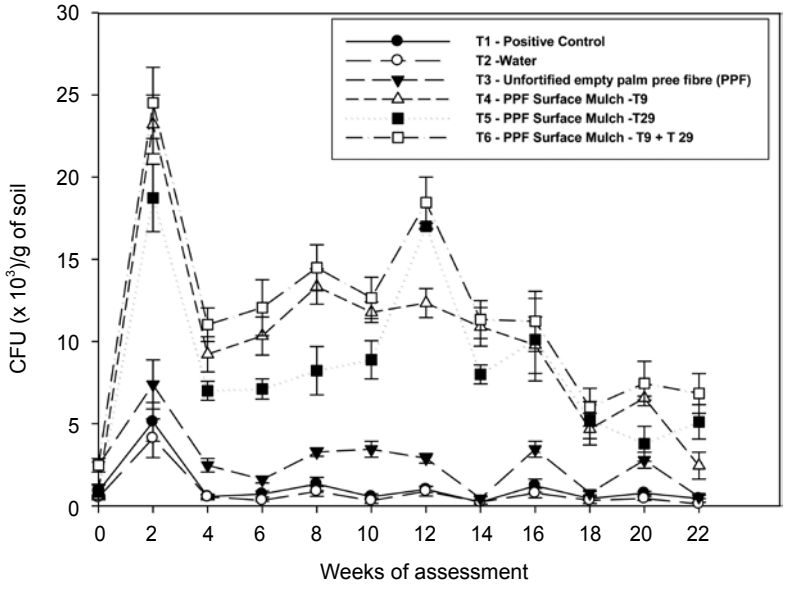

Figure 5. Population dynamic based on the colony forming unit (CFU g^{-1}) of Trichoderma *spp. isolated from soil samples at different sampling intervals. Vertical error bars indicate standard error with assessment according to Tukey's test (HSD) at $P \leq 0.05$.*

Leaf and Root Biomass

At the end of the 24 weeks, the leaves and roots from each treatment were separated from the seedlings. Positive control treatment (T1) that had neither the infection nor the *Trichoderma* – enriched treatment recorded the highest value with a significant difference ($P \leq 0.05$) for dry weight of leaf and root with 22.36 g and 6.02 g respectively, compared to the other treatments as specified in *Table 2*. Treatment using *Trichoderma* T9-PPF surface mulch (T4) and *Trichoderma* T29-PPF surface mulch (T5) recorded 14.73 g and 14.76 g values for leaf; 4.06 g and 4.75 g for root, respectively. These values significantly differed ($P \leq 0.05$) compared to the control treatment using water (T2), PPF (T3) and mixed inocula of T9-T29.

DISCUSSION

In the present study, more than 100 *Trichoderma* isolates were isolated from the sampled soil. However, only 15 *Trichoderma* isolates antagonised *G. boninense* with a PIRG of more than 60% under *in vitro* assay via dual culture. Mycelial interaction is a basic method to assess antagonistic properties of biocontrol agents (Rahman *et al.,* 2009). Two isolates namely; *T. asperellum*, T9 and *T. virens*, T29 were found to be potential biocontrol agents against *G. boninense* with highest PIRG (%) values. Further assessment confirmed that, the culture filtrates produced by *T. asperellum*, T9 and *T. virens*, T29 effectively inhibited the growth of *G. boninense*. The culture filtrate assay via poison food agar test proves the secretion of inhibitory anti-fungal compounds by the *Trichoderma* spp. particularly by T29. The ability of *Trichoderma* species to produce inhibitory anti-fungal compounds against microorganisms has been described by Dennis and Webster (1971); Jinantana and Sariah (1998); Rini and Sulochana (2007); Rahman *et al.* (2009). Both isolates were eventually selected to be tested as single and mixed inocula application through nursery assessment using PPF as surface mulches.

The application of single species of *Trichoderma* as PPF surface mulches suppressed the disease severity in plants infected with *Ganoderma* BSR.

TABLE 1. AREA UNDER DISEASE PROGRESSION CURVE (AUDPC), DISEASE SEVERITY, EPIDEMIC RATE AND DISEASE REDUCTION OF *Ganoderma* BASAL STEM ROT IN OIL PALM TREATED WITH *Trichoderma* AT 24 WEEKS AFTER *Ganoderma* INFECTION

Treatment	Disease severity (%)	AUDPC (unit)	Epidemic rate (R_L) (unit per week)	Reduction (%)
T2 - Water (control)	95.00 ± 2.88a	743.00 ± 18.55a	0.15 ± 0.024a	0
T3 - Empty palm press fibre (PPF)	96.67 ± 1.67a	780.00 ± 37.86a	0.16 ± 0.025a	1.75
T4 - PPF surface mulch-T9	58.33 ± 3.33bc	336.67 ± 46.67bc	0.04 ± 0.006b	38.59
T5 - PPF surface mulch-T29	47.67 ± 4.4c	293.33 ± 57.83c	0.03 ± 0.005b	50.87
T6 - PPF surface mulch-T9-T29	83.33 ± 3.33ab	580.00 ± 75.71ab	0.09 ± 0.015ab	12.28

Note: Means (± SE) in the same column followed by same letter are not significantly different ($P \leq 0.05$) according to Tukey's test (HSD) on their transformed values. Each value was computed on three replicates.

TABLE 2. LEAF AND ROOT BIOMASS OF OIL PALM SEEDLINGS TREATED WITH *Trichoderma* ENRICHED PALM PRESS FIBRE (PPF) HARVESTED 24 WEEKS AFTER INFECTION

Treatment	Biomass (g)	
	Leaf	Root
T1 - Positive control (healthy)	22.36 ± 0.903a	6.02 ± 0.161a
T2 - Water	6.24 ± 0.236c	1.36 ± 0.126c
T3 - Empty palm press fibre (PPF)	6.74 ± 0.521c	1.48 ± 0.304c
T4 - PPF surface mulch-T9	14.73 ± 1.14b	4.06 ± 0.481b
T5 - PPF surface mulch-T29	14.76 ± 1.391b	4.75 ± 0.594b
T6 - PPF surface mulch-T9+T29	9.42 ± 0.843c	1.93 ± 0.139bc

Note: Means (±SE) followed by same letter within the same column are not significantly different ($P \leq 0.05$) according to Tukey's test (HSD). Each value was computed on three replicates.

Disease suppression was achieved due to the presence of *Trichoderma* spp. as a single inocula application but failed to give higher disease suppression when applied as mixed inocula. Antagonistic interactions between *Trichoderma* and other fungi have traditionally been classified as antibiosis, mycoparasitism and competition for nutrients but these mechanisms are not mutually exclusive and a given antagonist mechanism can fall into several categories (Hjeljord and Tronsmo, 1998). Martin and Loper (1999) reported that *Trichoderma* spp. are early colonisers of substrates and reduce the activity of other fungi simply by substrate occupation and depletion and this might explain the present observation. The beneficial microorganisms compete with the pathogens for infection sites, leaving limited space for pathogens to proliferate or to secrete secondary metabolites on the plant surface, and also directly parasitise pathogens (Baker and Cook, 1982; Ma *et al.*, 2001; Weltzien 1991). Other studies using biocontrol agents against *Ganoderma* include Sapak *et al.* (2008) whereby *Ganoderma* infection was reduced by 76% in seedlings pre-inoculated with *Pseudomonas aeruginosa* (P3) while *Burkholderia cepacia* (B3) reduced incidence by 42% while the mixture of *P. aeruginosa* and *B. cepacia* reduced disease by 54%. The disease suppression was probably higher as the biocontrol agent used was endophytic which colonises within palm providing successful suppression of disease.

Isolate T9 and T29 performed better as single inocula with the latter being superior as biocontrol agent candidate against *G. boninense* BSR disease. Traditionally, mixed fungal cultures are avoided because the population dynamics of such populations are difficult to predict. However, the compatible combination of mixed cultures can exhibit synergy and significantly increase the efficacy of the end product (Bhuiyan *et al.*, 2003; Etebarian *et al.*, 2000). The present study attempted to investigate mixed inoculum of *Trichoderma* treatment (T9 + T29) but this was found to be unsuccessful in suppressing or delaying the disease development of BSR in oil palm. A similar study on mixed species applications by Sariah and Cheng (1999) was carried out using single and mixed isolates of *Trichoderma* spp. to reduce the incidence of collar rot of eggplant using UPM 23 (*T. virens*) and UPM 29 (*T. harzianum*). UPM 23 was found to be superior as single and compared to the mixed treatments. This suggests strong interspecific competition between both *Trichoderma* isolates which eventually resulted in reduced disease suppression. Research has shown that interference competition involves behavioural or chemical mechanism by which one organism limits another organism's access to the substrate, which results in both interspesific and intraspesific mycelia interactions (Wicklow, 1992). The non-synergistic effect of these T9 and T29 failed to give significant disease suppression due to strong antagonism between each isolate. While mixed inoculation of biocontrol agents cannot be completely disregarded, *in vivo* trials utilising the biocontrol agents requires investigation. Therefore, it is suggested that a combination of compatible isolates or species mixture is a crucial exploration in studies involving the effect of single and mixed *Trichoderma* isolates/species.

The present study used PPF, a biowaste produced after the crushing of the oil palm mesocarp as surface mulch. PPF was further colonised by *Trichoderma* (T9 or T29) and termed as *Trichoderma*-PPF surface mulches. PPF efficiently sustained the respective *Trichoderma* (T9 or T29) propagation in soil at an effective level as shown in *Figure 5*. Treatment that used PPF without the drenches of *Trichoderma* showed no sign of disease suppression. This was clearly attributed to the presence of the beneficial microorganism and this study it was *Trichoderma*. *Trichoderma* and other potential biocontrol fungi proliferate abundantly in various natural soils when added as young mycelium in intimate contact with a food base (Jefferson *et al.*, 2000). Previous findings by Siddiqui *et al.* (2008) revealed that, *Trichoderma*-enriched rice straw compost extracts promoted significant reduction in the incidence of Choanephora wet rot of okra and simultaneously promoted the plant growth (Siddiqui *et al.*, 2009). Similarly, highest disease suppression (61.6%) was obtained by the application of rice straw compost amended with *T. harzianum* on the management of damping-off and charcoal-rot of sunflower (Morsy and El-Korany, 2007). The authors concluded that, the amended compost with *T. harzianum* was found to accelerate rice-straw composting and simultaneously produced a highly disease suppressive compost. *T. aperellum* (T9), has been reported to be a potential biocontrol agent for various diseases (Cotxarreraa *et al.*, 2002; Shoresh *et al.*, 2005). *T. asperellum* (previously reported as *T. harzianum*) was also reported to penetrate the roots of cucumber seedlings and colonise the epidermis and outer root cortex (Yedidia *et al.*, 1999). These interactions also induced host plant resistance to pathogens (Yedidia *et al.*, 2003). *Trichoderma* root inoculation has been shown to be effective against different types of pathogens in a wide variety of plants (Shoresh *et al.*, 2005). Contact with pathogenic and non-pathogenic microorganisms triggers a wide range of defense mechanisms in plants that protect them against invasion but requires further investigation. The disease suppression attained in the present study was possibly due to the ability of *Trichoderma* conidia to germinate, colonised the oil palm rhizosphere and parasitised the targeted pathogen hyphae (Aryantha *et al.*, 2000; Scheuerell *et al.*, 2005). The accumulation of inhibitory culture filtrates produced by *Trichoderma* spp. in this study may have also contributed to the disease suppression.

CONCLUSION

This study indicates the potential use of PPF as surface mulch for the active propagation of *Trichoderma*. Significant disease suppression was achieved with the application of *Trichoderma* as surface mulches followed up with six fortnightly conidial drenching. The results from this study suggest PPF as suitable organic mulches that are effectively colonised by biocontrol agents and applied as biocontrol agents for disease management. This study provides preliminary evidence for further exploitation of PPF as food substrate/carrier for biocontrol agents. To further establish the possibility of the disease management using the combination of PPF as surface mulches with *Trichoderma*, treatments are currently being tested in the field. This will be carried out using a seedling bait technique (Sundram, 2012). Currently, research is also being undertaken to investigate the possibility of endophytic *Trichoderma* isolates from oil palm utilised as biocontrol agents against *Ganoderma* in oil palm. Defense mechanisms triggered by these *Trichoderma* isolates in oil palm could be potential area for further research.

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