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EFFICACY OF SINGLE AND MIXED TREATMENTS OF *Trichoderma harzianum* AS BIOCONTROL AGENTS OF *Ganoderma* BASAL STEM ROT IN OIL PALM

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ABSTRACT

Two Trichoderma harzianum *strains (FA 1132 and FA 1166) were tested as biocontrol agents for basal stem rot in oil palm seedlings artificially infected with the causal pathogen,* Ganoderma boninense*. The treatment was carried out by applying a* Trichoderma-*infused surface mulch and periodic applications of a conidial soil drench made from spore suspensions of the respective* Trichoderma *strains. A disease severity index (DSI) ranging from 0 to 100 was used to assess the disease severity. A single strain application of* T. harzianum, *FA 1132 gave the best disease suppression with the lowest DSI of 28.35 compared to the infected, non-treated control plants that gave the highest DSI of 86.67. However, FA 1166 as a single application was ineffective, so was the mixture of the two strains. The biological control property of* Trichoderma *was shown to be strain-specific and not species-specific. In addition, it was found that applying the mixed inocula significantly decreased the performance of FA1132, the choice strain.*

Keywords: oil palm, basal stem rot, *Trichoderma harzianum, Ganoderma boninense*.

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INTRODUCTION

Basal stem rot (BSR) is a major disease of oil palm, caused by the fungus *Ganoderma boninense* although other *Ganoderma* species have also been implicated (Ho and Nawawi, 1985). The disease management is based on the severity of the infected palm. The less severely diseased palms still yielding are mounded to encourage root growth to extend their productive life, and a trench dug around them to obviate infecting the healthy neighbouring palms.

There is, however, no known cure for the disease and as it progresses and the palms start dying, they are totally removed, including their roots (Idris, 1999). Chemicals may also be used but their efficacy remains arguable (Jollands, 1983; Khairudin, 1990; PORIM, 1997; MPOB, 2001; 2002; Idris, *et al.*, 2004). With no clear control against the disease and environmental hazards from the chemicals used, researchers are looking into using biocontrol instead.

Biocontrol agents for various diseases of annual and perennial crops have been studied extensively, one of them being fungus from the genus of *Trichoderma*. *In vitro* studies on the antagonistic properties of *Trichoderma* against *Ganoderma* in Malaysia have been carried out (Varghese, 1975; Abdullah *et al.*, 1999; Ilias, 2000). Abdullah *et al.* (2003a) found in the greenhouse that strain FA 1132 could control the pathogen both *in vitro* and *in vivo*. In this present study, two strains of *Trichoderma harzianum* - FA 1132 and FA 1166 - were tested for their effectiveness against BSR infection in oil palm seedlings. FA 1166 and FA 1132 were selected for their similar antagonistic property against *Ganoderma*

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based on *in vitro* dual cultures. Strain FA 1166 also produced a higher spore count in culture if compared to FA 1132 (Ilias, 2000). Traditionally, mixed fungal cultures are avoided because the population dynamics of such cultures are difficult to predict although a compatible mixed culture can exhibit synergy and greatly increase the efficacy of the end product. Roberts *et al.* (2004) reported that a formulation of *Trichoderma virens* with the bacterial species *Burkholderia cepacia* and *B. ambifania* significantly improved the control of damping off by *Rhizoctonia solani*. Another study by Etebarian *et al.* (2000) also proved that *T. harzianum* in combination with *T. virens* reduced disease severity in shoots and roots of potatoes 10 weeks after inoculation of the pathogen, *Phytophthora erythroseptica* that causes root and stem rot in tomato. Both isolates worked synergistically to control the disease in tomato. Bhuiyan *et al*. (2003) used two commercial *Trichoderma* spp. of Trichopel and Trichoflow and two isolates of *Penicillium citrnum* in controlling the conidial germination of macroconidia of *Claviceps africana*, the cause of ergot sugary disease of sorghum. They found that Trichopel that had the combination of *T. harzianum* and *T. viride* performed equally well with the single application of *Trichoderma* isolates as formulated in Trichoflow that had one *T. harzianum* species.

In view of the above, a formulation consisting of both strains, namely FA 1132 and FA 1166, was tested for possible synergistic performance in the control of disease development on oil palm seedlings artificially infected with *G. boninense* in a greenhouse trial.

MATERIALS AND METHODS

Fungal Cultures

Fungal cultures, FA 1132 and FA 1166, were obtained from the stock collection of the Mycology Laboratory, Department of Biology, Faculty of Science, Universiti Putra Malaysia (UPM). The pathogen, *G. boninense* (isolate FA 5201), was the same pathogen used by Abdullah *et al.* (2003b). The fungal mycelium was subcultured on potato dextrose agar (PDA) for further investigation.

Oil Palm Seedlings, Potting Media and Inoculum Preparation

A total of 75 four-month-old seedlings were used in this experiment. The seedlings were divided into three experimental and two control treatments and planted in garden pots containing a non-sterilized 3:2:1 mixture of soil, peat and sand, respectively, obtained from UPM, Serdang, Selangor. The pathogen inoculum was prepared on *Hevea brasiliensis* wood blocks of 6 x 6 x 12 cm size. These blocks were sterilized (121°C/1.04 kg cm^{-2} pressure) for 30 min and then inoculated with macerated FA 5201 culture at one plate per block. Completely colonized blocks at eight to 10 weeks after inoculation were ready for artificial infection of the oil palm seedlings. Details on the inoculum block preparation and artificial infection was according to Abdullah *et al.* (2001).

Preparation of *Trichoderma*-infused Surface Mulch

Surface mulch was prepared by incorporating *Trichoderma* pressed mesocarp fibre of oil palm (MFOP) fruits. Firstly, the MFOP was rinsed, placed in heat resistant polypropylene (PP) bags at about 500 g per bag and then sterilized (121°C/1.04 kg cm^{-2} pressure) for 45 min. The cooled MFOP was inoculated with macerated FA 1132 and FA 1166 according to the respective treatments at one culture plate per bag, followed by two weeks' incubation in the dark at room temperature. For the treatment using mixed inocula application, the colonized FA 1132 and FA 1166 MFOP were homogenously mixed manually in a container at 1:1 proportion and immediately divided equally between two pots and applied.

Preparation of *Trichoderma* Conidial Soil Drench

In addition to the surface mulch, the plants were also periodically given a conidial soil drench. Conidia from a 7-day-old culture plate of *Trichoderma* were harvested in 10 ml distilled water, filtered to remove mycelial debris and then topped up with distilled water to make into 1 litre of conidial suspension. This soil drench, whether of FA 1132 or FA 1166, was immediately applied at 1 litre per pot according to the treatment. For the mixed inocula treatment, 1 litre conidial suspensions from both the strains were homogenously mixed in a trough, divided equally between two pots and then applied at 1 litre per plant.

Artificial Infection of Oil Palm Seedlings

The fully colonized wood blocks were directly placed under the basal of the seedlings. Three roots were tied to the inoculum block using two rubber bands. The plant-cum-inoculum was placed into a pot filled only one-third with soil. More soil was then added to cover the roots and inoculum.

Experimental Design

The oil palm seedlings were divided into five groups with each group consisting of 15 seedlings in a complete randomized design (CRD). The groups

were separated into three sets of treatments (T1, T2 T3) and two sets of controls (C1 and C2). The C1 plants were absolute control for the trial whereby the seedlings were non-infected and non-treated while the C2 plants were artificially infected but non-treated. Seedlings of T1, were applied with FA 1132 *Trichoderma*-incorporated surface mulch followed by a soil drench of the same fungal strain at 1 litre per plant, according to the regime outlined at 0, 2, 4, 6, 10 and 14 weeks. As for T2, a similar application was carried out, with the MFOP and soil drench of FA 1166 instead. Similarly for T3, the MFOP and drench consisted of a 1:1 mixture of FA 1132 and FA 1166, prepared in the same manner as the above.

Application of *Trichoderma*-infused Surface Mulch and Conidial Soil Drench

As described earlier, the trial consists of three treatments (T1, T2 and T3) and two controls (C1 and C2). The *Trichoderma*-incorporated surface mulch prepared as described earlier was spread loosely on the soil and around the seedling at 1 bag per pot. Only one application of the respective MFOP was made - at the start of the experiment. The experimental and control plants were maintained in the greenhouse and watered twice a day at approximately 1 litre per plant up to week 24. Conidial soil drench was applied six times according to the set regime described earlier. No conidial soil drenches were given from week 14 onwards. The C2 control plants were artificially infected with *Ganoderma* with no application of *Trichoderma* conidial soil drench. All plants were observed for their disease symptoms at weekly basis until 24 weeks.

Numerical Assessment of Disease Signs and Symptoms

A disease severity index (DSI) for BSR with values from 0 to 100 was used as assessment in this study (Abdullah *et al.*, 2003a; Ilias, 2000). The DSI was derived from a mathematical formula based on observable signs and symptoms of 'disease class' of the infected plants which was translated from numerical values, ranging from 0 to 4 (*Table 1*) – disease symptoms were modified based on the BSR (Sherwood and Hagedorn, 1958). The DSI was determined every four weeks after the commencement of the experiment.

The disease class data were then calculated with the following formula to derive the DSI:

$$\text{Disease severity index (DSI)} = \frac{\Sigma(A \times B) \times 100}{\Sigma n \times 4}$$

Note: A = disease class (0, 1, 2, 3 or 4)
B = number of plants showing that disease class per treatment
n = total number of replicates (= 15 in this study) constant No. 4 represents the highest class of assessment.

All the DSI values for each sampling were checked and were subsequently transformed because of non-normality to accommodate the analysis of variance (ANOVA). Duncan's multiple range test (DMRT) was used to rank the relative statistical significance among the treatments.

Plant Biomass

All the seedlings were harvested in week 24. The roots were washed off the soil, and the seedlings were separated into shoots (leaves and stem) and roots. They were labelled, sun-dried for 24 hr and further oven-dried at 40°C to 60°C until constant weight was achieved.

Sustainability of *Trichoderma* Spores in Soil

The propagation of fungus in the soil from the inoculum applied was determined from the number of colony forming units (CFU). The determination of CFU (for *Trichoderma*) was done before any application of conidial suspension for baseline data and continued at two weeks intervals till week 24. The soil sampling was done at 5 and 15 cm depths in five randomly selected pots for each treatment/control. A stock solution of 10 g (fresh weight) soil in 10 ml distilled water was placed on an environmental orbital shaker at 100 rpm for 15 min.

TABLE 1. SIGNS AND SYMPTOMS OF INFECTION AND THEIR CORRESPONDING DISEASE CLASS NUMERICAL VALUES

Class	Associated signs and symptoms of infection
0	Healthy plant with green leaves with absolutely no mycelial development on any of the plant part.
1	Sign of infection based on the presence of 3 or more chlorotic leaves, but no observable symptom of fungal presence on the plant.
2	Appearance of white mass of mycelia on any part of the plant, with or without leaf chlorosis.
3	Formation of mycelial mass or basidioma initials whether at the stem or leaf base ofplant; with leaf chlorosis.
4	Formation of well-developed basidioma with at least 50% of total leaf number showing severe chlorosis and the plant drying up, is dying or is already dead.

Serial dilution at 1:10 was carried out and on the third dilution, 1 ml of the solution was pippetted into an empty sterile Petri plate followed by the pouring 9 ml sterilized molten Rose Bengal agar (RBA) over it. The plates were swirled and then incubated for 10 days at room temperature (28 ± 2ºC). Fungal colonies that appeared on the surface of the RBA were marked, counted and recorded. The colonies were prepared as slide cultures to be observed under light microscope to confirm their identity.

Soil Factors: Moisture Content and pH

Soil samples were taken from three randomly selected pots per treatment and control immediately before the scheduled conidial soil drench or just before watering in the case of the control plants. These soil samples were used for moisture content (MC) and pH determination. The samples per treatment/control were pooled, homogenously mixed, after which 10 g (fresh weight) weighed out and oven-dried at 40ºC to 60ºC to constant weight. The soil MC was calculated as shown below:

$$MC = \frac{\text{(fresh weight – dry weight (constant)}}{\text{fresh weight}} \times 100$$

For pH determination, each soil (10 g fresh weight) was placed in a conical flask to which was added 50 ml sterilized, distilled water. The flask was agitated on an orbital shaker at 100 rpm/10 min, left to settle and the pH taken using a pH meter.

RESULTS

Disease Severity Index

All the three treatments showed signs of disease infection. However, the best disease control was by T1 (FA 1132), which gave the lowest DSI of 28.35. Its first sign of disease was only observed at week 20 (DSI 8.33). However, treatments T2, T3 and C2 showed disease symptoms as early as week 12 with DSI of 3.30, 8.5 and 16.67, respectively. There was no significant difference between T2, T3 and C2 at week 24 (*Appendix 1*). The response of the treatment and control plants up to week 24 is illustrated in *Figure 1*.

Plant Biomass

The highest shoot and root biomass was observed in the uninfected control C1 (61.632 g and 21.160 g, respectively) while the lowest was from the infected, non-treated control C2 (21.356 g, 8.290 g, respectively). Among the treatments, T1 gave the highest and significant shoot and root biomass at 44.797 g and 14.828 g, respectively. Treatment T2 and T3 were not statistically different from each other nor with C2 either (*Figure 2*). *Figures 3, 4, 5, 6* and *7* are the actual plants of experiment for C1, C2, T1, T2 and T3 respectively after harvesting on the 24th week of observation.

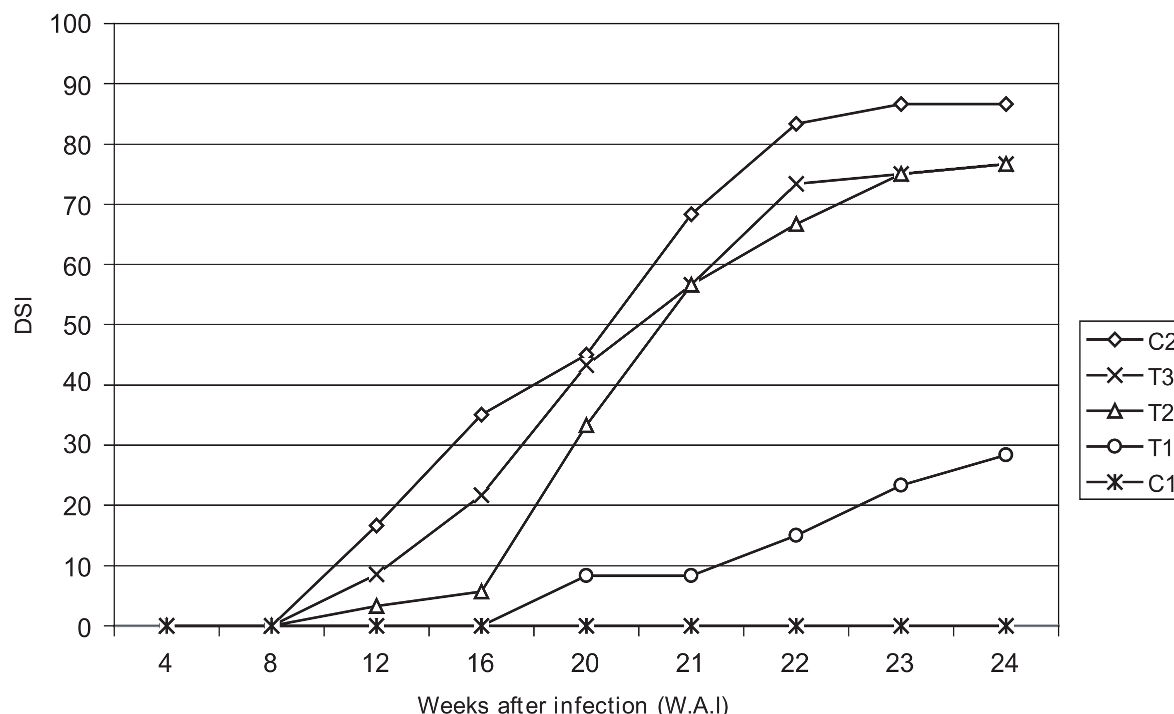

Figure 1. Disease severity index (DSI) of replicate plants in three treatments and two control experiments up to 24 weeks.

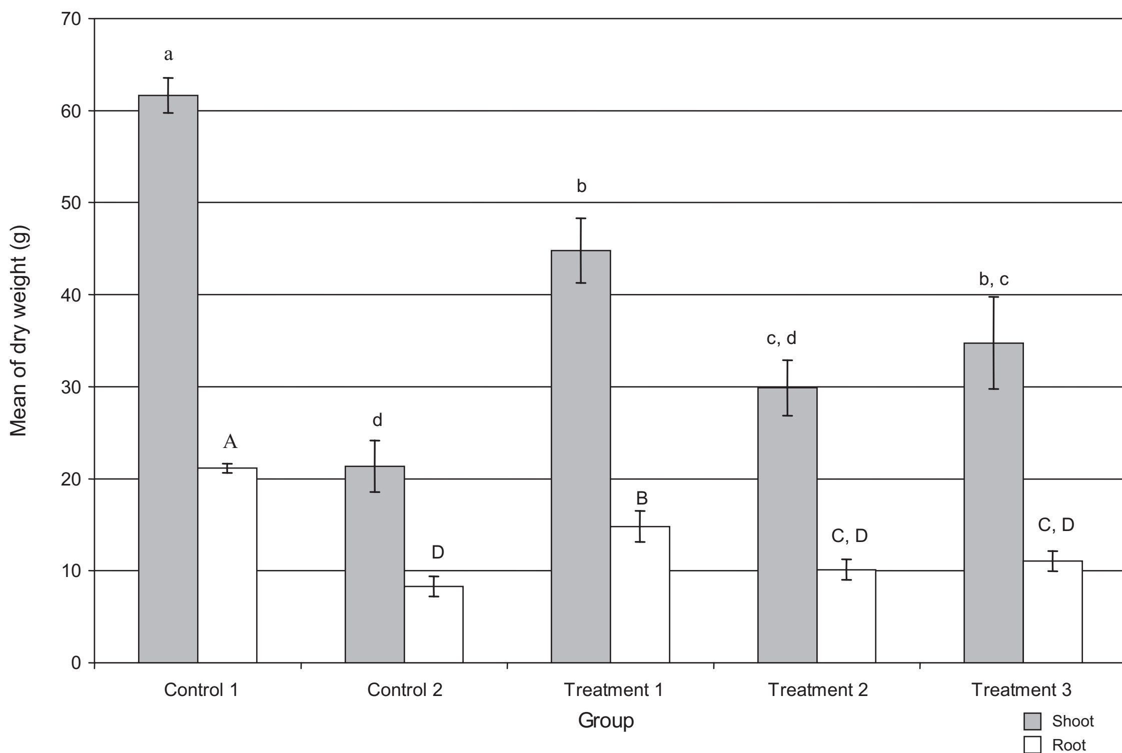

Figure 2. Mean oil palm dry weights at 24 weeks after treatment.

Legend:
Control 1 - absolute control with no infection and treatment.
Control 2 - artificially infected with FA 5201.
Treatment 1 - artificially infected with FA 5201 and treated with FA 1132.
Treatment 2 - artificially infected with FA 5201 and treated with FA 1166.
Treatment 3 - artificially infected with FA 5201 and treated with 1:1 mixture of FA 1132 and FA 1166.

Figure 3. C1 plants (uninfected and non-treated) at 24 weeks after infection showing healthy leaves and good root volume (DSI = 0).

Figure 4. C2 plants (infected and non-treated) at 24 weeks after infection with all plants infected with Ganoderma *at their bases. Almost 100% leaf necrosis with very poor root development (DSI = 86.67).*

Figure 5. T1 plants (infected and treated with FA 1132) at 24 weeks after infection showing good leaf and root volume for most with only a few showing leaf necrosis (DSI = 28.35).

Figure 6. T2 plant (infected and treated with FA 1166) at 24 weeks after infection showing poor leaf and root development with more than 50% seedlings succumbed to death (DSI = 76.67).

Figure 7. T3 plants (infected and treated with FA 1132 + FA 1166) at 24 weeks after infection with almost similar symptoms as T2 (DSI = 76.67).

Estimation of *Trichoderma* Spores: CFU g^{-1} Soil

There was no statistical difference between the numbers of CFU's found at 5 cm compared to 15 cm depth for all the treatments (*Figures 8* and *9*). Treatment T3 at 5 cm gave the highest CFU g^{-1} soil followed by T1 at 15 cm with 3.140×10^4 and 2.741×10^4 g^{-1} soil, respectively (*Appendix 2*). For T1, T2 and T3, the number of CFU's increased to a peak at week 18, after which there was a sharp decline. For C1 and C2, the CFU g^{-1} soil ranged from 0.20 to 0.29 for both soil depths.

Soil Moisture and pH

There was no extreme difference in soil moisture content at 5 and 15 cm depth between all the treatments and controls. According to *Table 2*, group T1 recorded the highest mean for MC (23.36%) at 15 cm depth. As for the pH readings, group T2 recorded the lowest pH mean at 5.95 for both 5 and 15 cm depth (*Table 3*). There was only a small difference for the pH mean recorded according to the sampling weeks.

DISCUSSION

This study showed that FA 1132 was able to significantly suppress BSR disease development based on its DSI of 28.35, compared to the untreated infected control seedlings which gave the highest DSI at 86.67. The single treatment with FA 1166 and the mixed treatments of the two strains were ineffective as they gave DSI values that were not statistically different from that of the C2 plants.

With this ability, FA 1132 also recorded the highest foliage and root biomass production among the three *Trichoderma* application treatments. A similar outcome for FA 1132 was also reported by Ilias (2000) on its maiden trial although the DSI was higher. However, in the trials of Abdullah *et al.* (2003a), FA

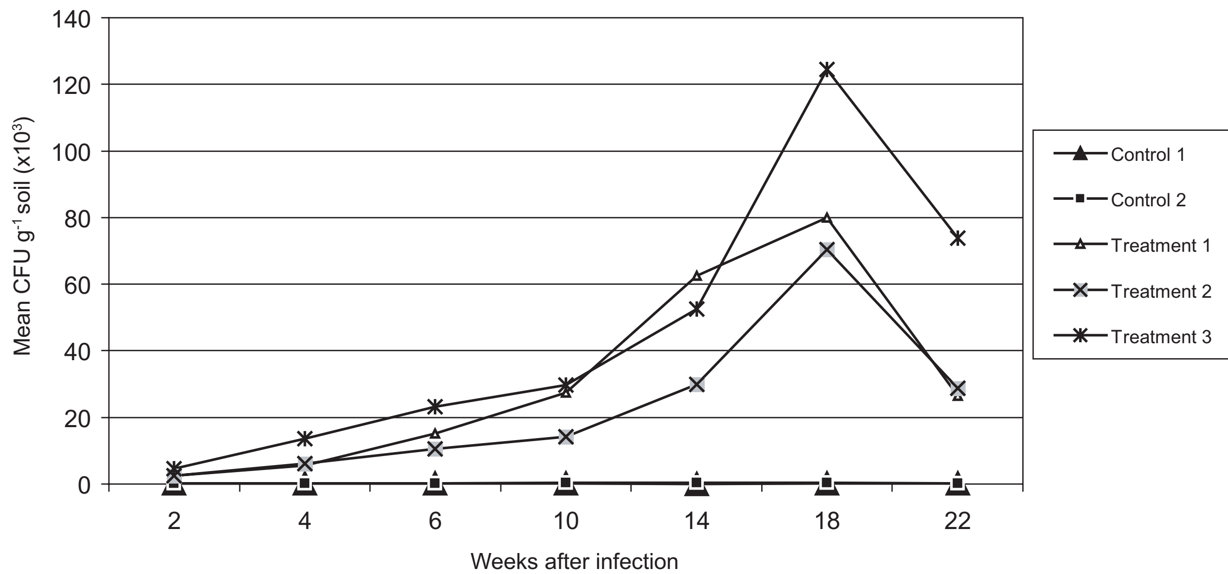

Figure 8. Mean colony forming units (CFU) g^{-1} soil of T. harzianum *at 5 cm depth from 0 to 22 weeks after injection.*

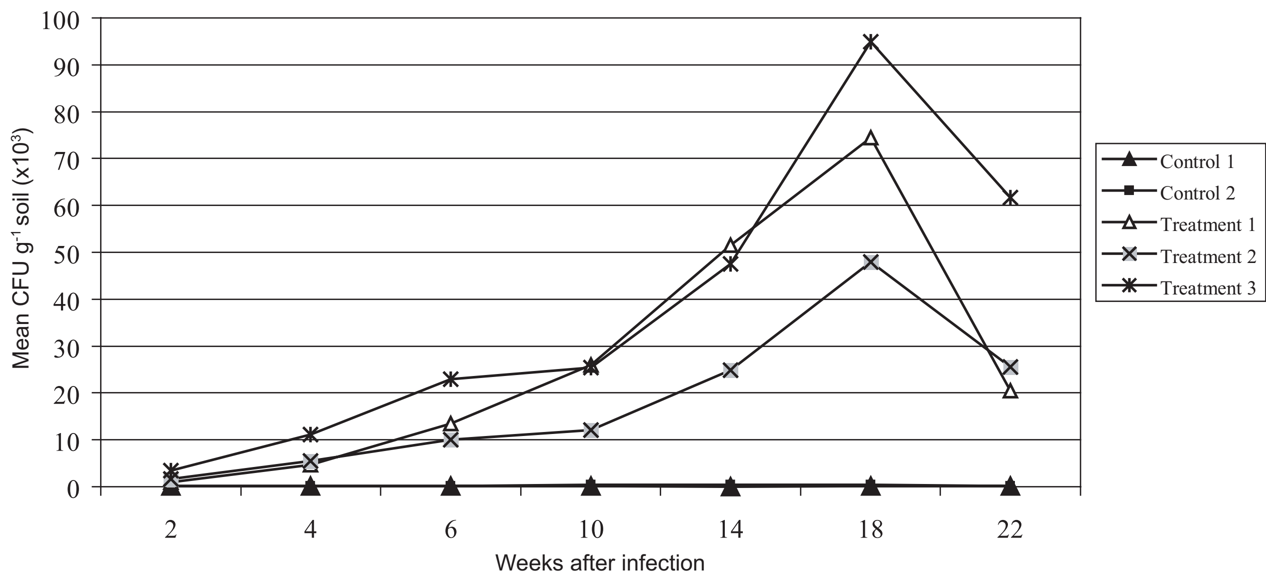

Figure 9. Mean colony forming units (CFU) g^{-1} soil of T. harzianum *at 15 cm depth from 0 to 22 weeks after injection.*

Legend:
Control 1 - absolute control with no infection and treatment.
Control 2 - artificially infected with FA 5201.
Treatment 1 - artificially infected with FA 1132 and treated with FA 1132.
Treatment 2 - artificially infected with FA 5201 and treated with FA 1166.
Treatment 3 - artificially infected with FA 5201 and treated with 1:1 mixture of FA 1132 and FA 1166.

1132 gave an even lower DSI value of 5.0. Both reports showed very promising biocontrol efficacy of FA 1132 with the difference in the DSI as an outcome of the different methods and frequencies of application of the fungus on the infected seedlings.

Strain FA 1166 showed good antagonistic properties against *G. boninense* in *in vitro* assays, but was not effective in the nursery trials. With a DSI of 76.67 at week 24, its control of the disease was not significantly different from that in the infected, untreated C2 control. Samuels (1996) stated that different strains of the same *Trichoderma* species do not necessarily confer the same level of biocontrol. Several other factors, such as abiotic factors, may also be involved in the making of a good potential biocontrol agent, and although good *in vitro* performance may be an indicator but it is not a guarantee of *in vivo* success.

Biocontrol agents inhibit their target organisms by parasitism, competition, antibiosis (by production of antibiotics, enzymes and toxins, *etc*.) or interaction with the host (Arras and Arru, 1997). However in a mixture of same species inoculum, FA 1132 performed very poorly compared to its single

TABLE 2. MEAN OF SOIL MOISTURE CONTENT (%) OVER 22 WEEKS AFTER INFECTION (W.A.I.) AT 5 AND 15 cm DEPTHS

Group (depth)	Sampling period (W.A.I.)								Mean of moisture (by group)
	0	2	4	6	10	14	18	22	
C1 – 5 cm	25.92 ± 0.69	25.92 ± 0.69	19.57 ± 0.59	20.50 ± 2.42	19.78 ± 1.10	20.70 ± 1.06	21.07 ± 0.41	20.61 ± 0.33	21.16^{*}
C1 – 15 cm	26.56 ± 0.60	26.56 ± 0.60	19.55 ± 0.58	21.78 ± 0.34	22.10 ± 2.27	19.67 ± 0.71	19.42 ± 0.65	21.04 ± 0.62	$21.44^{@\#}$
C2 – 5 cm	26.59±1.15	26.59 ± 1.15	19.19 ± 0.28	20.16 ± 0.81	23.44 ± 1.67	23.13 ± 0.52	22.75 ± 0.61	20.31 ± 1.07	22.08^{*}
C2 – 15 cm	25.05±0.61	25.05 ± 0.61	19.81 ± 0.44	23.38 ± 1.19	20.18 ± 0.55	22.42 ± 0.40	21.75 ± 0.67	20.10 ± 0.61	$21.81^{@\#}$
T1 – 5 cm	-	20.68 ± 0.37	24.48 ± 1.13	21.96 ± 0.31	23.19 ± 1.09	21.72 ± 1.49	19.85 ± 1.72	23.11 ± 0.38	22.14^{*}
T1 – 15 cm	-	24.96 ± 1.15	21.95 ± 0.89	23.76 ± 1.57	22.51 ± 0.99	22.91 ± 1.73	22.73 ± 0.97	24.03 ± 0.99	$23.36^{@}$
T2 – 5 cm	-	22.54 ± 1.032	24.12 ± 0.80	19.87 ± 0.86	18.78 ± 0.48	22.14 ± 1.02	19.87 ± 0.81	22.12 ± 0.61	21.23^{*}
T2 – 15 cm	-	1.22 ± 0.76	21.65 ± 2.02	22.12 ± 0.89	19.45 ± 0.55	19.98 ± 0.53	23.12 ± 0.84	21.01 ± 0.40	$21.22^{\#}$
T3 – 5 cm	-	22.12 ± 0.91	23.12 ± 0.42	21.17 ± 0.47	19.87 ± 0.88	19.78 ± 0.60	18.55 ± 0.37	20.15 ± 0.26	20.68^{*}
T3 – 15 cm	-	19.98 ± 0.50	21.22 ± 0.57	23.45 ± 0.62	19.71 ± 0.69	20.22 ± 0.92	19.78 ± 0.39	21.28 ± 1.05	$20.80^{\#}$
Mean 5 cm	By sampling weeks	23.21^{A}	21.78^{AB}	20.48^{B}	21.36^{AB}	21.55^{AB}	20.59^{B}	21.26^{AB}	-
Mean 15 cm		23.40^{a}	21.25^{b}	22.43^{ab}	21.52^{b}	21.30^{b}	22.06^{ab}	21.87^{ab}	

Notes: Means in the same columns/row with same superscripts denote no significant difference at $p \leq 0.05$ [means analysed according to sampling weeks (AB/ab)] and groups (*/$^{@\#}$) at 5 and 15 cm respectively (DMRT). The means are followed by their standard error (SE).

Legend:
C1 - absolute control with no infection and treatment.
C2 - artificially infected with FA 5201.
T1 - artificially infected with FA 5201 and treated with FA 1132.
T2 - artificially infected with FA 5201 and treated with FA 1166.
T3 - artificially infected with FA 5201 and treated with 1:1 mixture of FA 1132 and FA 1166.

TABLE 3. MEAN OF pH OVER 22 WEEKS AFTER INFECTION (W.A.I.) AT 5 AND 15 cm DEPTHS

Group (depth)	Sampling period (weeks)								Mean pH (by group)
	0	2	4	6	10	14	18	22	
C1 – 5 cm	5.91 ± 0.12	6.23 ± 0.02	6.17 ± 0.01	6.19 ± 0.01	6.23 ± 0.01	6.21 ± 0.03	6.19 ± 0.04	6.24 ± 0.01	6.21^{-}
C1 – 15 cm	6.20 ± 0.14	6.21 ± 0.04	6.19 ± 0.02	6.23 ± 0.02	6.06 ± 0.07	6.20 ± 0.02	6.14 ± 0.26	6.02 ± 0.02	$6.15^{\#}$
C2 – 5 cm	5.86 ± 0.06	5.91 ± 0.06	6.10 ± 0.02	6.20 ± 0.01	6.25 ± 0.04	6.20 ± 0.04	5.70 ± 0.16	5.70 ± 0.05	6.01^{-}
C2 – 15cm	6.11 ± 0.02	6.07 ± 0.02	6.14 ± 0.01	6.27 ± 0.09	6.28 ± 0.02	6.30 ± 0.07	5.91 ± 0.07	5.91 ± 0.01	$6.12^{\#}$
T1 – 5 cm	-	5.95 ± 0.09	6.08 ± 0.05	5.74 ± 0.11	5.87 ± 0.06	6.05 ± 0.91	6.22 ± 0.04	6.26 ± 0.04	6.02^{-}
T1 – 15 cm	-	5.88 ± 0.08	5.99 ± 0.21	5.82 ± 0.07	5.93 ± 0.01	6.19 ± 0.05	6.16 ± 0.11	6.22 ± 0.03	$6.03^{\#}$
T2 – 5 cm	-	5.89 ± 0.07	5.84 ± 0.15	5.98 ± 0.07	5.74 ± 0.18	6.23 ± 0.13	6.01 ± 0.12	5.99 ± 0.79	5.95^{*}
T2– 15 cm	-	5.98 ± 0.18	5.83 ± 0.18	5.92 ± 0.13	5.71 ± 0.15	6.23 ± 0.12	5.98 ± 0.09	6.01 ± 0.12	$5.95^{@}$
T3– 5 cm	-	6.12 ± 0.14	5.80 ± 0.17	6.24 ± 0.07	6.31 ± 0.12	6.12 ± 0.10	6.22 ± 0.07	6.02 ± 0.16	6.11^{-}
T3– 15 cm	-	5.89 ± 0.15	5.84 ± 0.14	6.21 ± 0.06	6.27 ± 0.51	6.01 ± 0.51	5.97 ± 0.15	6.87 ± 0.12	$6.15^{\#}$
Mean 5 cm	By sampling weeks	6.17^{A}	6.13^{A}	6.20^{A}	6.20^{A}	6.14^{A}	6.08^{A}	6.08^{A}	-
Mean 15 cm		6.21^{a}	6.13^{a}	6.22^{a}	6.19^{a}	6.16^{a}	6.07^{a}	6.22^{a}	

Notes: Means in the same columns/rows with the same superscripts denote no significant difference at $p \leq 0.05$ [means analysed according to sampling weeks (A/a)] and groups ($^{*-/@\#}$) at 5 and 15 cm respectively (DMRT). The means are followed by their standard errors (SE).

Legend:
C1 - absolute control with no infection and treatment.
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T3 - artificially infected with FA 5201 and treated with 1:1 mixture of 1132 and FA 1166.

application. Study by Sariah and Cheng (1999) also reported that the single treatment of *T. virens* was more effective than its combination with *T. harzianum* for the control of collar rot in eggplant. Wicklow (1992) proposed that intraspecific interference or competition may have limited one organism's access to the substrate, which could be accomplished by some form of behavioural or chemical mechanism. It has been suggested that biocontrol which act by antibiosis rather than by competition would be the most effective against pathogen (Rytter *et al*., 1989).

A combination of the right isolates may appear elusive, but getting them right can be rewarding. Vinale *et al*. (2004) reported that a mixture of *T. harzianum* (T22) with *T. atroviride* (P1) decreased the disease symptoms of *Fusarium* on tomato while, at the same time, improved their growth, in addition to improving the growth of other crops such as lettuce and pepper. This was similarly exhibited in the production of commercial BINAB–T, a combination of *T. harzianum* and *T. viride* (Samuels, 1996) for the control of internal decay of wood in trees.

In the event that an isolate is proven to be a good biocontrol agent, there are at least two more factors that might contribute towards its success. The first being the application frequency and second, the method of application. Ilias (2000) terminated the application of FA 1132 conidial soil drench at week 10 and found that the DSI was 40 at 24 weeks. In this study, the conidial soil drench was extended up to 14 weeks and the DSI was 28.35 at 24 weeks. Abdullah *et al*. (2003a) gave a continuous and consistent conidial soil drench every two weeks up to the end of their experiment; the DSI at 24 weeks was only 5.0. Further studies should be carried out on the threshold level of fungi in the soil at which further applications is no longer needed to be made or could be made at longer time intervals to confer effective protection.

The CFU count in soil for this study showed the proliferation of *Trichoderma* in all the applications. The CFU g^{-1} soil increased to a peak at 18 weeks after infection, then sharply declined thereafter, with cessation of application of the conidial suspension at week 14. This suggested that continuous conidial drenching is required for better protection against the pathogen. According to Chet (1987), the minimal effective amount of *Trichoderma* conidia is about 10^6 CFU g^{-1} soil. However, in this study, only 10^4 CFU g^{-1} soil was retrieved but, nevertheless, conferred promising biocontrol against basal stem rot. One of the important criteria as a good biocontrol agent is its proliferation in the rhizosphere. A study by Beagle and Papavizas (1985) suggested that populations of *Trichoderma* spp. increased 100-fold in soil with fermentor biomass added, and they concluded that sustaining the proliferation would give significant efficacy to any biological control agent in suppressing soil-borne disease. For this study, MFOP as surface mulch provided substrate for *in situ* proliferation of the *Trichoderma* isolates. This study also found that the *Trichoderma* population was higher at 5 cm than 15 cm soil depth. This observation was supported by Eastburn and Butler (1988) who also found that the density of *T. harzianum* tended to be highest nearer the soil surface.

The soil moisture content (MC) ranged from 18.55%-26.59%. An effective biological control agent of *Ganoderma* must be able to tolerate low moisture and higher soil acidity (pH). Jinantara (1995) found that *T. harzianum* also proliferated better at lower soil moisture content of 10%-25% compared to higher soil moisture content of 30%-35%. However, the MC in this study is within the preference of the fungus. Thus, this study was able to exhibit optimal proliferation for both the test isolates, namely, FA 1132 and FA 1166. MC has an important effect on the success of any biological control system as it determines the efficacy of the biocontrol agents (Ilias, 2000). However, it is usually not possible to ensure a suitable moisture content in the field due to abiotic factors, namely, temperature, rainfall, humidity and soil type. In this study, the soil was acidic with pH of 5.70 – 6.97, within the range in which *Trichoderma* is purported to thrive (2.0 – 6.0 with 4.0 as optimum) (Laszlo *et al*., 2003). Jinantara (1995) also found that *T. harzianum* grew well under acidic condition in pH 3.8 – 5.8. Therefore, the soil pH in the pots corresponded to the pH in oil palm fields of 4.0 - 6.5, with 5.0-5.5 being optimal.

CONCLUSION

This study found FA 1132 to be a good biocontrol agent against BSR disease of oil palm based on *in vitro* and nursery trials. The nursery trial used MFOP as the food substrate compared to compost by Abdullah *et al*. (2003a). However, applying FA 1132 together with FA 1166 was ineffective performing poorer than FA 1132 as single application. Although in the laboratory, FA 1166 gave good control against *G. boninense*, in the nursery trials it showed otherwise. This study also reinforced previous findings that the biocontrol property of *Trichoderma* is strain-specific, not species-specific. The nursery trials have provided drive for further studies on its application onto mature field palms in the very near future.

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Appendix 1

DISEASE SEVERITY INDEX (DSI) OF THE NURESRY TRIAL FROM 4 TO 24 WEEKS AFTER INFECTION (W.A.I.)

Group	Disease severity index[a]					
	4	8	12	16	20	24
Control 1 (C1)	0.00	0.00	0.00[a]	0.00[a]	0.00[a]	0.00[a]
Control 2 (C2)	0.00	0.00	16.67[b]	35.00[c]	45.00[b]	86.67[b]
Treatment 1 (T1)	0.00	0.00	0.00[a]	0.00[a]	8.33[a]	28.35[a]
Treatment 2 (T2)	0.00	0.00	3.30[a]	5.70[a, b]	33.30[b]	76.67[b]
Treatment 3 (T3)	0.00	0.00	8.50[a]	21.70[b]	43.30[b]	76.67[b]

Notes: [a] Data are backtransformed and analysed using DMRT.
[b]Means in the same column with different letters are significantly different ($p<0.05$) ($n=15$).

MEAN OF OIL PALM DRY WEIGHTS

Groups	Mean of dry weight (g)	
	Foliage	Root
Control 1 (C1)	61.632[a] ± 1.9	21.160[A] ± 0.5
Control 2 (C2)	21.356[d] ± 2.8	8.290[D] ± 1.1
Treatment 1 (T1)	44.797[b] ± 3.5	14.828[B] ± 1.7
Treatment 2 (T2)	29.882[c, d] ± 3.0	10.332[C, D] ± 1.1
Treatment 3 (T3)	34.760 [b, c] ± 5.0	11.060[C, D] ± 1.1

Notes: Means in each column with different superscripts are significantly different at $p > 0.05$ ($n=15$) (DMRT). The means are followed by their standard errors (SE).

Legend:
C1 - absolute control with no infection nor treatment.
C2 - positive control, artificially infected with FA 5201.
T1 - artificially infected with FA 5201, treated with FA 1132.
T2 - artificially infected with FA 5201, treated with FA 1166.
T3 - artificially infected with FA 5201, treated with 1:1 FA 1132 and FA 1166.

Appendix 2

MEAN OF CFU g^{-1} FOR *Trichoderma* ISOLATES FROM SOIL AT 5 AND 15 cm SOIL DEPTHS OVER 22 WEEKS AFTER INFECTION (W.A.I.)

Group	*Trichoderma* colony count at 10^3 g^{-1} soil								
	Depth (cm)	2	4	6	10	14	18	22	Mean (by group)
C1	5	0.20 ± 0.11	0.20 ± 0.11	0.20 ± 0.11	0.20 ± 0.11	0.20 ± 0.11	0.20 ± 0.11	0.20 ± 0.11	0.20^{C}
	15	0.20 ± 0.11	0.20 ± 0.11	0.20 ± 0.11	0.20 ± 0.11	0.20 ± 0.11	0.20 ± 0.11	0.20 ± 0.11	0.20^{c}
C2	5	0.20 ± 0.11	0.20 ± 0.11	0.20 ± 0.11	0.40 ± 0.16	0.40 ± 0.16	0.40 ± 0.16	0.20 ± 0.11	0.29^{C}
	15	0.20 ± 0.11	0.20 ± 0.11	0.20 ± 0.11	0.40 ± 0.16	0.40 ± 0.16	0.40 ± 0.16	0.00 ± 0.00	0.26^{c}
T1	5	2.46 ± 0.54	5.53 ± 0.98	15.06± 2.05	27.33 ± 4.28	62.60 ± 7.57	80.00 ± 22.20	26.53 ± 4.40	31.40^{AB}
	15	1.00 ± 0.24	4.73 ± 0.98	13.53 ± 0.91	26.00 ± 2.79	51.53 ± 7.36	74.46 ± 13.79	20.53 ± 3.27	27.41^{ab}
T2	5	2.53 ± 0.18	6.13 ± 0.235	10.53 ± 0.721	14.13 ± 0.638	29.86 ± 1.592	70.46 ± 2.50	28.73 ± 1.237	23.19^{ABC}
	15	11.60 ±0.364	5.53 ± 0.340	10.00 ± 0.613	12.13 ± 0.578	24.86 ± 0.547	48.00 ± 3.29	25.53 ± 1.237	18.24^{abc}
T3	5	4.53 ± 0.443	13.53±0.270	23.13 ±0.736	29.73 ±1.349	52.60 ±0.974	124.53 ±24.72	73.86 ±8.34	45.99^{A}
(FA 1132 + FA 1166)		(2.00/2.53)	(6.33/7.20)	(11.53/11.60)	(14.53/15.20)	(25.40/27.60)	(59.00/65.53)	(35.46/38.40)	
(FA 1132/FA 1166)	15	3.46 ±0.54	11.13 ± 1.145	23.00 ±0.421	25.46±0.455	47.53±0.767	94.86±2.01	61.73±0.561	38.17^{a}
		(1.60/1.86)	(5.21/5.82)	(11.13/11.86)	(13.53/12.06)	(23.13/23.40)	(49.73/45.13)	(30.60/31.13)	

Notes: Means followed by same alphabets denote no significant difference at $p \leq 0.05$ [means analysed according to groups at 5 cm (ABC) and 15 cm (abc) (DMRT)]. The means are followed by their standard error (SE).

Legend:
C1 - absolute control with no infection and treatment.
C2 - artificially infected with FA 5201.
T1 - artificially infected with FA 5201 and treated with FA 1132.
T2 - artificially infected with FA 5201 and treated with FA 1166.
T3 - artificially infected with FA 5201 and treated with 1:1 mixture of FA 1132 and FA 1166.