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In Planta EFFICACY OF LOCAL *Trichoderma* ISOLATES AND SELECTED COMMERCIAL BIOLOGICAL AGENTS AGAINST *Ganoderma boninense* IN OIL PALM

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ABSTRACT

Trichoderma *species are well-known biological control agents with significant antagonistic activity against various fungal phytopathogens. This study evaluated the* in planta *efficacy of local* Trichoderma *isolates (*Trichoderma virens *and* Trichoderma asperellum*) in comparison to commercial biological control agent (BCA) products in controlling* Ganoderma *disease in oil palm seedlings. The local* Trichoderma *isolates were applied either singly or as a mixture to the soils of both transplanted and* Ganoderma*-inoculated oil palm seedlings. Additionally, two commercial BCA products were tested as benchmarks, with a negative and a positive control. It was found that the local* Trichoderma *isolate, either applied singly or as a mixture, could reduce disease by 41.82%–57.73%, and be on par with the commercial BCA products. Untreated positive control showed a significant loss in physiological integrity, in terms of chlorophyll content, plant height, bole diameter, and the number of fronds, due to the* G. boninense *infection, meanwhile, the treated seedlings with local* Trichoderma *isolates and commercial BCAs were able to resist the infection significantly to a certain degree. These isolates are promising BCAs for the future management of* G. boninense *in oil palm.*

Keywords: biological control agents, *Ganoderma boninense*, oil palm, *Trichoderma asperellum*, *Trichoderma virens*.

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INTRODUCTION

The African oil palm (*Elaeis guineensis* Jacq.) is a major vegetable oil-producing crop contributing to 40% of the global vegetable oil supplies (United States Department of Agriculture [USDA], 2025). In Malaysia, oil palm is grown on more than 5.65 million hectares and produces more than 18 million tonnes of palm oil per year (Malaysian Palm Oil Board [MPOB], 2023). One of the major disease challenges associated with large-scale oil palm plantations in Malaysia is the threat of basal stem rot (BSR), also known as *Ganoderma* disease due to the pathogenic fungus, *Ganoderma boninense*. It has been estimated that the economic losses due to this disease can be as high as 68% (Assis et al., 2021). Nevertheless, integrated disease management is strongly recommended to minimise economic losses, such as by integrating biological control agents (BCAs), cultural practices, and chemical treatments. In this context, BCAs are considered a sustainable method to control plant diseases, as they are non-toxic and environmentally friendly (TariqJaveed, 2021).

To date, *Trichoderma* species are known for their effectiveness as a promising BCA for suppressing the growth of various soilborne phytopathogens, such as *Rhizoctonia solani*, *Fusarium oxysporum*, *G. boninense* and *Sclerotium rolfsii*, due to its ideal properties such as rapid growth, wide adaptation to different soil types and temperature ranges, good root colonisation and survivability under

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extreme conditions (Meena et al., 2017). In addition, *Trichoderma* species have various direct and indirect mechanisms of action for controlling phytopathogenic fungi (Ghorbanpour et al., 2018). *Trichoderma* species suppress the growth of pathogens directly through competition, antibiosis, the production of cellulases and other hydrolytic enzymes and mycoparasitism. *Trichoderma* species can suppress pathogens indirectly by inducing plant resistance (Saravanakumar et al., 2016). In numerous studies, *Trichoderma* species have been tested individually or in combination in nursery trials and have been effective in controlling *Ganoderma* disease in oil palm (Musa et al., 2017). However, the use of a single BCA often leads to inadequate performance in agriculture (Harshita et al., 2018), as BCAs tend to be highly disease-specific. Researchers are increasingly interested in combining BCAs with different mechanisms to improve the success of biological control (Rajeela et al., 2018). Compatible combinations of BCAs may have synergistic effects and significantly increase their efficacy. On the other hand, there are many commercial BCA products available in the market that are effective in controlling *Ganoderma* disease (e.g. combinations of *Trichoderma* species, mycorrhizal fungi and *Bacillus* species). Most of these commercial products are not locally sourced, so the introduction of foreign microorganisms can affect local agroecology (Myers & Cory, 2017). Importing products containing foreign microorganisms requires approval from the local authority, such as the Sabah Department of Agriculture, and also requires assistance with transportation costs. The use of local *Trichoderma* isolates makes the extraction and production of BCAs comparatively easier. It also helps to preserve the local ecosystem without harming some beneficial microorganisms found in natural habitats. Benchmarking the local *Trichoderma* isolates with commercial BCAs can provide useful information on the possibility of proposing the potential local *Trichoderma* isolates for commercial mass production.

Therefore, the study was conducted to evaluate the *in planta* efficacy of local *Trichoderma* isolates (*Trichoderma virens* and *Trichoderma asperellum*) in comparison to commercial BCA products in controlling *Ganoderma* disease in oil palm seedlings, in terms of disease and plant physiology assessments and soil and plant chemical properties.

MATERIALS AND METHODS

Preparation of Planting Materials

Oil palm seedlings used in an *in planta* study were 3-month-old, hybrid (*Dura* × *Pisifera*) grown in a nursery facility at MPOB Research Station, Lahad Datu, Sabah. A germinated seed was sown in a small polyethylene planting bag (15 × 23 cm) containing a soil mixture in a ratio of 2:1 (non-sterile soil: compost) for three months. Then, the same soil mixture was filled into a bigger polyethylene planting bag (30 x 38 cm) before being used for transplanting.

Preparation of *Ganoderma* Inoculum

Ganoderma inoculum was prepared on a 6 × 6 × 6 cm rubber wood block (RWB). The RWB were soaked in tap water overnight, and inserted into a heat-resistant polypropylene plastic bag. Later, 10 mL of potato dextrose broth (PDB) was added and autoclaved at 121°C for 25 min. Additionally, the RWB was supplemented with an additional 10 mL of sterile molten potato dextrose agar (PDA) and allowed to solidify. Six mycelia plugs (5 mm diameter) from a 7-day-old culture of *G. boninense* were placed on each side of the RWB aseptically, and the plastic was sealed to avoid contamination. The inoculated RWBs were incubated in the dark at room temperature (28°C ± 2°C) for 10 weeks to allow the fungal mycelium to fully colonise the block. The fully colonised and uncontaminated RWBs were applied as inoculum to oil palm seedlings (Nusaibah et al., 2016).

Artificial Inoculation

Inoculation was performed upon transplanting of the oil palm seedlings using the RWB sitting technique according to Rakib et al. (2015). The RWB inoculum was placed in the middle of the polybag containing soil mixture, and then the 3-month-old oil palm seedling was transplanted on top of the RWB inoculum to ensure direct contact between the roots and the inoculum.

Preparation of *Trichoderma* Conidial Suspension

The local *T. virens* and *T. asperellum* were previously isolated from the rhizosphere of the roots of healthy oil palm trees from a local oil palm estate in Sandakan, Sabah, Malaysia and both *Trichoderma* isolates were identified based on the internal transcribed spacer sequence (Darlis et al., 2023). The local *Trichoderma* isolates used as treatments were sub-cultured by plating an 8 mm of a 7-day-old mycelia plug onto a PDA plate and incubating at 25°C temperature for seven days. The culture was harvested with sterilised distilled water (10 mL) by carefully separating it with an L-shaped glass rod. The conidial suspension was filtered through a filter paper with 11 µm pore size and 9 cm diameter to remove

mycelial fragments. The concentration of conidia suspension was adjusted to 1 × 10^6 conidia mL^{-1} using a hemocytometer.

Application of *Trichoderma* Species and Experimental Design

Two local *Trichoderma* isolates, and two commercial BCA products (Product X and Y) were tested for their efficacy as a treatment to suppress *G. boninense* infection in oil palm seedlings. All seven treatments consisted of 10 replications (*Table 1*). The experimental units were arranged in a complete randomised design (CRD) in a nursery at MPOB Research Station, Lahad Datu, Sabah. Regular weeding and watering (twice a day) were performed and fertiliser was applied according to standard commercial practices (MPOB, 2016).

Disease Assessment

The data presented in this study were according to the records at nine months after inoculation. The disease incidence (DI) and the disease severity index (DSI) were based on the foliar symptoms and disease class (*Table 2*), the area under the disease progress curve (AUDPC) and percentage of necrotic primary roots were calculated according to Rakib et al. (2015). The DI, DSI, AUDPC, percentage of necrotic primary roots and percentage of disease reduction were calculated using Equations 1, 2, 3, 4 and 5, respectively. To complete Koch's postulates, the presence of *Ganoderma* was either confirmed visually (*Ganoderma* basidiocarp) or by employing *Ganoderma* selective media (GSM) plating of necrotic primary roots for non-visible *Ganoderma* presence (Rakib et al., 2015).

TABLE 2. NUMERICAL DISEASE CLASSES AND THEIR CORRESPONDING FOLIAR SYMPTOMS ON OIL PALM SEEDLINGS

Class	Symptoms
0	Healthy plants with green leaves and no fungal mass development on any part of the plant.
1	Appearance of 1–3 chlorotic leaves with no fungal mass development on any part of the plant.
2	Appearance of fungal mass with or without chlorotic leaves.
3	Appearance of >3 chlorotic leaves, necrotic leaves (dead leaves) with or without fungal mass development on any part of the plant.
4	At least 50% of the total leaf number shows severe chlorosis or necrosis with or without fungal mass.
5	Dead plants with or without fungal mass.

Source: Rakib et al. (2015).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected seedlings}}{\text{Total number of seedlings}} \times 100 \quad (1)$$

$$\text{Disease severity index (\%)} = \frac{\sum (A \times B)}{\sum n \times 5} \times 100 \quad (2)$$

where, A is the disease class (0 to 5), B is the number of seedlings showing the disease class per treatment, n is the total number of replications, and 5 is the constant representing the highest class of assessment.

$$\text{Area under disease progress curve unit} = \sum_{i}^{n-1} \left(\frac{Y_i + Y_{i+1}}{2} \right) (t_{i+1} - t_i) \quad (3)$$

TABLE 1. EXPERIMENTAL TREATMENTS AND DESCRIPTION

Code	Treatment	Descriptions
T1	Untreated (negative control)	Non-inoculated seedlings and without any treatment.
T2	Untreated (positive control)	*Ganoderma*-inoculated seedlings, and without any treatment.
T3	*Trichoderma virens*	*Ganoderma*-inoculated seedlings and treated with locally isolated *T. virens*. Conidial suspension of 10^6 was applied once by drenching 100 mL onto the soil after transplanting.
T4	*Trichoderma asperellum*	*Ganoderma*-inoculated seedlings and treated with locally isolated *T. asperellum*. Conidial suspension of 10^6 was applied once by drenching 100 mL onto the soil after transplanting.
T5	*T. virens* and *T. asperellum*	*Ganoderma*-inoculated seedlings and treated with a mixture of locally isolated *T. virens* and *T. asperellum*. Conidial suspension of 10^6 was applied once by drenching 100 mL onto the soil after transplanting.
T6	Commercial product X	*Ganoderma*-inoculated seedlings and treated with a commercial product containing a mixture of 12 species of arbuscular mycorrhizal fungi (250–300 spore/10 g product). Applied by broadcasting 40 g of the product once into the planting hole upon transplanting.
T7	Commercial product Y	*Ganoderma*-inoculated seedlings and treated with a commercial product containing a mixture of *T. viride*, *T. harzianum*, *Paecilomyces lilacinus*, *Pseudomonas fluorfecens* and *Bacillus subtilis*. Applied by broadcasting 5 g of the product once into the planting hole upon transplanting.

where, n is the number of assessment times, Y is the disease incidence, and t is the observation time.

$$\text{Necrotic primary roots (\%)} = \frac{\text{Number of necrotic primary roots}}{\text{Total number of roots per seedling}} \times 100 \quad (4)$$

$$\text{Disease reduction (\%)} = \frac{X1 - X2}{X1} \times 100 \quad (5)$$

where, X1 is the AUDPC of positive control (T2), and X2 is the AUDPC of the treated seedlings.

Plant Physiology Assessment

Chlorophyll content was recorded using a SPAD chlorophyll device (Rakib et al., 2019). Plant height was measured from the ground to the tip of the highest leaf by placing a steel tape measure next to the plant. The stem diameter was also measured using a digital caliper and the number of fronds was counted.

Soil Sampling and Chemical Properties Analysis

Soil samples were collected after nine months of nursery trials. Soil samples at a depth of 0–15 cm were collected from the growing medium in the planting bags. A total of 10 samples from each replication were bulked into one sample, homogenised, air-dried, ground and sieved through a 2 mm mesh. The dried and ground samples were stored in an air-tight polyethylene container for further analysis.

Soil pH was determined at a soil-to-distilled water ratio of 1:2.5 (w/v) using an Orion combination electrode pH (Eckert & Sims, 2013). The pH meter was calibrated before being used to read the pH value. Available nutrient elements were determined by leaching the soil sample with 100 mL of ammonium acetate (Castilho & Rix, 1993). The leachate was used for measuring the concentration of nutrients such as phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn) and copper (Cu) using an Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES).

Plant Sampling and Chemical Properties Analysis

Leaf samples were collected by identifying the frond 3, at 12 months of age in young palms (Sabri et al., 2019). The leaf samples were oven-dried at 80°C for 24 hr (constant weight), pulverised using an electrical grinder, and kept for further analysis. The leaf samples were analysed for N, P, K, Ca, Mg, Zn and Cu. The samples were extracted using the dry ashing method except for N (Enders & Lehmann, 2012). The concentration of these elements was measured using an ICP-OES, while N was measured using a CHN analyser.

Statistical Analysis

Analysis of variance (ANOVA) was performed and the means were compared using Tukey's test at the significance level of $p \leq 0.05$. Pearson's correlation was performed between the variables. All statistical analysis was performed using the SPSS statistical software (SPSS version 24).

RESULTS AND DISCUSSION

Disease Suppression in Oil Palm Seedlings

The results of the *in planta* nursery experiment showed that treatments by the local *Trichoderma* isolates and the selected commercial BCAs significantly reduced disease signs and symptoms in the infected oil palm seedlings with *G. boninense* compared to the untreated positive seedlings (T2). The local *Trichoderma* species (T3, T4 and T5) and the commercial BCAs (T6 and T7) showed similar efficacy in terms of the DSI (8.67%–21.33%), AUDPC (21.67 to 45.00 units) and percentage of necrotic primary roots (6.76%–17.73%). Application of the local *Trichoderma* species and commercial BCAs has resulted in a significant disease reduction ranging from 50.91%–76.36%. In terms of the DI, the positive control recorded 100%, while there was a significant reduction in the treated oil palm seedlings, where the local *T. virens* (T3), Product X (T6) and Product Y (T7) recorded the lowest DI. However, the DI was not significantly different among the local *T. virens* (T3), local *T. asperellum* (T4) and the mixture of both local isolates (T5) (*Figure 1*).

The application of *Trichoderma* isolates and arbuscular mycorrhizal fungi (AMF) can suppress *G. boninense*. The AMF mainly improves plant nutrition, altering the morphological structure of plant roots, regulating the synthesis of secondary metabolism, improving the microenvironment in the rhizosphere of plants, directly competing with pathogenic microorganisms for invasion sites and nutrients, and inducing resistance to plant diseases and the formation of a defense system (Tatsumi et al., 2021). *Trichoderma,* on the other hand, can control plant disease due to diverse antifungal activities such as mycoparasitism, antibiosis, competition for nutrients and induced systemic resistance in plants (Druzhinina et al., 2011).

It is noteworthy that the single application of *T. asperellum* and *T. virens* resulted in disease suppression against *G. boninense* with 41.82% and 57.73% disease reduction, respectively. The combination of these *Trichoderma* species did not

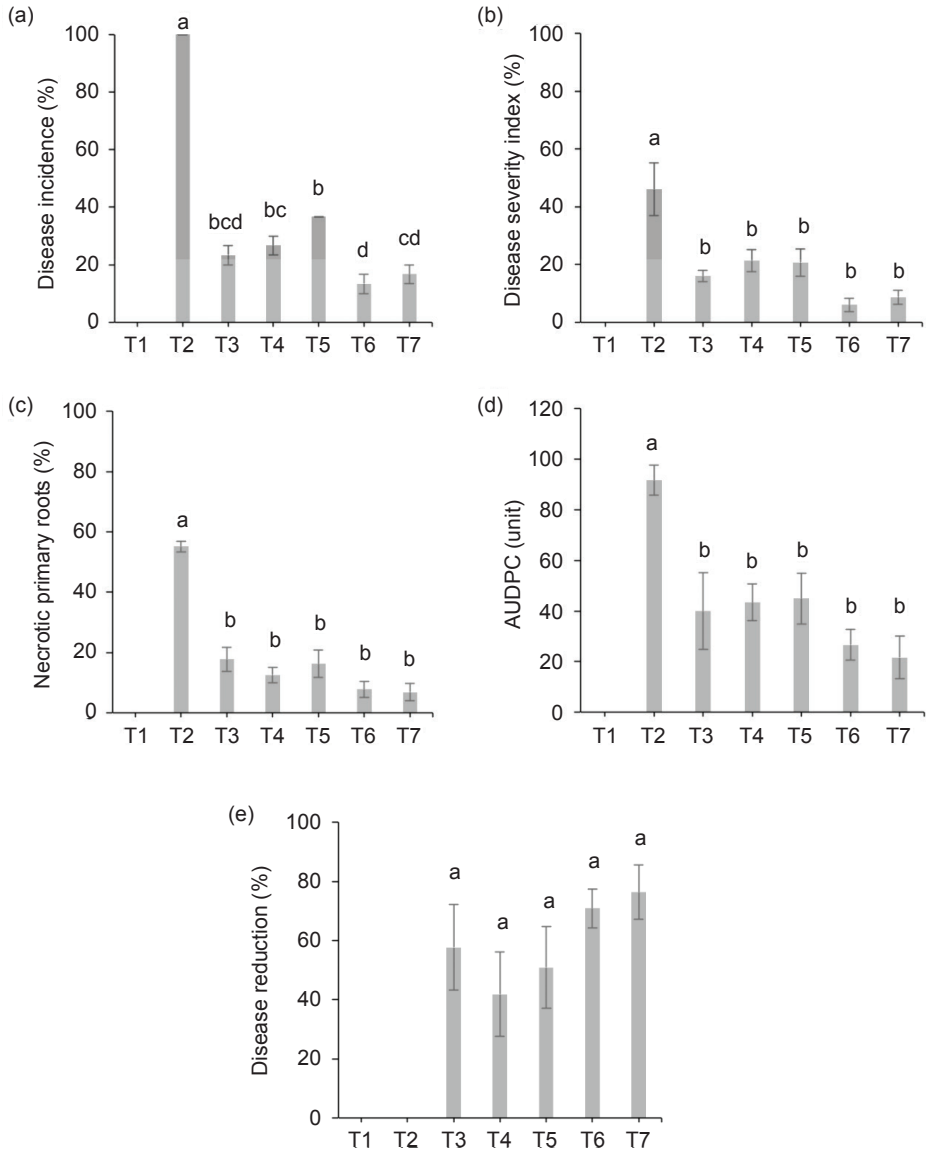

Figure 1. Effect of local Trichoderma *isolates and commercial BCAs against* G. boninense*, nine months after inoculation. (a) Disease incidence; (b) disease severity index; (c) area under progress curve (AUDPC); (d) percentage necrotic roots and (e) disease reduction. T1, Untreated (negative control); T2, Untreated (positive control); T3,* T. virens*; T4,* T. asperellum*; T5,* T. virens *and* T. asperellum*; T6, Commercial product X; and T7, Commercial product Y. Means (± standard error) followed with different letters were significantly different at p ≤ 0.05 by Tukey's test.*

show better results either. This could be due to some incompatibility as the *Trichoderma* species were selected for their antagonistic behaviour against *G. boninense in vitro*, and not for their combined efficacy. Similar to the findings in the current study, Shamala (2013) also reported these species as potential BCAs against *G. boninense* infection in oil palm nursery experiments. These *Trichoderma* species produce chitinase, cellulase, protease and β-glucanase, which play a crucial role in mycoparasitism in fungi (Musa, 2017) and reduce the integrity of the pathogen's cell wall by breaking the polysaccharides chitin and β-glucan (Howell, 2003). Furthermore, *Trichoderma* species outperform phytopathogens in terms of nutritional competition, and release siderophores to mobilise iron in the soil, which may have efficiently inhibited the growth of *G. boninense*, thus suppressing *Ganoderma* disease in oil palm seedlings due to iron uptake (Saha et al., 2013). Moreover, these *Trichoderma* species are also reported to produce volatile organic compounds, which may contribute to reducing the hyphal growth of *G. boninense* (Inayati et al., 2020).

In this study, commercial Product X also showed a disease reduction of 70.91%. Product X (T6) contains a mixture of 12 species of AMF, including fungi from the genera *Glomus*, *Sclerocystis*, *Acaulospora*, *Gigaspora* and *Scutellospora*. Weng et al. (2022) described the direct and indirect mechanisms of AMF as BCA, including enhancing lignification of the cell wall, and root branching. Furthermore, mycelial networks of the AMFs work as a barrier in the root epidermis, improving soil structure, trapping pathogens, killing pathogens by root

exudates, enhancing the proliferation of beneficial microorganisms and improving plant nutrition and water absorption (Weng et al., 2022). Meanwhile, the commercial Product Y (T7) has a mixture of *T. harzianum*, *T. viride* and a few other BCAs, which resulted in a 76.36% disease reduction. The disease suppression achieved with Product Y can be due to the compatible combination of mixed strains, which can show synergy and significantly increase the effectiveness of the product. In addition, this could be due to diverse toxic substances (gliotoxins) produced by the different *Trichoderma* species, which can aid in the growth of *Trichoderma* in the soil.

Effects on Physiology of Oil Palm Seedlings

The infection of *G. boninense* in oil palm seedlings has caused a significant loss in chlorophyll content, plant height, bole diameter and frond production. However, the application of the local *Trichoderma* isolates, either singly (T3 and T4) or as a mixture (T5) has provided significant disease resistance in the oil palm seedlings towards *G. boninense* infection, as attributed to the physiological responses, compared to the untreated positive control. Moreover, there was no significant difference among all BCA-treated oil palm seedlings (T3, T4, T5, T6 and T7), indicating the local *Trichoderma* isolates have similar efficacy in resisting the loss of plant physiological integrity. The percentage of reduction in terms of chlorophyll content, plant height and bole diameter in the treated oil palm seedlings ranged between 9.49%–19.40%, 11.41%–18.87% and 4.26%–18.82%, respectively, as compared to the untreated negative control (*Figure 2*). These findings were further validated by Pearson's correlation analysis which revealed a negative correlation between the disease variables and physiological responses as shown in *Table 3*, while there was only a positive correlation with disease reduction, suggesting that disease reduction by these BCAs in oil palm seedlings was directly correlated with the promotion of plant physiology.

These were consistent with other studies reported by Syafiq et al. (2021), in which they observed an enhanced growth response induced by *Trichoderma* species in infected *Ganoderma* oil palm seedlings. The finding supports the hypothesis that numerous antagonistic microbes,

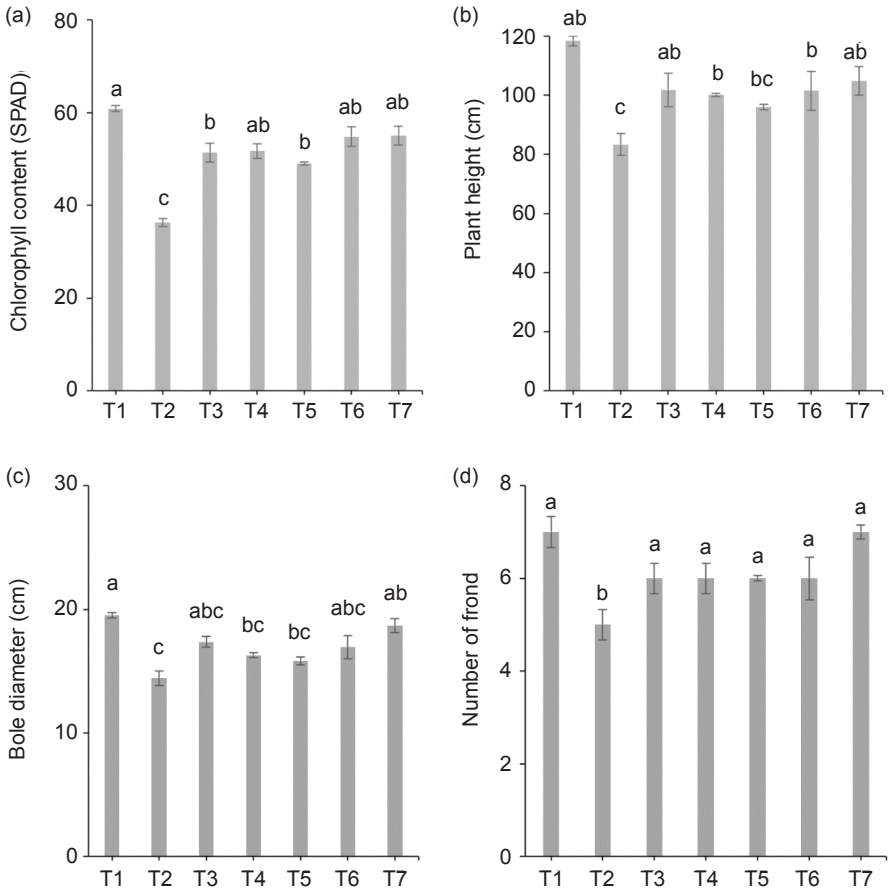

Figure 2. Effect of local Trichoderma *isolates and commercial BCAs on plant growth, nine months after inoculation. (a) Chlorophyll content; (b) plant height; (c) bole diameter and (d) number of fronds. T1, Untreated (negative control); T2, Untreated (positive control); T3,* T. virens*; T4,* T. asperellum*; T5,* T. virens *and* T. asperellum*; T6, Commercial product X; and T7, Commercial product Y. Means (± standard error) followed with different letters were significantly different at p ≤ 0.05 by Tukey's test.*

like *Trichoderma*, indirectly promote plant growth by mitigating the impacts of pathogens and reducing disease severity (Beneduzi et al., 2012). Interestingly, *T. virens* and *T. asperellum* have been reported to produce a phytohormone-like auxin, which significantly increased plant height, frond number and chlorophyll content in this study. This phytohormone promotes plant growth directly and indirectly by balancing other hormones like gibberellic acid (GA3). It also promotes plants by expanding lateral and adventitious roots, improving nutrient access and enhancing root exudation which offers more resources for soil microbe and root interactions (Spaepen & Vanderleyden, 2011). According to Brummel and Hall (1987), even at lower levels, this hormone can promote plant growth. This may contribute to increasing plant performance through *Trichoderma* treatment in nursery trials.

Effect on Soil and Plant Nutrients Uptake

This study also investigated the effect of the *Trichoderma* isolates and the commercial BCAs on soil nutrient availability and total nutrients in the oil palm seedlings by the end of the nursery trials. As compared to the untreated negative control, the treated soils in T3, T4, T5, T6 and T7 resulted in significantly higher availability of P (0.025–0.029 g kg^{-1}), K (0.128–0.131 g kg^{-1}), Ca (1.36–1.41 g kg^{-1}), Mg (0.119–0.124 g kg^{-1}) and Zn (6.60–7.55 mg kg^{-1}) (*Table 4*). This is consistent with a study by Khan et al. (2017) showed that the breakdown and adsorption of P, K, Ca, Mg, Cu, Fe, Mn and Zn in the soil can be greatly enhanced by BCAs such as *Trichoderma* species.

Furthermore, the higher availability of nutrients in the soil was directly attributed to the higher total nutrients in the leaves of oil palm seedlings. Generally, significantly higher P (1.85–1.87 g kg^{-1}), K (12.53–13.43 g kg^{-1}), Ca (17.51–18.60 g kg^{-1}), Mg (3.07–3.15 g kg^{-1}) and Zn (18.27–18.60 mg kg^{-1}) were recorded in the treated oil palm seedlings, as compared to the untreated negative control (*Table 5*).

Certain nutrients in the soil are present in a poorly soluble or insoluble form, limiting the usual nutrient cycle in the soil. *Trichoderma* species may secrete organic acids that alter the pH of the soil rhizosphere, thus improving the plants' uptake of macronutrients such as P and micronutrients such as Fe, Mn and Zn (Li et al., 2015). *Trichoderma* species also have a strong colonisation ability, which

TABLE 3. PEARSON'S CORRELATION ANALYSIS BETWEEN VARIABLES MEASURED FOR DISEASE AND PLANT PHYSIOLOGICAL RESPONSES

Variable	DI	DSI	AUDPC	PR	DR	CHL	HT	BD
DSI	0.96**	-	-	-	-	-	-	-
AUDPC	0.93**	0.98**	-	-	-	-	-	-
PR	0.99**	0.96**	0.93**	-	-	-	-	-
DR	-0.93**	-0.98**	-1.00**	-0.93**	-	-	-	-
CHL	-0.98**	-0.98**	-0.97**	-0.98**	0.96**	-	-	-
HT	-0.89**	-0.92**	-0.96**	-0.88**	0.95**	0.96 **	-	-
BD	-0.83*	-0.90**	-0.93**	-0.82*	0.93**	0.90**	0.95**	-
NF	-0.84*	-0.89**	-0.92**	-0.87*	0.92**	0.90**	0.90**	0.95**

Note: DI - disease incidence; DSI - disease severity index; AUDPC - area under disease progress curve; PR - percentage of necrotic primary roots; DR - percentage of disease reduction; CHL - chlorophyll content; HT - plant height; BD - bole diameter; NF - number of fronds. *Significantly correlated at $p \leq 0.05$. **Significantly correlated at $p \leq 0.01$.

TABLE 4. EFFECT OF LOCAL *Trichoderma* ISOLATES AND COMMERCIAL BIOLOGICAL CONTROL AGENTS ON SOIL NUTRIENT AVAILABILITY, NINE MONTHS POST INOCULATION

Treatment	pH	Available P (g kg^{-1})	Available K (g kg^{-1})	Available Ca (g kg^{-1})	Available Mg (g kg^{-1})	Available Zn (mg kg^{-1})	Available Cu (mg kg^{-1})
T1	6.61 ± 0.23^{a}	0.023 ± 0.00^{c}	0.125 ± 0.00^{c}	1.24 ± 0.00^{c}	0.119 ± 0.00^{b}	6.16 ± 0.04^{b}	1.22 ± 0.01^{a}
T2	5.23 ± 0.06^{b}	0.018 ± 0.00^{d}	0.111 ± 0.00^{d}	1.15 ± 0.00^{d}	0.111 ± 0.00^{c}	3.62 ± 0.13^{c}	0.93 ± 0.00^{b}
T3	7.53 ± 0.67^{a}	0.029 ± 0.00^{a}	0.130 ± 0.00^{a}	1.36 ± 0.00^{b}	0.124 ± 0.00^{a}	7.51 ± 0.20^{a}	1.25 ± 0.00^{a}
T4	6.68 ± 0.94^{a}	0.029 ± 0.00^{a}	0.130 ± 0.00^{a}	1.37 ± 0.01^{b}	0.124 ± 0.00^{a}	7.70 ± 0.09^{a}	1.25 ± 0.00^{a}
T5	6.37 ± 0.06^{a}	0.029 ± 0.00^{a}	0.131 ± 0.00^{a}	1.36 ± 0.01^{b}	0.123 ± 0.00^{a}	7.55 ± 0.22^{a}	1.25 ± 0.02^{a}
T6	7.01 ± 0.09^{a}	0.025 ± 0.00^{b}	0.128 ± 0.00^{b}	1.38 ± 0.00^{ab}	0.119 ± 0.00^{b}	6.60 ± 0.15^{b}	1.25 ± 0.00^{a}
T7	6.94 ± 0.06^{a}	0.027 ± 0.00^{ab}	0.129 ± 0.00^{ab}	1.41 ± 0.00^{a}	0.120 ± 0.00^{b}	6.74 ± 0.01^{b}	1.25 ± 0.00^{a}

Note: P - posphorus; K - potassium; Ca - calcium; Mg - magnesium; Zn - zinc; Cu - copper; T1 - Untreated (negative control); T2 - Untreated (positive control); T3 - *T. virens*; T4 - *T. asperellum*; T5 - *T. virens* and *T. asperellum*; T6 - Commercial product X; T7 - Commercial product Y. Means (± standard error) followed with different letters within a column were significantly different at $p \leq 0.05$ by Tukey's test.

TABLE 5. EFFECT OF LOCAL *Trichoderma* ISOLATES AND COMMERCIAL BIOLOGICAL CONTROL AGENT ON OIL PALM SEEDLING NUTRIENT CONTENT NINE MONTHS POST INOCULATION

Treatment	N (%)	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	Zn (g kg^{-1})	Cu (g kg^{-1})
T1	7.04 ± 0.32^{a}	1.75 ± 0.03^{b}	12.16 ± 0.10^{b}	15.52 ± 0.10^{d}	3.03 ± 0.01^{b}	16.40 ± 0.26^{b}	7.20 ± 0.06^{a}
T2	6.54 ± 0.01^{b}	1.16 ± 0.20^{c}	8.66 ± 0.30^{c}	14.67 ± 0.12^{e}	2.25 ± 0.00^{c}	11.17 ± 0.09c	3.37 ± 0.09^{b}
T3	7.12 ± 0.01^{a}	1.85 ± 0.02^{a}	12.85 ± 0.00^{ab}	18.60 ± 0.23^{a}	3.08 ± 0.01^{b}	18.50 ± 0.23^{a}	7.50 ± 0.06^{a}
T4	7.04 ± 0.07^{a}	1.87 ± 0.01^{a}	13.43 ± 0.19^{a}	17.54 ± 0.01^{c}	3.15 ± 0.02^{a}	18.60 ± 0.06^{a}	7.43 ± 0.24^{a}
T5	7.02 ± 0.08^{a}	1.86 ± 0.01^{a}	12.66 ± 0.01^{ab}	17.74 ± 0.07^{bc}	3.08 ± 0.01^{b}	18.60 ± 0.11^{a}	7.67 ± 0.15^{a}
T8	7.01 ± 0.01^{a}	1.86 ± 0.00^{a}	12.53 ± 0.24^{b}	17.51 ± 0.13^{c}	3.05 ± 0.01^{b}	18.27 ± 0.12^{a}	7.23 ± 0.09^{a}
T9	7.01 ± 0.07^{a}	1.86 ± 0.01^{a}	12.58 ± 0.26^{ab}	18.29 ± 0.08^{ab}	3.07 ± 0.02^{b}	18.37 ± 0.09^{a}	7.30 ± 0.06^{a}

Note: N - nitrogen; P - posphorus; K - potassium; Ca - calcium; Mg - magnesium; Zn - zinc; Cu - copper; T1 - Untreated (negative control); T2 - Untreated (positive control); T3 - *T. virens*; T4 - *T. asperellum*; T5 - *T. virens* and *T. asperellum*; T6 - Commercial product X; T7 - Commercial product Y. Means (± standard error) followed with different letters within a column were significantly different at $p \leq 0.05$ by Tukey's test.

can increase the root-soil contact area and improve access to nutrients as the root system grows and expands. In addition, several isolates of *Trichoderma* have been shown to produce bio-stimulant and hormone-like compounds that may promote plant nutrient uptake. However, this study did not evaluate the plant-promoting traits of *T. viren* and *T. asperellum*. These *Trichoderma* species have been shown to have various plant growth-promoting properties, including the ability to solubilise phosphate, and produce indole-3-acetic acid (IAA) and siderophere (Inayati et al., 2020; Muniroh et al., 2019). *Trichoderma* species can colonise and live endophytically in plant roots, similar to those of mycorrhizal fungi (Kleifeld & Chet, 1992), and IAA synthesis by these fungi can enhance plant root mass (López-Bucio et al., 2015). *Trichoderma* species' ability to dissolve phosphate and produce siderophores may have increased nutrient release for plant root uptake (Rudresh et al., 2005). Consequently, these *Trichoderma* species could significantly improve the physiological status of plants as well as their nutritional status.

CONCLUSION

Both local *T. virens* and *T. asperellum*, either applied singly or as a mixture, were able to reduce disease by 41.82%–57.73%, and at par with the commercial BCA products. The untreated oil palm seedlings (positive control) showed a significant loss in physiological integrity in terms of chlorophyll content, plant height, bole diameter, and number of fronds due to the *G. boninense* infection, meanwhile, the treated seedlings with local *Trichoderma* isolates and commercial BCAs were able to resist the infection significantly to a certain degree. The application of the local *Trichoderma* species and both commercial BCAs also generally improves the availability of macronutrients in the soil, as well as the nutrient content in the plants. Therefore, it was suggested to further study the synergy of the local *Trichoderma* isolates with other BCAs to further improve their efficiency in suppressing phytopathogens, as well as improving soil health, crop growth and yield. The performance of the local *Trichoderma* isolates is considered similar to that of commercial BCAs and can be further exploited for mass production and commercialisation.

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