Special Issue (UniSZA), Malaysia:
ICAFT 2023: 3rd International Conference on Agriculture, Animal Sciences & Food Technology

Trichoderma Species' *In Vitro* and *In Planta* Inhibition against *Ganoderma boninense*, a Causative Agent of Basal Stem Rot in Oil Palm (*Elaeis guineensis*)

Jumiati Asis[1], Nur Aainaa Hasbullah[1], Mohamadu Boyie Jalloh[1], Noor Khairani Mohamad Basri[1], Palanivell Perumal[2], Peter Mojiun[2] and Mohd. Rashid Mohd. Rakib[1]*

[1]Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, 90000 Sandakan, Sabah, Malaysia; [2]Eco Management Unit, Wilmar Plantations Sdn. Bhd. (formerly known as PPB Oil Palms Berhad), 90000 Sandakan, Sabah, Malaysia.

Abstract | *Trichoderma* species are well-known biological control agents (BCAs) that have significant antagonistic activity against various fungal phytopathogens. On the other hand, *Ganoderma boninense* has been identified as the phytopathogen causing basal stem rot (BSR), a devastating disease in oil palm crop (*Elaeis guineensis*). In this study, the *in vitro* and *in planta* inhibition of *G. boninense* using *Trichoderma* isolates were evaluated. A total of 20 *Trichoderma* isolates were collected. Among the isolates, T4RH and T8R were selected for further *in planta* experiments as they showed significant inhibitory activity against *G. boninense* via *in vitro* dual-culture and dual-plate assays. Isolates T4RH and T8R were identified as *Trichoderma virens* and *Trichoderma asperellum* by the internal transcribed spacer gene sequences. The *in-planta* experiment was conducted by inoculation of 3-month-old oil palm seedlings with rubber wood block inoculum. Single and mixture conidial suspensions 1×10^6 of T4RH and T8R were applied to the *Ganoderma*-inoculated plants. It was found that T4RH, T8R, and mixed *Trichoderma* treatments have significantly lower disease incidence, disease severity index, area under disease progress curve, and percentage of necrotic primary roots as compared to the positive control. The disease reduction was up to 57.70% and 43.86% when plants were treated with T4RH and T8R, respectively. Additionally, the *Trichoderma* treatment recorded significantly higher chlorophyll content, plant height, bole diameter, and number of fronds as compared to the positive control. The findings from this study suggested that *T. virens* T4RH and *T. asperellum* T8R have the potential to be used as promising BCAs against *G. boninense*.

Received | February 22, 2024; **Accepted** | October 11, 2024; **Published** | November 01, 2024
***Correspondence** | Mohd. Rashid Mohd. Rakib, Faculty of Sustainable Agriculture, University Malaysia Sabah, 90000 Sandakan, Sabah, Malaysia; **Email:** rakibmrm@ums.edu.my
Citation | Asis, J., N.A. Hasbullah, M.B. Jalloh, N.K.M. Basri P. Perumal, P. Mojiun and M.R.M. Rakib. 2024. *Trichoderma* species *in vitro* and *in planta* inhibition against *Ganoderma boninense*, a causative agent of basal stem rot in oil palm (*Elaeis guineensis*). *Sarhad Journal of Agriculture*, 40(Special issue 1): 173-185.
DOI | https://dx.doi.org/10.17582/journal.sja/2024/40/s1.173.185
Keywords | Biocontrol, Dual culture assay, Pathology, *Trichoderma virens*, *Trichoderma asperellum*, Volatile compounds

Introduction

The African oil palm (*Elaeis guineensis* Jacq.) is an oil-producing plant that is mainly cultivated in tropical regions, particularly in Indonesia and Malaysia, that accounts for 60% and 25% of the world's vegetable oil supply, respectively (USDA, 2022). The region's oil palm faces a severe threat from basal stem rot (BSR), also called *Ganoderma* disease, which is primarily caused by *Ganoderma boninense*, a destructive disease caused by the phytopathogenic basidiomycete fungus (Rees *et al*., 2012; Rakib *et al*., 2014). Oil palm trees that are infected usually experience decay at the base of the stem, leading to a collapse, and eventual death (Hushiarian *et al*., 2013; Alam *et al*., 2015). The disease is estimated to cause economic losses as high as 68% due to the loss of productive oil palm trees and the expenses associated with controlling the disease (Assis *et al*., 2021). Although BSR in oil palm was discovered more than 50 years ago, it remains a major threat to the sustainability of the industry despite numerous efforts to find prophylactic and curative treatments for the disease.

However, it is strongly recommended that integrated disease management be implemented to minimise economic losses due to the disease. This can be achieved through various disease control strategies, including biological control agents (BCAs), chemical treatments, and cultural practices (Susanto *et al*., 2005; Lim *et al*., 2013). In this regard, BCAs are considered a sustainable solution for addressing plant disease management as they are non-toxic and environmentally friendly (Cornejo *et al*., 2020; Javeed *et al*., 2021).

The genus *Trichoderma* is mentioned in most of the literature on the biological control of plant diseases. Its usefulness has been known since 1930 (Weindling, 1934). Today, modern technologies use *Trichoderma* for biological control of various diseases. According to Zin and Badaluddin (2020), *Trichoderma* species that are extensively utilised as BCAs in bio-fungicides include *T. asperellum*, *T. harzianum*, and *T. atrovitide*, and to a lesser extent are *T. virens*, *T. afroharzianum*, *T. gamsii*, and *T. polysporum*. For numerous years, *Trichoderma* species have been used as natural enemies to combat plant diseases because of their capability to exert antagonistic behavior (Harman, 2006).

Antagonistic activity is based on both direct and indirect processes, such as rivalry for resources and space, mycoparasitic activity, antibiosis, and the development of systemic resistance to combat pathogens and promote plant growth. They act at the soil, leaf, and root levels by producing and releasing various compounds that can induce local or systemic resistance to abiotic and biotic stress factors in plants (Zaidi *et al*., 2014; Birkenbihl *et al*., 2017). The ecological success of these fungi is the result of a combination of mechanisms that the plants activate (Harman *et al*., 2004).

The efficacy of *Trichoderma* species has been evaluated in both individual and combined, and laboratory and nursery trials, demonstrating its effectiveness in disease reduction. However, the performance of a BCA is often insufficient when only a single BCA or organism is used (Harshita *et al*., 2018). Traditionally, mixed fungal cultures have been avoided as populations are difficult to predict, although a compatible mixed culture can have synergies and significantly increase the efficacy of the product. Nowadays, more emphasis is placed on the use of combined biological control agents with multiple mechanisms that could increase the success of biological control (Collinge *et al*., 2022).

Despite this, the study was conducted to evaluate the antagonistic activity of local *Trichoderma* isolates against *G. boninense*, and the efficacy of selected local single, and mixed *Trichoderma* isolates in controlling the infection of *G. boninense*, *in planta* on oil palm seedlings.

Materials and Methods

Isolation of Trichoderma species

The feather roots of healthy oil palm trees collected randomly from a local oil palm estate located in Sandakan, Sabah, Malaysia, yielded *Trichoderma* species that were isolated from the rhizosphere. A total of 20 *Trichoderma* isolates were recovered using potato dextrose agar (PDA), following the isolation procedure described by Siddique *et al*. (2009). The pure culture of *Trichoderma* isolates was identified based on the characteristics described by Baca *et al*. (2022), including the formation of greenish to yellowish conidia, and concentric rings, with rapid mycelial growth and loosely arranged conidia.

In vitro antagonistic activities of Trichoderma species against Ganoderma boninense

Dual-culture assay: All the *Trichoderma* isolates were

screened for their inhibitory effects on the growth of *Ganoderma* via a dual-culture assay (Rahman *et al*., 2009). The isolate of *Ganoderma boninense* (G4) utilised in this research was acquired from a pure culture maintained in the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah (Malaysia), and identified previously as *Ganoderma boninense* based on internal transcribed spacer (ITS) gene sequences. Plugs of *Trichoderma* isolate and *G. boninense*, each 8 mm in diameter, were placed 2 cm from the periphery of a Petri dish with a 9 cm diameter, on opposite sides. A plate inoculated with *G. boninense*, without *Trichoderma* as the antagonist was served as the control. The plates were incubated at 28 °C temperature for seven days in darkness. The experiment was conducted in three replicates and arranged in a completely randomised design (CRD). The radial growth of *G. boninense* was measured, and the percentage inhibition of radial growth (PIRG) was calculated using Equation 1 (Bivi *et al*., 2010).

$$\text{PIRG (\%)} = \frac{(R1-R2)}{R1} \times 100 \quad \ldots(1)$$

Where R1 is the radial growth of *G. boninense* in control and R2 is the radial growth of *G. boninense* challenged with *Trichoderma* isolate.

Volatile organic compounds (VOCs) dual-plate assays

The VOCs assay was carried out to assess the inhibiting effect of *Trichoderma* isolates on *G. boninense* through their production of volatile compounds (Vila *et al*., 2017). The dual-plate assay was carried out by Rajani *et al*. (2021). Each *Trichoderma* isolate was infected with a mycelial plug (8 mm in diameter) in the centre of a Petri dish containing PDA (9 cm in diameter), and the dish was then incubated at 28 °C for seven days under dark conditions. Subsequently, a comparable-sized mycelial plug of *G. boninense* was inoculated onto a different PDA plate, positioned in an inverted manner above the *Trichoderma* isolates that were seven days old, and sealed using parafilm. The plates without *Trichoderma* isolate served as a control. The plates were incubated at 28 °C for seven days in darkness. The experiment was performed in a CRD arrangement with three replications. The diameter growth of *G. boninense* was measured, and the percentage inhibition of the diameter growth (PIDG) was calculated using Equation 2 (Ting *et al*., 2010).

$$\text{PIDG (\%)} = \frac{(D1-D2)}{D1} \times 100 \quad \ldots(2)$$

Where D1 is the diameter growth of *G. boninense* in the control plate, and D2 is the diameter growth of *G. boninense* with *Trichoderma* isolates.

Molecular identification of the potential Trichoderma isolates

The *Trichoderma* isolates that showed potential as BCA candidates based on the *in vitro* assays were identified based on the ITS gene according to the protocol described by Darlis *et al*. (2023), which include the genomic deoxyribonucleic acid (gDNA) extraction, polymerase chain reaction (PCR), and purification and sequencing of the PCR products. The sequence was employed to identify homologous sequences through the Basic Local Alignment Search Tool (BLAST) hosted on the National Center for Biotechnology Information (NCBI) website (https://www.ncbi.nlm.nih.gov/).

In planta antagonistic activities of Trichoderma species against Ganoderma boninense

Among the isolates, T4RH and T8R were selected for further *in planta* experiments based on their significance in inhibition against *G. boninense* via *in vitro* dual-culture and VOCs dual-plate assays. The efficacy of both *Trichoderma* isolates in suppressing *G. boninense* infection in oil palm seedlings (*in planta*) was evaluated singly and in combination with untreated negative and positive controls. A 3-month-old (*dura* × *pisifera*) oil palm seedling was inoculated with a 6×6×6 cm *Ganoderma*-colonised rubber wood block inoculum prepared as described by Nusaibah *et al*. (2016). The inoculation was performed by placing the inoculum in a planting hole in a polyethylene planting bag (30×38 cm) containing a soil mixture (non-sterile soil and organic matter in a 2:1 ratio) before transplanting the seedling on top of the inoculum (Rakib *et al*., 2015). *Trichoderma*' conidia suspension prepared according to the protocol described by Jinantana and Sariah (1998) was immediately applied after the inoculation. All five treatments in this experiment are summarised in Table 1. Each treatment has three replications, and each replicate consisted of 10 seedlings, which resulted in 150 experimental units. All experimental units were arranged in a completely randomised design (CRD) in a nursery facility at the Malaysian Palm Oil Board (MPOB) Research Station, Lahad Datu, Sabah. The experiment was conducted for nine months. The seedlings were watered twice a day, and basal fertilizer was applied monthly according to the recommended practices (MPOB, 2016).

Table 1: *Description of treatments.*

Code	Treatment	Description
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.

Table 2: *Classes of diseases in oil palm seedlings and their associated symptoms can be identified based on a numerical value.*

Class	Symptoms
0	An oil palm seedling that is healthy, with green leaves and no signs of fungal growth on any part of the plant.
1	The presence of 1-3 chlorotic leaves on the oil palm seedling, and there is no fungal mass development on any part of the plant.
2	The fungal mass may be present with or without leaves showing chlorosis (yellowing).
3	The oil palm seedling may exhibit more than 3 yellowish (chlorotic) leaves and dead (necrotic) leaves, which may or may not have fungal mass on any part of the plant.
4	Severe chlorosis or necrosis is present in at least 50% of the total leaf count, with or without fungal mass.
5	Oil Palm seedling that are dead, with or without a fungal mass.

Source: *Rakib et al. (2015).*

Data collection

Disease signs and symptoms of the *Ganoderma* infection were assessed at monthly intervals over a 9-month period. Disease incidence (DI), disease severity index (DSI) based on the disease class described in Table 2, the area under disease progress curve (AUDPC), and the percentage of necrotic primary roots were recorded at the end of the 9-month experiment and 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 3 to 7. In addition, the oil palm seedlings physiological data were recorded. The plant height, bole or stem diameter, number of fronds, and chlorophyll content were recorded at the end of the nursery trials. The chlorophyll content was recorded using a single photon avalanche diode (SPAD) chlorophyll device (Rakib *et al.*, 2019).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected seedlings}}{\text{Total number of seedlings}} \times 100 \quad \ldots (3)$$

$$\text{Disease severity index (\%)} = \frac{\sum (A \times B)}{\sum n \times 5} \times 100 \quad \ldots (4)$$

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.

$$\text{Area under disease progress curve unit)} = \sum_{i}^{n-1} \left(\frac{Y_i + Y_{i+1}}{2} \right) (t_{i+1} - t_i) \quad \ldots (5)$$

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

$$\text{Necrotic primary roots (\%)} = \frac{\text{Number of necrotic primary roots}}{\text{Total number of primary roots}} \times 100 \quad \ldots (6)$$

$$\text{Disease reduction (\%)} = \frac{(X1\text{-}X2)}{X1} \times 100 \quad \ldots (7)$$

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

Statistical analysis

All the data were subjected to a one-way analysis of variance (ANOVA), and the differences among the means were determined for significance at $p \leq 0.05$ level using Turkey's test. The statistical analysis was performed using SPSS statistical software (version 26).

Results and Discussion

In vitro antagonistic activities of Trichoderma species against Ganoderma boninense

All 20 *Trichoderma* isolates were successfully isolated

from the rhizosphere of feather roots of healthy oil palms. Generally, the mycelium of *Trichoderma* species appeared transparent (hyaline) or white on PDA, and the reverse usually with a pale yellowish colour. The colony primarily appeared green and yellow, with rapid growth, similar to those described by Baca *et al.* (2022). It was found that all *Trichoderma* isolates inhibited the mycelial growth of *G. boninense*, with a PIRG (%) value greater than 60% as shown in Table 4. Notably, two *Trichoderma* isolates, T4RH and T8R had the highest PIRG values of 92.08% and 91.09%, respectively. Both *Trichoderma* isolates are shown in Figure 1, which varied in terms of their appearance on PDA. The isolate T4RH has dense mycelia with greenish-to-yellowish conidia, while T8R has hyaline mycelium with greenish conidia. Molecular identification confirmed the identity of the isolate T4RH as *Trichoderma virens*, and T8R as *Trichoderma asperellum* as summarised in Table 3. Similar findings were reported by Shamala (2013), where *Trichoderma* isolates inhibited the mycelial growth of *G. boninense* by more than 60% and, recorded the highest PIRG values for *T. virens* and *T. asperellum*. This finding also corresponds to various studies that have reported that *Trichoderma* species can suppress the growth of a range of phytopathogens, including *Rhizoctonia solani, Alternaria alternata, Curvularia lunata, Fusarium oxysporum, G. boninense, Sclerotium. rolfill, Sclerotinia sclerotiorum*, and *Sclerotium cepivorum* (Shamala, 2013; John *et al.*, 2015; Hirpara *et al.*, 2017).

Figure 1: *(A) Trichoderma virens T4RH, and (B) Trichoderma asperellum T8R culture of selected Trichoderma isolates on PDA.*
\

Trichoderma species have shown promising effectiveness in controlling fungal phytopathogens through direct mechanisms like antibiosis and mycoparasitism, as well as indirect mechanisms such as competing for nutrients, enhancing plant defense responses, and promoting plant growth (Dukare *et al.*, 2022). The strong effectiveness of *Trichoderma* isolates against the pathogenic fungal strains responsible for BSR in oil palm was confirmed in the current study. All *Trichoderma* isolates inhibited *G. boninense*, grew at a faster rate, and showed varying levels of inhibition, likely attributable to genetic variability (Debbi *et al.*, 2018). The cause of these antagonistic behaviors may be mycoparasitism and competition for space and nutrients, as demonstrated by the dual culture assay. Mycoparasitism is one of the mechanisms of *Trichoderma* sp. to protect plants against pathogen attack, which involves hyphal interaction and parasitism. The different hyperparasitic potentials of various isolates of *Trichoderma* have been reported (Prasad and Rageswaram, 1999; Sankar and Sharma, 2001; Pan and Bhagat, 2007). This is because the individual species or isolates produce different amounts of hydrolytic enzymes when they attack the mycelium of the pathogen. The ability of *Trichoderma* bioagents to break down the hyphae of phytopathogenic fungi may be attributed to various external enzymes, including chitinases, and cellulases. These enzymes aid in the penetration of *Trichoderma* bioagents into the hyphae of phytopathogens (Bhat, 2017; Rajani *et al.*, 2021). *Trichoderma* species exhibit highly competitive biological activities due to their rapid colonization rates, which in turn reduce the presence of competing microbes (Bizos *et al.*, 2020). In addition, the proliferation of *G. boninense* might be inhibited by the metabolites generated by *Trichoderma* species, as they are known for synthesizing various antibiotics such as trichodernin and herzianolides using secondary metabolites, which are crucial in the antibiosis mechanism (Küçük and Kıvanç, 2004; Khan *et al.*, 2020; Tomah *et al.*, 2020).

Table 3: *Identity of the isolates T4RH and T8R based on the similarity between sample sequences and the sequences in GenBank.*

Isolate code	Accession number (NCBI)	Identity	ITS fragment length	Homology	BLAST bit score
T4RH	KJ739790.1	*Trichoderma virens*	615 base pairs	99.84%	1104
T8R	MF774876.1	*Trichoderma asperellum*	603 base pairs	100%	1088

Table 4: *Percentage inhibition of radial growth (PIRG) and Percentage inhibition of diameter growth (PIDG) of Ganoderma boninense based on in vitro dual-culture and volatile organic compounds (VOCs) dual-plate assays.*

Trichoderma isolates	PIRG in dual-culture assay (%)	PIDG in dual-plate assay (%)
T3R	87.13 ±1.98abcd	66.07 ±6.48abc
T5R	82.18 ±1.71abcdef	50.89 ±6.58bc
T6R	85.15 ±2.97abcdef	53.57 ±5.70abc
T8R	91.09 ±1.71ab	42.41 ±9.87c
T11R	86.14 ±2.62abcde	79.91 ±2.05a
T12R	78.22 ±0.99cdef	46.88 ±5.26bc
T15R	75.25 ±2.62f	53.57 ±1.61abc
T1RH	83.17 ±0.99abcdef	52.68 ±1.18abc
T3RH	88.12 ±1.71abc	50.89 ±5.15bc
T4RH	92.08 ±2.62a	75.45 ±1.95ab
T5RH	81.19 ±0.99bcdef	70.09 ±7.35abc
T7RH	84.16 ±0.99abcdef	41.52 ±7.82c
T9RH	82.18 ±1.71abcdef	45.09 ±5.41c
T10RH	85.15 ±1.71abcdef	58.48 ±5.41abc
T11RH	77.23 ±0.99def	63.84 ±6.14abc
T3S	64.36 ±3.43g	63.84 ±4.31abc
TSF	76.24 ±2.97ef	66.96 ±1.95abc
G18R	86.14 ±0.99abcde	42.41 ±7.73c
TFPL	81.19 ±0.99bcdef	41.52 ±3.13c
TThai	90.10 ±2.62ab	57.59 ±0.45abc

Note: *Means (± Standard error) followed by different letters within the column were significantly different at p ≤ 0.05 by Tukey's test.*

The inhibitory potential of *Trichoderma* isolates against *G. boninense* was also evaluated using a volatile compounds (VOCs) assay. The dual-plate assay was used to evaluate the effect of volatile antifungal compounds released by *Trichoderma* species (Vila *et al.*, 2017). The study discovered that production of volatile compounds effectively by *Trichoderma* inhibited the growth of *G. boninense*. Isolate T4RH demonstrated the highest inhibition of *G. boninense* at 75.45%, whereas isolate T8R showed a lower inhibition at 45.4%. Previous research has reported comparable findings, demonstrating that the growth of various phytopathogens was inhibited by the volatile compounds generated by *Trichoderma* species (Shamala, 2013; Marques *et al.*, 2018; Baiyee *et al.*, 2019).

This production is an antibiosis mechanism within the *Trichoderma* species. The production released by *Trichoderma* species can benefit the host plant by exerting an antifungal effect and providing protection against plant pathogens. The production of volatile compounds such as pyrones and sesquiterpenes has been described as a possible mechanism by *Trichoderma* sp. (Reino *et al.*, 2008). In addition, the VOCs phenlethyl alcohol (PEA) have been reported to be produced by several *Trichoderma* species, such as *T. alroviride*, *T. harzianum*, *T. spirale*, and *T. virens*, which exhibit antifungal abilities against various plant pathogens (Stoppacher *et al.*, 2010; Siddiquee *et al.*, 2012; Liu *et al.*, 2014; Baiyee *et al.*, 2019). PEA also has strong antifungal activity against *G. boninense* (Angel *et al.*, 2016). Siddiquee *et al.* (2012) reported a few other fungal volatile metabolites with antifungal efficacy against pathogenic fungi including aromatic metabolites, pyrones, terpenes, butenolides, and polyketides. The metabolites could inhibit the production of ergosterol (the primary sterol in fungi) and also impede the synthesis of macromolecules, resulting in toxicity to plant pathogens (Ghannoum and Rice, 1999).

This study discovered that different *Trichoderma* isolates may produce various amounts and toxicity of VOCs, resulting in varying levels of pathogen inhibition. Even while both *T. virens* T4RH and *T. asperellum* T8R have demonstrated the potential ability to inhibit the growth of *G. boninense* G4 *in vitro*, the results are insufficient to conclude that these isolates are suitable BCAs for application in the field. Therefore, both T4RH and T8R were selected for further examination *in planta* to gain comprehensive and conclusive data as screening methods for selecting potential BCAs.

In planta antagonistic activities of Trichoderma species against Ganoderma boninense

The results of the in plant trials in the nursery showed that treatment with the local *Trichoderma* isolates T3, T4, and T5 significantly reduced the signs and symptoms of disease in seedlings infected with *G. boninense* compared to the untreated positive seedlings (T2). The untreated negative control (T1) showed no signs and symptoms of disease. The application of a single *Trichoderma* species, either *T. virens* or *T. asperellum*, showed similar efficacy *in planta* inhibition of *G. boninense* infection. Compared to the positive control, both *T. virens* and *T. asperellum* significantly reduced DI, DSI, AUDPC, and the percentage of necrotic primary roots by 73.33–76.67%, 30–34%, 41.67–55.00 unit, and 41.05–42.81%, respectively, when applied singly. While mixed applications of *T.*

virens and *T. asperellum* have lower efficacy than *T. virens* alone. This was evident in the decrease of DI and DSI by 13.34% and 8%, respectively, as shown in Figure 2.

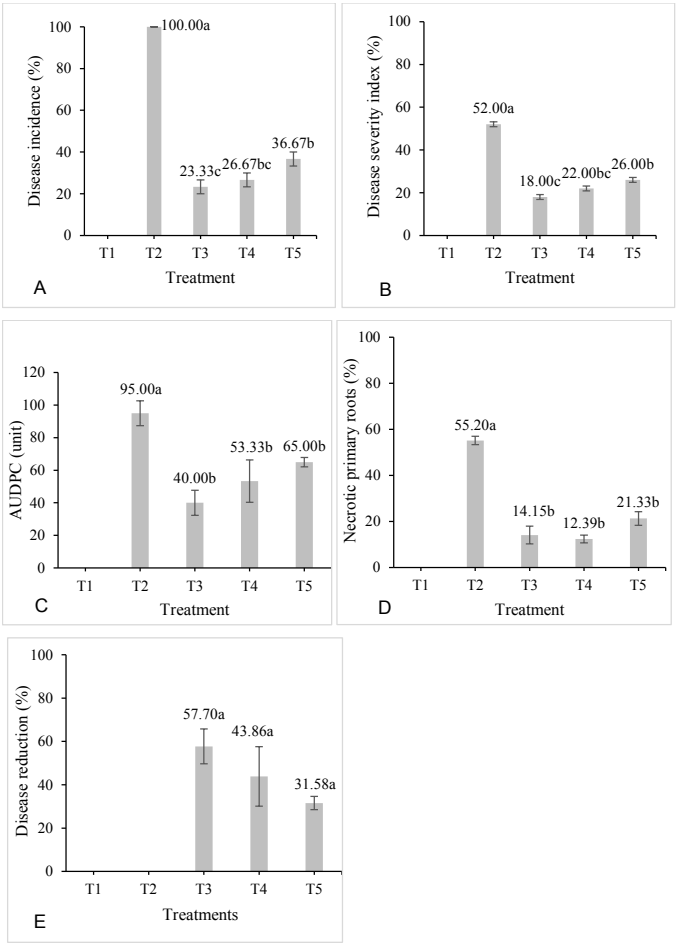

Figure 2: *(A) Disease incidence, (B) disease severity index, (C) area under disease progress curve (AUDPC), (D) percentage of necrotic primary roots, and (E) percentage of disease reduction in oil palm seedlings after 9 months after inoculated with Ganoderma boninense.* **Note:** *T1: Untreated (Negative control), T2: Untreated (Positive control), T3: T. virens, T4: T. asperellum and T5: T. virens and T. asperellum. Means (± Standard error) followed by different letters within the column were significantly different at p ≤ 0.05 by Tukey's test.*

This finding is consistent with the work of You *et al.* (2016), who attributed the ability of *Trichoderma* to control plant diseases to various mechanisms, including competition, mycoparasitism, the formation of a restrictive structure, antibiosis, secondary metabolite formation, as well as the induction of resistance in plants. The antagonistic mechanisms are not mutually exclusive, and a specific mechanism may belong to multiple categories. Additionally, *Trichoderma* species have been reported to have rapid growth, thus colonizing the substrate faster, and reducing the activity of other fungi by occupying and depleting the substrate (Martin and Loper, 1999; Bizos *et al.*, 2020). The beneficial microorganisms fight against infections at infection sites, reducing the chances for pathogens to proliferate, and produce secondary metabolites on the plant surface, and allowing them to parasitise directly. Moreover, *Trichoderma* species are known to produce various enzymes, such as chitinases, proteases, and cellulases, that can degrade pathogen cell walls and reduce pathogen virulence (Musa *et al.*, 2017). Some strains of *Trichoderma* can also produce secondary metabolites, such as peptaibols, gliotoxin, harzianic acid, and trichokonins, which exhibit antifungal activity against a wide range of plant pathogens (Harman *et al.*, 2004). Furthermore, *Trichoderma* sp. can induce plant systemic resistance which is a long-lasting and broad-spectrum resistance against a variety of pathogens. This resistance is achieved by activating the plant's defense mechanisms, such as the production of phytoalexins, pathogenesis-related proteins, and lignin (Harman *et al.*, 2004).

The relatively lower efficacy reported in this study in inhibiting *G. boninense* infection by the mixed application of two *Trichoderma* species is shown in Figure 1. This is consistent with a study conducted by Shamala (2013), which was associated with interspecific competition between the two isolates. Behavioral or chemical mechanisms that restrict one organism's access to the substrate are known as interference competition, and they can result in mycelial interactions within or between different species (Wicklow, 1992). The non-synergistic effect of *T. virens* and *T. asperellum* did not result in significant disease suppression due to the strong antagonism between the isolates. Although mixed inoculation of biological control agents cannot be completely disregarded, it is suggested that a combination of compatible isolates or a mixture of species plays a crucial role in studying the effect of single or mixed *Trichoderma* isolates. The use of compatible isolates or a mixture of species may be necessary to achieve the desired effect in controlling diseases in crops.

As shown in Table 5, the suppression of *G. boninense* infection by *Trichoderma* species (in T3, T4, and T5) has been attributed to significantly higher chlorophyll content, plant height, bole diameter, and number of fronds compared with untreated positive control (T2). However, no significant difference was found among different *Trichoderma* treatments. The highest levels of chlorophyll content (60.83%), plant height (118.33 cm), and palm diameter (19.50 cm), were

Table 5: *Chlorophyll content, plant height, bole diameter, and number of fronds of oil palm seedlings after nine months of inoculated with Ganoderma boninense.*

Treatment	Chlorophyll content (SPAD value)	Plant height (cm)	Bole diameter (cm)	Number of fronds
T1: Untreated (Negative control)	60.83±0.64a	118.33±1.59a	19.50±0.21a	7±0.33a
T2: Untreated (Positive control)	36.26±2.09c	83.33±0.67c	14.43±0.59c	5±0.33c
T3: *T. virens*	51.37±1.67b	101.77±1.19b	17.37±0.52b	6±0.58ab
T4: *T. asperellum*	51.72±1.62b	98.82±1.69b	16.27±0.20b	6±0.58ab
T5: *T. virens* and *T. asperellum*	49.50±0.37b	96.00±3.06b	15.83±0.24bc	6±0.33ab

Note: *Means (± standard error) followed by different letters within the column were significantly different at p≤ 0.05 by Tukey's test.*

recorded in the uninfected and untreated seedlings (T1). The relatively better physiological characteristics of *Trichoderma*-treated oil palm seedlings may be related to several factors, including direct colonisation, increased positive interaction with the plant, and increased nutrient uptake by the plant due to reduced activity of the pathogen (Harman, 2000). Studies have shown that oil palm seedlings inoculated with *T. virens* and *T. asperellum* have increased leaf and root biomass compared to the control (Shamala, 2013). The capability *Trichoderma* species to secrete plant growth-promoting hormones like auxins, gibberellins, and cytokinins may attributed to this phenomenon, as these substances promotes plant growth and development (Cornejo *et al.*, 2009). In addition, *Trichoderma* can also indirectly promote plant growth by inducing systemic resistance to pathogens. The induction of systemic resistance can increase the plant's defenses against pathogenic attacks, resulting in healthier and more vigorous plants (Benhamou, 1996; Yu *et al.*, 2022). The production of volatile organic compounds and hydrolytic enzymes by *Trichoderma* can also stimulate plant growth by enhancing nutrient acquisition and root growth (Hermosa *et al.*, 2013).

It is noteworthy that the isolated *T. virens* and *T. asperellum* were able to increase plant growth of oil palm seedlings in terms of height, stem diameter, number of fronds, and chlorophyll content in the present work. These findings agreed with those reported in other studies (Nusaibah *et al.*, 2016; Shamala, 2013; Muniroh *et al.*, 2019). *T. virens* and *T. asperellum* can produce IAA, the most active auxin (Li *et al.*, 2018; Muniroh *et al.*, 2019; Inayati *et al.*, 2021). IAA is a phytohormone that promotes plant development and growth both directly and indirectly, primarily through the modulation of other phytohormones, such as gibberellic acid (Stewart and Hill, 2014). Directly, IAA enhances root system development by increasing the number of lateral and adventitious roots, improving nutrient uptake, and stimulating root exudation, thereby providing additional resources for soil microbes to interact with the roots (Gamalero and Glick, 2011). In addition to improving the root system, IAA even in lower concentrations can directly enhance growth by promoting cell division and elongation (Brummell and Hall, 1987), and it has also been reported to enhance the fitness of plant-microbe interactions (Patten and Glick, 2002). This might contribute to an increase in plant performance in nursery trials. Therefore, it would be very useful to use these *Trichoderma* strains, which have been shown to be effective in controlling *Trichoderma* disease and promoting plant growth.

Conclusions and Recommendations

In conclusion, this study has identified two *Trichoderma* species, namely *T. virens* (T4RH) and *T. asperellum* (T8R), as potential BCAs against *G. boninense*. The promising results of *in vitro* assays revealed that both *T. virens* and *T. asperellum* exhibited high levels of inhibition against *G. boninense*. Moreover, *in planta* assessment of the local *Trichoderma* isolates showed significant suppression of *G. boninense* infection. The outcomes of this study suggest that *T. virens* T4RH and *T. asperellum* T8R can be considered effective BCAs to manage *G. boninense* infection. These findings contribute to the advancement of sustainable and environmentally friendly strategies for mitigating the detrimental effects of *G. boninense* on oil palm cultivation. However, further analyses, characterisation, and validation assays are necessary to substantiate and confirm the potential of the local *Trichoderma* isolates as effective BCAs against *G. boninense*.

Acknowledgments

Special thanks go to the Universiti Malaysia Sabah

(UMS) and Wilmar Plantations Sdn. Bhd. (formerly known as PPB Oil Palms Berhad) for their support in granting research funding through the Research Collaboration Grant (GKP0026-2019). The author also would like to thank the Malaysian Palm Oil Board (MPOB) Lahad Datu Research Station for allowing to use of their research facility to conduct the nursery trial and the Sabah Agriculture Department for providing oil palm planting material.

Novelty Statement

Local isolates of *Trichoderma* species from Sabah, Malaysia, were identified as potential biological control agents against *Ganoderma boninense*, a phytopathogen causing basal stem rot in oil palm. These potential BCAs could be mass-produced to further developed as a commercial product.

Author's Contribution

Nur Aainaa Hasbullah, Mohamadu Boyie Jalloh, Palanivell Perumal, Peter Mojiun, and Mohd. Rashid Mohd. Rakib: Conceptualised the ideas.
Jumiati Asis, Nur Aainaa Hasbullah, Mohamadu Boyie Jalloh, and Mohd. Rashid Mohd. Rakib: Designed the methodology.
Jumiati Asis: Wrote the original draft, performed the statistical analysis, investigation
Mohd. Rashid Mohd. Rakib, Palanivell Perumal and Peter Mojiun: Provided the research materials, reviewed and edited the draft.
Jumiati Asis, Noor Khairani Mohamad Basri: Reviewed and edited the draft.
Mohd. Rashid Mohd. Rakib and Nur Aainaa Hasbullah: supervised the research.
Mohd. Rashid Mohd. Rakib: Administered the project, acquired research funds.
All authors read and approved the final manuscript.

Conflict of interest
The authors have declared no conflict of interest.

References

Alam, A.F., A.C. Er and H. Begum. 2015. Malaysian oil palm industry: Prospect and problem. J. Food, Agric. Environ., 13(2): 143-148.

Angel, L.P.L., M.T. Yusof, I.S. Ismail, B.T.Y. Ping, I.N.A. Mohamed Azni, N.H. Kamarudin and S. Sundram. 2016. An *in vitro* study of the antifungal activity of *Trichoderma virens* 7b and a profile of its non-polar antifungal components released against *Ganoderma boninense*. J. Microbiol., 54(11): 732-744. https://doi.org/10.1007/s12275-016-6304-4

Assis, K., C.K. Phin, I.A. Seman, D. Gabda and H.C. Mun. 2021. Estimating the yield loss of oil palm due to *Ganoderma* basal stem rot disease by using bayesian model averaging. J. Oil Palm Res., 33(1): 46-55.

Baca, M.A.M, C.U. García, S.P. Álvarez, M.A.F. Córdova, C.M.E. Bonilla, M.A.M. Tapia and E.S. Chávezd. 2022. Morphological and molecular characterization of a new autochthonous Trichoderma sp. isolate and its biocontrol efficacy against *Alternaria* sp. Saudi. J. Biol. Sci., 29(4): 2620-2625. https://doi.org/10.1016/j.sjbs.2021.12.052

Baiyee, B., C. Pornsuriya, S.I. Ito and A. Sunpapao. 2019. *Trichoderma spirale* T76-1 displays biocontrol activity against leaf spot on lettuce (*Lactuca sativa* L.) caused by *Corynespora cassiicola* or *Curvularia aeria*. Biol. Cont., 129: 195-200. https://doi.org/10.1016/j.biocontrol.2018.10.018

Benhamou, N., 1996. Elicitor-induced plant defense pathways. Trends. Plant Sci., 1(9): 233-240. https://doi.org/10.1016/S1360-1385(96)86901-0

Bhat, K.A., 2017. A new agar plate assisted slide culture technique to study mycoparasitism of Trichoderma sp. on *Rhizoctonia solani* and *Fusarium oxysporium*. Int. J. Curr. Microbiol. Appl. Sci., 6(8): 3176-3180. https://doi.org/10.20546/ijcmas.2017.608.378

Bio-Pesticide Data Base. 2021. Bio-Pesticide Database, University of Hertfordshire, Hatfield, Hertfordshire, UK (http://sitem.herts.ac.uk/aeru/bpdb/). Retrieved on Sep 20, 2022.

Birkenbihl, R.P., S. Liu and I.E. Somssich. 2017. Transcriptional events defining plant immune responses. Curr. Opin. Plant Biol., 38: 1-9. https://doi.org/10.1016/j.pbi.2017.04.004

Bivi, M.R., M.S.N. Farhana, A. Khairulmazmi and A. Idris. 2010. Control of *Ganoderma boninense*: A causal agent of basal stem rot disease in oil palm with endophyte bacteria *in vitro*. Int. J. Agric. Biol., 12(6): 833-839.

Bizos, G., E.M. Papatheodorou, T. Chatzistathis, N. Ntalli, V.G. Aschonitis and N. Monokrousos. 2020. The role of microbial inoculants on

plant protection, growth stimulation, and crop productivity of the olive tree (*Olea europea* L.). Plants, 9(6): 743. https://doi.org/10.3390/plants9060743

Brummell, D.A. and J.L. Hall. 1987. Rapid cellular responses to auxin and the regulation of growth. Plant, Cell Environ., 10(7): 523-543. https://doi.org/10.1111/j.1365-3040.1987.tb01833.x

Collinge, D.B., D.F. Jensen, M. Rabiey, S. Sarrocco, M.W. Shaw and R.H. Shaw. 2022. Biological control of plant diseases. What has been achieved and what is the direction? Plant Pathol., 71: 1024-1047. https://doi.org/10.1111/ppa.13555

Cornejo, H.A.C., L.M. Rodríguez, C.C. Penagos and J.L. Bucio. 2009. *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in *Arabidopsis*. Plant Physiol., 149(3): 1579-1592. https://doi.org/10.1104/pp.108.130369

Cornejo, H.A.C., L.M. Rodríguez, E. del-Val and J. Larsen. 2020. Interactions of *Trichoderma* with plants, insects, and plant pathogen microorganisms: chemical and molecular bases. Co-evolution of secondary metabolites. 1st ed. Springer, Mexico.

Darlis, D., M.B. Jalloh, C.F.S. Chin, N.K.M. Basri, N.A. Besar, K. Ahmad and M.R.M. Rakib. 2023. Exploring the potential of Bornean polypore fungi as biological control agents against pathogenic *Ganoderma boninense* causing basal stem rot in oil palm. Sci. Rep., 13: 10316. https://doi.org/10.1038/s41598-023-37507-0

Debbi, A., H. Boureghda, E. Monte and R. Hermosa. 2018. Distribution and genetic variability of *Fusarium oxysporum* associated with tomato diseases in Algeria and a biocontrol strategy with indigenous *Trichoderma* spp. Front. Microbiol., 9: 282. https://doi.org/10.3389/fmicb.2018.00282

Dukare, A.S., R.K. Singh, R.K. Jangra and B. Bhushan. 2022. Non-fungicides-based promising technologies for managing post-production *Penicillium* induced spoilage in horticultural commodities: A comprehensive review. Food Rev. Int., 38(3): 227-267. https://doi.org/10.1080/87559129.2020.1727497

Gamalero, E. and B.R. Glick. 2011. Mechanisms used by plant growth-promoting bacteria. Bacteria in agrobiology: Plant nutrient management. 1st edn. Springer, Italy. https://doi.org/10.1007/978-3-642-21061-7_2

Ghannoum, M.A. and L.B. Rice. 1999. Antifungal agents: Mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. Clin. Microbiol. Rev., 12(4): 501-517. https://doi.org/10.1128/CMR.12.4.501

Harman, G.E., 2000. Myths and dogmas of biocontrol changes in perceptions derived from research on *Trichoderma harzinum* T-22. Plant Dis., 84(4): 377-393. https://doi.org/10.1094/PDIS.2000.84.4.377

Harman, G.E., 2006. Overview of mechanisms and uses of *Trichoderma* spp. Phytopathology, 96(2): 190-194. https://doi.org/10.1094/PHYTO-96-0190

Harman, G.E., C.R. Howell, A. Viterbo, I. Chet and M. Lorito. 2004. *Trichoderma* species opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol., 2(1): 43-56. https://doi.org/10.1038/nrmicro797

Harshita, A., J.B. Sinha, S. Khan, A. Trivedi and G. Rao. 2018. Compatibility of fungal and bacterial bio-agents and their antagonistic effect against *Fusarium oxysporum* f. Sp. Lycopersici. Int. J. Curr. Microbiol. Appl. Sci., 7(7): 2305-2316. https://doi.org/10.20546/ijcmas.2018.707.269

Hermosa, R., M.B. Rubio, R.E. Cardoza, C. Nicolás, E. Monte and S. Gutiérrez. 2013. The contribution of *Trichoderma* to balancing the costs of plant growth and defense. Int. Microbiol., 16(2): 69-80.

Hirpara, D.G., H.P. Gajera, H.Z. Hirpara and B.A Golakiya. 2017. Antipathy of *Trichoderma* against *Sclerotium rolfsii* Sacc.: Evaluation of cell wall-degrading enzymatic activities and molecular diversity analysis of antagonists. Microb. Physiol., 27(1): 22-28. https://doi.org/10.1159/000452997

Hushiarian, R., N.A. Yusof and S.W. Dutse. 2013. Detection and control of *Ganoderma boninense*: Strategies and perspectives. Springer Plus, 2: 1-12. https://doi.org/10.1186/2193-1801-2-555

Inayati, A., L. Setyowati, L.Q. Aini and E. Yusnawan. 2021. Plant growth promoter produced by *Trichoderma virens* and its effect on mungbean (*Vigna radiata* (L.) Wilczek) seedling. IOP Conf. Ser. Earth Environ. Sci.,

803: 012013. https://doi.org/10.1088/1755-1315/803/1/012013

Javeed, M.T., T. Farooq, A.S. Al-Hazmi, M.D. Hussain and A.U. Rehman. 2021. Role of *Trichoderma* as a biocontrol agent (BCA) of phytoparasitic nematodes and plant growth inducer. J. Invertebr. Pathol., 183: 107626. https://doi.org/10.1016/j.jip.2021.107626

Jinantana, J. and M. Sariah. 1998. Potential for biological control of *Sclerotium* foot rot of chilli *Trichoderma* spp. Pertanika J. Trop. Agric. Sci., 21(1): 1-10.

John, N.S., I.P. Anjanadevi, V.S. Nath, S.A. Sankar, M.L. Jeeva, K.S. John and R.S. Misra. 2015. Characterization of *Trichoderma* isolates against *Sclerotium rolfsii*, the collar rot pathogen of Amorphophallus. A polyphasic approach. Biol. Cont., 90: 164-172. https://doi.org/10.1016/j.biocontrol.2015.07.001

Khan, R.A.A., S. Najeeb, S. Hussain, B. Xie and Y. Li. 2020. Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic fungi. Microorganisms, 8(6): 817. https://doi.org/10.3390/microorganisms8060817

Küçük, C. and M. Kıvanç. 2004. *In vitro* antifungal activity of strains of *Trichoderma harzianum*. Turk. J. Biol., 28(2): 111-115.

Li, Y.T., S.G. Hwang, Y.M. Huang and C.H. Huang. 2018. Effects of *Trichoderma asperellum* on nutrient uptake and Fusarium wilt of tomato. Crop Prot., 110: 275-282. https://doi.org/10.1016/j.cropro.2017.03.021

Lim, K.H., S.S. Lim, F. Parish and R. Suharto. 2013. RSPO manual on best management practices (BMPs) for existing oil palm cultivation on peat. 1st ed. RSPO, KL, Malaysia.

Liu, P., Y. Cheng, M. Yang, Y. Liu, K. Chen, C.A. Long and X. Deng. 2014. Mechanisms of action for 2-phenylethanol isolated from *Kloeckera apiculata* in control of Penicillium molds of citrus fruits. BMC Microbiol., 14(1): 1-14. https://doi.org/10.1186/s12866-014-0242-2

Malaysian Palm Oil Board (MPOB). 2016. MPOB code of practice - code of good nursery practice for oil palm nurseries. 2nd ed. MPOB, Malaysia.

Marques, E., I. Martins and S.C.M.D. Mello. 2018. Antifungal potential of crude extracts of *Trichoderma* spp. Biota Neotrop., 18(1): 1676-0611. https://doi.org/10.1590/1676-0611-bn-2017-0418

Martin, F.N. and J.E. Loper. 1999. Soilborne plant diseases caused by *Pythium* spp.: Ecology, epidemiology, and prospects for biological control. Crit. Rev. Plant Sci., 18(2): 111-181. https://doi.org/10.1016/S0735-2689(99)00389-5

Muniroh, M.S., S.A Nusaibah, G. Vadamalai and Y. Siddique. 2019. Proficiency of biocontrol agents as plant growth promoters and hydrolytic enzyme producers in *Ganoderma boninense* infected oil palm seedlings. Curr. Plant Biol., 20: 100116. https://doi.org/10.1016/j.cpb.2019.100116

Musa, H., M.A. Hassan, M.S. Isyaku, J. Halidu and A.S. Suleiman. 2017. Antagonistic potential of Trichoderma species against Ganoderma disease of oil palm. Niger. J. Agric. Food Environ., 13(2): 60-67.

Nusaibah, S.A., A.S.N. Akmar, A.S. Idris, M. Sariah and Z.M. Pauzi. 2016. Involvement of metabolites in early defense mechanism of oil palm (*Elaeis guineensis* Jacq.) against *Ganoderma* disease. Plant Physiol. Biochem., 109: 156-165. https://doi.org/10.1016/j.plaphy.2016.09.014

Pan, S. and S. Bhagat, 2007. Antagonistic potential of *Trichoderma* and *Gliocladium* spp. from West Bengal. J. Myco. Plant Pathol., 37(2): 235-239.

Patten, C.L. and B.R. Glick. 2002. Role of Pseudomonas putida indoleacetic acid in development of the host plant root system. Appl. Environ. Microbiol., 68(8): 3795-3801. https://doi.org/10.1128/AEM.68.8.3795-3801.2002

Prasad, R.D. and R. Rangeshwaran, 1999. Granular formulation of *Trichoderma* and *Gliocladium* spp. in biocontrol of *Rhizoctonia solani* of chickpea. J. Mycol. Plant Pathol., 29(20): 222-226.

Rahman, M.A., M.F. Begum and M.F. Alam. 2009. Screening of *Trichoderma* isolates as a biological control agent against *Ceratocystis paradoxa* causing pineapple disease of sugarcane. Mycobiol., 37(4): 277-285. https://doi.org/10.4489/MYCO.2009.37.4.277

Rajani, P., C. Rajasekaran, M.M. Vasabthakumari, S.B. Olsson, D. Ravikanth and R.U Shaanker. 2021. Inhibition of plant pathogenic fungi by endophytic *Trichoderma* spp. through mycoparasitism and volatile organic compounds. Microbial Res., 242: 126595. https://doi.org/10.1016/j.micres.2020.126595

Rakib, M.R.M., A.H. Borhan and A.N. Jawahir. 2019. The relationship between SPAD chlorophyll and disease severity index in

Ganoderma-infected oil palm seedlings. J. Bangladesh Agric. Univ., 17(3): 355-358. https://doi.org/10.3329/jbau.v17i3.43211

Rakib, M.R.M., C.F.J. Bong, A. Khairulmazmi and A.S. Idris. 2014. Genetic and morphological diversity of *Ganoderma* species isolated from infected oil palms (*Elaeis guineensis*). Int. J. Agric. Biol., 16(4): 691-699.

Rakib, M.R.M., C.F.J. Bong, A. Khairulmazmi and A.S. Idris. 2015. Aggressiveness of *Ganoderma boninense* and *G. zonatum* isolated from upper- and basal stem rot of oil palm (*Elaeis guineensis*) in Malaysia. J. Oil Palm Res., 27(3): 229-240.

Rees, R.W., J. Flood, Y. Hasan, M.A. Wills and R.M. Cooper. 2012. *Ganoderma boninense* basidiospores in oil palm plantations: evaluation of their possible role in stem rots of *Elaeis guineensis*. Plant Pathol., 61(3): 567-578. https://doi.org/10.1111/j.1365-3059.2011.02533.x

Reino, J.L., R.F. Guerrero, R. Hernandez-Galan and I.G. Collado. 2008. Secondary metabolites from species of the biocontrol agent *Trichoderma*. Phytochem. Rev., 7: 89-123. https://doi.org/10.1007/s11101-006-9032-2

Sankar, P. and R. Sharma. 2001. Management of charcoal rot of maize with *Trichoderma viride*. Indian Phytopath., 55(3): 390-391.

Shamala, S., 2013. The effects of *Trichoderma* in surface mulches supplemented with conidial drenches in the disease development of *Ganoderma* basal stem rot in oil palm. J. Oil Palm Res., 25(3): 314-325.

Siddiquee, S., B.E. Cheong, K. Taslima, H. Kausar and M.M. Hasan. 2012. Separation and identification of volatile compounds from liquid cultures of *Trichoderma harzianum* by GC-MS using three different capillary columns. J. Chromatogr. Sci., 50(4): 358-367. https://doi.org/10.1093/chromsci/bms012

Siddiquee, S., U.K. Yusuf, K. Hossain and M.S. Jahan. 2009. *In vitro* studies on the potential *Trichoderma harzianum* for antagonistic properties against *Ganoderma boninense*. J. Food Agric. Environ., 7: 970-976.

Stewart, A. and R. Hill. 2014. Applications of *Trichoderma* in plant growth promotion. Chapter 31: 415-428. In: Biotechnology and biology of *Trichoderma*. Elsevier, New Zealand. https://doi.org/10.1016/B978-0-444-59576-8.00031-X

Stoppacher, N., B. Kluger, S. Zeilinger, R. Krska and R. Schuhmacher. 2010. Identification and profiling of volatile metabolites of the biocontrol fungus *Trichoderma atroviride* by HS-SPME-GC-MS. J. Microbiol. Methods, 81(2): 187-193. https://doi.org/10.1016/j.mimet.2010.03.011

Susanto, A., P.S. Sudharto and R.Y. Purba. 2005. Enhancing biological control of basal stem rot disease (*Ganoderma boninense*) in oil palm plantations. Mycopathologia, 159(1): 153-157. https://doi.org/10.1007/s11046-004-4438-0

Ting, A.S.Y., S.W. Mah and C.S. Tee. 2010. Identification of volatile metabolites from fungal endophytes with biocontrol potential towards *Fusarium oxysporum* F. sp. cubense race 4. Am. J. Agric. Biol. Sci., 5(2): 177-182. https://doi.org/10.3844/ajabssp.2010.177.182

Tomah, A.A., I.S. Abd Alamer, B. Li and J.Z. Zhang. 2020. A new species of *Trichoderma* and gliotoxin role: A new observation in enhancing biocontrol potential of *T. virens* against *Phytophthora capsici* on chili pepper. Biol. Cont., 145: 104261. https://doi.org/10.1016/j.biocontrol.2020.104261

United States Department of Agriculture (USDA). 2022. Oilseeds: World markets and Trade. Washington, United States (www.usda.gov). Retrieved on Sep 20, 2023.

Vila, A.G., N. Teixidó, A.D. Francesco, J. Usall, L. Ugolini, R. Torres and M. Mari. 2017. Antifungal effect of volatile organic compounds produced by *Bacillus amyloliquefaciens* CPA-8 against fruit pathogen decays of cherry. Food Microbiol., 64: 219-225. https://doi.org/10.1016/j.fm.2017.01.006

Weindling, R., 1934. Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. Phytopathology, 24(11): 1153-1179.

Wicklow, D.T., 1992. Interference competition. The fungal community: Its organization and role in the ecosystem: 2nd ed. USA.

You, J., J. Zhang, M. Wu, L. Yang, W. Chen and G. Li. 2016. Multiple criteria-based screening of *Trichoderma* isolates for biological control of *Botrytis cinerea* on tomato. Biol. Cont., 101: 31-38. https://doi.org/10.1016/j.biocontrol.2016.06.006

Yu, Y., Y. Gui, Z. Li, C. Jiang, J. Guo and D. Niu. 2022. Induced systemic resistance for improving plant immunity by beneficial microbes. Plants, 11(3): 386. https://doi.

org/10.3390/plants11030386

Zaidi, N.W., M.H. Dar, S. Singh and U.S. Singh. 2014. *Trichoderma* species as abiotic stress relievers in plants. In: Biotechnology and Biology of *Trichoderma*. Elsevier, India. Chapter 38: 515-525. https://doi.org/10.1016/B978-0-444-59576-8.00038-2

Zin, N.A. and N.A. Badaluddin. 2020. Biological functions of *Trichoderma* spp. for agriculture applications. Ann. Agric. Sci., 65(2): 168-178. https://doi.org/10.1016/j.aoas.2020.09.003