

Original Research Article

Total Phenolic Content, Peroxidase and Polyphenoloxidase Activities in *Ganoderma* Infected Oil Palm Seedlings – Inoculated with Arbuscular Mycorrhiza Fungi (AMF)

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Abstract: Basal stem rot disease caused by *Ganoderma boninense* is the most predominant disease of oil palm plantations in Southeast Asia. Although an effective method for controlling the disease is elusive, research towards early detection and remedy for disease control measures remains crucial for plantation operation. Arbuscular mycorrhiza fungi (AMF) offers a practical alternative that should be seriously considered and implemented. This study evaluated the enzyme activities (TPC, PPO, PO activity) in oil palm seedlings inoculated with arbuscular mycorrhizal fungi (AMF) and infected with *G. boninense*. Artificial inoculation was conducted using oil palm germinated seeds in the nursery, and plant responses were analysed at 8, 12, 16, and 20 weeks of post-inoculation (WPI). At 20 WPI, the highest accumulation of total phenolic content (TPC) was recorded in T3 (AMF + *G. boninense*) with 6.09 mg/g compared to T1 (untreated), T2 (*G. boninense*), and T4 (AMF), which recorded TPC of 3.24 mg/g, 4.19 mg/g and 3.87 mg/g respectively with no significant difference indicating the natural presence of phenol compound in healthy tissue at concentration enough for defense, whether as free compound or in the conjugated form which released after the attack. Both T2 and T3 showed higher peroxidase (PO) levels with 122.8 unit/g tissue and 116.5 unit/g tissue compared to T1 (73.63 unit/g tissue) and T4 (80.6 unit/g tissue). The polyphenoloxidase (PPO) activity was also higher in both T2 (142.3 unit/g tissue) and T3 (111.7 unit/g tissue) compared to T1 (55.3 unit/g tissue) and T4 (36.3 unit/g tissue). Seedlings treated with AMF also showed increased plant growth compared to untreated seedlings. It was observed that T3 seedlings with AMF prophylactic treatment showed the highest chlorophyll content even with *G. boninense* infection.

Keywords: arbuscular mycorrhiza fungi; *Ganoderma boninense*; total phenolic content; peroxidase; polyphenoloxidase

Received: 5th August 2020

Received in revised form: 12th June 2021

Accepted: 16th June 2021

Available Online: 1st July 2021

Citation: Saallah, S., Md Noh, N., Mohd. Azmi, M. A., *et al* (2021). Total phenolic content, peroxidase and polyphenoloxidase activities in *ganoderma* infected oil palm seedlings – inoculated with arbuscular mycorrhiza fungi (AMF) *Adv Agri Food Res J* 2021; In-Press: a0000235. <https://doi.org/10.36877/aafrj.a0000235>

1. Introduction

Oil palm (*Elaeis guineensis* Jacq) is among the essential perennial crops globally, with Malaysia and Indonesia dominating approximately 85% of world palm oil productions (Gunarso *et al.*, 2013). However, for decades, its cultivation was constrained by *Ganoderma boninense* basal stem rot (BSR) disease. A severe disease infection could destroy thousands of hectares of oil palm plantations. Roslan and Idris (2012) reported that the *Ganoderma* attack could lead to fresh fruit bunches (FFB) yield reduction between 0.04 t and 4.34 t/ha on ten years to 22 years of planting, respectively. Based on the annual growth of *Ganoderma* incidence rate, it was estimated that in 2020, a total of 400 thousand hectares (65.6 million of palm trees) could be affected. The current approach primarily relies on chemical treatments, including organic and inorganic, to create hazardous effects on the environment and human beings. Researchers are actively exploring an eco-friendly approach to eliminate any toxic elements which cause potential harm to beneficial microbes and the soil environment (Aziz & Campus, 2018). Therefore, the application of arbuscular mycorrhiza fungi (AMF) is one option that might benefit oil palm cultivation in both agronomic plant health and ecosystems (Phosri *et al.*, 2010).

AMF are abundant and known to perform many ecosystem functions with associations over 90% of all plant species (Bonfante & Genre, 2010). Most terrestrial plants have a mutualistic association with arbuscular mycorrhizal fungi (AMF) by growth inside the plant roots' cortical cells and give beneficial values (Auliana & Kaonongbua, 2018). AMF role as bio-fertilizer as well as bio-enhancer of plant growth has been studied; AMF-inoculated plants such as patchouli (Arpana *et al.*, 2008), soybean (Meghvansi *et al.*, 2008), tomato (Salvioli *et al.*, 2008), and groundnut (Pawar *et al.*, 2018) had showed increased in vegetative plant growth compared to control. Oil palm seedlings inoculated with single species *Glomus etunicatum* resulted 80.36 % increase in growth response compared to the two species — consortium inocula (Sundram, 2010).

Although the importance of AMF symbiosis for bio-protection in plants is evident (Smith & Read 1997; Gianinazzi *et al.*, 2010) however, the mechanisms of action are still poorly understood because the observed effects are more likely to result from different factors that act together (Rodriguez *et al.*, 2001) which related to the competition for colonisation's and the improvement of plant defense systems (Diagne *et al.*, 2020). Borowicz *et al.* (2001) reported that AMF reduced pathogen growth in ~50% of studies included in χ^2 tests; the effects of AMF on pathogen are most likely indirect and resulted from improved nutrition or

altered physiology of the host. Healthier mycorrhizal plants directly or indirectly produce more vigorous seedlings with higher and stronger internal resistance to ward off disease; higher density roots produced in the presence of mycorrhiza can compensate for the loss of roots caused by disease infections; significant Ca deposition in mycorrhizal cells creates a physical barrier to disease advancement in the palm roots while higher deposition of secondary metabolites by mycorrhizal roots inhibits pathogen spread in the palm roots (Azizah, 2003).

In this study, the activities of plant defense-related enzymes such as peroxidase (PO), polyphenoloxidase (PPO), and total phenolic content (TPC) that act as anti-microbial, structural barrier, growth inhibitor of invader, modulator of pathogenicity, and activators of plant defense genes (Rao *et al.*, 2015) in oil palm seedlings treated with AMF but infected with *G. boninense* were investigated.

2. Materials and Methods

2.1 Source of *G. boninense* Isolate and AMF

Ganoderma boninense isolate PER71 obtained from *Ganoderma* & Diseases Research of Oil Palm Unit Laboratory, MPOB was used in this experiment. The culture was maintained at the Crop Protection Unit, Sime Darby Plantation, R&D Centre, Banting, Selangor, Malaysia. *Ganoderma* pathogenicity test, as described by Idris *et al.* (2006), was used. Meanwhile, the AMF product named “ARMYCORR” produced by Crop Protection Unit, Sime Darby Plantation, R&D Centre, was used in this study. The product contains a mixture of five AMF species *viz.* *Glomus* species, *Glomus clarum*, *Acaulospora tuberculata*, *Kuklospora columbiana*, and *Paraglomus* species, with about 200–250 spores per 10 grams of the product.

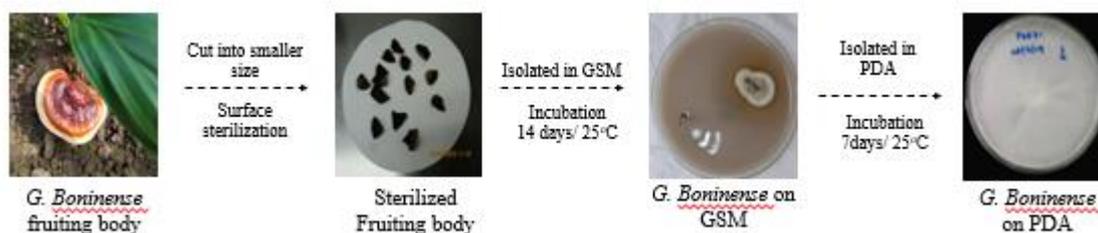


Figure 1. Isolation and preparation of *G. boninense* pure culture.

2.2 Rubber Wood Block Preparation

The *Ganoderma* fungus was grown on the rubber woodblock (RWB) size 6 x 6 x 6 cm

as an artificial inoculum of *Ganoderma* BSR. All RWBs were washed and dried in an oven at 80°C overnight, autoclaved at 121 °C for one hour. Then, each RWB was placed in an autoclavable plastic bag, and 120 ml of malt extract broth was added. Bags were sealed and autoclaved at 121 °C for 15 minutes and left overnight to solidify. A 7-day-old *Ganoderma* grown on a PDA plate was cut into four parts, and one-half of agar blocks were inoculated onto the RWB. The inoculated RWBs were incubated at 25–28°C with 60–70% relative humidity for about 90 days. By the end of this time, the *Ganoderma* mycelia would have completely covered the RWB and used it in this experiment.

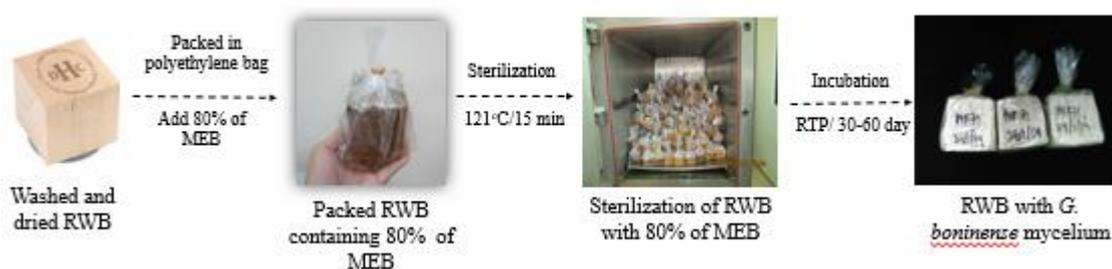


Figure 2. Preparation of RWB as *G. boninense* substrate.

2.3 AMF and *Ganoderma* Inoculation

The trial was laid out in randomised complete block design (RCBD) with three replications using oil palm germinated seeds cultivar (*Dura* × *Pisifera*) supplied by Sime Darby Plantation Seeds and Agricultural Services Sdn. Bhd., Banting, Selangor, Malaysia. AMF inoculum was applied during seeds sowing. The AMF product at a rate of 50 grams per seed was placed at the center of the polybag (15 x 23 cm²); the seeds were then placed on top of the inoculum before being covered with the topsoil (up to 1 cm from the surface). The seed radicle should be in direct contact with the AMF inoculum. No AMF was applied to T1 and T2 treatments.

Meanwhile, for T2 and T3 treatments, the germinated seeds were subjected to *Ganoderma* inoculated RWB using modified sitting technique as described by Idris *et al.* (2001); Besides inoculation of the *Ganoderma* – RWB inoculum, 50 grams of AMF was used during seeds sowing for treatment T3. Destructive sampling for enzyme analysis was carried out at 8, 12, 16, and 20 weeks of post-inoculation (WPI). Based on observation, it was found that 8-weeks of post-inoculation is the most appropriate time to sample the leaves for the analysis. All seedlings were grown in the nursery following Breton *et al.* (2009).

Table 1. Treatment details

Treatments	Descriptions
T1	Untreated / Control
T2	+ <i>Ganoderma boninense</i>
T3	50 gram AMF + <i>Ganoderma boninense</i>
T4	+ 50 gram AMF

2.4 Biochemical Analysis

2.4.1 Determination of total phenolic content (TPC)

The concentration of TPC in oil palm plantlets was measured using Folin's method (Goh *et al.*, 2016). The concentration was expressed as gallic acid equivalent (GAE)g⁻¹ of the leaves. Different concentration of gallic acid (0.0 mg/g, 0.2 mg/g, 0.4 mg/g, 0.6 mg/g, 0.8 mg/g and 1.0 mg/g) were used as a standard. Before TPC determination, the net absorbance value at 765 nm was compared against the standard curve.

2.4.2 Preparation of crude protein extraction for enzyme assays

The crude protein was isolated as Musa *et al.* (2018) described with slight modifications. The samples and chemicals used were maintained in ice cubes along the extraction process. A freshly harvested leaves were cut and macerated in liquid nitrogen using pestle and mortar to produce a fine powder and placed in ice cubes. To form a thick paste, one gram of sample powder was homogenised in 2 ml of cold sodium phosphate buffer (pH 5). The homogenate was then filtered through three layers of cheesecloth and followed by 20 min centrifuged (4,000 rpm for 20 min at four °C). The supernatant for each sample was collected and stored at -80 °C for PO and PPO analysis.

2.4.3 Determination of peroxidase (PO)

The crude protein extract (50 µl) was added into a tube containing 750 µl of reaction substrate (80 µl of 0.1 M sodium phosphate buffer [pH 6] and 250 µl of 1 mM hydrogen peroxide). About 500 µl of guaiacol was added to the tube. The solution mixture was incubated at room temperature for 30 minutes. The change in color intensity of the sample was measured based on absorbance reading at 470 nm using a spectrophotometer. The mixture of reaction substrate and guaiacol served as a blank. One unit of enzyme activity is defined as 0.001 changes in absorbance per min. The blank was prepared from the reaction substrate without adding the extract as a control (Kokkinakis & Brook, 1979; Musa *et al.*,

2018).

2.4.4 Determination of polyphenoloxidase (PPO)

The activity of PPO was verified through the changes of colour intensity of pyrrol products (Musa *et al.*, 2018). About 50 µl of crude protein extract was placed in a 2 ml tube containing 750 µl of 0.2 M sodium acetate buffer (pH 5) at four °C. Then, 100 µl of 0.02 M pyrogallol was added. The mixture was measured using a spectrophotometer at 410 nm against blank. The blank was prepared from the reaction substrate without adding the extract as a control (Kokkinakis & Brook, 1979; Musa *et al.*, 2018).

Enzyme activity was calculated as follow:

$$\text{PO or PPO Activity} = \frac{(\text{Abs}_{\text{final}} - \text{Abs}_{\text{initial}})}{0.001 \times t}$$

Where Abs_{final} is the final absorption of the sample, Abs_{initial} is the initial absorption of the sample, and t is the reaction time in minutes.

2.5 Assessment on the Plant Growth (Fresh weight and Plant height)

The effect on the growth of AMF on oil palm seedlings concerning *Ganoderma* disease was evaluated. The plant height (cm) was measured from ground level to the tip of leaves using measuring tape placed alongside the seedling. In addition, to determine the effect of all treatments on fresh weight, a random destructive sampling was carried out at 8, 12, 16, and 20 weeks of post inoculation (WPI). The total fresh weight (gram) was recorded using a digital weighing balance.

2.6 Assessment on the Chlorophyll Content

The content of chlorophyll a, b, and total chlorophyll was conducted as described by Arnon *et al.* (2018). One gram of leaves was cut into small pieces for each treatment and macerated in 80% of acetone (10 ml) using pestle and mortar. The mixture was stood on chillers for four hours, followed by centrifugation at 4000 rpm for 15 minutes. The supernatant was transferred into a volumetric flask while precipitate was re-dissolved in 10 ml of 80% acetone until the supernatant's color became colorless. The supernatant was pooled, and the absorbance was measured using a spectrophotometer at 480 nm, 645 nm, and

664 against the solvent. The chlorophyll content was calculated based on Arnon *et al.*, (2018) equations:

$$\text{Chl a: } 12.7 A_{663} - 2.69 A_{645} \quad (1)$$

$$\text{Chl b: } 22.9 A_{645} - 4.68 A_{663} \quad (2)$$

$$\text{Total Chl: } 20.2 A_{645} + 8.02 A_{663} \quad (3)$$

2.7 Statistical analysis

Statistical analysis of the data was carried out via analysis of variance (ANOVA) using MINITAB 14 software. The means were separated using Tukey's multiple range tests at $p < 0.05$, where the F-value was significant.

3. Results and Discussions

This study determined the enzyme activities in oil palm seedlings inoculated with arbuscular mycorrhizal fungi (AMF) with the presence of *G. boninense* disease. The main objectives targeting understanding the early activities of plant defense-relative enzymes in relation to Ganoderma disease development and AMF roles in oil palm were met; however, the Ganoderma disease symptom was not observed in seedlings T2 and T3 treatments within 20 weeks of trial assessment. Thus, the percentage of *Ganoderma* disease severity was not shared here.

3.1 Total Phenolic Content (TPC) Activity

At 20 weeks post-inoculation (WPI), there was a significant difference in TPC activity. The highest accumulation of total phenolic content (TPC) was recorded in T3 (AMF + *G. boninense*) with 6.09 mg/g compared to T1 (untreated), T2 (*G. boninense*), and T4 (AMF), which recorded a TPC of 3.24 mg/g, 4.19 mg/g and 3.87 mg/g respectively (Figure 3).

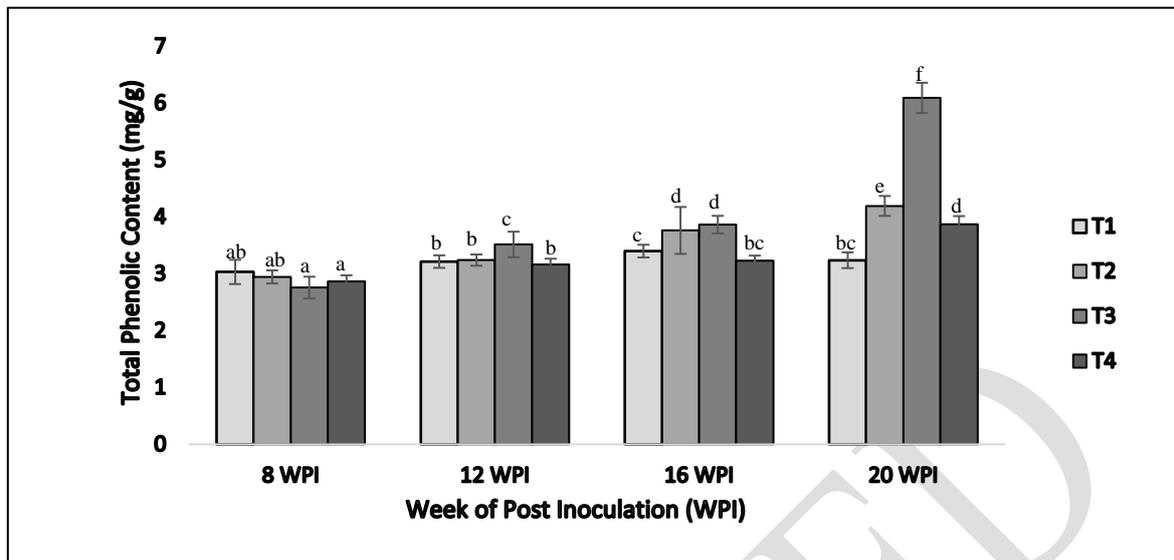


Figure 3. Total phenolic content of the treated plants; T1 (Untreated), T2 (+*G. boninense*), T3 (AMF + *G. boninense*), and T4 (+AMF) at 8, 12, 16, and 20 WPI. The bar represents the mean \pm standard deviation of three biological replicates. Using Tukey's test, different letters above each bar with the same color indicate significant differences between means ($P < 0.05$).

There was an increased pattern for TPC activity in T2 and T3 started at 12-weeks or third months' post-inoculation with *G. boninense*. This response indicates that oil palm seedlings produced and accumulated phenol to counter the *Ganoderma* attack. The production of phenol was highest in T3 because AMF helped the seedlings produce and accumulate more phenol to demonstrate the plant systemic defense. At the same time, it was observed that TPC activity in either T1 or T4 remains at a low level without a fungal attack. However, phenolic content in T4 is higher than in T1.

The phenolic is naturally present in healthy tissue at concentrations enough for defense, whether as free compounds or in conjugated form released after the fungal attack (Strack, 1997). These findings are consistent with the early study of cowpea infected with *Rhizoctonia* and *Fusarium* attacked chickpea and pigeon-pea due to the secretion of phenolic content from the cell wall structure during the infections (Datta & Lal, 2012). The increment of phenolic content in AMF inoculated seedlings was also concomitant with a previous study of *Ganoderma* infected oil palm seedlings inoculated with *Trichoderma spp.* (Musa *et al.*, 2018) which indicates the potential of treatment applied to suppress *G. boninense* activity.

3.2 Peroxidase (PO) Activity

At 20 WPI, both T2 and T3 showed higher peroxidase (PO) levels with 122.8 unit/g tissue and 116.5 unit/g tissue compared to T1 (73.63 unit/g tissue) and T4 (80.6 unit/g tissue)

(Figure 2). Likewise, it was observed that without *Ganoderma* infection, PO activity in either T1 or T4 remains at a low level with no significant difference. Thus, it was noted that the PO enzyme was increased in oil palm seedlings due to plant systemic defense against the *Ganoderma* infection.

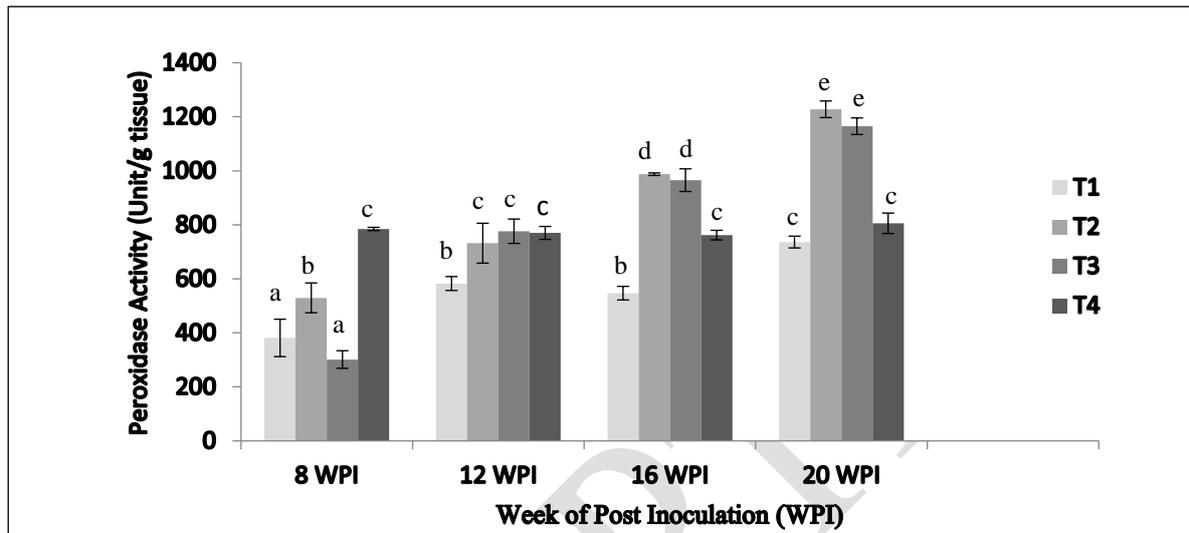


Figure 4. Peroxidase (PO) activity of the treated plants; T1 (Untreated), T2 (+*G. boninense*), T3 (AMF + *G. boninense*), and T4 (+AMF) at 8, 12, 16, and 20 WPI. The bar represents the mean \pm standard deviation of three biological replicates. Using Tukey's test, different letters above each bar with the same color indicate significant differences between means ($P < 0.05$).

The lowest PO activity was observed at 8-weeks for T3 treatment. This response could be due to the interaction between AMF and *Ganoderma* in oil palm seedlings as the result of inoculation at the same time. Azizah (2003) reported that most of the studies on AMF — pathogen interactions suggest pre-establishment (at least 3-months) of AMF in the roots of oil palm seedlings prior to *G. boninense* exposure AMF to start colonising the roots first before the pathogen. This is critical since both symbiont and pathogen fight for the same infection site on the rhizoplane and rhizosphere of the oil palm seedlings (Jalali & Jalali, 1991). Rodriguez *et al.* (2001) reported that when AMF colonisation is successful in plants, some fungal strategies of self-camouflage may occur, such as cell wall modification during the colonisation process or suppression of the induced plant defense mechanisms. Nevertheless, in this study, it was noted that AMF could compete with *Ganoderma* for the infection sites in the roots of oil palm seedlings. The findings support our recommendation that AMF could be utilised to combat the *Ganoderma* disease via prophylaxis application during the seeds stage.

3.3 Polyphenoloxidase (PPO) Activity

At 20 WPI, the polyphenoloxidase (PPO) activity was considered higher in both T2 (142.3 unit/g tissue) and T3 (111.7 unit/g tissue) compared to T1 (55.3 unit/g tissue) and T4 (36.3 unit/g tissue). (Figure 3). Likewise, it was observed that without *Ganoderma* infection, PPO activity in either T1 or T4 remains at a low level with no significant difference.

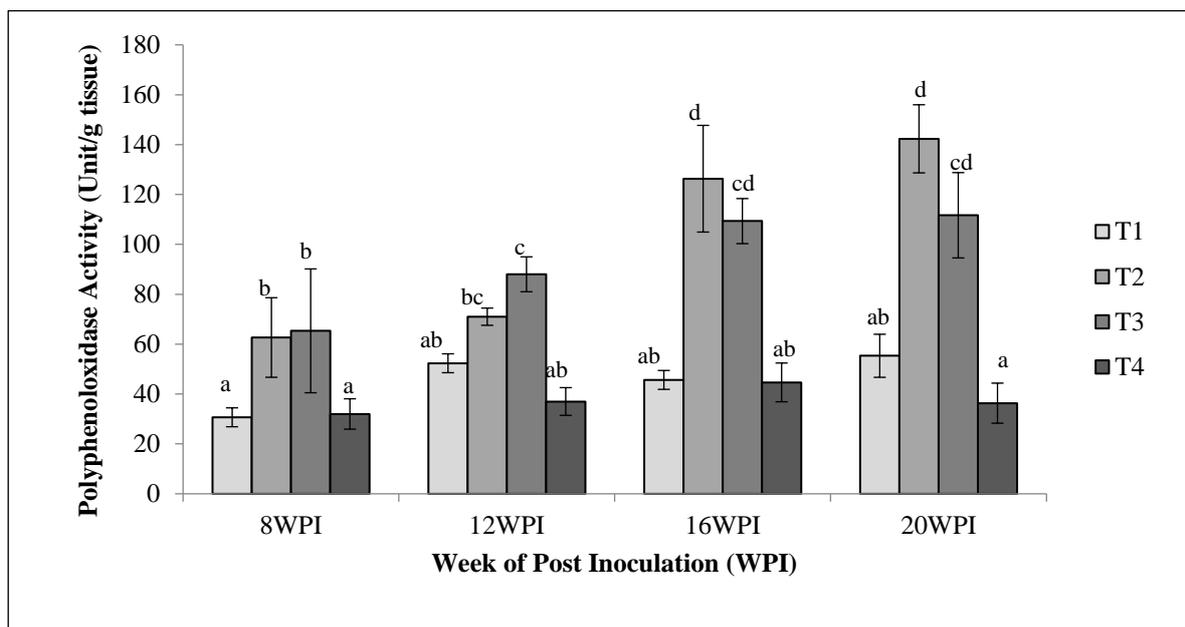


Figure 5. Polyphenoloxidase activity of the treated plants; T1 (Untreated), T2 (+*G. boninense*), T3 (AMF + *G. boninense*), and T4 (+AMF) at 8, 12, 16, and 20 WPI. The bar represents the mean \pm standard deviation of three biological replicates. Using Tukey's test, different letters above each bar with the same color indicate significant differences between means ($P < 0.05$).

The PO and PPO activities in plants are generally the defense mechanisms reaction by the plants responded to either pathogen attack or elicitor (biological control agents) treatment where various physical, chemical, and environmental stresses factors might also have contributed to the plant's reaction (Rodriguez *et al.*, 2001). These coincide with studies on hybrid poplar plants infected by *Melampsora medusae* (Miranda *et al.*, 2007) and *Jasminum grandiflorum* infected with *Uromyces hobsoni* (Jite & Tressa, 1999). Both PO and PPO activities were lower in T3 treatment than T2 treatment at 20 WPI. This could also be related to the AMF delaying the *Ganoderma* disease progression in the oil palm seedlings. However, the defense mechanism developed subsequent to microorganism penetration is difficult to be studied since all plants have a similar repertoire against pathogen attack (Rodriguez *et al.*, 2001).

In this study, our results showed that TPC, PO, and PPO activities were significantly in response to *G. boninense* infection in oil palm. With the presence of AMF and *Ganoderma* in the roots, oil palm will produce and accumulate more phenol to demonstrate the plant systemic defense. Our findings also indicated that AMF initially provoked a like-defense response in oil palm, subsequently suppressed. The result was indicated by the lower PO and PPO activities in oil palm roots at T4 treatment.

Indeed, it was suggested that there was higher compatibility between AMF and oil palm seedlings because of the lower induction of both PO and PPO enzymes in the AMF colonised plants. This result also corresponding with the study that mycorrhizal colonisation began on the first day after germination (Rodriguez *et al.*, 2001) and could be observed after two weeks of growth; the proliferation of the fungal mycelia become more vigorous after three to five months; where more colonisation activity was detected on new oil palm roots with AMF intercellular hyphae were predominant on the secondary roots rather than the primary roots (Normahnani *et al.*, 2013). Therefore, applying AMF during seeds sowing is highly recommended to protect the oil palm roots against *Ganoderma* infection.

3.4 Effect of AMF on the Plant Growth

At 20 WPI, both T3 and T4 seedlings treated with AMF showed increased plant growth (based on fresh weight and height measurement) compared to the untreated seedlings in T1 and T2 treatments (Table 2 and 3).

Table 2. Fresh weight (g) of the treated oil palm seedlings; T1 (Untreated), T2 (+ *G. boninense*), T3 (AMF + *G. boninense*), and T4 (+AMF) at weeks 8, 12, 16, and 20 of post inoculation (WPI).

Week of Post-Inoculation (WPI)	T1	T2	T3	T4
8	6.14 ± 0.57a	4.94 ± 0.26a	5.46 ± 0.88a	6.14 ± 0.76a
12	7.16 ± 0.82a	5.78 ± 0.33a	6.67 ± 1.27a	8.10 ± 0.30a
16	30.67 ± 2.08bc	31.00 ± 5.12bc	36.67 ± 4.16bc	37.67 ± 3.51bc
20	35.33 ± 10.12bc	39.68 ± 20.9c	43.67 ± 8.14cd	51.00 ± 10.0cd

*Data are mean values of three replicates ± standard deviation followed by the same alphabet are not significantly different $p < 0.05$ using Tukey test.

Table 3. Height (cm) of the treated oil palm seedlings; T1 (Untreated), T2 (+ *G. boninense*), T3 (AMF + *G. boninense*), and T4 (+AMF) at week 8, 12, 16, and 20 of post inoculation (WPI).

Week of Post- Inoculation (WPI)	T1	T2	T3	T4
8	15.59 ± 1.45a	12.55 ± 0.66a	13.87 ± 2.24a	15.59 ± 1.92a
12	24.11 ± 3.41ab	24.38 ± 2.93ab	26.71 ± 1.96ab	28.30 ± 1.73ab
16	35.47 ± 6.76b	35.05 ± 7.44b	37.10 ± 3.12b	41.40 ± 1.11c
20	47.47 ± 3.37cd	49.27 ± 14.9cd	48.87 ± 9.27cd	54.23 ± 1.89d

*Data are mean values of three replicates ± standard deviation followed by the same alphabet are not significantly different $p < 0.05$ using Tukey test

Although the results presented here are not significantly different due to only a small number of samples being measured, the result showed that AMF treated seedlings in T4 treatment were the heaviest fresh weight and tallest height among all the treatments. The AMF — *Ganoderma* seedlings in T3 treatment showed better growth than the *Ganoderma* infected seedlings in T2 treatment. The result suggested that AMF do play a role in promoting plant growth, and the growth of AMF colonised seedlings are less affected by the *Ganoderma* infection. The improvement can explain this finding in plant nutrition due to the formation of the AMF hyphal network in plant roots. Furthermore, colonisation of AMF improves the accessibility of roots to a large soil surface area for better absorption of inorganic nutrients for plant growth (Begum *et al.*, 2019). Studies have shown that AMF-inoculated plants such as patchouli (Arpana *et al.*, 2008), soybean (Meghvansi *et al.*, 2008), tomato (Salvioli *et al.*, 2008), oil palm (Sundaram *et al.*, 2010), and maize (Zare-maivan *et al.*, 2017).

3.5 Effect of AMF on the Chlorophyll Content

At 20 WPI, it was observed that T3 seedlings with AMF prophylactic treatment showed the highest chlorophyll content with 21.2 mg/ml chlorophyll a, 7.99 mg/ml chlorophyll b, and 29.18 mg/ml total chlorophyll (Table 3).

Table 3. Chlorophyll content (mg/ml) on leaf tissue of the treated oil palm seedlings; T1 (Untreated), T2 (+ *G. boninense*), T3 (+AMF + *G. boninense*), and T4 (+ AMF) at weeks 8, 12, 16, and 20 of post inoculation (WPI).

Samples	T1			T2			T3			T4		
	a	b	Total	a	b	Total	a	b	Total	a	b	Total
8 WPI	9.24a	3.35a	13.22a	10.79b	3.63ab	15.14b	9.19a	2.87a	12.65a	11.63c	4.93cd	17.43c
12 WPI	12.57d	4.02b	16.59c	12.97d	4.12b	17.09c	11.26c	3.54ab	14.79b	13.6de	4.89cd	18.55d
16 WPI	15.1e	5.09d	20.18e	12.95d	4.44c	18.39d	13.7de	4.75cd	18.43d	14.4de	5.06d	19.09d
20 WPI	16.09f	5.41d	21.49f	13.7de	5.22d	18.98d	21.2g	7.99f	29.18g	15.35e	5.98e	21.33f

*Data are mean values of three replicates ± standard deviation followed by the same alphabet are not significantly different $p < 0.05$ using Tukey test

At 20 WPI, the level of chlorophyll a, chlorophyll b, and a total of chlorophyll content in T2 treatment is lower than seedlings in T3 treatment. The lower chlorophyll content in the *G. boninense* infected seedlings could reflect the *Ganoderma* disease progression that has started to affect the vascular tissue of the oil palm seedlings. The invasion of pathogenic fungi on roots caused a restriction of nutrient absorption as the infections developed, thus inhibiting chlorophyll's metabolism (Vincenzo & Veronica, 2015). Likewise, in the T3 seedlings, AMF colonisation in plant roots continue to improve the water and uptake of nutrient even with the presence of *G. boninense* infection.

4. Conclusion

It was concluded that TPC, PO, and PPO activities responded significantly to *G. boninense* infection in oil palm. This study's findings suggested higher compatibility between AMF and oil palm seedlings because of lower stimulation of PO and PPO enzymes in the AMF colonised plants. Results also showed that the AMF inoculated seedlings infected with *Ganoderma* will produce and accumulate more phenol to demonstrate the plant systemic defense. There was an increment of plant growth in AMF treated seedlings whereby the growth of the AMF inoculated seedlings was less affected by *Ganoderma* infection. Similarly, the *Ganoderma* infected seedlings showed an effect on the chlorophyll content, but in the AMF colonised seedlings, the chlorophyll content was less affected. In this study, it was noted that AMF shows its benefits in oil palm. Therefore, it is highly recommended that the oil palm planters start applying the Arbuscular mycorrhiza fungi (AMF) during seeds sowing to protect the oil palm roots against the *Ganoderma* BSR disease.

Author Contributions: Conceptualisation, S.S*, N.M.N and M.A.M.A.; investigation, S.S, and M.A.M.A.; writing-original draft preparation, S.S, writing-review, and editing, S.S, and N.M.N, M.A.M.A*

Funding: This research was funded by The Corporate Research of Economy, Science and Technology (CREST) and Sime Darby Plantation Research, Malaysia.

Conflicts of Interest: The authors declare no conflict of interest.

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