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Ligninolytic enzymes profiling in association with the aggressiveness of *Ganoderma boninense* isolates

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ABSTRACT

Aims: This study was designed to examine the enzyme activity of selected virulent isolates of *Ganoderma boninense* against oil palm. In a separate *in vitro* assessment, the effect of macronutrients on the mycelial growth of four selected *Ganoderma* spp. was also tested.
Methodology and results: The study involved a comparison of ligninolytic enzymes; lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac) profiling of eight isolates of *G. boninense*, categorized into three levels of aggressiveness, with two control isolates (*G. boninense* PER71 and *G. tornatum* NPG1) using solid-state fermentation (SSF). The Principal Component Analysis (PCA) revealed that the isolates had a significant production of ligninolytic enzymes on day 80. The most aggressive isolate, ET61 had the highest Lac production. As for the macronutrient test, mycelial growth for all the *Ganoderma* spp. was highly affected by potassium (K).
Conclusion, significance and impact of study: The findings of this study elucidated the characteristics of *G. boninense* in relation to enzyme production for the degradation of oil palm lignin and the identification of essential nutrients involved in the survival and growth of *Ganoderma* spp. The study provides vital information on the pathogenic characteristics of *G. boninense* isolates involved in biomass degradation along with the role of nutrient on the growth of *Ganoderma* spp. that may influence basal stem rot (BSR) management in the field.

Keywords: Basal stem rot, *Ganoderma boninense*, ligninolytic enzyme, macronutrients, oil palm

INTRODUCTION

The productivity of oil palm is severely affected by a disease known as BSR, caused by *Ganoderma boninense* Pat. The disease causes lignin and cellulose degradation in the basal of the trunk and roots of the palms. The crop is entering its 4th generation planting in Malaysia and has seen the incidences increasing over the years. A surveillance study conducted between 2013 to 2017 on small-scale oil palm plantations in Malaysia found that a total area of 3450.75 ha from 37359.81 ha (9.24%) surveyed mature palms were infected by *G. boninense* (Shukri *et al.*, 2020).

The pathogen, *G. boninense* is a white-rot fungus that is able to degrade the lignin of oil palm wood. This degradation process involves enzymes such as peroxidases (LiP and MnP), Lac, cellulases and xylanases (Elisashvili *et al.*, 2008; Subramaniyam and Vimala, 2012) and they mineralise the polymers into carbon dioxide (CO_2) (Valaskova *et al.*, 2007; Baldrian, 2011) and eventually cause plant tissues to rot (Latiffah *et al.*, 2005; Naher *et al.*, 2015). Although the process of delignification is similar in palms, *G. boninense* isolates exhibit different degrees of rotting due to the differences in aggressiveness (Rees *et al.*, 2007) and it has never been investigated, causing the lack of information on the delignification profiles within the isolates.

Meanwhile, agrochemical use in the oil palm industry plays a pertinent role on the commodity crop for optimal growth. These nutrients have a significant role in plant metabolism and affect the anatomy, morphology, and chemical composition, thereby influencing disease tolerance and resistance (Huber *et al.*, 2012). However, the direct effect of these macronutrients on the growth of *Ganoderma* spp. received very little attention.

Bearing the above in mind, this study aims to decipher the ligninolytic enzyme profiles among the *G. boninense* isolates and the possibility of its association with

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aggressiveness levels. Additionally, the direct effect of macronutrients on the growth among the *Ganoderma* spp. will shed light on the understanding of fungus development. Based on this study, we anticipate that the findings will provide new insight into understanding the epidemiology of BSR disease development and better management strategies in the future.

MATERIALS AND METHODS

Culture and substrate preparation

GanoID (https://ganoidmpob.arkgene.com/) is an online *Ganoderma* database that compiles information on the culture collection of *Ganoderma* isolates. The portal and the culture collection are managed by the Malaysian Palm Oil Board (MPOB, Bangi) with an archive of 434 isolates that were sampled from various locations within Malaysia. Among these cultures, this study utilised the sporocarps of *G. boninense* from four different locations within Peninsular Malaysia: Perak, Terengganu, Selangor and Johor. The pure cultures were obtained from Plant Pathology and Biosecurity Laboratory, MPOB cultured on Potato Dextrose Agar (PDA; Difco, France). Periasamy *et al.* (2019) conducted pathogenicity trials of 12 isolates using the seed germination technique as described by Idris *et al.* (2004) and Breton *et al.* (2006) at MPOB-UKM nursery, Bangi. Eight isolates were shortlisted and categorised into three groups based on the percentage of infection during the pathogenicity trial: a) most aggressive (40%-60%: ET61 and WS91), b) moderately aggressive (10%-30%: ET32, NP63, NP61 and SJ 22) and c) least aggressive (less than 10%: NP21 and SJ33) based on the study conducted. Two isolates, namely the pathogenic *G. boninense* PER71 and the non-pathogenic *G. tornatum* NPG1 were used as standard controls (Idris, 2000) to compare the ligninolytic enzyme profiling. These isolates are commonly used as reference isolates for *Ganoderma* research in Malaysia (Jing *et al.*, 2015; Isaac *et al.*, 2018; Wong *et al.*, 2019). Both cultures are maintained by Plant Pathology and Biosecurity Laboratory, MPOB. The list of isolates corresponding to aggressiveness, BSR symptoms of the palm and location is shown in Table 1.

Mycelium plugs of the isolates were cultured on potato dextrose agar (PDA; Difco, France) and incubated in the dark at 28 °C for 7 days. Meanwhile, the disease-free oil palm trunk was cut into small pieces and autoclaved at 121 °C for 15 min. The blocks were oven-dried overnight at 60 °C until a constant weight was achieved. These blocks were arranged in polypropylene plastic bags (15 cm × 33 cm × 0.5 mm thick). Thirty mL of malt extract agar (MEA; Merck KGaA, USA) was then added to each bag and autoclaved again at 121 °C for 20 min. The sterilized blocks were inoculated with mycelium plugs excised from the actively growing peripheral margin (1 cm × 1 cm) of the *Ganoderma* isolates and incubated in the dark at 28 °C for 20, 40, 60 and 80 days for SSF (Sundram *et al.*, 2015). Upon completion of the incubation period, the blocks were subjected to enzyme extraction.

Extraction of ligninolytic enzymes

The method was partially modified from Hariharan and Nambisan's (2013) study. The pH of the 50 mM sodium acetate buffer varies according to the type of enzyme; pH 3 for LiP and pH 4.5 for manganese peroxidase and Lac (Hariharan and Nambisan, 2013; Naidu *et al.*, 2017). The mixture was filtered using filter paper (Whatman 11 µm, United Kingdom). Subsequently, the filtrate was subjected to centrifugation at 10000 rpm for 30 min at 4 °C (Eppendorf Centrifuge 5804 R, Germany) and the supernatant was collected. The supernatant was then subjected to LiP, MnP and Lac quantification by measuring the absorbance of the ligninolytic enzymes using a Thermo Scientific UV-Vis spectrophotometer (GENESYS 10S, USA). The total enzyme activity was expressed in U/mL. The enzyme assay experiment was repeated twice to validate the consistency of the results.

Ligninolytic enzyme assay

The LiP activity was determined through the oxidation of veratryl alcohol (3, 4-dimethoxybenzyl alcohol) to veratraldehyde (ε 310, 9300 M^{-1} cm^{-1}) as described in previous literature (Krik *et al.*, 1990; Hariharan and Nambisan, 2013). The reaction mixture consisted of 0.25 mL of enzyme solution, 0.25 mL of 1 mM veratryl alcohol, 0.2 mM H_2O_2 and 0.5 mL of 0.1 M citrate buffer (pH 3). The activity was measured at 310 nm.

The MnP was quantified based on the oxidation of 2,6-dimethoxyphenol (DMP; ε 469, 49600 M^{-1} cm^{-1}). The mixture consisted of 25 µL of 20 mM 2,6-DMP, 25 µL of 20 mM $MnSO_4 \cdot H_2O$ and 300 µL of enzyme extract. About 50 µL of 4 mM H_2O_2 was added to the mixture to initiate the reaction. The absorbance of the product was measured at a 469 nm wavelength (Naidu *et al.*, 2017).

The Lac activity involved oxidation of 2,2'-azino-bis(3-ethylbenzthiazoline)-6-sulfonate (ABTS; ε 420, 36000 M^{-1} cm^{-1}). The activity was determined spectrophotometrically at 420 nm. The reaction mixture was prepared by adding 0.1 mL of 0.3 mM ABTS in 300 µL of 100 mM citrate buffer (pH 4.5) and finally, 0.6 mL of the enzyme was added (Bonugli-Santos *et al.*, 2010; Hariharan and Nambisan, 2013).

Effect of macronutrients on mycelial growth of *Ganoderma* spp.

An *in vitro* preliminary study was conducted to examine the effect of macronutrients; nitrogen (N), phosphorus (P), potassium (K) and magnesium (Mg) on the radial growth of *G. boninense*, *G. miniatocinctum*, *G. zonatum* and *G. tornatum*. The N, P, K and Mg are the major macronutrients applied as agrochemicals to provide optimum growth for the oil palm (Tarmizi and Tayeb, 2006). The four isolates were obtained from the Plant Pathology and Biosecurity Unit, MPOB. The specific selection of these four *Ganoderma* spp. is mainly due to their variation in infectivity in the oil palm as reported by

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Table 1: Description of palms and details of the selected *Ganoderma* isolates.

Description	ET61	WS91	ET32	NP61	NP63	SJ22	NP21	SJ33	PER71	NPG1
Degree of aggressiveness	High	High	Moderate	Moderate	Moderate	Moderate	Least	Least	Control	Control
Age of palm	22	19	22	27	27	27	27	27	NA	NA
Generation	Second	Second	Second	Second	Second	Second	Second	Second	NA	NA
Symptoms	Skirting, multiple spears, basal stem is hollow and 70-90% of infection	Healthy, basal is not hollow, 70-90% infection	Healthy, basal area not hollow and 50-70% infection	Skirting, multiple spears, hollow and 10-30% infection	Skirting, multi spears, basal stem hollow, 10-30% infection	Rotting one side, leaf skirting and no unopened spears	Healthy, basal area not hollow and 50-70% infection	Skirting	NA	NA
Location	Coastal	Coastal	Coastal	Inland	Inland	Inland	Inland	Inland	NA	NA
Identity of isolate	*G. boninense* strain UPMLD1806	*G. boninense* strain GbHap1	*G. boninense* strain GBLS	*G. boninense* strain GB001	*G. boninense* strain A4FB1	*G. boninense* strain GBLS	*G. boninense* strain KU20020.21	*G. boninense* strain TARI_R210222	*G. boninense* PER71	*G. tornatum* NPG1

Note: *Ganoderma boninense* PER71 and *Ganoderma tornatum* were obtained from the Plant Pathology and Biosecurity (PPB) Unit, Malaysian Palm Oil Board. NA refers to not applicable.

Table 2: Composition of the media prepared to test the effect of macronutrients on the growth of *Ganoderma* spp.

Content	Treatments/Media composition (g/L)				
	Complete	Nitrogen free	Phosphorus free	Potassium free	Magnesium free
Glucose	40.00	40.00	40.00	40.00	40.00
Magnesium sulphate	1.25	1.25	1.25	1.25	-
Potassium dihydrogen phosphate	2.50	2.50	-	-	2.50
Potassium nitrate	5.00	-	5.00	-	2.50
Potassium chloride	-	5.00	-	-	-
Potassium sulphate	-	-	2.50	-	2.50
Sodium dihydrogen phosphate	-	-	-	5.00	-
Sodium nitrate	-	-	-	5.0	-
Agar	20.00	20.00	20.00	20.00	20.00

Source: Seelan (2004).

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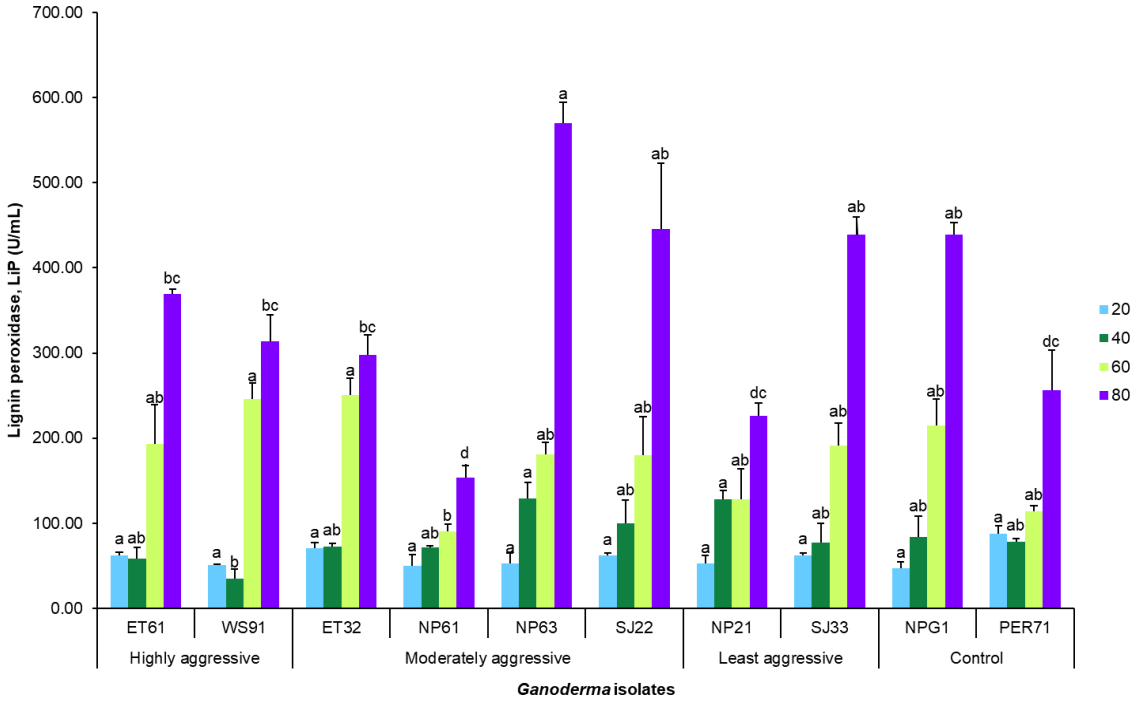

Figure 1: Production of total lignin peroxidase (LiP) by *Ganoderma boninense* isolates (ET61, WS91, ET32, NP61, NP63, SJ22, NP21, SJ33, PER71) and *Ganoderma tornatum* (NPG1). The legend represents incubation periods at 20, 40, 60 and 80 days. Vertical error bars indicate standard errors. The different alphabets represent statistically significant variables.

Idris *et al.* (2000). In the current study, the influence of macronutrients on the growth of *Ganoderma* spp. was assessed by measuring of radial growth in modified plates (R2) and calculating it against the radial growth in control plate (R1). A complete nutrient media served as the control. The details of the treatments or media are tabulated in Table 2. The percentage inhibition of radial growth (PIRG) of *Ganoderma* spp. was calculated using the following formula used by Sundram (2013).

$$(R1 - R2)/R1 \times 100\%$$

Statistical analysis

The raw data of enzyme profiling were square root transformed and PIRG data were arcsine transformed before subjected to Analysis of Variance (ANOVA) using SAS version 9.0 (SAS Institute Inc., USA). The significance was accepted at the 0.05 level of probability (p<0.05) and the Tukey test was used for the mean separation test.

RESULTS

Ligninolytic enzyme activity of selected *Ganoderma boninense* isolates

The production of LiP was significantly triggered on day 80 of SSF (p<0.05). LiP activity of isolates were reported in the following order, isolate NP63 (570.27 U/mL) > SJ22 (445.54 U/mL) > NPG1 (439.43 U/mL) > SJ33 (493.04 U/mL) > ET61 (369.22 U/mL) secreted the highest level of LiP on day 80, as shown in Figure 1. The secretion of LiP increases over time, with NP63 increasing 10-fold from day 20 to day 80. Meanwhile, SJ22, NPG1, SJ33 and ET61 had a 7-, 9-, 7- and a 5-fold increase in LiP secretion, respectively.

It was observed that isolates SJ22 > SJ33 > NP63 > ET61 > NPG1 had the highest MnP production on day 80 in descending order (Figure 2). Interestingly, the listed isolates produced the highest LiP, although the sequence is not similar. In general, the production of MnP has increased gradually from day 20 to day 80. The top 5 isolates (SJ22, SJ33, NP63, ET61 and NPG1) secreted MnP at 2-, 3-, 2-, 1- and 2-fold from day 20 to day 80. The MnP production on the 80th day is highly significant (p<0.05) compared to other days.

The variability in Lac production in the SSF condition is shown in Figure 3. Isolate ET61 (highly aggressive) produced the highest significant amount of Lac (12.35 U/mL), followed by NP63 > SJ22 > SJ33 > NP21 after 80 days of the SSF period (Figure 3). The Lac production of ET61 was also noted to increase 6-fold from day 20 to day 80. Similar to LiP and MnP production, Lac production is highly significant on day 80 (p<0.05).

The data on enzyme secretion was further analysed to establish a possible association between enzyme production and aggressiveness. The data were subjected

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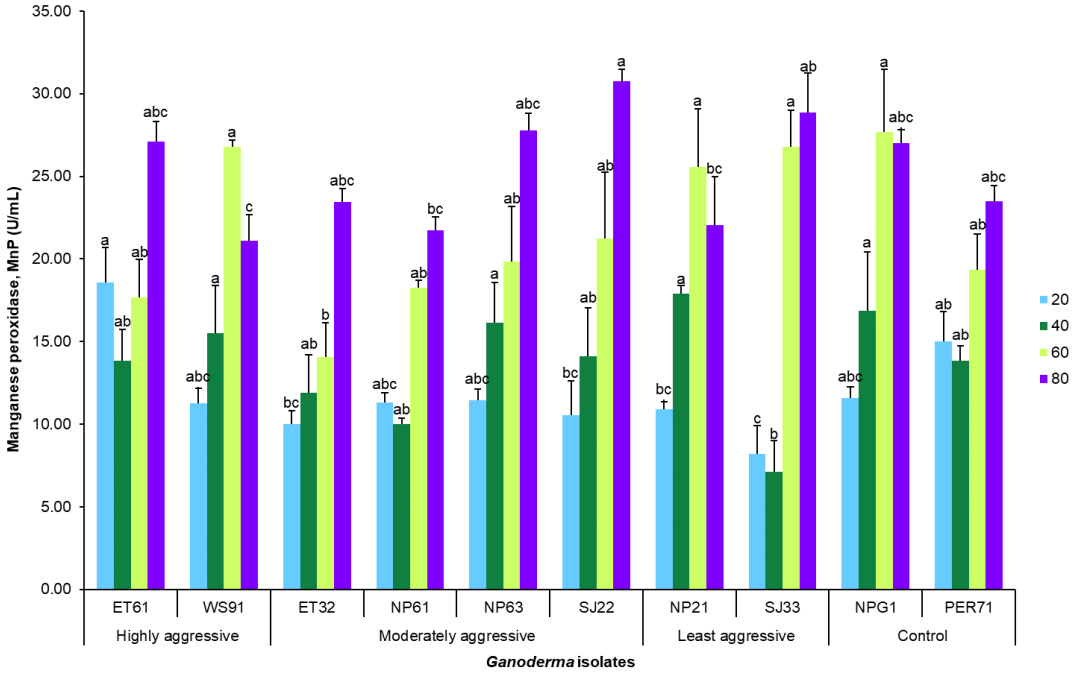

Figure 2: Production of total manganese peroxidase (MnP) by *Ganoderma boninense* isolates (ET61, WS91, ET32, NP61, NP63, SJ22, NP21, SJ33, PER71) and *Ganoderma tornatum* (NPG1). The legend represents incubation periods at 20, 40, 60 and 80 days. Vertical bars indicate standard errors. The different alphabets represent statistically significant variables.

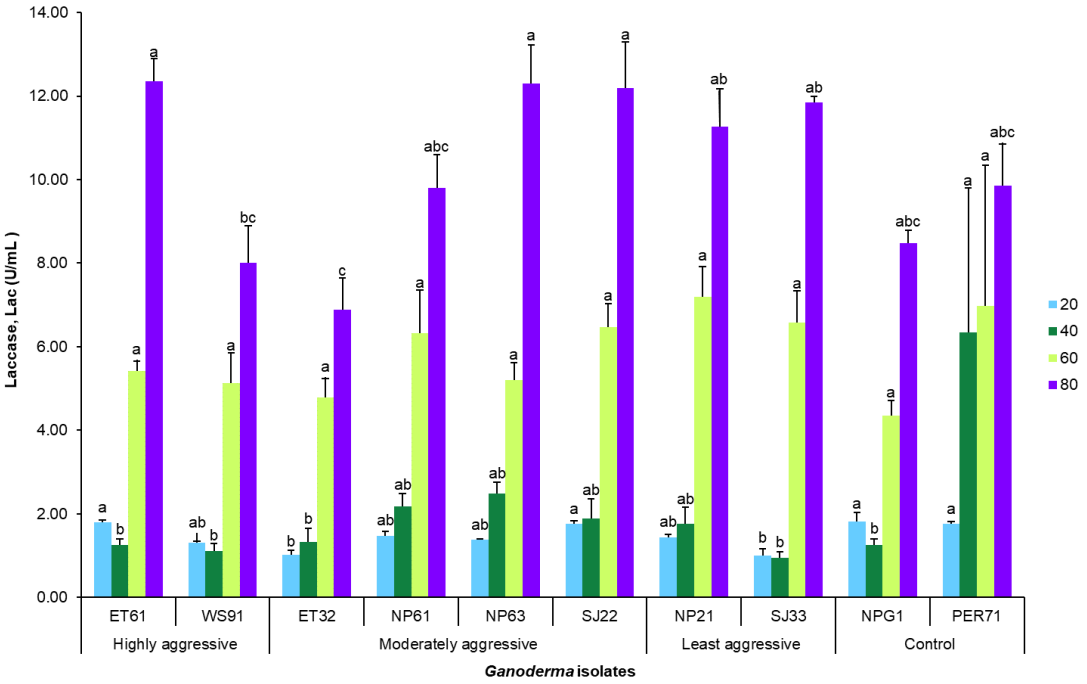

Figure 3: Production of total laccase by *Ganoderma boninense* (ET61, WS91, ET32, NP61, NP63, SJ22, NP21, SJ33, PER71), *Ganoderma tornatum* (NPG1). The legend represents incubation periods at 20, 40, 60 and 80 days. Vertical bars indicate standard errors. The different alphabets represent statistically significant variables.

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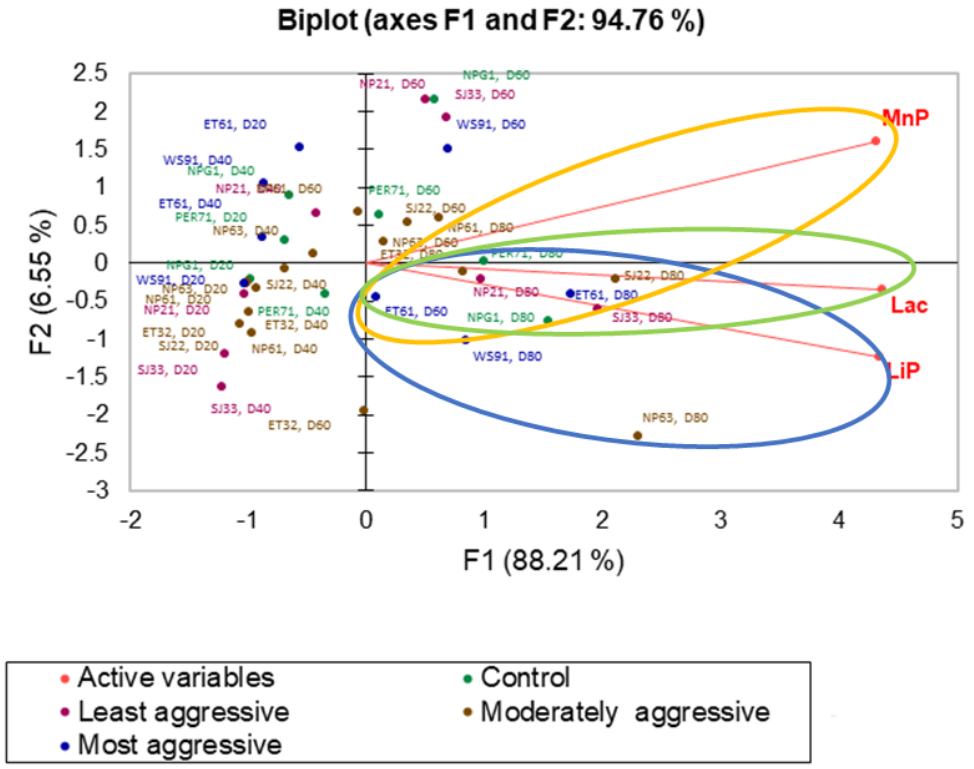

Figure 4: Principal component analysis (Biplot) of the solid-state fermentation of *Ganoderma boninense* isolates (ET61, WS91, ET32, NP61, NP63, SJ22, NP21, SJ33, PER71) and *Ganoderma tornatum* (NPG1). The dots represent the observations and day (D20=Day 20, D40=Day 40, D60=Day 60 and D80=Day 80). Red lines indicate the active variables (lignin peroxidase, manganese peroxidase and laccase). The circles indicate the isolates that had high enzyme production (Blue=LiP, Yellow=MnP and Green=Lac).

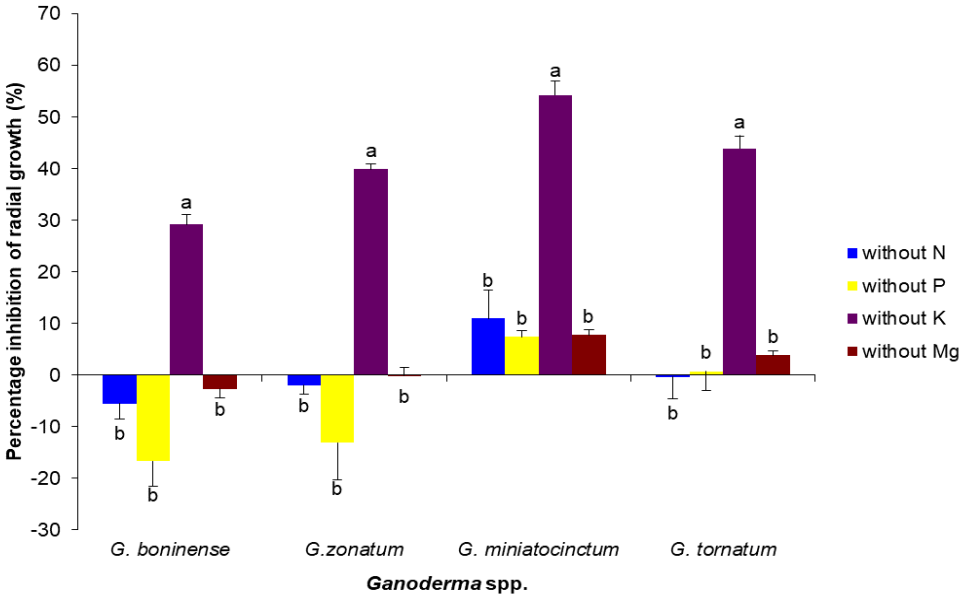

Figure 5: Growth of *Ganoderma* spp. on nutrient manipulated media. Each value represents a mean of five replicates. The legend represents media without each macronutrient respectively (N: Nitrogen; P: Phosphorus; K: Potassium; Mg: Magnesium). Vertical bars indicate standard errors. The different alphabets represent statistically significant variables.

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Table 3: Correlation matrix [Pearson (n)] for principal component analysis of the solid-state fermentation condition comprises of ligninolytic enzymes.

Variables	LiP	MnP	Lac
LiP	1	0.553	0.719
MnP	0.553	1	0.728
Lac	0.719	0.728	1

Note: The correlation test was conducted at a significance level of alpha=0.05.
Abbreviations: LiP=Lignin peroxidase; MnP=Manganese peroxidase; Lac=Laccase.

Table 4: Categorization of isolates according to the quantity of enzymes produced using solid-state fermentation.

Enzymes	Quantity of production	
	Highest	Lowest
LiP	NP63 ++	NP61 ++
MnP	NPG1 +	WS91 +++
Lac	ET61 +++	ET32 ++

Note: LiP=Lignin peroxidase; MnP=Manganese peroxidase; Lac=Laccase; +++ = most aggressive, ++ = moderately aggressive, + = least aggressive and control isolates.

to PCA analysis and based on the results, it was found that the active variables Lac have a strong positive correlation with LiP and MnP, where the r value is more than 70% (Table 3). The first two principal components, F1 (88.21%) and F2 (6.55%) are shown in Figure 4, delivering 94.76% variability. The optimum production of LiP, MnP and Lac occurred towards the end of the biodegradation period, which was 80 days as shown in Figure 4 (right side). Among the isolates, NP63, SJ22, SJ33 and ET61 significantly contributed to all three ligninolytic enzymes on day 80. In contrast, the production of the three enzymes is lower on day 20 and day 40.

Table 4 summarises the isolates according to the highest and least enzyme production on day 80 of SSF. Lac production shows an interesting association with the degree of aggressiveness. Isolate ET61, which was identified as the most aggressive isolate based on the pathogenicity test conducted by Periasamy *et al.* (2019), secreted the highest amount of Lac on day 80. The finding of this study is consistent with the reports by previous studies that proved Lac is being responsible for the degradation of oil palm lignin by *G. boninense* (Goh *et al.*, 2014; Christina, 2016). This is also the first report that relates ligninolytic enzyme activities and the levels of aggressiveness in *G. boninense*. Nonetheless, this is a preliminary result and requires further in-depth investigation to establish the enzyme activity and the degree of aggressiveness within the *Ganoderma* isolates.

Effect of macronutrient on radial growth of *Ganoderma* spp.

The effects of the four macronutrients, N, P, K and Mg on the radial growth of *Ganoderma* spp. are shown in Figure 5. It is noted that media without the presence of K significantly (p<0.05) reduced the radial growth of *G. boninense* by 29.20%, *G. zonatum* by 39.93%, *G. miniatocinctum* by 54.31% and *G. tornatum* by 43.90%. On the contrary, media without N, P and Mg showed an inconsistent trend of radial growth among the tested *Ganoderma* spp. The growth profiles affected by each macronutrient through the radial growth inhibition of the respective *Ganoderma* spp. are illustrated in Figure 5.

DISCUSSION

Ligninolytic enzymes secretion in association with the aggressiveness of *Ganoderma boninense* isolates

White-rot fungi are commonly known for their ability to degrade the lignocellulose components of wood, such as lignin, cellulose and hemicellulose. Lignin is the first defence barrier protecting plants from biotic and abiotic factors. The destruction of the first defence barrier exposes internal tissues and starch reservoirs to infection by pathogens and environmental factors (Yang *et al.*, 2018). Lignin degradation is a complex process assisted by the activity of ligninolytic enzymes such as LiP, MnP, Lac and other enzymes.

A previous study (Khaledi *et al.*, 2017) revealed that the production of ligninolytic enzymes influences the aggressiveness of a pathogen in causing disease in a plant. The study reported that the *Fusarium* strains exhibit higher levels of cell wall degrading enzymes and aggressively infect wheat in the pathogenicity trials. According to Ramzi *et al.* (2019), the release of cell wall degrading enzymes during saprophytic and necrotrophic stages of infection determines the pathogenicity of white-rot fungi. Ramzi *et al.* (2019) highlighted that further research is required at the molecular level to prove the relationship between cell wall degrading enzymes and the pathogenesis of *G. boninense.*

Ganoderma boninense is a hemibiotroph and lives an intermediate lifecycle between biotrophs and necrotrophs (Bahari *et al.*, 2018). It degrades the host's cell wall extensively during the transition from the biotrophic to the necrotrophic phase (Goh *et al.*, 2014). This stipulates that *G. boninense* once in contact with a compatible host initiates the infection by aggressively attacking the host through the release of cell wall-degrading enzymes. In

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other words, the optimum release of cell wall degrading enzymes have to be achieved if the host responds by activating the plant defence factors (Bahari *et al.*, 2018). The isolates in the present study were inoculated on dead oil palm blocks instead of a living host for enzyme profiling. This condition eliminates the plant and pathogen interaction, whereby the transition from the biotrophic to the necrotrophic phase is absent. At this stage, *Ganoderma* acts merely as a saprophyte. Besides, Adaskaveg *et al.* (1990) reported that different isolates of the same white-rot species have variations in degrading the lignin. This may have affected the quantity and types of enzymes secreted and the correlation with the degree of aggressiveness of the *G. boninense* isolates.

Peroxidase and laccase play a major role in the delignification process and most of the white-rot fungi have high laccase activity. Previous studies show that *G. boninense* produces a significant amount of laccase and lower peroxidase (Goh *et al.*, 2014; Surendran *et al.*, 2018). However, Hariharan and Nambisan (2013) and Sudarson *et al.* (2014) reported that white-rot fungi, including *Ganoderma* spp., have very low laccase activity compared to peroxidase. Similar results were obtained in the present study, where the relative amount of LiP and MnP are significantly higher compared to Lac. Zhou *et al.* (2013) reported that not all *Ganoderma* spp. produces lignin-modifying enzymes (LiP, MnP and Lac) at the same time. They reported that some *Ganoderma* strains were only able to produce only one or two of the enzymes. Besides the type of substrates, fermentation conditions and detection substrate (Krik and Farrell, 1987), it may be the onset timing of the peroxidase secretions (Arora and Gill, 2001; Naidu *et al.*, 2017) that could be the factors influencing the enzyme production.

Fungus grown on woody substrates produces more Lac due to the composition of syringyl and guaiacyl-lignin of the substrate (Schwarze, 2007; Paterson *et al.*, 2008). Previous studies show that *G. boninense* produces a significant amount of laccase and lower peroxidase when rubber wood block is utilized as a substrate (Goh *et al.*, 2014; Surendran *et al.*, 2018). Guaiacyl wood lignin is more resistant to fungal degradation than syringyl-composed lignin. Hence, white-rot fungi will require a high concentration of enzymes to degrade the lignin of woody plants (Hatakka and Hammel, 2010). However, the oil palm blocks utilised in the current study to imitate the actual host system and the degradation process contain a large proportion of non-condensed syringyl and a small amount of guaiacyl (Schwarze, 2007; Paterson *et al.*, 2008). Different substrate utilisation might be the possible reason for a low quantity of Lac secretion in the present study.

The SSF condition causes carbon starvation in the system and eventually encourages the white-rot fungus to efficiently degrade the lignin to access the cellulose component in order to cope with starvation (Vasina *et al.*, 2017; Ho *et al.*, 2020). This justifies the secretion of a low level of ligninolytic enzymes at the beginning of the incubation periods with a slow increase over time (day 20 and day 40). According to Hariharan and Nambisan (2013), buffer, pH, temperature and agitation also influence the quantity and quality of the enzymes being produced. This study was designed by standardising the condition of incubation without the above-mentioned factors influencing the ligninolytic enzyme secretion. Therefore, the investigated parameters should be selected carefully based on the objective/s of the study to achieve the desired results.

The onset timing of enzyme production is responsible for the variation in quantity and type of enzyme produced (Naidu *et al.*, 2017). In this study, the quantity of peroxidase, especially LiP is the highest regardless of the incubation period. According to Lankinen (2004), LiP was not produced at early cultivation (5 days) of *Phlebia radiata* but gradually increased over time (in a week). However, the profiling of peroxidase enzymes of *Trametes hirsuta* showed that the production was triggered as early as days 3 and 5 (Vasina *et al.*, 2017). This explains that the onset of peroxidase enzymes differs among the white-rot basidiomycetes. Hence, long-term exploration of the enzyme secretion could provide a different perspective concerning the aggressiveness of the *G. boninense* isolates.

Besides, delignification is not the effect of a single enzyme but the synergy of ligninolytic enzymes, which differ between fungi (Arora and Gill, 2001; Naidu *et al.*, 2017). Our present findings reported a positive correlation between the three enzymes (LiP, MnP and Lac), with more than a 70% correlation. *Ganoderma boninense* isolates of ET61, NP63, SJ22 and SJ33 are the isolates that produced a high quantity of all three enzymes.

In summary, the production of the Lac demonstrates association with the aggressiveness of *G. boninense* isolates in the SSF condition, with results indicating the most aggressive isolate, ET61 secreted the highest amount of Lac. Meanwhile, LiP and MnP secretion showed no association with the aggressiveness of *Ganoderma* isolates. It needs to be stated that the current experimentation utilised dead material as a substrate source. There is a possibility that living biological material may result in different profiling of enzymes' activity, but this requires further exploration.

The effect of macronutrients on radial growth of *Ganoderma* spp.

Most of the previous studies have focused on the effect of nutrients on the development of plant defence responses (Armengaud *et al.*, 2004; Wang *et al.*, 2013; Nuranis *et al.*, 2016; Zhang *et al.*, 2017; Ogden *et al.*, 2018) than the direct effect of the nutrients on suppression of *Ganoderma* spp. The current study focuses on the direct effect of the nutrients on the radial growth of *Ganoderma* spp. For instance, Peng *et al.* (2019) studied the effect of the environment (pH and temperature) and nutritional conditions (carbon and nitrogen) on the mycelial growth of *G. boninense*. They reported that the mycelial growth of *G. boninense* was better when supplied with glucose or fructose as a carbon source. This study however used glucose as the carbon source in every medium, which

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eliminates the effect of carbon sources on the mycelial growth of *Ganoderma* spp.

The current study indicates that K has the most significant effect in reducing the growth of *Ganoderma* spp., implying that K is crucial in *Ganoderma* spp. mycelial growth. According to Kim *et al.* (2002), *Paecilomyces sinclairii* had the highest mycelial growth when grown in K-containing media. Correspondingly, media supplemented with potassium dihydrogen phosphate enhanced the mycelial growth of *Ganoderma applanatum* (Jo *et al.*, 2009). This study has proven that K has a significant effect on the growth of *Ganoderma* spp. where the absence of K in media has significantly restricted the mycelial growth. Thus, the elimination of K in the current study has caused the suppression of *Ganoderma* spp. growth. However, it would be interesting to test different commercially available sources of K applications, such as muriate of potash on the growth of *G. boninense* and the compound combination of other nutrients (urea, rock phosphate and silica) on *Ganoderma* spp. growth.

The influence of N, P and Mg on the growth of *Ganoderma* spp. is still very vague (Ariffin *et al.,* 2000; Gransee and Fuhrs, 2013). Previous studies suggested that organic or inorganic form, time of application and solubility and availability of the nutrients have a significant contribution to the growth of fungal mycelia (de Miranda and Harris, 1994; Gransee and Fuhrs, 2013; Peng *et al.,* 2019). However, the effect of the nutrients on the growth of microorganisms is always tested by supplying different forms or/and quantities of nutrients (Deshmukh *et al.*, 2012; Ceci *et al.*, 2018; Liu *et al*., 2018; Peng *et al.*, 2019; Lin *et al*., 2020) but this information was lacking in *Ganoderma* research. This requires an extensive study to understand the relationship between nutrients and survival as well as the growth of microorganisms.

The current study is preliminary testing on the effect of essential nutrients on the growth of *Ganoderma* spp. Extensive studies need to be conducted to validate the role of nutrients in the radial growth of *Ganoderma* spp. This will provide new insight into nutrient management in the field to reduce BSR disease development.

CONCLUSION

There is an association between Lac secretion and aggressiveness of *Ganoderma* isolates. The finding proved that a highly aggressive isolate, ET61, had the highest Lac activity but relatively in smaller quantity compared to other enzymes. This can be attributed to the fact that the experimental materials used were non-living. The correlation between aggressiveness and ligninolytic enzyme secretion could be more evident when utilising a living plant (oil palm) to imitate a real microenvironment representing the disease triangle.

It is proven that K is a crucial element in limiting the growth of *Ganoderma* spp. The lack of K causes significant suppression of radial growth of *Ganoderma* spp. However, this is a preliminary study conducted under laboratory conditions and requires further nursery and field investigations to validate this finding using commercially available K products for clarity in managing the fertiliser application and eventually reducing the cost of agrochemical inputs.

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