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Full Length Article

Inhibition of Lignin Degrading Enzymes of *Ganoderma* spp.: An Alternative Control of Basal Stem Rot Disease of Oil Palm

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Abstract

Basal stem rot (BSR) disease is one of the greatest threat to oil palm producing countries. The disease is caused by *Ganoderma* spp. *Ganoderma* is a white rot fungus that degrades the oil palm lignin completely. Lignin degradation is a rate limiting step in the infection procedure. Changing the lignin degrading activity of *Ganoderma* could be a possible approach to alleviate the spread of *Ganoderma* in future. Therefore, a study was carried out to regulate the production and inhibition of lignin degrading enzymes namely lignin peroxidase, manganese peroxidase and laccase by *Ganoderma* sp. in the presence of enzyme inhibitors such as thioglycolic acid, EDTA, 2-mercaptoethanol. Different *Ganoderma* sp. namely, (*G. boninense* (PER71), *G. miniatocinctum, G. zonatum, G. tornatum*) were cultured on plate amended with Remazol Brilliant Blue R (RBBR) to assess their lignolytic ability by decolorization of the dye. All the *Ganoderma* spp. had potential to degrade RBBR dye with diverse effectiveness. *G. boninense* (PER71) produce the highest percentage of RBBR decolorization at 90.21% followed by *G. miniatocinctum* (71.11%), *G. zonatum* (54.63%) and *G. tornatum* (38.51%). The quantification of the three lignolytic enzymes revealed that the isolate having least RBBR decolorizing efficiency, showed the presence of lignin peroxidase, manganese peroxidase and laccase activities in significantly lesser quantities. The maximum activities were perceived in *G. boninense* (PER71) with 0.069, 0.075, 0.606 U mL^{-1} protein for lignin peroxidase, manganese peroxidase and laccase activities, respectively. The effect of various enzymatic inhibitors was in following descending order EDTA > TGA > mercaptoethanol. The chemical compounds causing high inhibition of enzymatic production could be developed as chemical control strategy in integrated management of basal stem rot in field applications.

Keywords: *Ganoderma*; Lignin peroxidase; Manganese peroxidase; Laccase; Enzyme inhibitors

Introduction

The oil palm (*Elaeis guineensis* Jacq), a native to West Africa, is a major commodity of global trade and source of leading vegetable oil. Palm oil alone account for quarter of world consumption and roughly 60% of international trade in vegetable oils. Around 85% of the world palm oil production is solely contributed by Indonesia and Malaysia (Ong and Chai, 2011).

In past decade, due to increased global population and oil consumption in food and industrial products, demand for palm oil is soaring and expected to be double by 2020. Replanting of oil palm has been found to be one of the stratagems to recover oil palm productivity. This is usually carried out when palms attain 25–30 years of fruiting age. The existing oil palm cultivated areas are consistently under threat due to Basal Stem Rot (BSR) disease caused by *Ganoderma* species namely, *G. boninense, G. zonatum, and G. miniatocinctum*, replanting dose not contribute much. Therefore, to meet the global demand, an expansion of 43% in area and cultivation of approximately 15 million ha throughout the world has been reported (Koh and Wilcove, 2008). However, such expansion comes at the cost of tropical rain forests. Large scale conversion of tropical rainforest to oil palm plantations had devastating effect on biodiversity and climate (Koh and Wilcove, 2008).

The total affected area due to *Ganoderma* in Malaysia was estimated at 151, 208 ha in 2009 with a probable loss of RM 1.3 billion and reported to increase up to 400,000 ha in Malaysia in near future (Roslan and Idris, 2012). Generally, lignin a most important structural component of woody and non-woody plants is responsible for giving strength and

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rigidity to plant cell, linked to cellulose and hemicelluloses forming a physical barrier around the latter two components preventing microbial attack (Sun *et al*., 2013). Due to the protective lignin barrier, lignocellulose degradation is an intricate, complex process, which requires the synergistic action of several extracellular enzymes. However, *Ganoderma* white-rot fungi are unusual organisms capable of degrading lignin and revealing celluloses and hemicelluloses as accessible hydrolysable nutrients causing substantial mineralization of lignin (Paterson, 2007). The fungus precisely degrades the lignin constituent of wood by exploiting the group of extracellular enzymes collectively termed as "ligninases" while leaving the white cellulose exposed making it vulnerable to further saprophytic microbial attack. Although the complete lignolytic system of this fungus is still not known, two families of lignolytic enzymes are generally thought of playing an important role in the enzymatic degradation: 1) phenol oxidase (laccase) and 2) peroxidases (lignin peroxidase and manganese peroxidase) (Srebotnik and Hammel, 2000).

Though the BSR disease has significant impact on present nation-wide total oil palm production, it is likely that it might further spread to a greater extent since no measure has yet been devised for the protection of these oil palms from the aforementioned disease. Flood *et al.* (2005) suggested that the debris left in the field is an important source of infection. Reduction in the infection source during field clearing would be advantageous. Oil palm waste generated during felling activity has been burned previously but this activity is banned in Malaysia due to zero burning law under Environmental Quality Act 1974 (E.Q.A., 1974). On the other hand, breeding/selection for high, or more resistant, lignin containing palms and using white rot fungi to disintegrate oil palm generated wastes on plantation floor are suggested (Paterson *et al*., 2008; Naidu *et al*., 2015, 2017). One of the significant features of *Ganoderma* spp. relates to the use of its lignolytic potential. Studies of lignolytic enzymes in this fungus and possible enzymatic inhibition which may be used as potential control method of the disease are still not completely known. Realizing the dearth of systematic information in this area, a stepwise study was undertaken to investigate the production of lignolytic enzyme by *Ganoderma* spp. and effect of enzyme inhibitors (EDTA, TGA and mercaptoethanol) on ligninolytic activity as a potential effective chemical treatment of *Ganoderma* infection.

Materials and Methods

Four *Ganoderma* spp. strains namely *G. boninense* (PER71), *G. miniatocinctum, G. zonatum, and G. tornatum* originally isolated from infected oil palm were obtained from research laboratory culture collection, Department of Plant Protection, Universiti Putra Malaysia. All the isolates were maintained in malt extract agar medium (MEA) 2% (w/v) slants at 25℃.

Remazol Brilliant Blue R (RBBR) Decolorization Test

Agar plugs (2 mm diameter) obtained from 7 days old culture of each of the actively growing test isolates, were inoculated in petri plates (90 mm diameter) containing 2% MEA added with 0.05% RBBR (Sigma) in quadruplets. Petri plates with no RBBR served as control. Culture plates were incubated in dark at 25℃ for 14 days. All the culture isolates with RBBR were then evaluated for their lignolytic potential by their ability to decolorize the dye.

RBBR decolorization in liquid culture medium was carried out to verify the results. Each of the strain was cultured in liquid culture medium (2.5% malt extract; 0.05% (w/v) RBBR in 100 mL of sterilized distilled water). The cultures were incubated at 25℃, in the dark, under continuous shaking (130 rpm). Decolorization was assessed at 14 days by observing the decrease in the absorbance peak at 595 nm, UV – VIS spectrophotometer (UV-1700 PharmaSpec, SHIMADZU ®). The mycelia biomass was filtered and weighed; quantification was carried out by gravimetric method. Briefly, the filtered mycelium was oven dried at 70^0C until constant weight was attained *(*Kornilłowicz-Kowalska and Rybczyńska, 2012). Percent decolorization was defined as

$$\text{Percent Decolorization} = \frac{[\text{Absorbance in control} - \text{Absorbance in treated}]}{\text{Absorbance in control}} \times 100$$

Enzyme Analyses

Three (3) mycelial discs (9 mm) of each fungal strain cultures on MEA were transferred to Erlenmeyer flasks containing 2.5% (w/v) malt extract medium. The cultures were incubated for 7 days at 30^0C under continuous agitation (130 rpm). The cultures were then filtered and filtrates were utilized for lignin peroxidase, manganese peroxidase and laccases activities.

The lignin peroxidase activity was determined by spectrometric absorption of veratryl aldehyde production during veratryl alcohol oxidation ($^{TM}_{310}$ = 9300 M^{-1} cm^{-1}). The reaction mixture contained (375 μL of 0.33M sodium tartrate buffer, pH 3.0; 125 μL of 4 m*M* veratryl alcohol; 50 μL of 10 m*M* hydrogen peroxide; 450 μL of distilled water and 250 µL of culture medium) (Khindaria *et al*., 1995).

The manganese peroxidase was assessed by phenol red oxidation at 610 nm ($^{TM}_{610}$ = 4460 M^{-1} cm^{-1}) according to Kuwahara *et al.* (1984). The reaction mixture (1 mL) contained (100 μL of phenol red; 100 μL of 250 m*M* sodium lactate; 200 μL of 0.5 % bovine albumin; 50 μL of 2 m*M* manganese sulfate; 50 μL of 2 m*M* hydrogen peroxide and 500 μL of culture medium). The reactions took place at 30^0 C for 5 min and were disrupted by the adding 2 N NaOH (40 μL) (Vares *et al.*, 1995).

While the laccase activity was measured by oxidation of o-dianisidine at 525 nm ($^{TM}_{525}$ = 65, 000 M^{-1} cm^{-1}). The reaction mixture (1 m*M*) consists of (200 μL of 0.5 *M* citrate-phosphate buffer, pH 5.0; 100 μL of 1 m*M* o-

dianisidine solution, 100 μL of hydrogen peroxide and 600 μL culture medium). The boiled cultured medium for each isolate was used as a control. For all the enzymes determined, one activity unit was expressed as the amount required to oxidize 1 μmoL substrate per minute (Vares *et al.,* 1995). The specific activity was expressed as units per microgram of protein (Um L^{-1}). Each value represented the mean of four replicates.

Inhibition of Enzymatic Production

The effect of various enzymatic inhibitors: thioglycolic acid (TGA), ethylenediaminetetraacetic acid (EDTA), and 2-mercaptoethanol (Merck), were tested for inhibition of manganese peroxidase, lignin peroxidase and laccase activity. All the isolates were cultured in liquid culture medium as mentioned previously. The inhibitory activity of the enzymes was achieved by co-incubation of each of the culture isolates with each of the inhibitor substance for a specific period of time (usually 2–10 min) to confirm complete inhibition (Majcherczyk *et al.,* 1999). The inhibitors at 40, 80 and 160 $mg.kg^{-1}$ were used in the inhibition assay. Enzyme activities were recorded as described above. Isolates cultured in liquid medium where inhibitor compound was not added, was conducted in parallel as a control treatment.

The inhibition percentage was derived by using the formula:

$$I = 100 (A_{CT}-A_{IT})/A_{CT}$$

Where, I: inhibition (in %), A_{IT}: absorbance in inhibition test after 2 min, and A_{CT}: observed absorbance in control test after 2 min.

Statistical Analyses

All experiments were conducted in Completely Randomized Design (CRD). Each treatment was replicated four times. Experiments were carried out at least twice unless otherwise stated. Data were subjected to analysis of variance (A.N.O.V.A.) and tested for significance by Least Significant Difference (L.S.D.) using PC-SAS (PC-S.A.S. software V8.2, S.A.S. Institute, Cary, N.C., U.S.A.). A P value of ≤ 0.05 was considered significant.

Results

Remazol Brilliant Blue R (RBBR) Decolorization Test

The RBBR dye decolorization ability of *Ganoderma* indicated the production of lignin degrading enzymes. All the *Ganoderma* spp. tested in this study showed their potential to produce lignolytic enzymes through RBBR dye decolorization with different efficacies when grown on solid and liquid culture medium (Table 1 and 2). The decolorization process started much earlier in the *G. boninense* plates compared to the other strains. *G. tornatum* decolorized the dye without a clear decolorization halo and was less efficient, by approximately 59% in decolorizing compared to *G. boninense.*

G. boninense (PER71) showed average growth velocity higher than or equal to 1 cm day^{-1} (Table 2). In contrast, the *G. zonatum* has the growth velocity of 0.5 cm day^{-1} that is half the value of *G. boninense* (PER71) when maintained at the same cultural condition. The slowest growing species in the study was *G. tornatum*. Similarly, the mycelial biomass production was four fold and two fold higher in *G. boninense* when compared to *G. tornatum* and *G. zonatum*, respectively.

The correlation coefficient (r) between the analysis between the biomass and the decolonization potential of *Ganoderma* spp. are highly correlated in an uphill manner (Table 3). *G. boninense* (PER71) has retained the maximum percentage of decolorization of RBBR dye as the maximum biomass production followed by *G. miniatocinctum, G. zonatum* and *G. tornatum*.

Enzymatic Analysis

The *Ganoderma* spp. selected in this study produced variable concentrations of both peroxidases and laccase enzymes. In general, the laccase production was almost 10 folds higher than that of the peroxidase enzymes production (Fig. 1, 2 and 3 at 0 $mg.kg^{-1}$). The peroxidase enzymes such as manganese peroxidase and lignin peroxidase were produced in considerable same units. The amount of enzyme produced was species specific and hence no particular pattern was observed for enzyme produced.

The maximum amounts of peroxidase enzymes were produced by *G. boninense* (PER71) (Fig. 1 and 2 at 0 $mg.kg^{-1}$). Whereas, *G. miniatocinctum* got upper hand of *G. boninense* in laccase production (Fig. 3). *G. zonatum* has the lowest production of peroxidase enzymes and laccase at the given environment.

Inhibition of Lignolytic Enzymes

The inhibitor potential of two reducing agents and a chelating agent toward the lignin degrading enzymes was evaluated. Not all the three chemicals significantly inhibited the lignin degrading enzymes. The three inhibitors exceptionally inhibited the peroxidase enzyme more than laccase. For instance, TGA completely inhibited the manganese peroxidase (Fig. 1) and lignin peroxidase (Fig 2) at 160 $mg.kg^{-1}$ however, laccase enzyme was active in the presence of 160 $mg.kg^{-1}$ of TGA (Fig. 3).

The inhibitory potential of a compound not only depends on the concentration but also on the source of the enzymes. The TGA inhibited 10.14% of the laccase activity produced by *G. boninense* (PER 71) followed by 1.21% in *G. miniatocinctum*. A complete contrasting picture was displayed by *G. tornatum*, indeed TGA at 40 $mg.kg^{-1}$ increased the activity of the laccase.

Table 1: Decolorization of Remazol Brilliant Blue R (RBBR) by different species of *Ganoderma* and their radial mycelial growth on malt extract agar

Species	Decolorization scale[a]	Radial growth rate[b]
G. boninense (PER71)	+++++	+++++
G. miniatocinctum	++++	+++
G. zonatum	+++	+++
G. tornatum	+	++

[a] Decolorization scale measured at 10th day of cultivation on MEA medium containing 0.5% of RBBR: + diameter of decolorized zone 0-20 mm, ++ diameter of decolorized zone 21-30 mm, +++ diameter of decolorized zone 31-50 mm, ++++ diameter of decolorized zone 51-70 mm, +++++ diameter of decolorized zone 71-100 mm
[b] Mycelium radial growth rate measured at 10th day of cultivation on MEA medium containing 0.5% of RBBR: + diameter of mycelial colony 0-20 mm, ++ diameter of mycelial colony 21 – 30 mm, +++ diameter of mycelial colony 31- 30 mm, ++++ diameter of mycelial colony 51-70 mm, +++++ diameter of mycelial colony 71 to 100 mm.

Table 2: Remazol Brilliant Blue R (RBBR) decolorization, mycelia extension rate and fungal biomass of white rot fungi (*Ganoderma* spp.) on 10th day of cultivation on Malt Extract Agar (MEA 2%) and Malt Extract Broth (MEB 2%)

Isolates	*Mycelial extension rate (cm day^{-1})	**RBBR decolorization (%)	**Biomass (g)
G. boninense (PER71)	1.02 ± 0.17	90.21a	0.189 ± 0.07
G. miniatocinctum	0.85 ± 0.11	71.11b	0.145 ± 0.05
G. zonatum	0.59 ± 0.11	54.63c	0.087 ± 0.02
G. tornatum	0.37 ± 0.09	38.51d	0.043 ± 0.01

Each value is the mean of four replicates (n=4) ± Standard Error according to Least Significant Difference (L.S.D.) at $P \leq 0.05$. Means with a same letter in a column are not significantly different at $P \leq 0.05$ using LSD test. * MEA; ** MEB

Table 3: Correlation analysis for the growth rate and RBBR dye decolorization (%)

	Mycelial Extension rate(r^2)	RBBR decolorization (%)(r^2)	Total biomass production (r^2)
Mycelial extension rate	1		
RBBR decolorization (%)	0.99	1	
Total biomass production	0.96	0.95	1

Table 4: Inhibition percentage of three lignolytic enzymes produce by different species of *Ganoderma* by three inhibitors TGA, EDTA and mercaptoethanol at different concentrations

Ganoderma species	Inhibitors								
	TGA			EDTA			Mercaptoethanol		
	40 mg.kg^{-1}	80 mg.kg^{-1}	160 mg.kg^{-1}	40 mg.kg^{-1}	80 mg.kg^{-1}	160 mg.kg^{-1}	40 mg.kg^{-1}	80 mg.kg^{-1}	160 mg.kg^{-1}
	Inhibition of Manganese Peroxidase (%)								
G. boninense (PER71)	31.04b	53.21c	100a	13.06a	91.29b	100a	-3.54b	11.63a	22.56a
G. miniatocinctum	35.86b	59.31c	100a	-0.42c	91.91b	100a	2.28a	-2.13b	18.80b
G. tornatum	39.11b	81.66b	100a	15.63a	93.30a	100a	-2.45b	-0.52b	-0.32c
G. zonatum	83.44a	100a	100a	11.11b	94.35a	100a	-33.90c	-39.94c	-24.45d
	Inhibition of lignin peroxidase (%)								
G. boninense (PER71)	30.16b	94.37a	100a	18.23a	94.37b	100a	5.80b	24.27	31.16a
G. miniatocinctum	12.41c	92.17b	100a	7.66b	92.17b	100a	-3.06c	5.76	16.16b
G. tornatum	34.14b	93.29b	100a	0.37c	93.29b	100a	23.12a	26.75	32.41a
G. zonatum	90.09a	99.24a	100a	5.45b	99.24a	100a	-53.26d	-41.69	-40.46c
	Inhibition of Laccase (%)								
G. boninense (PER71)	10.14a	13.39a	13.37b	11.07a	95.66a	100a	10.73a	26.34a	38.06a
G. miniatocinctum	1.21b	6.57b	17.49b	8.48b	96.70a	100a	-10.85c	9.18b	27.28c
G. tornatum	-12.90c	3.46b	19.11b	1.70c	95.34a	100a	-0.95b	6.49b	23.27c
G. zonatum	3.28b	12.25a	16.73b	5.05b	99.40a	100a	-84.32d	-59.12c	42.33b

Means with similar letter in each column for each enzyme at each concentration is not significantly different at $P \leq 0.05$ using LSD test. Each value is the mean of four replicates (n=4)

When the three inhibitors were compared at 40 mg.kg^{-1} TGA was considered as a premier inhibitor followed by EDTA (Table 4). However, mercaptoethanol at the same concentration increased the production of lignolytic enzymes. As the concentration doubled EDTA excelled its activity over TGA. At 80 mg.kg^{-1} the inhibitory range of EDTA was about 91.2–99.4% whereas the TGA has a wide range of inhibition between 3.5–100%. When the concentration increased further to 120 mg.kg^{-1}, EDTA has completely inhibited all the three lignolytic enzymes produced by all the *Ganoderma* species tested. At 120 mg.kg^{-1} the TGA absolutely inhibited only the peroxidase enzymes (Manganese peroxidase and lignin peroxidase) whereas laccase enzyme activity was retained in all the species tested. Merchaptoethanol was unable to inhibit the lignin degrading enzymes produced by *G. zonatum*, instead it has elevated the enzyme activity.

Discussion

The genus *Ganoderma* is ubiquitous around the world. In 1930, *G. lucidum* was reported as a causative agent for BSR (Thompson, 1931). Later six new strains were also reported in Malaysia and Indonesia namely *G. boninense*, *G.*

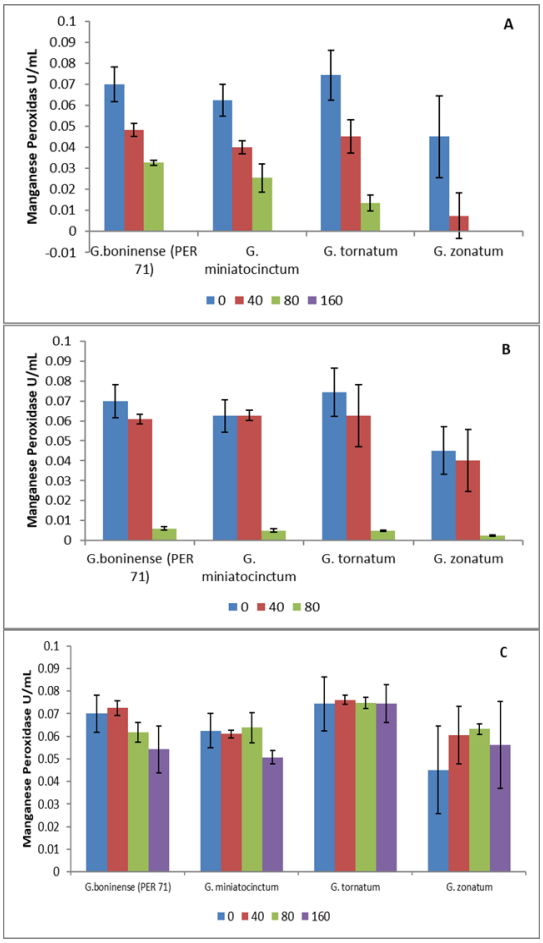

Fig. 1: The quantification of manganese peroxidase produced by various species of *Ganoderma* in the presence of inhibitors. (**A**) TGA (**B**) EDTA and (**C**) Mercaptoethanol. Each value is the mean of four replicates (n=4). Vertical bars indicate standard error

tornatum, G. miniatocinctum, G. zonatum and G. xylonoides (Steyaert, 1967), of which *G. boninense* is the most virulent strain till date (Ho and Nawawi, 1986). Hence, most of the studies were focused only on *G. boninense* and very limited number of studies is conducted on other species of *Ganoderma* causing BSR in oil palm. Even though other *Ganoderma* spp. except *G. boninense* are less virulent, their potential as a causal pathogen of BSR disease in oil palm should not be over looked.

White rot fungi are known to degrade lignin by means of their extracellular lignolytic systems to carbon dioxide and water (Kirk and Farrell, 1987). Lignin degradation by *Ganoderma* spp. is evident in this study by the presence of clear decolorized zone on the RBBR dye amended test plates. Various lignolytic fungi decolorize azo dyes using lignolytic enzymes namely, laccases, lignin peroxidase and manganese peroxidases. Generally, the fungal metabolism of azo dyes began by a reductive cleavage of the azo bond, resulting in the formation of colorless amines. These reductive procedures are defined for some aerobic microorganisms, which can grow with azo compounds.

The structure of anthraquinone-based dyes is similar to lignin backbone, and thus proficiently decolorized by the white rot fungi such as *Ganoderma*. It has been reported that the observation of RBBR decolorization halo in solid medium is a rapid and reliable validation of presence of the enzymatic activity responsible for the degradation of the dye and other xenobiotics (Machado *et al.*, 2005). The main enzymes responsible for decolorization of RBBR involve phenol and peroxidase enzymes. *Ganoderma* spp. are efficient white rot fungi that yield potential laccase (Revankar and Lele, 2007; Senthilkumar *et al*., 2014). It was reported that degree of dye decolorization by *Ganoderma* spp. was very high compared to the most extensively used strains such as *Trametes versicolor* and *Phanerochaete chrysosporium* (Revankar and Lele, 2007). More recently, Murugesan *et al*. (2009) showed that *G. lucidum* (KMK2) produced laccase as a key extracellular enzyme during solid-state fermentation on wheat bran and was recognized as thermostable enzyme effectively decolorizing different reactive dyes.

G. boninense (PER71) showed average growth velocity higher than or equal to 1 cm day^{-1} (Table 2). This was in accordance with the study conducted by Surendran *et al.* (2017). The higher growth rate of the fungus is one of the desirable characteristics in colonization by the pathogenic fungi making them more virulent with the native soil biota. Our result was in accordance to the study conducted to evaluate the pathogenicity of *G. boninense* and *G. zonatum* conducted by Rakib *et al.* (2015). It clearly states that the growth of *G. boninense* is far higher than *G. zonatum*. According to this study, *G. miniatocinctum* has the intermediate growth velocity between *G. boninense* and *G. zonatum*. *G. miniatocintum* is also a known causal agent of BSR and should not be overlooked for their virulence.

In general, the *Ganoderma* spp. produced laccase approximately 10 times higher than that of the other two peroxidase enzymes. This might be due to the high glucose and nitrogen content in the medium which is hindrance for the production of peroxidase enzymes and laccase is insensitive to the C and N levels (Paterson, 2007). The production of laccase plays an important role in the pathogenicity. The laccase plays crucial role in lignin degradation and aids the pathogen to overcome the toxic substance produced by the host. Hence the laccase is an important factor in the plant-pathogen interaction (Baldrian, 2006). This supports the idea of inhibition of lignin degrading enzymes can slow down the infection process. The findings in this study could be well corroborated with those who stated that the quality and the quantity of the lignolytic enzyme produced is species specific (Jong *et al.*, 1999). For instance, at the given similar conditions *G. boninense* produced 0.072 U mL^{-1} of manganese peroxidase whereas *G. zonatum* produced only 0.045 U mL^{-1}. It has

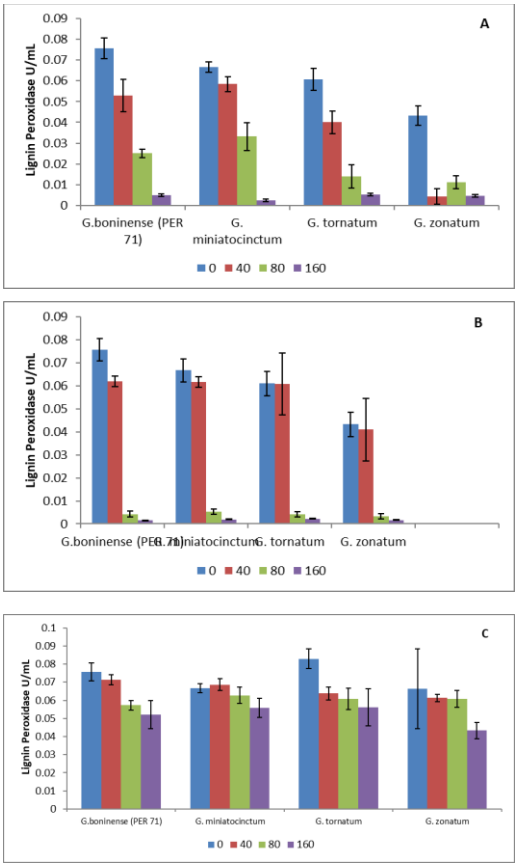

Fig. 2: The quantification of lignin peroxidase of various species of *Ganoderma* in the presence of inhibitors. (**A**) TGA, (**B**) EDTA and (**C**) mercaptoethanol. Each value is the mean of four replicates (n=4). Vertical bars indicate standard error

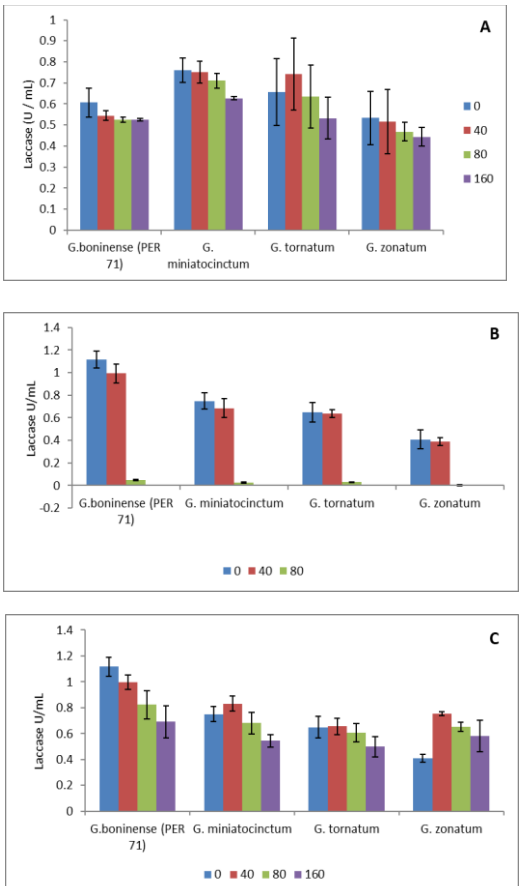

Fig. 3: The quantification of laccase produced by various species of *Ganoderma* in the presence of inhibitors. (**A**) TGA, (**B**) EDTA and (**C**) mercaptoethanol. Each value is the mean of four replicates (n=4). Vertical bars indicate standard error

been well documented that nitrogen levels and aeration influence enzyme production by white rot fungi (Woude *et al.*, 1993; Eggert *et al.*, 1997; D'souza *et al.*, 1999), while in others N-sufficiency (low C:N) results in enhanced enzyme production (Kaal *et al.*, 1993).

The major enzymatic activity detected in the supernatant from *G. boninense* was laccases (benzenediol: oxygen oxidoreductase) which is involved in the biodegradation of lignin (Eggert *et al.*, 1997). The lignolytic enzymes are widely used in various industries. These enzymes have the wide potential application in the biotechnology fields such as bio-bleaching, increasing the strength of cellulose fibers, textile dye or stain bleaching, and bioremediation (Johannes *et al.*, 1996; Alexandre and Zhulin, 2000; Wong *et al.*, 2000; Martins *et al.*, 2002). Due to their ability to degrade aromatic compounds, these enzymes are employed to degrade the industrial waste, allowing them to be considered as potential bioremedial agents. Even though *G. zonatum* produced the lignolytic enzymes in the lesser quantity their inhibition percentage by the three inhibitors are far low when compared to the other species. This indicates that *G. zonatum* lignolytic enzymes can be potential agents to use in the industrial sectors.

TGA, EDTA and mercaptoethanol are commonly used to degrade the proteins in proteomics studies. However, no studies are available on the influence of these chemicals on production of lignin degrading enzymes by *Ganoderma* spp. It was observed that TGA and EDTA are the potential inhibitors for the lignolytic enzymes of *Ganoderma*. In this study, we used two different agents, first is the chelating agent and the next is the reducing agent. EDTA is considered as a strong chelating agent for copper ions. The strong inhibition of the lignolytic enzymes by EDTA states that these enzymes have copper ions in their active site (Rodriguez *et al.*, 1996). When EDTA removes the copper ions from the active site, it leads to the conformational change in active site of the enzymes and hence inactivated. It has been reported that type-II copper ions can be reversibly removed from any protein by EDTA, such that the copper depletion protein is in an inactive form states that type- II copper ions has enzymatic functional role in the protein (Jaiswal *et al.*, 2014).

The other two inhibitors such as TGA and mercaptoethanol are categorized as reducing agents. Two types of inhibition have been reported by the reducing agents. The first type, the reducing agents might change the oxidative state of the copper ions in the active site and results in inactivation of the enzymes. The second mechanism is by removing the disulfide bonds and causing the conformational change. The reducing agent removes the OH molecules in the disulfide bond present the cysteine. This is the indication of presents of cysteine in the active site and responsible for the enzyme activity (Chefetz *et al.*, 1998). The results obtained in this study are in acceptance with studies conducted on lignolytic enzymes of *Fomes annosus* influenced by TGA (Harrs and Huttermann, 1980).

Chemical control of BSR has not been effective and long lasting even though *in vitro* screening has identified several chemicals that are effective against *Ganoderma* (Hashim, 1990). The effective use of chemical control for treatment of *Ganoderma* - infected palms is limited by the fact that both visibly infected and subclinical palms may harbour established infections by the time treatment is applied. However, preliminary results on trunk injection of fungicides into BSR-infected oil palms have indicated that Traidimenol® increased their economic life span as treated palms remain alive 52 months after the original BSR diagnosis (Chung *et al*., 1988). Similar findings by (Ariffin and Idris, 1993) indicated that systemic fungicides (Tridemorph® and Dazomet®) also limited the spread of infection and further concluded that the chemical moved systemically downwards into the roots when injected into plants. This *in vitro* study revealed that the chelating and reducing agents used had inhibitory effect on production of lignolytic enzymes by *Ganoderma* spp. that might have a great role in BSR development. These agents can be used as chemical treatment using high-pressure injections resulting in the passage of chemicals straight into the trunk or infection sites.

Conclusion

The sustainability of oil palm cultivation is of direct concern for farmers and producers and eventually the countries involved in palm oil production. *Ganoderma* spp. are known to degrade the lignin component of the palm wood engaging an arsenal of extracellular enzymes to allow the energy rich cellulose to be utilized. When the cellulose is degraded the palm is vulnerable to collapse. If the lignin barrier is maintained or not allowed to be degraded could be advantageous in management of this devastating pathogen and disease. This approach of BSR control involving inhibition of lignin degradation enzymes utilizing reducing and chelating agents could be a possible chemical control of *Ganoderma* on oil palm. Further nursery and field trials, and investigation on the environmental impact of using these chemicals in the field, needs consideration as they are often toxic. But this is a good initial investigation in search of suitable chemical control of BSR.

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