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AGGRESSIVENESS OF *Ganoderma boninense* AND *G. zonatum* ISOLATED FROM UPPER- AND BASAL STEM ROT OF OIL PALM (*Elaeis guineensis*) IN MALAYSIA

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ABSTRACT

Currently, the most known devastating oil palm disease throughout South-east Asia is basal stem rot (BSR) caused by Ganoderma boninense. *However, there is insufficient information on* G. zonatum *which also has been associated with the BSR, and upper stem rot (USR). Thus, this study reports pathological symptoms and degree of aggressiveness amongst* G. zonatum *and* G. boninense *of USR and BSR on oil palm seedlings. All the* Ganoderma *isolates tested showed positive signs of infection on the seedlings at 12 and up to 24 weeks after inoculation. However, the symptoms of infection on the seedlings were indistinguishable amongst the* Ganoderma *species tested. In fact, they showed significantly different degree of aggressiveness in terms of area under disease progress curve (AUDPC), epidemic rate, severity of foliar symptoms (SFS), disease severity index (DSI), stem bole necrosis and primary roots necrosis. The present findings suggested that* G. zonatum *of USR was the most aggressive, followed by* G. zonatum *and* G. boninense *of BSR, and* G. boninense *of USR was the least aggressive. Hence, a new mechanism of control strategies is urgently required to contain the disease from spreading especially for USR and also for* G. zonatum *in Malaysia.*

Keywords: basal stem rot, *Ganoderma boninense*, *Ganoderma zonatum*, pathogenicity, upper stem rot.

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INTRODUCTION

Oil palm (*Elaeis guineensis*) is an oil producing crop which contributes 39% of the world's vegetable oils and fats supply, making it as the most important oil producing crop in the world (USDA, 2014). Oil palms are mostly planted in the South-east Asia region, especially in Malaysia and Indonesia. The sustainability of production in the region is threatened by a basidiomycete fungus, *Ganoderma* species. The most prominent disease is basal stem rot (BSR) caused by *G. boninense* which reduces the yield and shortens the economic life of oil palm (Flood *et al*., 2000; Corley and Tinker, 2003). However, little is known about the threats by other species of *Ganoderma* in the oil palm plantations such as *G. zonatum* which is also associated with BSR of oil palm in Malaysia (Idris *et al*., 2000). Besides that, similar pathogens were also found to be closely associated with upper stem rot (USR) of oil palm (Hasan *et al*., 2005; Pilotti, 2005; Rees *et al*., 2012; Rakib *et al*., 2014a).

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Occurrence of USR of oil palm in Malaysia has been reported since 1937 and it usually occurs on deep peat and inland valley soils. USR is not considered as a major disease of oil palm at the earlier time (Thompson, 1937) but recently, USR has begun to gain more attention when a few cases of the disease were observed to severely infect oil palm with the presence of *Ganoderma* species in Sabah, Malaysia (Abdullah *et al*., 1999), Papua New Guinea (Pilotti, 2005) and Indonesia (Rees *et al*., 2012). There is little information about USR due to the lack of studies on epidemiology and etiology of the causal pathogen.

There were several reports on aggressiveness of *G. boninense* which cause BSR in oil palm (Rees *et al*., 2007; Sariah *et al*., 2007; Kok *et al*., 2013). On the other hand, the degree of aggressiveness of *Ganoderma* species isolated from USR-infected oil palms still remains unknown. Moreover, until recently, there has been no comparative study between *G. boninense* and *G. zonatum*. Understanding the variation in aggressiveness level amongst *Ganoderma* species may contribute towards a vital role in strategising the disease control programmes because disease intensity and rate of infection progression of USR and BSR could be different.

Assessment of infection symptoms by using an appropriate variable could be used to evaluate the aggressiveness of *Ganoderma* species which corresponds to the intensity of damage caused by the pathogen. Furthermore, pathogenicity and aggressiveness of the pathogen were positively correlated (Breton *et al*., 2006). Artificial inoculation of *Ganoderma* species has been used to evaluate the infection with various inoculum types and sizes tested on oil palm germinated seeds or seedlings. This technique has been adapted in several studies to test the pathogenicity of *Ganoderma* species (Idris *et al*., 2006; Sariah *et al*., 2007), the aggressiveness within species of *Ganoderma* (Rees *et al*., 2007; Kok *et al*., 2013), the susceptibility of different varieties of oil palm against *Ganoderma* species (Idris *et al*., 2004; Breton *et al*., 2006; Chong *et al*., 2012a), and the effectiveness of bio-control agents against *Ganoderma* species (Shamala *et al*., 2008; Zaiton *et al*., 2008).

USR disease and *G. zonatum* should not be overlooked by the researchers as well as the estate managers because, in the long run, this disease and pathogen may destroy the estates and seriously affect the oil palm industries. As such, information on the pathological data and aggressiveness of *Ganoderma* species is very important to effectively address the problem. Thus, the objectives of this study are to evaluate the pathological symptoms of *Ganoderma* infection and aggressiveness among *G. zonatum* and *G. boninense* of USR- and BSR-infected oil palms.

MATERIALS AND METHODS

Ganoderma Samples and Planting Materials

A total of 45 isolates of *Ganoderma* was obtained from USR- and BSR-infected palms from oil palm plantations in Sarawak, Malaysia (Rakib *et al*., 2014a). This study includes 20 and 13 isolates of *G. zonatum*, and nine and three isolates of *G. boninense* from USR- and BSR-infected palms, respectively. Planting materials used in this study were 3-month old oil palm (*Dura* × *Pisifera*) seedlings in polyethylene bags (15 × 20 cm) which were obtained from the Sarawak Oil Palm Berhad (SOPB) nursery in Sarawak, Malaysia.

Preparation of *Ganoderma* Inoculum and Inoculation on Oil Palm Seedlings

Methods for preparation of *Ganoderma* inoculums on 6 × 6 × 12 cm rubber wood block (RWB) were adopted and modified from Breton *et al*. (2006), Rees *et al*. (2007) and Kok *et al*. (2013) as described by Rakib *et al*. (2014a). RWB were initially soaked overnight in water and cleaned. They were then placed into autoclavable polypropylene plastic bags individually. Ten millilitres of 0.2% malt extract were added and autoclaved for 6 hr at 121ºC. The RWB was then added with additional 10 ml of sterile molten malt extract agar. They were left to solidify, and mycelia plugs (15 mm) were placed on each of the four different sides of the block. Finally, the inoculated RWB was sealed and then incubated for nine weeks in the dark and at a room temperature of 27±2°C for the fungal mycelia to fully colonise the block.

The inoculum was applied upon transplanting of the 3-month old oil palm seedlings to bigger polyethylene planting bags (20 × 30 cm) containing unsterile soil medium (2:1 soil to sand mixture). The seedlings were seated onto the inoculum to ensure the roots were in direct contact with the inoculum (Rees *et al*., 2007; Sariah *et al*., 2007; Kok *et al*., 2013). Each *Ganoderma* isolate was tested with three seedlings per replication in four blocks for a total of 12 seedlings per isolate. Uninoculated RWB served as a negative control. A total of 552 (including control) experimental units were arranged in a randomised complete block design (RCBD). The trial was conducted in a netted plant house facility in the Universiti Putra Malaysia Bintulu, Sarawak Campus (UPMKB) with temperature and relative humidity ranging from 25°C-35°C and 60%-80%, respectively.

Assessment of Pathogenicity Trial

Aggressiveness of the *Ganoderma* species was assessed based on external symptoms recorded every four weeks over a period of 24 weeks, which were: i) area under disease progress curve (AUDPC); ii) epidemic rate (percent per week); iii) severity of foliar symptoms (SFS); and iv) disease severity index (DSI).

AUDPC and epidemic rate were derived from disease incidence, which is referred to as the percentage of seedlings visually observed as infected (either with chlorotic or necrotic leaves, with or without appearance of basidiocarp) from the total number of seedlings per treatment. The AUDPC was calculated according to Shaner and Finney (1977), where n is the number of assessment times, *Y* is the disease incidence, and t is the observation time.

$$\text{AUDPC} = \sum_{i}^{n-1} \left(\frac{Y_i + Y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

The epidemic rate was obtained from the slope of regression curve derived from arcsine transformed data of disease incidence. The SFS was assessed according to Sariah and Zakaria (2000), where *a* is the number of necrotic (browned) leaves, *b* is the number of chlorotic (yellowing) leaves, *c* is the total number of leaves, and 1 and 0.5 are the constant index representing necrotic and chlorotic leaves, respectively.

$$\text{SFS (\%)} = \left(\frac{(a \times 1) + (b \times 0.5)}{c} \right) \times 100$$

The DSI was assessed according to Ilias (2000), where it was referred as visual observable symptoms of infected seedlings based on numerical values of disease class, which ranged from 0 to 5 in this study (*Table 1*), where *A* is the disease scale (0 to 5), *B* is the number of seedling showing the disease scale per treatment, *n* is the total number of replication, and 5 is the constant representing the highest scale of assessment.

$$\text{DSI (\%)} = \frac{\sum(A \times B)}{\sum n \times 5)} \times 100$$

The aggressiveness of *Ganoderma* species was also assessed based on internal symptoms. Destructive sampling was carried out at the end of the trial (24 weeks after inoculation). The seedling was detached from the RWB inoculum and soil medium carefully and cleaned by using running water. It was then cut cross-sectioned at the root base and stem bole, and assessed for internal symptoms, which were the percentage of necrotic primary root and stem bole necrosis area (using 1 mm grids). The presence of *Ganoderma* was either verified visually (presence of *Ganoderma* basidiocarp) or by using *Ganoderma* selective medium (GSM) plating of necrotic primary roots and stem bole tissues for non-visible *Ganoderma* presence to complete the Koch's postulate (Idris *et al*., 2006; Rees *et al*., 2009). Furthermore, the average percentage of disease incidence was recorded based on appearance of fungal mass, foliar symptoms, and stem and root necrosis to note the infection progression on an oil palm seedling.

Data Analysis

Data from each isolate was pooled based on the species of *Ganoderma* (either *G. zonatum* or *G. boninense*) and disease type (either USR or BSR). All the data were subjected to arcsine transformation prior to statistical analysis. Analysis of variance (ANOVA) and the Duncan's new multiple range test (DNMRT) were performed on each variable tested for mean comparisons. Variables recorded in this study were also subjected to Pearson's correlation analysis to cross validate all the variables used and evaluate any relationship between them. All statistical analysis was performed using the Statistical Analysis System (SAS version 9.2) program.

RESULTS

External and Internal Symptoms of *Ganoderma*-infected Oil Palm Seedlings

All 45 *Ganoderma* isolates tested showed positive signs of disease infection on the oil palm seedlings. Plating of the necrotic roots and stem bole tissues onto GSM constantly showed the presence of *Ganoderma* as they yielded white colony with the presence of brown halo around the colony. The uninoculated seedlings (negative control) did not show any sign of disease infection. Therefore, all 45 *Ganoderma* isolates were confirmed to be pathogenic to oil palm seedlings.

G. zonatum and *G. boninense* of both USR and BSR showed similar external and internal symptoms of infection on oil palm seedlings. Generally, the external symptoms appeared at 12 weeks after inoculation (WAI) and in several seedlings, the symptoms appeared as early as 8 WAI. External infection symptoms observed include leaf chlorosis (yellowing leaf) which eventually became necrotic (brown or dead leaf). Mycelia mass of *Ganoderma* appeared at the base of the seedlings which progressively developed into small white buttons that eventually formed bracket shape *Ganoderma* fruiting body. Severe leaf symptoms were observed as the infection progressed over time and eventually the seedlings died, either with or without visible above ground fungal mass on any part of the seedlings (*Figure 1*). Internal symptoms of infected primary roots and stem bole showed necrosis whereas healthy (negative control) seedlings showed no sign of necrotic root and stem bole (*Figure 2*).

At the end of the trial, it showed that significantly high number of oil palm seedlings had necrotic roots,

TABLE 1. NUMERICAL DISEASE SCALES AND THEIR CORRESPONDING SYMPTOMS

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

which accounted for 73.34% of the total inoculated seedlings. This was followed by stem bole necrosis and external foliar symptoms, which accounted for 64.28% and 62.33% of the total inoculated seedlings, respectively. However, they were not significantly different. Significantly low number of oil palm seedlings had above ground fungal mass, either white mycelia, small white button, or bracket shape *Ganoderma* which accounted for 49.21% of the total inoculated seedlings (*Figure 3*). Out of the total 540 inoculated seedlings, 13.33% (72 seedlings) were dead due to infection of *Ganoderma* throughout the trial period.

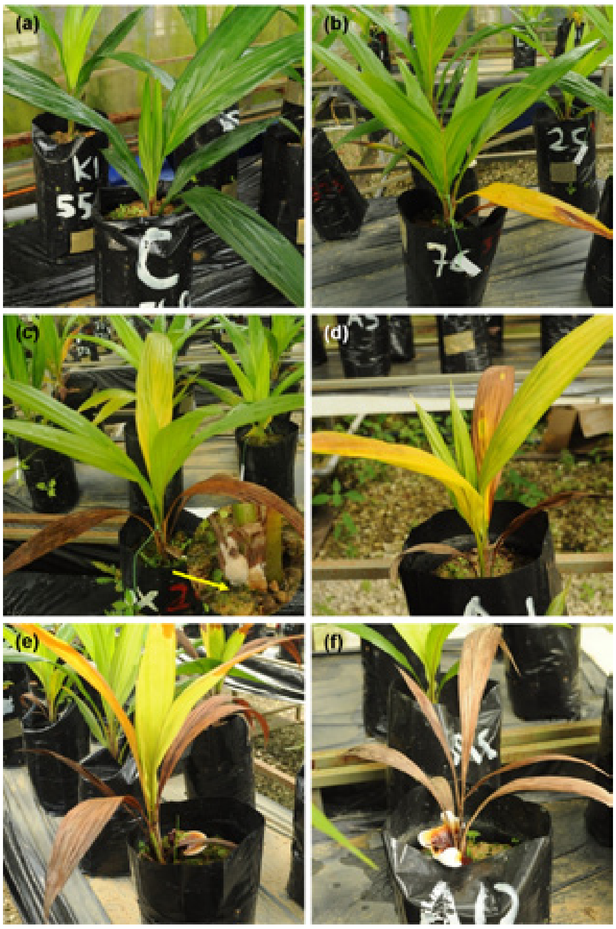

Figure 1. Infection progression of Ganoderma*-inoculated oil palm seedlings exhibits external symptoms. (a) Healthy seedling with no sign of infection; (b) seedling started to show leaf chlorosis or yellowing of the leaf either with or without fungal mass; (c) more leaves became chlorotic and eventually became necrotic (dead leaves), and fungal mass appeared at the base of a seedling's bole (arrow); (d) severe leave chlorosis and necrosis; (e) basidiocarp of* Ganoderma *formed with severe leaf symptoms on a dying seedling; (f) desiccated dead seedling with well-developed* Ganoderma *basidiocarp.*

Aggressiveness of *Ganoderma* Species

Although all the *Ganoderma* isolates tested showed indistinguishable external and internal symptoms amongst *G. zonatum* and *G. boninense* of USR and BSR, they however significantly exhibited different degree of aggressiveness level against the oil palm seedlings. Aggressiveness as evaluated

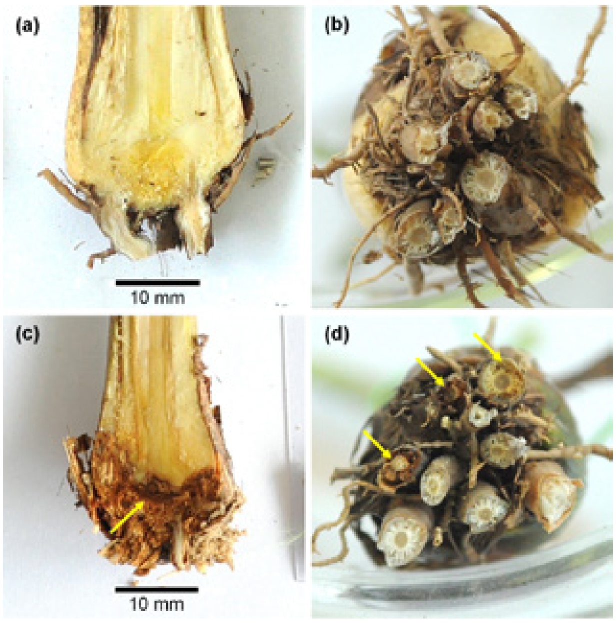

Figure 2. Oil palm seedlings exhibits internal symptoms of the bole (stem) and root (root base). (a) Control (uninoculated) sample with healthy bole with no sign of necrosis; (b) control (uninoculated) sample with healthy primary root base with no sign of necrosis; (c) Ganoderma*-inoculated sample with necrosis of internal bole tissue (arrow); (d)* Ganoderma*-inoculated sample with necrosis of primary root base (arrows).*

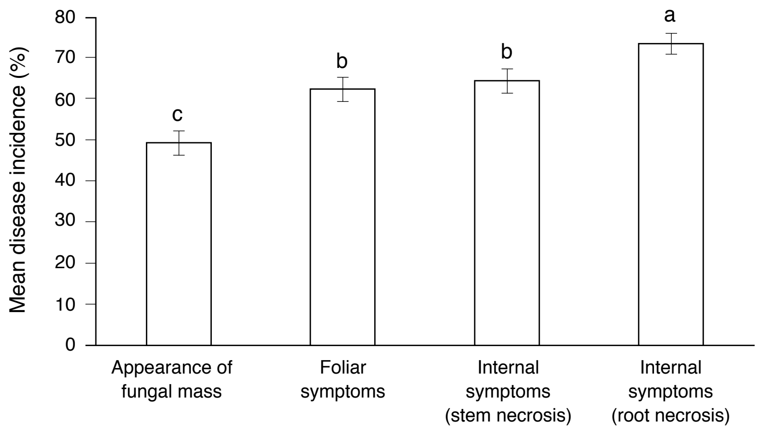

Figure 3. Mean (± standard error) percentage of disease incidence (DI) of all Ganoderma-*inoculated oil palm seedlings based on four different evaluations. Mean with different alphabets were significantly different at* $p < 0.05$ *by Duncan's new multiple range test (DNMRT).*

by the AUDPC indicates the amount of disease developed, where higher AUDPC corresponds to more aggressive isolate and *vice versa*. AUDPC value of *G. zonatum* of USR- and BSR-infected palms ranged from 300 – 983 units^2 and 200 – 900 units^2, respectively, which were generally higher compared with *G. boninense* of USR- and BSR-infected palms that ranged from 133 – 692 units^2 and 167 – 600 units^2, respectively. Moreover, epidemic rate indicated that *G. zonatum* of USR-infected palms (0.015% – 0.081% per week) and BSR-infected palms (0.019%-0.091% per week) had more rapid infection rate on the seedlings compared with *G. boninense* of USR-infected palms (0.015%-0.049% per week) and BSR-infected palms (0.021%-0.041 % per week) (*Table 2*).

Mean SFS of *G. zonatum* of USR-infected palms was significantly higher compared with those from BSR-infected palms, which was 5.25% and 1.46% at 12 WAI for USR and BSR, respectively. The mean increased to 33.18% and 23.69% at 24 WAI for USR and BSR, respectively (*Figure 4a*). *G. boninense* which was isolated from USR-infected palms showed significantly lower SFS compared with those from BSR-infected palms at 16 WAI (1.77% for USR and 4.89% for BSR) and 20 WAI (5.68% for USR and 14.91% for BSR). However, there was no significant difference between *G. boninense* of USR- and BSR-infected palms at 24 WAI which was 12.7% and 19.83%, respectively (*Figure 4b*). Furthermore, *G. zonatum* of USR-infected palms was significantly more aggressive than *G. boninense* of USR-infected palms (*Figure 4c*) while no significant difference was observed between *G. zonatum* and *G. boninense* of BSR-infected palms (*Figure 4d*).

Mean DSI of *G. zonatum* of USR-infected palms (6.84%) was significantly higher than those from BSR-infected palms (2.39%) at 12 WAI, and up to 43.78% and 34.08% at 24 WAI for USR and BSR, respectively (*Figure 5a*). DSI of *G. boninense* from USR was significantly lower (2.84%) compared with that from BSR (7.64%) at 16 WAI, but at 24 WAI, there was no significant difference in DSI caused by *G. boninense* of USR and BSR (*Figure 5b*). *Figure 5c* shows that within the USR palms, *G. zonatum* was significantly more aggressive than *G. boninense* but for BSR palms, there was no significant difference in aggressiveness between *G. zonatum* and *G. boninense* (*Figure 5d*).

A comparison between *G. zonatum* of USR and BSR (*Figure 6a*) and a comparison between *G. zonatum* and *G. boninense* of BSR (*Figure 6d*) in terms of stem bole and primary root necrosis showed that they were not significantly different. Stem bole necrosis between *G. boninense* of USR (20.51%) and *G. boninense* of BSR (30.04%) did not differ significantly, but primary root necrosis was significantly lower for *G. boninense* of USR (25.75%) compared with those isolated from BSR-infected palms (42.55%) as shown in *Figure 6b*. There was significantly higher stem bole necrosis caused by *G. zonatum* (42.20%) compared with *G. boninense* (31.29%) isolated from USR-infected palms, and similarly, *G. zonatum* of USR (48.55%) caused significantly more damage compared with *G. boninense* of USR (38.78%) in terms of primary root necrosis (*Figure 6c*).

Furthermore, Pearson's correlation analysis indicated that all of the variables were positively correlated ($p < 0.05$), in which Pearson's correlation coefficient, R ranged from 0.307 to 0.964 (*Table 3*).

DISCUSSION

Both *G. zonatum* and *G. boninense* isolated from USR and BSR palms were confirmed to be pathogenic to oil palm through pathogenicity test conducted and Koch's postulate was fulfilled. Besides the two species of *Ganoderma*, Idris *et al*. (2000; 2006) also reported that *G. miniatocinctum* as one of the pathogens causing BSR of oil palm through artificial inoculation. This present study showed similar external and internal infection symptoms on oil palm seedlings as reported in other studies on *Ganoderma*

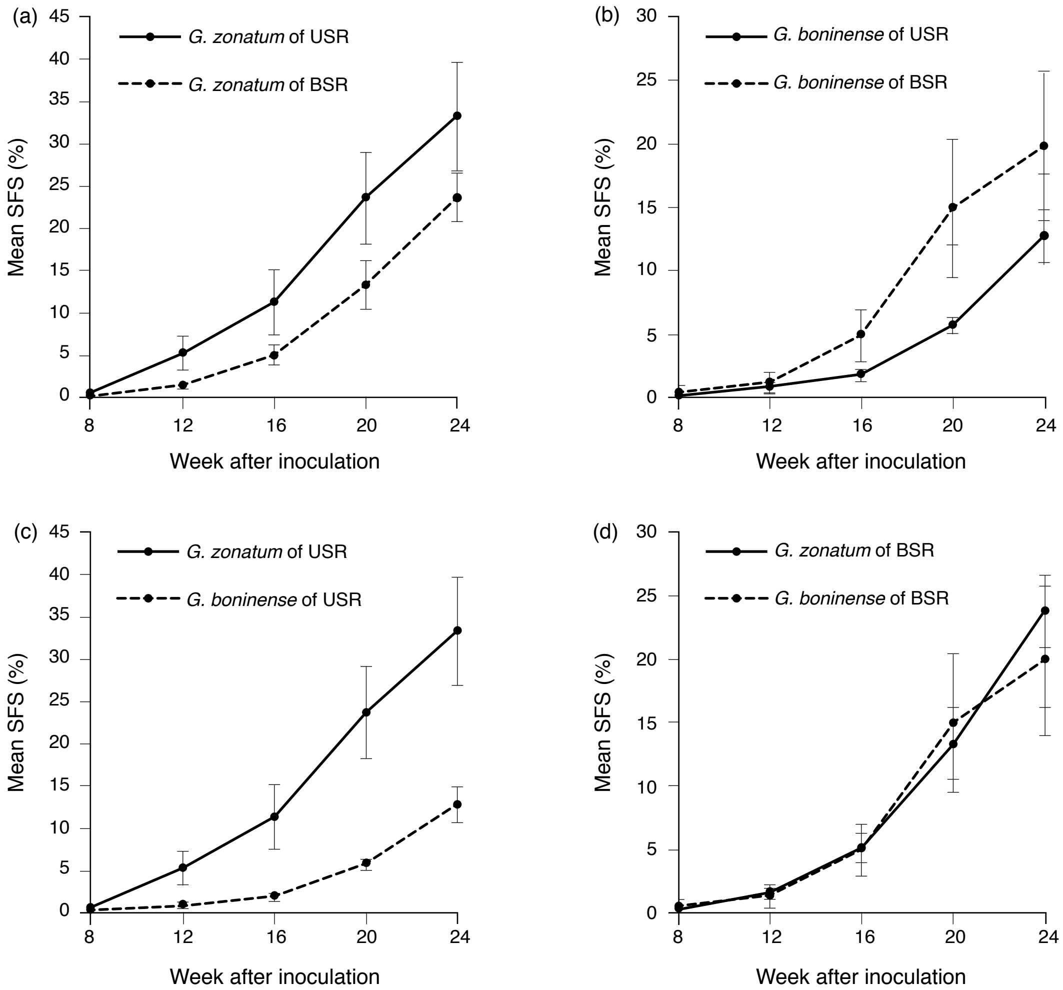

Figure 4. Mean (± standard error) severity of foliar symptoms (SFS) at four weeks intervals throughout 24 weeks after inoculation with G. zonatum *and* G. boninense *from upper stem rot (USR) and basal stem rot (BSR) infected oil palms. (a) Between* G. zonatum *from USR and BSR, (b) between* G. boninense *from USR and BSR, (c) between* G. zonatum *and* G. boninense *from USR, (d) between* G. zonatum *and* G. boninense *from BSR.*

boninense of BSR (Rees *et al.*, 2007; 2009; Shamala *et al.*, 2008; Zaiton *et al.*, 2008; Kok *et al.*, 2013).

Most infection symptoms on the seedlings were recorded based on the primary roots necrosis, followed by stem bole necrosis and foliar symptoms and lastly by basing on the appearance of fungal mass. This indicated that in general, the infection of *Ganoderma* species on oil palm seedlings was initiated at the root as the root and inoculum source were in direct contact. The infection later progressed to the stem bole. Foliar symptoms would appear once the stem bole was infected as the damaged internal stem bole was unable to sustain nutrients required for the infected seedlings (Rees *et al.*, 2009). Eventually, *Ganoderma* fungal mass appeared on the stem bole of the infected seedlings. Detailed infection progression through similar infection technique was reported by Rees *et al.* (2009) whereby the infection by *Ganoderma* species on the root's surface occurred from root contact with the hyphae. The hyphae then penetrates the root's epidermis and exodermis and eventually infects the stem bole, which was confirmed through plating on GSM and observation under electron microscopy (Rees *et al.*, 2009). In other reports, the infection mode through contact between *Ganoderma* inoculum and oil palm's root was further supported by Flood *et al.* (2000; 2005). Progressive desiccation of leaves and emergence of white fungal mass which developed to small white button and bracket shape basidiocarp were similar to those reported in other studies (Breton *et al.*, 2006; Sariah *et al.*, 2007; Kok *et al.*, 2013).

The present findings suggested that internal and external symptoms amongst the *G. zonatum* and *G. boninense* of USR and BSR on oil palm seedlings were indistinguishable, but varied in level of aggressiveness in terms of AUDPC, epidemic rate, SFS, DSI, stem bole necrosis and primary root necrosis. A wide range of variation within *Ganoderma* species in terms of AUDPC and epidemic rate could be attributed to the *Ganoderma* isolates which were collected from different sources and different geographical origins as reported in several studies (Breton *et al.*, 2006; Rees *et al.*, 2007;

TABLE 2. AREA UNDER DISEASE PROGRESS CURVE (AUDPC) AND EPIDEMIC RATE FOR INFECTION OF *G. zonatum* AND *G. boninense* OF UPPER STEM ROT (USR) AND BASAL STEM ROT (BSR) ON OIL PALM SEEDLINGS OVER A PERIOD OF 24 WEEKS

Species	Disease	Isolate*	AUDPC (units²)	Epidemic rate** (% per week)	Coefficient of determination (R2)**
G. zonatum	USR	G3	983	0.064	0.88
		G4	867	0.081	0.90
		G6	382	0.027	0.89
		G11	900	0.051	0.95
		G12	709	0.040	0.92
		G14	764	0.058	0.94
		G23	507	0.028	0.99
		G25	369	0.031	0.86
		G26	300	0.027	0.86
		G27	308	0.027	0.76
		G28	618	0.040	0.96
		G29	585	0.035	0.97
		G30	345	0.025	0.93
		G31	440	0.022	0.65
		G32	760	0.075	0.87
		G33	554	0.035	0.97
		G34	883	0.046	0.96
		G35	380	0.015	0.92
		G36	583	0.040	0.94
		G37	300	0.019	0.94
	BSR	G15	900	0.061	0.97
		G16	500	0.040	0.87
		G17	533	0.044	0.85
		G18	633	0.053	0.92
		G19	400	0.022	0.97
		G20	367	0.035	0.82
		G21	382	0.023	0.93
		G41	400	0.041	0.80
		G42	600	0.038	0.96
		G43	200	0.019	0.69
		G44	333	0.020	0.94
		G45	400	0.037	0.86
		G46	680	0.091	0.75
G. boninense	USR	G1	508	0.019	0.93
		G2	550	0.031	0.95
		G5	692	0.049	0.95
		G7	450	0.033	0.93
		G8	267	0.026	0.80
		G9	267	0.033	0.68
		G10	215	0.022	0.77
		G13	383	0.033	0.88
		G22	133	0.015	0.69
	BSR	G38	600	0.041	0.95
		G39	431	0.021	0.93
		G40	167	0.022	0.62

Note: * Isolate number as in Rakib *et al*. (2014a).
** Values were based on regression analysis of arc sine transformed data of disease incidence.

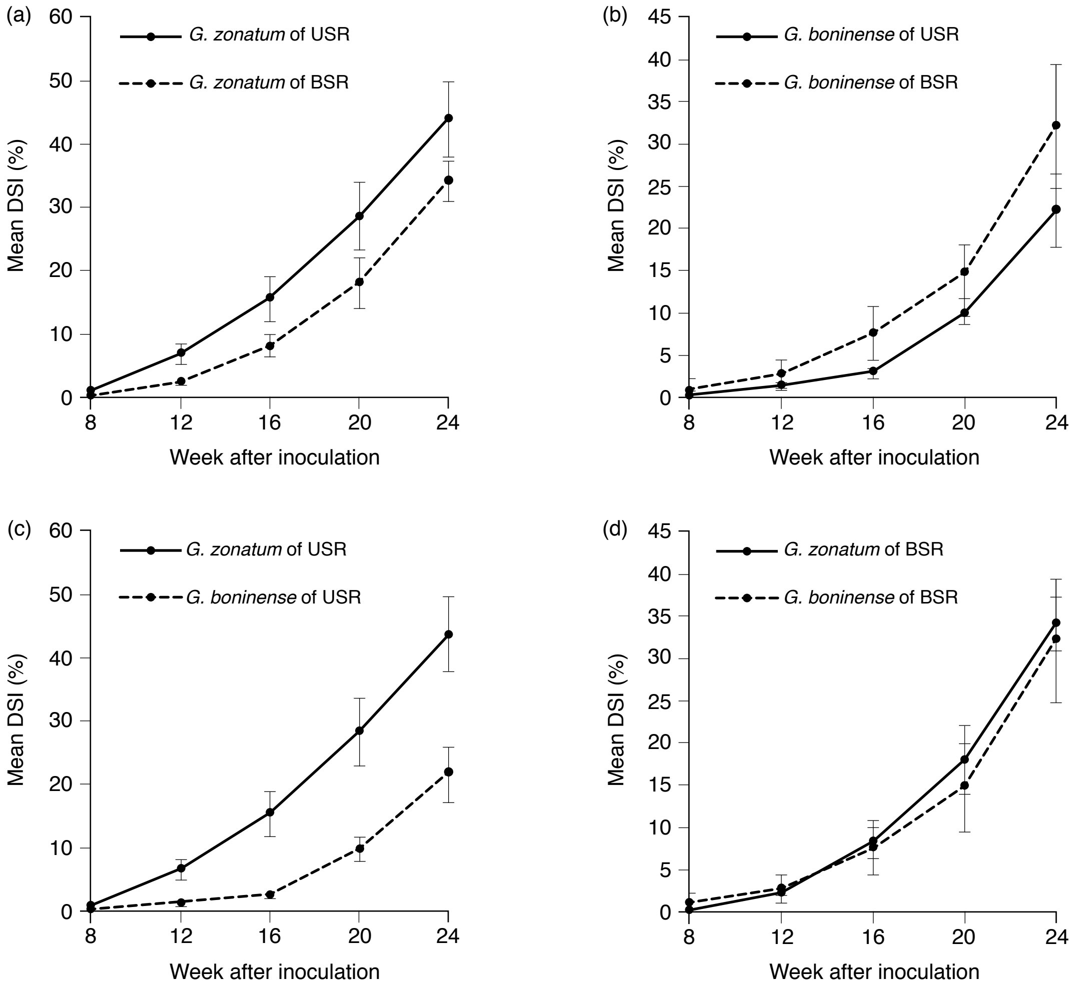

Figure 5. Mean (± standard error) disease severity index (DSI) at four weeks intervals throughout 24 weeks after inoculation with G. zonatum *and* G. boninense *from upper stem rot (USR) and basal stem rot (BSR) infected oil palms. (a) Between* G. zonatum *from USR and BSR, (b) between* G. boninense *from USR and BSR, (c) between* G. zonatum *and* G. boninense *from USR, (d) between* G. zonatum *and* G. boninense *from BSR.*

Kok *et al*., 2013), and these were related to genetic heterogeneity in the pathogenic fungal isolate that leads to differences in level of aggressiveness (Campanile *et al*., 2004). Genetic heterogeneity of *Ganoderma* species was proven with various molecular approaches such as amplified fragment length polymorphism (AFLP) (Nusaibah *et al*., 2010), random amplified microsatellite (RAMS) (Latiffah *et al*., 2005; Rees *et al*., 2012), restriction fragment length polymorphisms (RFLP) (Miller *et al*., 1999), and random amplified polymorphic DNA (RAPD) (Latiffah *et al*., 2005). Moreover, several studies have also demonstrated genetic heterogeneity through incompatibility of *Ganoderma* species (Miller *et al*., 1999; Pilotti *et al*., 2003; Pilotti, 2005; Nusaibah *et al*., 2010; Rakib *et al*., 2014a).

Generally, *G. zonatum* of USR-infected palms exhibits higher degree of aggressiveness, while *G. zonatum* and *G. boninense* of BSR-infected palms do not differ in degree of aggressiveness, and *G. boninense* of USR-infected palms was the least aggressive. Although *G. zonatum* which was isolated from USR-infected palms exhibited higher degree of aggressiveness, *G. zonatum* which was isolated from BSR-infected palms was less aggressive. Similarly, *G. boninense* isolated from BSR-infected oil palms was found to be more aggressive compared with those isolated from USR-infected palms. The factors that may attribute to differences in degree of aggressiveness of *Ganoderma* species from USR- and BSR infected palm on the seedlings were unclear. However, it could be related to complex host-pathogen interactions in the basal and upper stems of oil palm where the *Ganoderma* species were isolated in terms of bio-compounds composition such as phenolic compounds, lignin, antioxidant, and enzymes (Diaz *et al*., 2001; Lattanzio *et al*., 2006; Paterson *et al*., 2009; Chong *et al*., 2012b). The upper stem of oil palm may have different composition of bio-chemical compounds or different growing environment compared with the basal stem which may alter the genetic traits of the *Ganoderma* species and influence the aggressiveness. This phenomenon has been reported in ascomycete fungi, where its

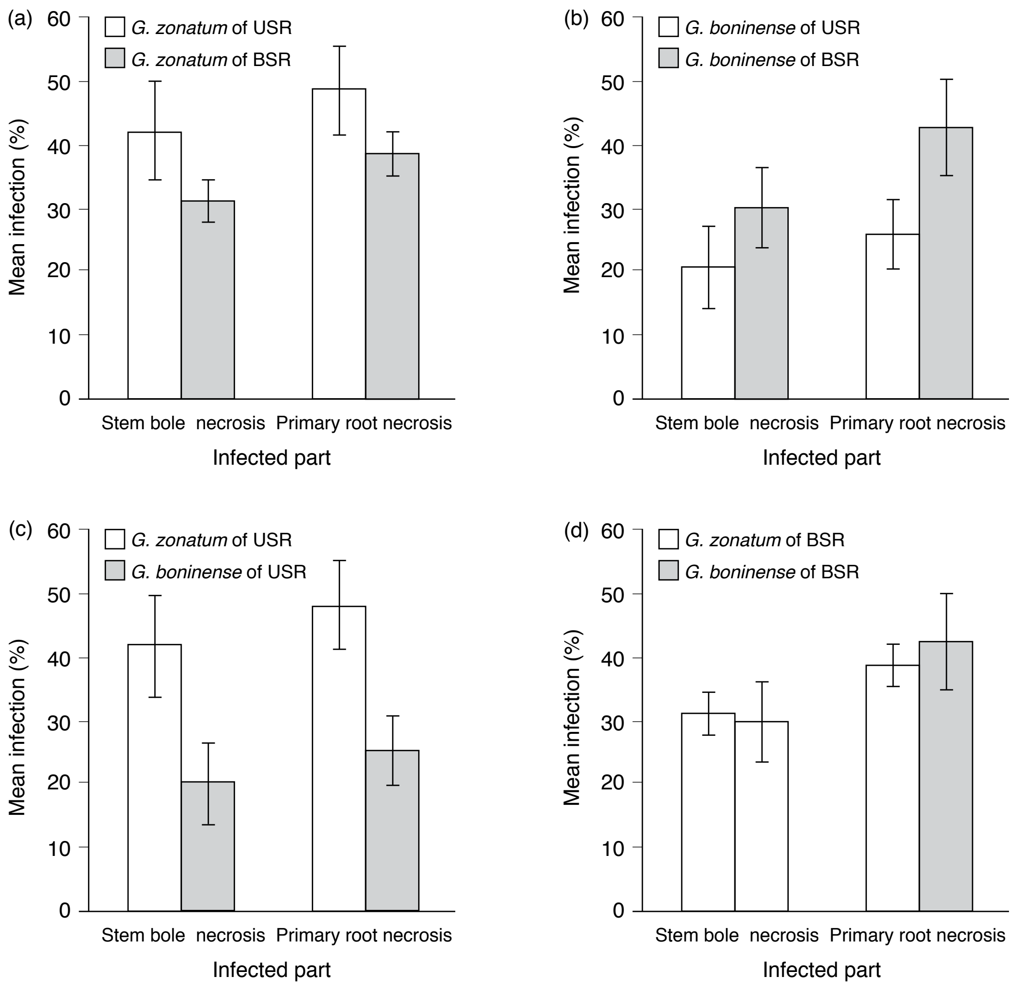

Figure 6. Mean (± standard error) stem bole necrosis and primary root necrosis at 24 weeks after inoculation with G. zonatum *and* G. boninense *from upper stem rot (USR) and basal stem rot (BSR) infected oil palms. (a) Between* G. zonatum *from USR and BSR, (b) between* G. boninense *from USR and BSR, (c) between* G. zonatum *and* G. boninense *from USR, (d) between* G. zonatum *and* G. boninense *from BSR.*

TABLE 3. PEARSON'S CORRELATION ANALYSIS BETWEEN VARIABLES TESTED TO ASSESS DISEASE INFECTION OF *Ganoderma* SPECIES ON OIL PALM SEEDLINGS

Variable	SFS	DSI	Stem bole necrosis	Primary root necrosis	AUDPC
DSI	0.958**	–	–	–	–
Stem bole necrosis	0.954**	0.940**	–	–	–
Primary root necrosis	0.923**	0.922**	0.964**	–	–
AUDPC	0.559**	0.520**	0.494**	0.439**	–
Epidemic rate	0.365*	0.351*	0.368*	0.307*	0.787**

Note: SFS - severity of foliar symptoms, DSI - disease severity index, AUDPC - area under disease progress curve.
*Significantly correlated at $p < 0.05$.
**Significantly correlated at $p < 0.01$.

genetic traits have been altered in response to the environmental stress (Gasch, 2007).

Similar level of aggressiveness between *G. zonatum* and *G. boninense* of BSR reported in this study correspond to findings by Idris *et al*. (2006) of which *G. zonatum* and *G. boninense* of BSR have caused similar degree of damage to oil palm seedlings in terms of external foliar symptoms but it was based on limited number of *Ganoderma* samples of each species which may be affected by high variation within the species. Variability in aggressiveness within the same species of *Ganoderma* has suggested that relying on limited number of isolates in the studies such as to evaluate the aggressiveness of different *Ganoderma* species, the effectiveness of bio-control agents, and the tolerance of planting material against *Ganoderma* species may be unreliable. It is crucial to incorporate more than one isolate into any of the studies regarding *Ganoderma* species. Hence, similar finding in this present study re-confirms the finding by Idris *et al*. (2006) as more samples were tested.

Some *G. boninense* of BSR from east Malaysia (Sarawak and Sabah) were stated to be less aggressive compared with *G. boninense* of BSR from west Malaysia (Peninsular) (*Chong et al*., 2011). This assumption was based on lower BSR incidence in east Malaysia compared with west Malaysia because in east Malaysia, most of the plantations are still the first generation of oil palm. Meanwhile in west Malaysia, most of the plantations have undergone second or third re-planting (Chong *et al*., 2011). Re-planting of oil palms seems to cause an increase in disease incidence in an oil palm plantation due to an increase of inoculum source that remains in the plantation (Flood *et al*., 2000; 2005). However, the hypothesis that *G. boninense* in east Malaysia is found to be less aggressive when compared with that in west Malaysia require further investigation to prove it.

Furthermore, higher aggressiveness of the *G. zonatum* of USR as reported in this study could be related to the higher occurrence of USR compared with BSR, as well as rapid infection of the disease in the plantation with higher occurrence of *G. zonatum* as reported in our previous study (Rakib *et al*., 2014b). In addition, *G. zonatum* was also reported to have relatively higher lignin degrading capability compared with *G. boninense* which may have attributed to higher aggressiveness of *G. zonatum* (Wong, 2013). Therefore, it was assumed that the higher occurrence of *G. zonatum* may lead to higher probability of the pathogen to cause the diseases, especially USR. Besides the prominent *G. boninense* causing BSR (Idris *et al*., 2004; Pilotti, 2005; Rees *et al*., 2009), the role of *G. zonatum* as a pathogen in oil palm plantations should not be overlooked. Thus, it is recommended that more emphasis should also be given to USR and *G. zonatum* for better disease management in oil palm plantations. Although both diseases were associated with similar pathogens, more specific control strategies for USR and *G. zonatum* should be taken into consideration such as those that are related to bio-control agents and disease resistant plant development as most previous disease control strategies focused only on BSR and *G. boninense* (Idris *et al*., 2004; Breton *et al*., 2006; Shamala *et al*., 2008; Chong *et al*., 2012a).

The positive and significant correlation amongst the variables (SFS, DSI, stem bole necrosis and primary root necrosis) as reported in this study indicated that any of the variables will affect proportionally among them. This also validate that any of the variables could be used to quantify the degree of aggressiveness of *Ganoderma* species on oil palm seedlings.

CONCLUSION

All the *Ganoderma* isolates tested have shown positive signs of infection on the seedlings at 12 and up to 24 WAI. This result provides strong indication that they are pathogenic species of *Ganoderma*, the causal pathogen of both USR and BSR in oil palm plantation in Malaysia. However, the symptoms of infection on the seedlings were indistinguishable amongst the groups of *Ganoderma* tested. In fact, they showed significantly different degree of aggressiveness where *G. zonatum* of USR was the most aggressive, followed by *G. zonatum* and *G. boninense* of BSR with similar aggressiveness, and *G. boninense* of USR was the least aggressive.

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REFERENCES

ABDULLAH, F; LIEW, S B and MALIK, N (1999). Upper stem rot of oil palms (*E. guineensis*) in Langkon, Sabah. *Sustainable Crop Protection Practices in the Next Millennium* (Sidek, Z; Bong, S L; Vijaya, S K; Ong, C A and Husan, A K eds.). Malaysian Plant Protection Society. p. 101-103.

BRETON, F; HASAN, Y; HARIADI; LUBIS, Z and De FRANQUEVILLE, H (2006). Characterization of parameters for the development of an early screening test for basal stem rot tolerance in oil palm progenies. *J. Oil Palm Res. Special Issue*: 24-36.

CAMPANILE, G; GIOVE, S L and LUISI, N (2004). Genetic and morphologic variability of *Phellinus torulosus isolates* in some oak woods of Southern Italy. *J. Plant Pathology, 86*: 105-115.

CHONG, K P; LUM, M S; FOONG, C P; WONG, C M V L; ATONG, M and ROSSALL, S (2011). First identification of *Ganoderma boninense* isolated from Sabah based on PCR and sequence homology. *African J. Biotechnology, 10*: 14718-14723.

CHONG, K P; MARKUS, A and ROSSALL, S (2012a). The susceptibility of different varieties of oil palm seedlings to *Ganoderma boninense* infection. *Pakistan J. Botany, 44*: 2001-2004.

CHONG, K P; ATONG, M and ROSSALL, S (2012b). The role of syringic acid in the interaction between oil palm and *Ganoderma boninense*, the causal agent of basal stem rot. *Plant Pathology, 61*: 953-963.

CORLEY, R H V C and TINKER, P B (2003). *The Oil Palm*. Fourth edition. Blackwell publishing. 592 pp.

DIAZ, J; BARNAL, A; POMAR, F and MERINO, F (2001). Induction of shikimate dehydrogenase and peroxidase in pepper (*Capsicum annuum* L.) seedlings in response to copper stress and its relation to lignifications. *Plant Science, 161*: 179-188.

FLOOD, J; HASAN, Y; TURNER, P D and O'GRADY, E B (2000). The spread of *Ganoderma* from infective sources in the field and its implications for management of the disease in oil palm. Ganoderma *Diseases of Perennial Crops* (Flood, J; Bridge, P D and Holderness, M eds.). Oxford, UK, CABI Publishing, p. 101-112.

FLOOD, J; KEENAN, L; WAYNE, S and HASAN, Y (2005). Studies on oil palm trunks as sources of infection in the field. *Mycopathologia, 159*: 101-107.

GASCH, A P (2007). Comparative genomics of the environmental stress response in ascomycete fungi. *Yeast, 24*: 961-976.

HASAN, Y; FOSTER, H L and FLOOD, J (2005). Investigations on the causes of upper stem rot on standing mature oil palms. *Mycopathologia, 159*: 109-112.

IDRIS, A S; ARIFFIN, D; SWINBURNE, T R and WATT, T A (2000). The identity of *Ganoderma* species responsible for basal stem rot (BSR) disease of oil palm in Malaysia – morphological characteristics. *MPOB Information Series*.

IDRIS, A S; KUSHAIRI, A; ISMAIL, S and ARIFFIN, D (2004). Selection for partial resistance in oil palm progenies to *Ganoderma* basal stem rot. *J. Oil Palm Res. Vol. 16*: 12-18.

IDRIS, A S; KUSHAIRI, D; ARIFFIN, D and BASRI, M W (2006). Technique for inoculation of oil palm germinated seeds with *Ganoderma*. *MPOB Information Series*.

ILIAS, G N M (2000). *Trichoderma and its Efficacy as a Bio-control Agent of Basal Stem Rot of Oil Palm* (Elaeis quineensis, Jacq.). Ph.D thesis, Universiti Putra Malaysia, Selangor, Malaysia.

KOK, S M; GOH, Y K; TUNG, H J; GOH, K J; WONG, W C and GOH, Y K (2013). *In vitro* growth *of Ganoderma boninense* isolates on novel palm extract medium and virulence on oil palm (*Elaeis guineensis*) seedlings. *Malaysian J. Microbiology, 9*: 33-42.

LATIFFAH, Z; HARIKRISHNA, K; TAN, S G; FARIDAH, A and HO, Y W (2005). Random amplified polymorphic DNA (RAPD) and random amplified microsatellite (RAMS) of *Ganoderma* from infected oil palm and coconut stumps in Malaysia. *Asia Pacific J. Molecular Biology and Biotechnology, 13*: 23-34.

LATTANZIO, V; LATTANZIO, V M T and CARDINALI, A (2006). Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. *Phytochemistry: Advances in Research* (Imperato, F ed.). Kerala, India, Research Signpost, p. 23-67.

MILLER, R N G; HOLDERNESS, M; BRIDGE, P D; CHUNG, G F and ZAKARIA, M H (1999). Genetic diversity of *Ganoderma* in oil palm plantings. *Plant Pathology, 48*: 595-603.

NUSAIBAH, S A; RAJINDER, S and IDRIS, A S (2010). Somatic incompatibility and AFLP analysis of four species of *Ganoderma* isolated from oil palm. *J. Oil Palm Res. Vol. 22*: 814-821.

PATERSON, R R M; SARIAH, M and NELSON, L (2009). The feasibility of producing oil palm with altered lignin content to control *Ganoderma* disease. *J. Phytopathology, 157*: 649-656.

PILOTTI, C A; SANDERSON, F R and AITKEN, E A B (2003). Genetic structure of a population of *Ganoderma* boninense on oil palm. *Plant Pathology, 52*: 455-463.

PILOTTI, C A (2005). Stem rots of oil palm caused by *Ganoderma boninense*: pathogen biology and epidemiology. *Mycopathologia, 159*: 129-137.

RAKIB, M R M; BONG, C F J; KHAIRULMAZMI, A and IDRIS, A S (2014a). Genetic and morphological diversity of *Ganoderma* species isolated from infected oil palms (*Elaeis guineensis*). *International J. Agriculture and Biology, 16*: 691-699.

RAKIB, M R M; BONG, C F J; KHAIRULMAZMI, A and IDRIS, A S (2014b). Occurrence and spatial distribution of *Ganoderma* species causing upper and basal stem rot in oil palm. *J. Food, Agriculture and Environment, 12*: 360-364.

REES, R W; FLOOD, J; HASAN, Y and COOPER, R M (2007). Effects of inoculum potential, shading and soil temperature on root infection of oil palm seedlings by the basal stem rot pathogen *Ganoderma boninense*. *Plant Pathology, 56*: 862-870.

REES, R W; FLOOD, J; HASAN, Y; POTTER, U and COOPER, R M (2009). Basal stem rot of oil palm (*Elaeis guineensis*); mode of root infection and lower stem invasion by *Ganoderma boninense*. *Plant Pathology, 58*: 982-989.

REES, R W; FLOOD, J; HASAN, Y; WILLS, M A and COOPER, R M (2012). *Ganoderma boninense* basidiospores in oil palm plantations: evaluation of their possible role in stem rots of *Elaeis guineensis*. *Plant Pathology, 61*: 567-578.

SARIAH, M and ZAKARIA, H (2000). The use of soil amendments for the control of basal stem rot of oil-palm seedlings. Ganoderma *Diseases of Perennial Crops* (Flood, J; Bridge, P D and Holderness, M eds). Oxford, UK, CABI Publishing. p. 89 -100.

SARIAH, M; HUSSIN, M Z; MILLER, R N G and HOLDERNESS, M (2007). Pathogenicity of *Ganoderma boninense* tested by inoculation of oil palm seedlings. *Plant Pathology, 43*: 507-510.

SHAMALA, S; FARIDAH, A; ZAINAL, A M A and UMI, K Y (2008). Efficacy of single and mixed treatments of *Trichoderma harzianum* as biocontrol agent of Ganoderma basal stem rot in oil palm. *J. Oil Palm Res. Vol. 20*: 470-483.

SHANER, G and FINNEY, R E (1977). The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology, 67*: 1051-1056.

THOMPSON, A (1937). Observations on stem rot of the oil palm. *Straits Settlements and F.M.S. Science Series*.

USDA (2014). *Oilseed: World Markets and Trade*. Foreign Agricultural Service, United States Department of Agriculture (USDA), Washington, USA.

WONG, L C (2013). *Identification* of Ganoderma *Species Isolated from Basal Stem Rot Infected Oil Palms in Sarawak*. M.Sc. thesis, Universiti Putra Malaysia, Selangor, Malaysia.

ZAITON, S; SARIAH, M and ZAINAL, A M A (2008). Effect of endophytic bacteria on growth and suppression of *Ganoderma* infection in oil palm. *International J. Agriculture and Biology, 10*: 127-132.