

GANODERMA SPECIES OF BASAL AND UPPER STEM ROT IN OIL PALM (*ELAEIS GUINEENSIS*) IN SARAWAK, MALAYSIA

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Abstract

Oil palm is one of the most important plantation crops in Malaysia. The sustainability of oil palm is threatened by Ganoderma species. It is commonly known that Ganoderma boninense is the causal pathogen for basal stem rot (BSR) in oil palm. However, little is known about the threats by other species of Ganoderma or upper stem rot (USR), which is associated with a similar pathogen. A total of 46 isolates of Ganoderma were isolated from BSR and USR infected oil palms. The isolates were identified using a multiplex PCR, and its genetic heterogeneity was determined using a somatic compatibility test. It was found that BSR and USR coexisted in the plantations, and USR emerged as one of the major diseases. The diseases were associated with similar pathogens, namely G. zonatum (71.7%), followed by G. boninense (26.1%), and G. miniatocinctum (2.2%). Somatic compatibility test indicated that all the isolates were genetically heterogeneous. These results show that G. zonatum and the transmission of the diseases through basidiospores play a vital role in the epidemiology of the diseases. Thus, USR should not be overlooked, and more emphasis should be given to G. zonatum and its mode of transmission for more effective disease management.

Keywords: *Ganoderma species, basal stem rot, upper stem rot, oil palm diseases, genetic heterogeneity*

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1. Introduction

Oil palm (*Elaeis guineensis*) is one of the most important oil producing plantation crop in Malaysia. Until recently, more than 5.7 million hectares of land in Malaysia has been cultivated with oil palm with an output of 17.3 million tonnes of crude palm oil, which supplies 32% of the world vegetable oils and fats (USDA, 2016; MPOB 2017). Furthermore, the planted area in Sarawak is rapidly expanding and is the second largest hectareage after Sabah.

However, the sustainability of oil palm production in Malaysia as well as in South-East Asia is seriously threatened by *Ganoderma* species, a basidiomycete fungus that causes stem rot disease in oil palm (Hasan *et al.*, 2005; Rees *et al.*, 2012). The disease reduces the yield and shortens the economic life of an oil palm. It is common knowledge that *Ganoderma boninense* is the causal pathogen for basal stem rot (BSR) in oil palm. However, little is known about the threats given by other species of *Ganoderma* in oil palm plantations, such as *G. zonatum* and *G. miniatocinctum*, which are also implicated in BSR (Idris *et al.*, 2000; Wong *et al.*, 2012). In addition, *Ganoderma* species also has been associated with upper stem rot (USR) in oil palm, where the infection of *Ganoderma* is on the upper portion of the stem or trunk of an oil palm instead of at the base as in BSR (Hasan *et al.*, 2005; Rakib *et al.*, 2014). Little is known about USR as it was considered as a minor disease of oil palm earlier (Thompson, 1937) until it gained more attention when few cases of the disease were reported in Sabah, Malaysia (Abdullah *et al.*, 1999), Papua New Guinea (Pilotti, 2005) and Indonesia (Rees *et al.*, 2012).

Turner (1981) listed 15 species of *Ganoderma* from different parts of the world associated with BSR. Among them, eight species were reported in Peninsular Malaysia, namely *G. boninense*, *G. zonatum*, *G. miniatocinctum*, *G. tornatum*, *G. lucidum*, *G. chaliceum*, *G. applanatum* and *G. pseudoferreum*. An earlier study in Peninsular Malaysia by Ariffin *et al.* (1989) assumed that all *Ganoderma* isolates from BSR-infected palms were *G. boninense*. Later, Idris *et al.* (2000) identified four species of *Ganoderma* associated with oil palm in Malaysia, namely *G. boninense*, *G. zonatum*, *G. miniatocinctum* and *G. tornatum*. The first three species were proven to be pathogenic to oil palm causing BSR, while *G. tornatum* was non-pathogenic.

Effective disease management is an important aspect to sustain the oil palm industry. Lack of knowledge on the diseases and pathogens may lead to inaccurate disease control strategies. Thus, the objectives of this study were to investigate the occurrence of BSR and USR and ascertain which species of *Ganoderma* are associated with the diseases.

2. Materials and Methods

2.1 Descriptions of study sites

The study was conducted in two selected oil palm plantations in Betong (total area of 873 ha) and Miri (total area of 2801 ha), located in Sarawak, Malaysia, with the study areas covering 15 hectares and 16 hectares, respectively. Both plantations were commercial first generation plantations, planted with Tenera (DxP) planting material, with a density of 160 palms per hectare, on previously secondary peat swamp forest located near to the coast. Average annual rainfall in Betong and Miri were 3333 and 3224 mm, respectively. The oil palms' ages were around 18 years old in Betong and 11 years old in Miri.

2.2 Census of basal and upper stem rots in the oil palms

All oil palm trees within the 15 ha and 16 ha study plots in Betong and Miri, respectively, were examined palm-to-palm for external disease signs and symptoms of BSR and USR (Hasan *et al.*, 2005; Pilotti, 2005). The number of BSR- and USR infected palms, and symptomless palms were recorded. Then, the percentage of occurrence or disease incidence was calculated.

2.3 Collection and isolation of *Ganoderma* species

A total of 46 samples of *Ganoderma* basidiomata were collected randomly from basal stem rot (BSR) and upper stem rot (USR) infected oil palm from the plantations in Betong and Miri. The samples were then isolated using *Ganoderma* selective medium (GSM) as described by Ariffin and Idris (1992). Pure cultures of *Ganoderma* isolates obtained were maintained on potato dextrose agar (PDA) for further analysis.

2.4 Identification of *Ganoderma* species

All 46 isolates of *Ganoderma* were identified using a multiplex polymerase chain reaction (multiplex PCR) technique. Sample preparation and DNA extraction was done using the DNeasy extraction kit (Qiagen) according to Karakousis *et al.* (2006) and Wong *et al.* (2012). Multiplex PCR was conducted using *Ganoderma*4 Genotyping kit (Seeplex) supplied by the Malaysian Oil Palm Board (MPOB). Four species of *Ganoderma* based on the band sizes, which are 656 bp for *G. boninense*, 415 bp for *G. zonatum*, 331 for *G. tornatum*, and 242 for *G. miniatocinctum*, were identified (Idris *et al.*, 2010; Wong *et al.*, 2012).

2.5 Assessment of genetic similarity using somatic compatibility test

Compatibility of the isolates was tested using a modified method by Miller *et al.* (1999), using PDA instead of 1% malt extract agar. Somatic compatibility was tested by pairing the isolates in all combinations and self-pairing as control. Mycelia plugs (8 mm) were transferred onto standard 9 cm PDA plates, and placed 2 cm apart. The plates were incubated for 14 days in the dark at room temperature (25-30 °C), and assessed and rated as either compatible or incompatible. Compatible isolates merged into single colony, whereas incompatible isolates formed inhibition zone or barrage.

3. Results and Discussion

3.1 Occurrence of basal and upper stem rots due to *Ganoderma* species

Both BSR and USR coexisted in both study sites. Relatively lower USR as compared with BSR was recorded in Betong, and vice versa in Miri (Table 1). In Betong, it was found that 4.01% and 1.32% of the total oil palm were BSR and USR infected, respectively, with a total of 6.33% of infection by *Ganoderma* species. On the other hand, the plot in Miri showed 3.78% and 6.02% of BSR and USR, respectively, with a total of 9.80% of infection by *Ganoderma* species. Ratios of USR to BSR were 1:3.03 in Betong and 1.59:1 in Miri. No specific pattern was detected in the ratios.

Hasan *et al.* (2005) reported the ratio of BSR to USR ranged from 1:1 to 10:1, and in some areas, the ratio was reversed. The lower occurrence of USR as compared with BSR in Betong could be related to closer canopy in the older palms, while the canopy for the younger palms in Miri was not well closed. *Ganoderma* basidiospores are airborne and their dispersal has been related to air movement (Agnieszka, 2010), while Pilotti (2005) reported that low USR was detected in a closed canopy plantations because it restricts upward movement of basidiospores and thus a majority of the basidiospores settle on the ground. The basidiospores that have settled on the ground may be attributed to higher BSR incidences as observed in Betong.

Table 1. Percentage of basal stem rot (BSR) and upper stem rot (USR) due to *Ganoderma* species in the oil palm plantations in Betong and Miri.

| | % of <i>Ganoderma</i> infection | |
|-------|---------------------------------|------|
| | Betong | Miri |
| BSR | 4.01 | 3.78 |
| USR | 1.32 | 6.02 |
| Total | 6.33 | 9.80 |

3.2 Species of *Ganoderma* associated with basal and upper stem rots

Based on the random sampling and identification of the 46 isolates of *Ganoderma* using a multiplex PCR, where three species of *Ganoderma* were found, namely *G. boninense*, *G. zonatum*, and *G. miniatocinctum* which were amplified at 656 bp, 415 bp, and 242 bp, respectively. The population of *G. zonatum* was the highest in both diseases. *G. miniatocinctum* was only found in USR infected oil palm, and no *G. tornatum* or other species were found.

It was found that *G. zonatum* is the most dominant species in BSR (81.3%), followed by *G. boninense* (18.7%) as shown in Figure 1(a). Similarly, *G. zonatum* was the most dominant species in USR, followed by *G. boninense* and *G. miniatocinctum*, which accounted for 66.7%, 30% and 3.3%, respectively (Figure 1b). Overall, *G. zonatum* comprised 71.7%, *G. boninense* 26.1%, and *G. miniatocinctum* 2.2% of the total (Figure 1(c)).

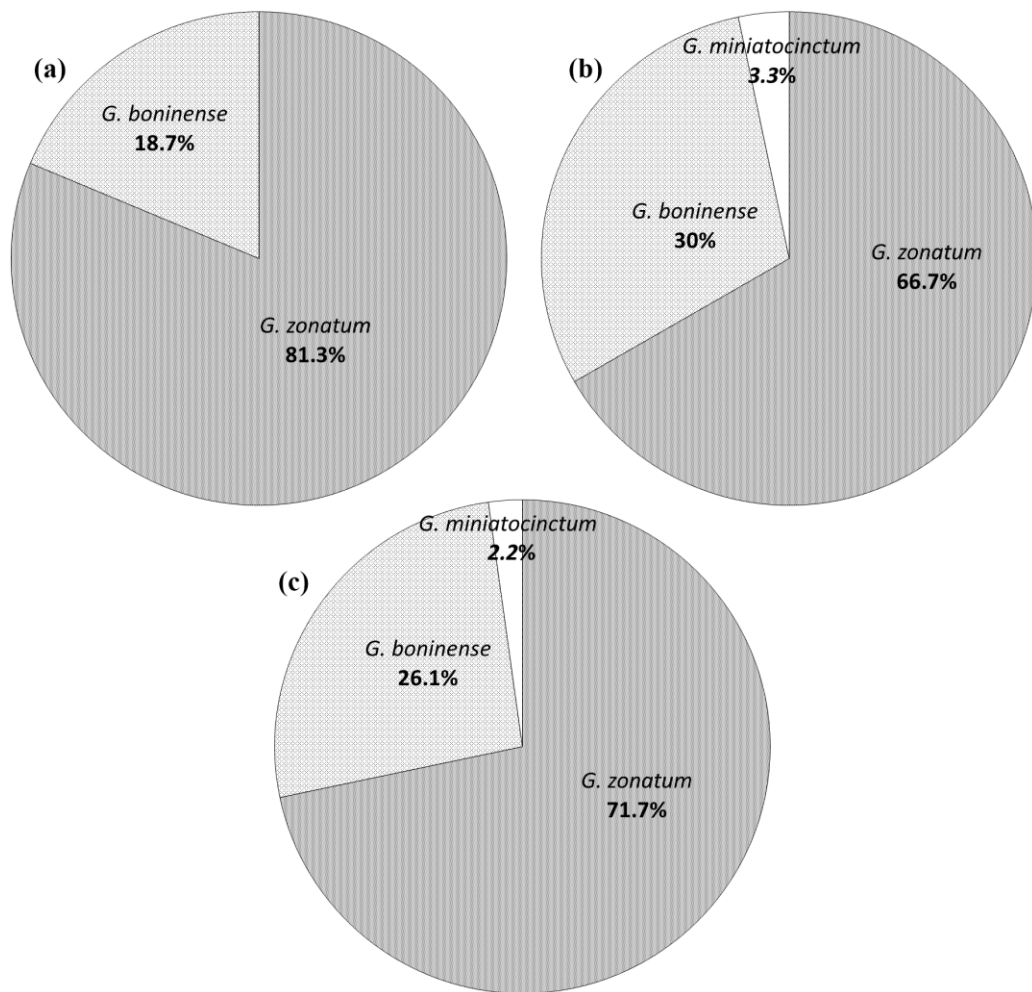


Figure 1. Distribution of *Ganoderma* species of stem rots in oil palm. (a) *Ganoderma* species of basal stem rot; (b) *Ganoderma* species of upper stem rot; (c) Overall distribution of *Ganoderma* species.

The three species of *Ganoderma* found to be associated with BSR and USR in this study were reported to be pathogenic to oil palm through a pathogenicity test, where *G. zonatum* of USR was found to be the most aggressive in terms of infection severity, followed by *G. boninense* and *G. zonatum* of BSR with similar aggressiveness, and *G. zonatum* of BSR was the least aggressive (Rakib *et al.*, 2015). The dominance of *G. zonatum* indicates that it may play a more vital role in the epidemiology of the disease than previously believed (Ariffin *et al.*, 2000; Idris *et al.*, 2004; Sapak *et al.*, 2008; Rees *et al.*, 2009). The current belief that *G. boninense* is the major causative pathogen of the disease may need to be reviewed to give more emphasis to studies on *G. zonatum* for more effective management of the disease. In addition, more studies should be conducted in other sites to further build upon the findings in this study.

3.3 Genetic heterogeneity of *Ganoderma* species

All possible pairings of the 46 isolates of *Ganoderma* resulted in somatic incompatibility, where it formed an inhibition zone or barrage between the paired isolates, except in self-

pairing isolates (control) (Figure 2). This indicates that they were genetically distinct and originated from different inoculum. The somatic incompatibility also included those in intra-species isolates. Latiffah *et al.* (2005) reported that genetic variation occurred in pathogens originating from the same species or closely related species. Compatibility between different samples of *Ganoderma* species in oil palm plantation were rare because this occurred only in very few samples and sometimes none of them were compatible (Miller *et al.*, 1999; Pilotti, 2005; Latiffah and Ho, 2005; Nusaibah *et al.*, 2010). *Ganoderma* species in the oil palm plantations were genetically heterogeneous due to sexual reproduction that involved dikaryotization of basidiospores and produced distinct individuals (Chan *et al.*, 2011). This suggests that dispersal of basidiospores plays an important role in the epidemiology of BSR and USR as the basidiospores become infective through the build-up of inoculum source parent (Abdullah, 2000; Hasan *et al.*, 2005). Hence, preventive measures to minimize spore spread should be taken for better disease management, such as removing as much inoculum sources (infected plant tissues) as possible in and around the planting areas that later develop into basidiomata, and removing existing basidiomata.

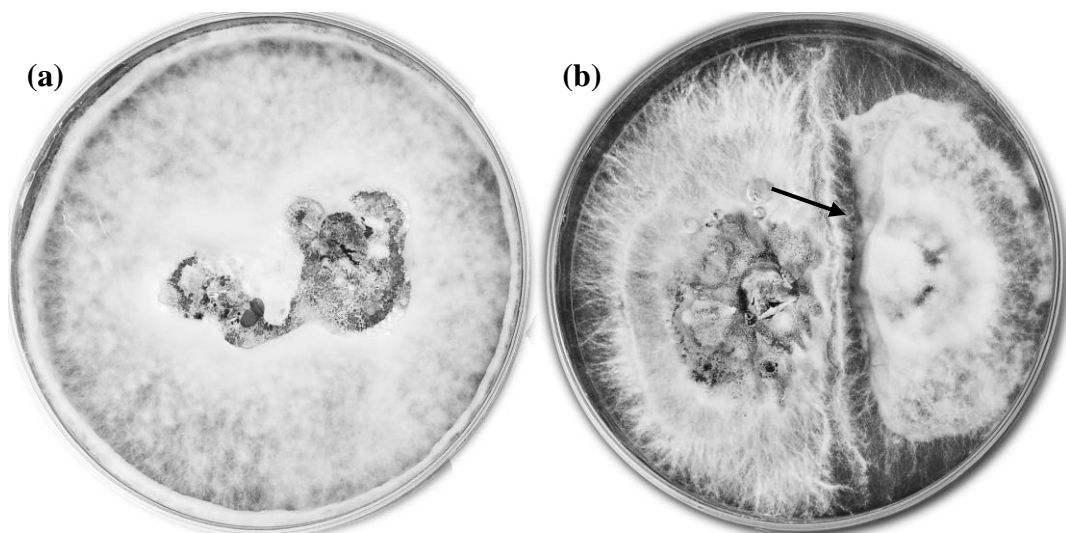


Figure 2. Somatic compatibility. (a) Compatible reaction of self-pairing or same isolate (control) where their mycelia merged into a single colony; (b) Incompatible reaction between two different isolates showing formation of inhibition zone or barrage (arrow).

4. Conclusion

Basal and upper stem rots coexisted in both oil palm plantations, and USR is the major disease in Miri. Three pathogenic species of *Ganoderma* were associated with the diseases, namely *G. zonatum*, *G. boninense* and *G. miniatocinctum*. Among the three species, *G. zonatum* (71.7%) was the most dominant species followed by *G. boninense* (26.1%) and *G. miniatocinctum* (2.2%). Besides that, the genetic heterogeneity of the *Ganoderma* isolates indicated that dispersal of basidiospores plays an important role in the epidemiology of BSR and USR.

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