Early Detection and Management of *Ganoderma* Basal Stem Rot Disease: A Special Report from Sabah

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ABSTRACT: Basal Stem Rot (BSR) disease caused by *Ganoderma* spp. is the most devastating disease of oil palm in Southeast Asia. This paper discusses sustainable approaches in managing BSR disease particularly on early detection and control of *Ganoderma* with some examples from oil palm estates in Sabah. New detection methods such as ergosterol analysis and *Ganoderma* signature via Fourier Transform Infrared Spectroscopy (FTIR) are emphasized. Latest disease control methods with great potentials such as combination of biological control agents (BCAs), enhancing defense mechanism of oil palm through enviro-friendly approach, potential biomarkers for selection of resistant breeding materials and utilization of eco-friendly fungicide were also discussed.

KEYWORDS: *Ganoderma*, Oil Palm, Basal Stem Rot, Detection, Control

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INTRODUCTION

Basal Stem Rot (BSR) disease caused by *Ganoderma* is an important disease of oil palm and caused losses up to RM 1.5 billion a year in Malaysia (Roslan & Idris, 2012). The loss is either directly with dead of palms or yield reduction from the standing palms. The failure in BSR controls may be due to the infected palms was not detected at the early stage of infection in the plantation and the necessary action be taken. Numerous attempts in early detection and controlling this disease have been reported but with no conclusive remedy. Currently diagnostic tools for early detection of *Ganoderma* infection are by using *Ganoderma* Selective Media (GSM) (Ariffin & Idris, 1992), enzyme-linked immunosorbet assay (ELISA) (Madihah *et al*., 2014), polymerase chain reaction (PCR) (Idris *et al*., 2003), colorimetric method with ethlylene diaminetetraacetic acid (EDTA) (Natarajan et al. 1986), tomography images (Shu'ud *et al*., 2007), statistical modeling to classify canopy spectra into *Ganoderma* attack severity levels (Lelong *et al*., 2010) and Molecularly Imprinted Polymer (MIP) sensors (Abdullah *et al*., 2012) show many uncertainties. For the time being, though it is impossible to manage a field free of *Ganoderma*, considerable reduction of the disease can be achieved through a proper management and advancement in research. Management of BSR includes soil mounding, surgery, good field sanitation, ploughing and harrowing, fallowing, planting legume cover crops, chemical treatments, application of fertilizers, biological control and screening for resistant planting materials (as reviewed by Hushiarian *et al*., 2013). As early detection is still under debate, it is not surprising that finding a practical, successful disease control system for BSR disease appears to be equally complicated, demanding a variety of techniques and strategies. This paper discusses some

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possible approaches in detection and control of *Ganoderma* with some examples from experiments conducted in Sabah estates.

UNDERSTANDING *Ganoderma boninense*

Lack of understanding on *Ganoderma* itself probably contributes to failure in controlling the *Ganoderma* pathogen. *Ganoderma*, a wood decaying fungi with hard fruiting bodies belong to the family of Polyporaceae (*Ganodermataceae*). Among all *Ganoderma* species, *Ganoderma boninense*, a white rot fungus is well known pathogen in oil palm plantation, causing BSR disease. However, different types of *Ganoderma* have been reported to cause BSR. In nature, *Ganoderma* sp. colonizes particular soil debris (Alexander & Chong, 2014). Planting oil palm on that particular soil will introduce new host to *Ganoderma* and eventually infecting the oil palm. Different from *Ganoderma* natural host, oil palm will suffer BSR disease when infected by *Ganoderma*. It is believed that *Ganoderma* produces certain metabolites that are unable to be tolerated by the oil palm and eventually cause the disease. Studies on the presence of important metabolites might be crucial for fundamental studies on their pathogenicity, infection and for disease control purposes. The presence of some important metabolites in *Ganoderma boninense* such as fatty acid and lipid derivatives; this includes terpenoid and steroidal compounds (Abdullah *et al*., 2016; Alexander *et al*., 2016; Ismail *et al*., 2014). Fatty acids and Fatty Acid Methyl Esters (FAME) in fungi species were previously been studied for species differentiation and characterization. Some of the reported metabolites are also known to contribute to the fungal defence mechanisms and for synthesis of many potent bioactive compounds including triterpenoids and lanostanes (Ismail *et al*., 2014). In early work, two compounds of terpene and sterols derivatives ((3β,22E)-Ergosta-5,22-dien-3-ol acetate and 3-hydroxy-(3β,5α,14β,20β,22β,25R)-Spirost-8-en-11-one) were also reported. It is known that (3β,22E)-Ergosta-5,22-dien-3-ol acetate is an esterified form with addition of lanostane backbone into ergosterol, a sterol highly specific for fungi (Chong *et al*., 2012). This compound is abundantly distributed along the fungal cell wall and being used by researchers to validate *G. boninense* infection in oil palm tissue (Chong *et al*., 2012; Alexander & Chong, 2014). Meanwhile, 3-hydroxy-(3β,5α,14β,20β,22β,25R)-Spirost-8-en-11-one is another derivatives of terpene with lanostane and lactone backbone. It is understood that the presence of steroidal compounds is intermediate and by products of terpene and squalene metabolisms. These published literatures not only provide important information in understanding *Ganoderma* through its unique metabolites profile but also provide some fundamental information for future research related to this pathogen and BSR. The metabolites reported in early work could become a key factor in developing new strategies in managing BSR and *Ganoderma* infection in oil palm plantation.

DETECTION

Fourier Transform Infrared Spectroscopy (FTIR)

The possibility of using FTIR for detection and identification of *Ganoderma boninense* in oil palm has been reported by Dayou *et al.* (2014) and Alexander *et al.* (2014b). It was found that FTIR is capable of showing presence of *G. boninense* in oil palm tissue with good sensitivity (Alexander *et al*., 2014a). Functional group of C-O-C content (1300-1000 cm^{-1}) was detected in the infected oil palm spectra. Interestingly, this peak was not detected in healthy palm spectra and cross-reference

with *G. boninense* spectra show resemblance of the latter peak. The C-O-C content which detected in this region (1300-1000 cm^{-1}) could possibly associate with *G. boninense*.

Ergosterol-Thin Layer Chromatography (TLC) Technique

The use of High Performance Liquid Chromatography (HPLC) to quantify ergosterol from *G. boninense* which infected oil palm was first reported by Chong *et al*. (2009c). In several studies the same method was also employed in detecting and quantifying level of infection of *Ganoderma* in oil palm (As'waad *et al*., 2011; Toh *et al*., 2011; Chong, 2012). The detection of ergosterol in the field was made possible with the assistance of TLC (Chong & Alexander, 2014). Ergosterol compound fluoresce under 365 nm wavelength indicated the presence of *Ganoderma* in oil palm tissue. However, the collection of tissue from the palms is very crucial to avoid faulty result as ergosterol is also present in other fungi.

Changes in Oil Palm Cell Wall Lipid during G. boninense Infection

The cell wall is a dynamic structure that often determines the outcome of the interactions between pathogens and plants. It is the first physical barrier that pathogens need to breach to colonize the plant tissue. Following a pathogen attack, host cell wall may undergo multiple changes due to defence responses, they may modify their cell wall to strengthen structural support and release defence associated metabolites. Lipid influence pathogenesis and resistance mechanisms associated with plant-microbe interactions. The lipid profile (using Gas Chromatography Mass Spectrometry, GCMS) from oil palm roots cell wall which potential to be used for detection of infection and/or physical damage due to *G. boninense* and to understand many metabolic processes that occur in the oil palm roots cell wall during the pathogenesis is shown in Table 1. Lipid compounds listed under infected oil palm are not detected in healthy oil palm and vice versa.

Table 1. Composition of cell wall lipid components present in high abundance based on Gas Chromatography Mass Spectrometry (GCMS) analysis extracted from infected and healthy oil palm. Lipid compounds listed under infected oil palm are not detected in healthy oil palm and vice versa.

Infected	Healthy
• 9-Octadecenoic acid, methyl ester • Tetracosanoic acid, methyl ester • 4,5,7-tris(1,1-dimethylethyl)-3,4-dihydro-1,4 Epoxynaphthalene-1(2H)-methanol • 3,7-dihydro-3-methyl-7-n-propyl-8-(2-ethoxycarbonyl ethyl)thio-1H-Purine-2,6-dione • 3,5-Bis(2,5-dimethylphenyl)- 2,3-dihydro-1H-inden-1-one • 6-trifluoromethyl-4-[2-(3-ethoxy-4-methoxypheny l)ethenyl]- Pyrimidin-2(1H)-one • Tetraacetato dichromium • Ergost-5-en-3-ol, (3.beta.)- • O-2,3,4,6-tetra-O-metmethyl-.bet a.-D-galactopyranosyl-(1.fwdarw.6) -2,3,4,5-tetra-O-methyl- D-Galactose • methyl 2,3,4,6-tetra-O-methyl-.alpha.-D-Glucopyranoside • Hexacosanoic acid, methyl ester • 5,14-dihydroxy- 6,13-Pentacenedione, • 10,13-Octadecadienoic acid, methyl ester • 1,2:3,4-di-O-isopropylidene-, L-Threitol • (Z)-11-Octadecenoic acid, methyl ester	• Hexadecanoic acid, 2-hydroxy-,methyl ester • 2-(2-Phenoxythiinyl)imidazolo[1,2-a]pyridine • 2-Phenyl-4,6-di(2-hydroxyphenyl)pyrimidine • Anthracene, 9,10-diethyl-9,10-dihy dro- • Campesterol • N-Methyllaurotetanine • 2,6-bis(1,1-dimethylethyl)-4-[(4-hydroxy-3,5-dimethylphenyl) methyl]- Phenol • 3-hydroxy -19-Norpregna-1,3,5(10),17(20)-tetraene-20-carboxylic acid, methyl ester • (Z)-9-Octadecenoic acid, methyl ester • 2(5H)-Thiophenone • Hexahydro- 4,8-Ethano-4H-1,3-benzodioxin • N-methoxycarbonyl-l-Alanine, isohexyl ester • Pentadecanoic acid, methyl ester

Volatile Organic Compounds (VOCs)

Early detection of *Ganoderma* infection via investigation on biomarkers from volatile organic compounds has been explored. Generally, plant tissues may produce some similar VOCs when they undergo some similar metabolic processes. However, if the tissues are infected by pathogen, there is a potential, tissues may start to produce different VOCs in comparison to the healthy tissues. Detection of these compounds may start from extraction of the infected tissues using suitable solvents and undergone a specific condition of GCMS analysis before the identity of these compounds is confirmed using the National Institute of Standards and Technology (NIST). Other more efficient technology may use specific instrument to trap the VOCs being released from the oil palm during the infection in an established estate. Fatty Acid Methyl Esters (FAME) such as dodecanoic acid, methyl ester; 9-Octadecenoic acid (Z)-, methyl ester and methyl stearate have been reported potential as biomarkers for detection of *Ganoderma* infection (Guo and Chong, 2016), while some FAME such as benzoic acid, methyl ester; hexadecanoic acid, methyl ester; 9,12-octadecadienoic acid (Z,Z)-, methyl ester and 9-octadecenoic acid (Z)-, methyl ester and phenols also were reported to play roles in defence mechanism of oil palm against the pathogen and potential to be used as biomarkers for screening resistant breeding materials in the future (Rozlianah *et al.*, 2015).

CONTROL

Biological Control Agents (BCA)

BCA have been widely used in controlling *G. boninense.* Lim *et al.* (2015) reported BCA isolated from Crocker Range soil identified as *Penicillium simplicissimum, Trichoderma harzianum, Aspergillus spp., Streptomyces sundarbansensis, Streptomyces spp., and Pseudomonas aeruginosa* showed to have more than 70% of Percentage Inhibition of Radial Growth (PIRG) against *G. boninense.* Combinations of several BCA in three microbial based products were also evaluated for the control of the *G. boninense* colonization in oil palm nursery and estate (Alexander & Chong, 2014). The products are Living Soil Microbes®, a combination of *Bacillus* spp. and *Trichoderma* spp.; High Technology Yield®, combination of *Bacillus* spp., *Pseudomonas* spp. and *Aspergillus* sp. and Agriorganica®, a combination of three food-associated microorganisms, *Lactobacillus*, *Nattobacillus* and *Saccharomyces cerevisiae*. All the three microorganisms were able to reduce *G.boninense* colonization in nursery and established estate compared to control. However, Living Soil Microbes® was found to be more effective in both nursery and field trial. Cocktails of biocontrol agents may have the advantages with broad spectrum activity, enhanced efficacy more reliable as the BCAs can communicate with each other as to maximize the biocontrol efficacy.

Enhancing the Defense Mechanism

Literatures on the utilization of chitin or chitosan in management of *Ganoderma* infection were limited. Some researchers report the chitanese enzymes activities during the infection. However the idea of utilization of crustacean shells as a source of chitin in enhancing defence mechanism of oil palm from *Ganoderma* infection has been proposed in some smaller estates. The potential of chitosan in enhancing defence mechanism and reducing the severity of *Ganoderma* infection in oil palm seedlings has been reported (Chong *et al*. 2012; Guni & Chong, 2012; Guni *et al*., 2012). Chitosan was reported to enhance accumulation of phenolic acids as a defence against *Ganoderma*

infection (Chong et. al., 2012). The application of chitosan also reduces the colonization of *Ganoderma* in infected seedlings and also with the chances in reducing potential of infection by this pathogen prior to infection (Guni & Chong, 2012; Guni *et al*., 2012). The role of phenolics in defence of oil palm against *Ganoderma* has been reported (Chong, 2012; Chong *et al*., 2012; Mohamad *et al*., 2007). Oil palm seedlings with higher total phenolic content were reported to be more tolerant to *Ganoderma* infection. Syringic acid, caffeic acids and 4-hydroxybenzoic acid from oil palm were also reported to restrict the *Ganoderma* growth and killed the pathogen at the concentration of 0.5 mgL^{-1} (Chong *et al*., 2009a). Synergistic effect of the three phenolics was also reported to be highly toxic to *Ganoderma* with the same concentration (Chong et al., 2009b). However, *Ganoderma* was reported to have the ability to metabolize the phenolic acids when the phenolic acids are present at lower concentration (Chong *et al*., 2012). Combination of syringic, caffeic and 4-hydroxybenzoic acids at higher concentration was reported to reduce the colonization of *Ganoderma* in infected oil palms as the pathogen failed to develop resistance to this combination (Jee & Chong 2014; Jee & Chong, 2015a; Jee & Chong, 2015b).

CONCLUSION

Detecting and managing the Basal Stem Rot disease caused by *Ganoderma* need continuous efforts from many parties and with different approaches. An insight understanding of this pathogen may help in searching the most suitable early detection and management methods of the disease. The future research for early detection of the infection should explore other newer technologies which may have not been investigated before while managing the disease should give priority to approach which is more environmental friendly such as biological control and enhancing the self-defense of the crop.

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