Relation of *Ganoderma* Ergosterol Content to Basal Stem Rot Disease Severity Index

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A R T I C L E   I N F O

A B S T R A C T

Background: Basal Stem Rot (BSR) caused by *Ganoderma boninense* remains the most devastating disease of oil palm in South East Asia. Numerous attempts in inventing early detection have been reported but with no conclusive answer. Many of the detection techniques are costly, impractical and difficult. A faster and more practical detection may by detecting the *Ganoderma* ergosterol in the infected oil palm. Ergosterol is fungal sterol found in *Ganoderma* and most fungi. However, the technique in tissues collection for analysis makes it reliable in quantifying the amount of *Ganoderma* biomass presence in oil palm. **Objective:** In this paper, the study on the relation of *Ganoderma* ergosterol to the disease severity index of BSR will be presented. **Results:** The amount of fungal biomass presence in the oil palm has a good relation (R^2=0.8158) with the visual disease severity index of Basal Stem Rot**.** Higher amount of *Ganoderma* sterol found in palms severely infected or with higher index and vice versa**. Conclusion:** The strong correlation between the ergosterol results and disease severity index suggested that ergosterol analysis could be utilised in future early detection and quantification of BSR disease progress.

INTRODUCTION

Oil palm is the most efficient oilseed crop in the world. One hectare of oil palm plantation is able to produce up to ten times more oil than other leading oilseed crops. Indonesia and Malaysia produce about 85% of the world's palm oil. Other producer countries include Thailand, Columbia, Nigeria, Papua New Guinea and Ecuador [1]. However, Basal Stem Rot (BSR) caused by *Ganoderma boninense* has hampered the production of palm oil for years in South East Asia. The loss estimated up to USD 500 million a year with no remedy is reported [2]. In most of the time, plantation managers failed to detect the disease or infection due to unavailability of detection method to be employed at large scale in their plantations where most of the detections are merely depend on the observation of foliar symptoms. However, the development of the foliar symptoms is slow and confusing with other symptoms of nutrient deficiency. Detection of *G. boninense* based on their ergosterol content is a technology possible to be used in a large scale of detecting and quantifying the amount of fungal biomass in infected palms. Ergosterol is the predominant sterol component in the plasma membrane of fungi, and is similar to cholesterol of mammals in the structure and functions. It is a compound that belongs to the steroid family. Ergosterol is labile, and therefore rapidly degraded after the death of fungal hyphae [3]. The lipid ergosterol membrane is found almost exclusively in fungi, and is frequently used by microbiologists as an indicator of living fungal biomass. Although, ergosterol is not a specific indicator for *Ganoderma* but experience and protocol of oil palm tissues samplings had proven this marker to be reliable in detecting and quantifying the amount of *Ganoderma* which presence in oil palm [2,4,5,6,7,8,9]. Detection or qualitative analysis can be conducted by estate personals using Thin Layer Chromatography Technique (TLC) at their own estates, while quantitative analysis can be conducted with the assistance of High Performance Liquid Chromatography (HPLC). However, to date, there is no study on the relation of this amount of *Ganoderma*

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ergosterol to the visual disease severity index of BSR. Therefore in this paper, we will present the study on the relation of this *Ganoderma* ergosterol to the disease severity index of BSR.

MATERIALS AND METHODS

Visual disease severity index:

A total of 60 healthy and infected oil palms were selected from a commercial oil palm estate in Kota Marudu, Sabah, Malaysia. The palms were scored using the disease severity index as in Table 1, to group them into different severity.

Table 1: Disease severity index for oil palm infected by Ganoderma boninense based on visual symptoms

Index	Description	Characteristic
0	Healthy	No foliar symptom observed. Trunk tissue tested on GSM* must be negative
1	Moderate Healthy	Foliar symptoms observed on leaves (Yellowish to pale). Trunk tissue tested on GSM must be positive
2	Moderate Severe	Foliar symptoms observed on leaves (Yellowish to pale). Skirting leaves and/or unopened new spears. Trunk tissue tested on GSM must be positive
3	Severe	Symptoms as index 2 but with fruiting bodies growing on the trunk of the oil palm.

*GSM denotes Ganoderma Selective Medium

Preparation of Ganoderma Selective Medium (GSM):

Ganoderma Selective Medium (GSM) was prepared as described by Ariffin and Idris [10]. GSM consists two parts; part A and B which contain antibiotic: streptomycin sulphate and chloramphenicol; and fungicides: Pentachloronitrobenzene (PCNB), Ridomil and Benlate to avoid the growth of unwanted bacteria and fungi but to allow *Ganoderma* to grow. This medium was used to isolate *Ganoderma* from oil palm tissue throughout this project.

Ergosterol analysis and quantification of Ganoderma biomass:

Infected and uninfected (as controls) oil palm trunk tissues were extracted as described by Chong *et al*. [7]. Tissues (100 mg) were extracted in methanol using bead beating to physically crush the sample. Polyvinylpyrrolidone (PVP) was added (10% w∕v) to the methanol to precipitate phenolic compounds. The extract was centrifuged at 15 000 g for 5 min and the supernatant was made up to 1.5 mL before being filtered through a 0.45 µm acetate syringe tip filter. An Agilent Series 1200 Chromatography System was used comprising: degasser G1313B, Quat Pump G131A (with a flow rate of 1.5 mL min^{-1}) and an autosampler ALS G1329A. Detection was achieved with an Agilent G1313B HPLC VWD detector set to 282 nm, with ChemStation data manipulation software. A reverse-phase Eclipse XDB-C18 4.6 mm x 150 mm with 5 µm particle-size column was used for separation. The isolated peak eluted at a retention time 5.5-5.8 min was identified as ergosterol, based on its co-chromatography and identical absorption spectrum with a pure standard. The system was run isocratically with 100% methanol. A serial dilution of the ergosterol standard, with a concentration range of 5–500 µg mL^{-1}, was injected into the HPLC system to develop a standard curve, which was then used for ergosterol quantification from oil palm root extracts.

Statistical analysis:

Data were statistically analysed by one-way analysis of variance and significant differences between index were detected by a Tukey test. Analyses used the Statistical Package for Social Sciences (SPSS) version 21. Correlation was studied between the disease severity index and ergosterol content.

Results:

Basal Stem Rot disease severity index:

All 60 selected palms were scored as described in Table 1. The result of the score is illustrated in Table 2.

Ergosterol content in oil palm trunk tissue:

Tissues from palms with different index were subjected to ergosterol analysis via the HPLC system. No ergosterol was found in tissues from healthy palms. The amount of ergosterol found in tissues from respective index is illustrated in Figure 1.

Table 2: Scores of the selected 60 palms based on the disease severity index

Index	Description	Characteristic
0	15	No foliar symptom was observed. No growth of *Ganoderma* was recorded when trunk tissues were isolated on GSM*
1	15	Yellowish to pale leaves symptoms were observed. Growth of *Ganoderma* was recorded when trunk tissues were isolated on GSM
2	15	Yellowish to pale leaves symptoms were observed. Skirting leaves and/or unopened new spears were recorded. Growth of *Ganoderma* was recorded when trunk tissues were isolated on GSM
3	15	Yellowish to pale leaves symptoms were observed. Skirting leaves and/or unopened new spears were recorded. Growth of *Ganoderma* was recorded when trunk tissues were isolated on GSM. Fruiting bodies were found growing on the trunk of the oil palm.

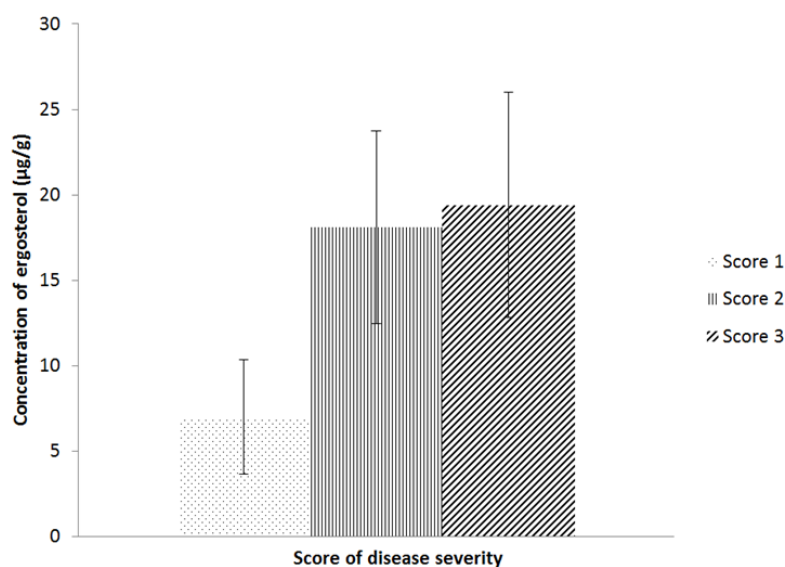*GSM denotes Ganoderma Selective Medium

Fig. 1: The amount of ergosterol with the different disease severity index.

Isolation of Ganoderma on GSM:

  Ganoderma from palm tissues with different index were isolated on GSM. No *Ganoderma* was isolated from healthy palm tissues (Figure 2). The growth of *Ganoderma* from tissues of palms with index 1 to 3 is illustrated in Figure 3.
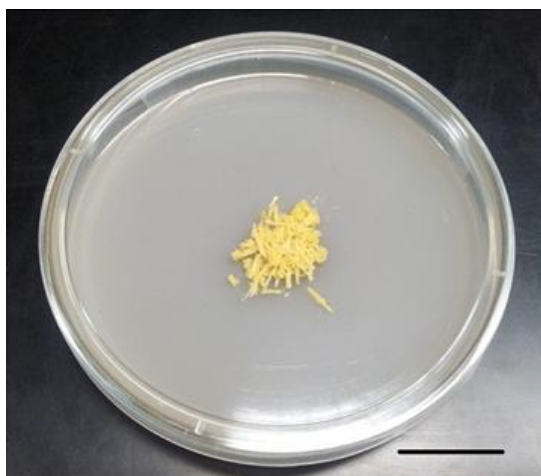

Fig. 2: No *Ganoderma* growth was observed on GSM when healthy palms tissues (Index) were incubated for five days. Bar: 2cm
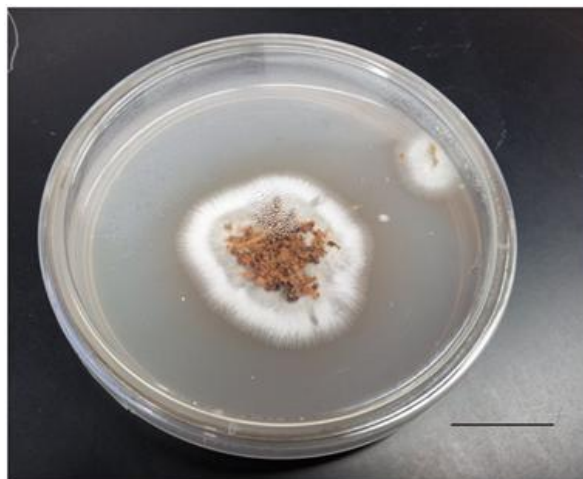
Fig. 3: *Ganoderma* growth from tissues with Index 1 to 3 on GSM after incubated for five days. Bar: 2cm

Correlation between ergosterol analysis and disease severity index:

Both the ergosterol analysis and disease severity index provided results for level of infection caused by *G. boninense* in the field. The results for these two paramaters were correlated and a strong correlation was shown between the two assessment methods (Figure 4) with $R^2 = 0.8158$.
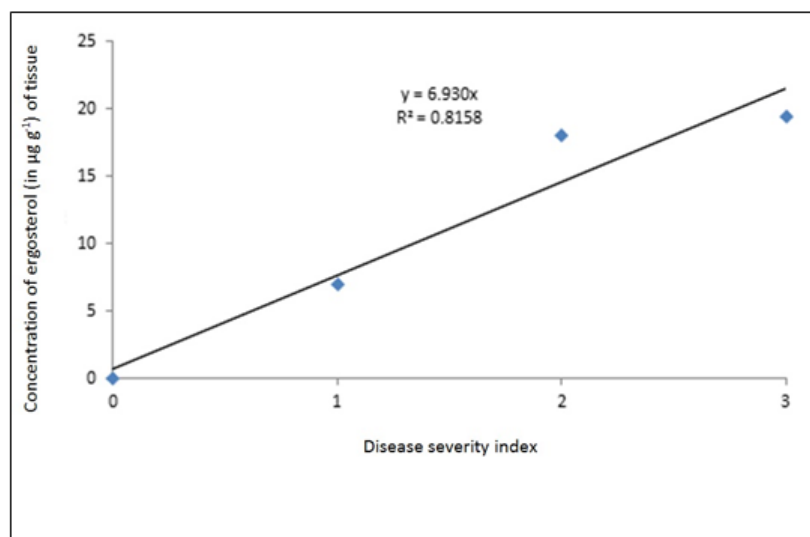

Fig. 4: Correlation between ergosterol contentt and disease severity index.

Discussion:

The most common techniques currently employed to detect the presence of *G. boninense* use ELISA or PCR [11,12,13,14,15]. The ELISA technique provides a cheaper detection for large number of samples but was reported to be non-specific, having cross reaction with other microorganisms [13,14,15]. ELISA is mainly used for detection purposes and it is not easy to employ as a tool for monitoring quantitative progress of disease development in the field. PCR is a more specific method for detecting *G. boninense* but it is rather expensive and needs some well-trained personel and thus may not provide a fast, reliable method for the oil palm planters. The indication of the presence of *G. boninense* using the ergosterol assay may not be as specific as molecular markers but it is a fast and reliable method of quantifying the possible presence of the fungus. The membrane lipid ergosterol is found almost exclusively in fungi, and is frequently used by microbiologists as an indicator of living fungal biomass, based on the assumption that ergosterol is labile, and therefore rapidly degraded after the death of fungal hyphae [3]. The main advantage with ergosterol compared to other biomarkers, such as chitin and ATP, is its specific association with fungi. Low amounts of ergosterol can be found in algae and protozoa [16,17], but generally it is safe to use it as a specific biomarker for fungi [18]. The disease severity index is mainly based on visualization and may be very subjective between different observers, however, this is the most common index used by plantation managers in monitoring BSR in their estates. Nevertheless, palms without

obvious early symptoms have hampered the effort of these managers in detecting and controlling the disease effectively. The strong correlation between the ergosterol results and disease severity index (Figure 4) suggested that ergosterol analysis could be utilised in future early detection and quantification of BSR disease progress. With the assistance of HPLC and a proper collection of trunk tissues, ergosterol analysis will help planters to detect and monitor the progress of this disease as early as possible. Alternatively, planters may consider using some simpler chromatography system such as Thin Layer Chromatography (TLC) as it is more affordable and may serve similar purpose.

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REFERENCES

[1] Oil World, 2013. Available online with updates at www.oilworld.biz

[2] Chong, K.P., M. Atong and S. Rossall, 2012d. The susceptibility of difference varieties of oil palm seedlings to *Ganoderma boninense* infection. Pakistan Journal of Botany, 44(6): 2001-2004.

[3] Mille-Lindblom, C., E. Von Wachenfeldt and L.J TranvikJ, 2004. Ergosterol as a measure of living biomass: persistance in environmental samples after fungal death. Journal of Microbiological Methods., 59: 253-262.

[4] Chong, K.P., 2012. An evaluation of Ganoderma fungal colonisation using Ergosterol analysis and quantification. *Planter*. 88(1034): 311-319.

[5] Chong, K.P., M. Atong and S. Rossall, 2012a. Antimicrobial activity of chitosan-induced phenolics acids in oil palm roots against Ganoderma boninense. Journal of Sustainability and Science Management, 7(2): 170-178.

[6] Chong, K.P., M. Atong and S. Rossall, 2012b. The Roles of syringic, caffeic and 4- hydroxybenzoic acids in Ganoderma-oil palm interaction. Asian Journal of Microbiology, Biotechnology and Environmental Science, 14(2): 157-166.

[7] Chong, K.P., M. Atong and S. Rossall, 2012c. 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.

[8] Guni, O.B. and K.P. Chong, 2012. The possible of utilizing environmental friendly and biodegradable chitosan in suppressing Ganoderma infection of oil palm. Ecology and Environmental Conservation, 18(3): 431-436.

[9] Guni, O.B., J.A. Naga and K.P. Chong, 2012. The potential of chitosan in suppressing *Ganoderma boninense* infection in oil palm seedlings. Journal of Sustainability and Science Management, 7(2): 186-192.

[10] Ariffin, D. and A.S. Idris, 1992. *The Ganoderma selective medium (GSM)*. PORIM Information Series, Bangi, Selangor.

[11] Chong, K.P., S. Abdullah, and T.L. Ng. 2013. Molecular fingerprint of Ganoderma spp from Sabah, Malaysia. International Journal of Agriculture and Biology, 15(6): 1112-1118.

[12] Chong, K.P., C.P. Foong, C.M.V.L. Wong, S. Rossall and M. Atong, 2011. First identification of *Ganoderma boninense* isolated from Sabah based on PCR and sequence homology. African Journal of Biotechnology, 10(66): 14718-14723.

[13] Idris, A.S. and A.R. Rafidah, 2008. *Polyclonal antibody for detection of Ganoderma.* MPOB Information Series No. 430. MPOB TT No. 405: 4.

[14] Shamala, S. and A.S. Idris, 2009. *Trichoderma as a biocontrol agent against Ganoderma in oil palm.* MPOB Information Series No. 463 MPOB TT No. 422.

[15] Utomo, C. and F. Niepold, 2000. The development of diagnostic tools for Ganoderma in oil palm. In: Flood, J., P.D. Bridge and M. Holderness. (eds.). *Ganoderma Diseases of Perennial Crops*. CABI Publishing, Wallingford. Pp: 235-248.

[16] Peeler, T.C., M.B. Stephenson, K.J. Einspahr and G.A. Thompson, 1989. Lipid characterization of an enriched plasma membrane fraction of *Dunaliella salina* grown in media of varying salinity. *Plant Physiology*. 89: 970-976.

[17] Raederstorff, D. and M. Rohmer, 1987. Sterol biosynthesis via cycloartenol and other biochemical features related to photosynthetic phyla in the amebas *Naegleria lovaniensis* and Naegleria gruberi. European Journal of Biochemistry. 164: 427-434.

[18] Newell, S.Y., 1992. Estimating fungal biomass and productivity in decomposting leaf litter. In: Carol, G.C. and D.T. Wicklow (eds.). *The Fungal Community: Its Organization and Role in the Ecosystem*. 2nd edition. Marcel Dekker, New York. pp: 521-561.