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Ganoderma Colonization in Oil Palm Tissues and Soil after Treated with Phenolic Acids

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ABSTRACT

Basal Stem Rot (BSR) disease caused by *Ganoderma boninense* is thriving rapidly within oil palm plantations, especially in Indonesia and Malaysia. This study illustrates the potential of combination of three phenolic acids at the ratio of 1:1:1 (w/w); caffeic acid (CA), syringic acid (SA) and 4-hydroxybenzoic acid (4-HBA) in suppressing *G. boninense* colonization in mature oil palms. Field trial was conducted to evaluate the effectiveness of four different concentrations of phenolic acids (ranging from 1 to 4 g of each syringic acid (SA), caffeic acid (CA), 4-hydroxybenzoic acid(4-HBA)) combined at the ratio of 1:1:1 (w/w). In this trial, infected palms with no statistical different in ergosterol content, similar age, soil topography and condition were chosen. *Ganoderma* progression was assessed based on ergosterol content, possible isolation of the pathogen from infected tissues on *Ganoderma* Selective Medium (GSM) after phenolic acids treatments and Colony Forming of *Ganoderma* on GSM from phenolic acids treated soil. Ergosterol was not detected in healthy palms at the beginning till the end of this study, but, detected in infected oil palm tissues although treated with fungicide hexaconazole and different concentrations of phenolic acids. Infected palms (without any treatment) showed significantly higher mean difference of ergosterol (20.4211 µg g^{-1}) compared to healthy palms and palms treated with hexaconazole or different concentrations of phenolic acids. Combinations of phenolic acids at 4.0 g of each SA, CA and 4-HBA had the lowest mean difference of ergosterol (-18.6888 µg g^{-1}). *Ganoderma* was isolated on GSM from all infected oil palm tissues either treated or untreated with the hexaconazole or phenolic acids. However, there was no colony of *Ganoderma* form on the GSM from the soil samples collected after incubated for one month.

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INTRODUCTION

The oil palm industry in Indonesia and Malaysia is seriously threatened by Basal Stem Rot disease. This destructive disease is caused by the wood rotting fungus *Ganoderma boninense*. The matured oil palms which affected by the BSR disease are normally incurable. This disease has been reported to cause large amount of economic losses up to 500 Million USD a year [2]. Many researches have been conducted to manage the disease, however most shown unsatisfactory result. There are also reports on the role of specific phenolic acids in suppressing the *Ganoderma* infection in oil palm [6,7,8]. Phenolic compounds are secondary metabolites of plants which generally exhibit a wide range of physiological properties, such as involved in defense against ultraviolet radiation or aggression by pathogens. These compounds are derivatives of the shikimate, pentose phosphate and Phenylpropanoid pathways in plants [15]. They are often synthesized and accumulated in the subepidermal layers of plant tissues exposed to biotic or abiotic stress and pathogen attack [10,16]. Factors, such as season and different stages of growth and development will affect the concentration of a particular phenolic compound within the plant tissue [13,14,17]. To date, there is little information on the role of phenolic compounds against the infection of *G. boninense* being evaluated in the field. In this study, we report the findings on the effect of three phenolic acids; caffeic acids (CA), syringic acid (SA) and 4-hydroxybenzoic acid

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(4-HBA) in suppressing the *Ganoderma* invasion within the infected oil palm trees and their surrounding soil in estate.

MATERIALS AND METHODS

Oil Palm Tissues Collection:

The oil palm tissues collection was conducted in an oil palm estate severely affected by BSR in Sawit Kinabalu's Langkon Estate, Sabah. Trunk tissues were collected from both infected and uninfected palms (for control). Selection of palm for tissues collection was based on visual symptoms. The tissues collection of oil palm trees was based on the ergosterol analysis as described by Chong [4]. The drill bit used for tissues collection was first sterilized by immersing them into 70% of ethanol for five minutes. Approximate two cm of the oil palm trunk tissues were drilled using the drill bit, half feet high from the ground to remove any unwanted saprophytic fungi which may presence on the tissues. The drill bit was later sterilized again in ethanol before further collection of tissues (approximate 10 cm into the trunk) at the same point for the collection of tissues for analysis of ergosterol. The same steps were repeated for another three points of tissues collection with angle of 90^{o} to each other. The tissues which collected then were mixed, transferred to the laboratory and homogenized in a commercial blender. Homogenized tissues from the same palms were taken randomly for ergosterol analysis.

Extraction of Ergosterol:

The extraction of ergosterol was as described by Chong *et al.* [5]. Homogenized tissues (100 mg) from each infected and uninfected palms were extracted in methanol using bead beating to physically crush the sample. The extract was centrifuged at 13,000 rpm for five minutes and the supernatant was made up to 1.5 mL before being filtered through a 0.45 µm acetate syringe tip filter.

Analysis and Quantification of Ergosterol:

The analysis and quantification of ergosterol was as described by Chong *et al*. [5]. The ZORBAX SB-C_{18} 4.6mm x 250mm, 5µm column was utilized with an Agilent Series 1200 HPLC system for this analysis. The wavelength of UV detector was set at 282 nm, and the isolated peak elution at about 7.0-8.0 min retention time was identified as ergosterol based on its co-chromatography and identical absorption spectrum with pure standard (20 µg mL^{-1}) from Sigma at the flow rate of 1.5 mL min^{-1}. The system was run isocratically with 100% methanol. A serial dilution with a range of 200-1000 µg mL^{-1} of the ergosterol standard was injected into the HPLC system to develop a standard curve. The developed standard curve (not shown) was then used for further ergosterol quantification from oil palm tissues extracts.

Preparation and Application of Phenolic Acids and Hexaconzole to Infected Palms:

Three phenolic acids; CA, SA and 4-HBA were prepared in a final concentration of 1.0 g, 2.0 g, 3.0 g and 4.0 g of each of the individual phenolic acids (at ratio 1:1:1 w/w) per Liter of aqueous alcoholic solution. Four holes were dug for each infected palm with depth of 15 cm and a distance of 30 cm from the infected palm, and 90° to each other. The combinations of phenolic acids were applied by pouring the well-mixed phenolic acids into the holes surrounding the infected palms. The phenolic acids were applied twice at six months interval. The same procedures were also applied to hexaconazole which served as positive control.

Assessment on Ganoderma Colonization based on Ergosterol Content:

The colonization of *Ganoderma* was assessed twice at six months interval after treatments using the same technique for ergosterol quantification and analysis as described by [5].

Isolation of Fungus on Ganoderma Selective Media (GSM):

Trunk tissues from healthy and infected palms were collected using drill and drill bit as described earlier were cultured on GSM. The preparation of GSM was as described by Ariffin and Idris [3].

Ganoderma Colony Forming Unit:

The number of *Ganoderma* that are capable in forming colonies on GSM after exposure to the phenolic acids in soil was determined using the plate count technique. The spread plate technique was used to conduct these assessments. Knowing the dilution factor, volume plated, and number of colonies on the plate (or average from the duplicate plates), the equation as described by Ahmed and Carlstrom [1] was used to calculate count of microorganisms in the samples. The colony formed on GSM was later transferred and maintained on PDA and further confirmed using molecular technique as described by Chong *et al*. [6].

Statistical Analysis:

Completely Randomized Design (CRD) was chosen as the experimental design for this field trial. One-Way ANOVA was calculated at 5% significance level using SPSS 20.0 (Statistical Package for Social Science software) for the data analysis. Data was subjected to analysis variance, differences were compared using Tukey test at a significance of $P<0.05$.

Results:

Ergosterol Analysis:

Ergosterol was not detected in healthy palms (Figure 1) at the beginning till the end of the experiment for healthy palms. In contrast, ergosterol was detected in infected tissues before and after the treatments although treated with hexaconazole or varies concentrations of phenolic acids (Figure 2). There was a significant difference between amount of ergosterol in the control infected palms (without treatment) and those treated with hexaconazole or different concentrations of phenolic acids (Figure 3).

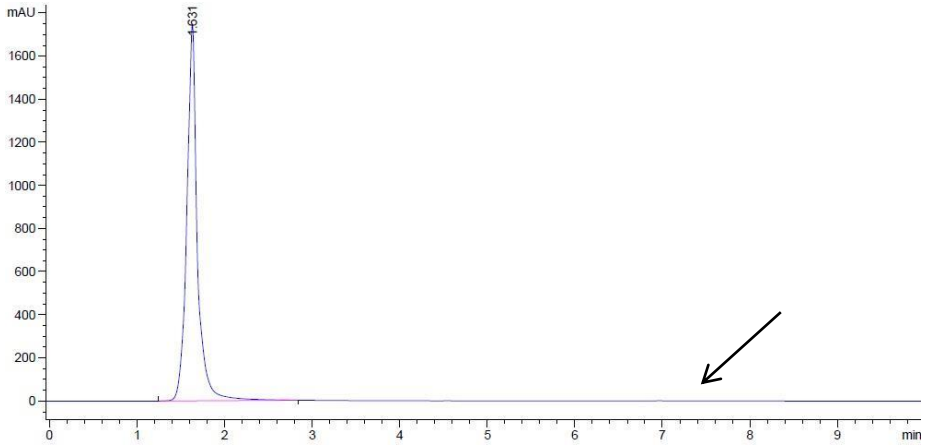

Fig. 1: Chromatogram showing the absence of ergosterol in trunk tissue from the healthy palms. No peak was found at the retention time of 7.0-8.0 min which corresponding to ergosterol (arrowed). The first peak is from methanol.

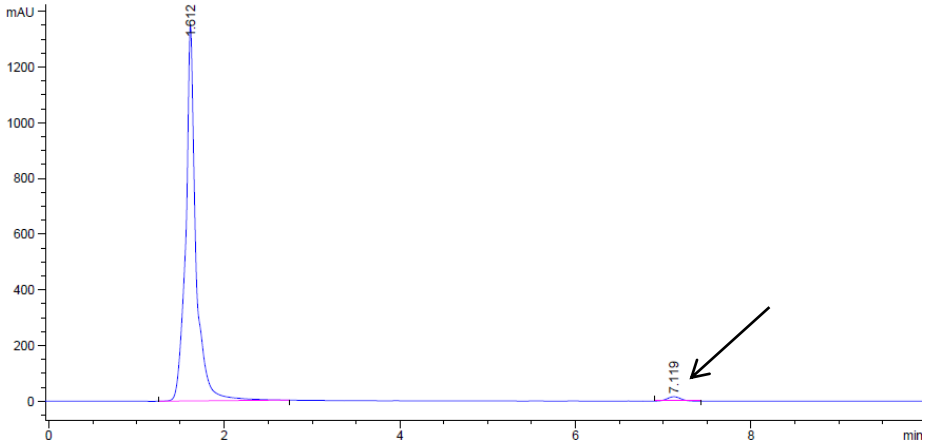

Fig. 2: Chromatogram showing the detection of ergosterol from trunk tissue of the infected palms at retention time of 7.119 min. The peak corresponding to ergosterol is arrowed. The first peak is from methanol.

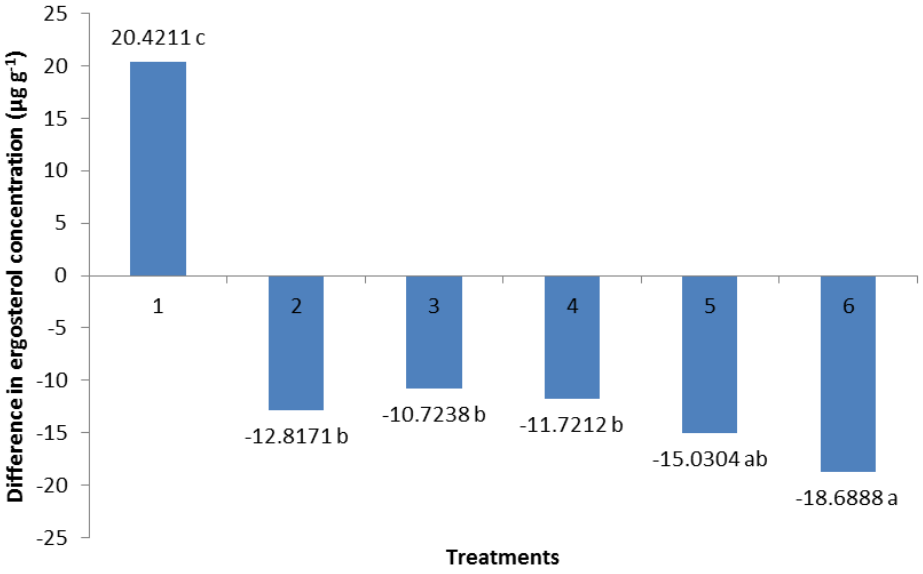

Fig. 3: The difference in ergosterol concentration (µg g^{-1}) of oil palm infected trunk tissues before and after 12 months of treatments where the treatments were applied twice at six months interval. No ergosterol was detected in healthy palms tissues. Mean with the same letter are not significantly different at $p<0.05$.

1 denotes Control (Infected without treatment), 2: Hexaconazole, 3: Phenolic acids (CA:SA:4-HBA) with 1.0 g each, 4: Phenolic acids (CA:SA:4-HBA) with 2.0 g each, 5: Phenolic acids (CA:SA:4-HBA) with 3.0 g each, 6: Phenolic acids (CA:SA:4-HBA) with 4.0 g each.

The infected palms which were not treated had significantly higher mean difference of ergosterol (20.4211 µg g^{-1}) at $p<0.05$ compared to palms treated with hexaconazole or different concentrations of phenolic acids. The combinations of phenolic acids with 4.0 g of each CA:SA:4-HBA reduced the colonization of *Ganoderma* to minimum, followed by 3.0 g of phenolic acids, hexaconazole, 2.0 g of phenolic acids and 1.0 g of phenolic acids. Palms treated with the highest amount of phenolic acids reduced the *Ganoderma* ergosterol in palm tissues the most (-18.6888 µg g^{-1}) and there was a significant difference between this concentration with hexaconazole and other concentrations of phenolic acids at $p>0.05$.

Isolation of Ganoderma on GSM:

Ganoderma was successfully isolated on the GSM after one week of incubation from all infected tissues either from treated or untreated tissues with hexaconazole or different concentrations of phenolic acids (Figure 4).

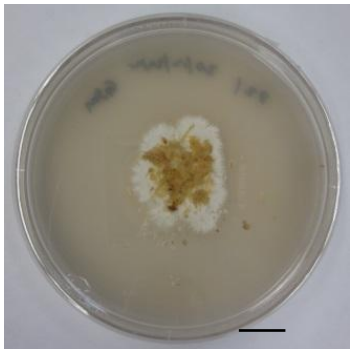

Fig. 4: Mycelia of *Ganoderma* from infected tissues grown on GSM. Similar results obtained from tissues treated with hexaconazole or different concentrations of phenolic acids. Bar: 6cm

Ganoderma Colony Forming:

There was no colony of *G. boninense* form on the GSM for both soil treated and untreated with the hexaconazole or combination of the phenolic acids after incubation of one month.

Discussion:

The infected palms which were not treated showed the highest detectable ergosterol (20.4211 µg g^{-1}) compared to other palms. This may due to the progress of *G. boninense* infection in the palm tissues if the palms left untreated. This is in correlation with the finding as reported by Idris *et al*. [12] where infected palms left untreated will not survive after a certain period of time. The results shown in Figure 3 clearly support the potential of the right combination of phenolic acids with the right concentration such as SA, CA and 4-HBA as a field control of *Ganoderma* infection in matured infected palms. On another hand, reports from Chong *et al*. (2009 a and b) [7,8,9] also proven CA, SA and 4-HBA to be toxic to *G. boninense* while the synergy effect of the combinations of them against this pathogen were also reported to have higher toxicity effect against the pathogen.

Ganoderma Selective Medium (GSM) is a classical method to further confirm the presence of *Ganoderma* besides extraction of ergosterol from the infected tissues. GSM was first reported as a useful tool in isolating *Ganoderma* from oil palm infected tissues. GSM potential in eliminating unnecessary microbes based on its unique combination of antibiotics and fungicides [3]. In the current experiment, *Ganoderma* was successfully isolated from the infected tissues, while no *Ganoderma* was found in healthy tissues confirming the ergosterol extracted during the ergosterol analysis was originated from the *Ganoderma* which infecting the oil palm tissues. Idris *et al*. [11] described the surface of the culture of *G. boninense* usually white in colour, while the reverse had a darkened region. However, GSM is unable to differentiate among the species of *Ganoderma* within the Genus or with some other basidiomycetes. Therefore, identification of *Ganoderma* isolated from the infected tissues using PCR and sequence homology as described by Chong *et al*. [5] was also conducted to further confirm the identity of this pathogen. No colony was form on the GSM although using the soil samples collected from the area which believed to be the most active area of *Ganoderma*, which is 15 cm in depth from the ground, where the oil palm roots are most abundant at this level. This may due to very little *Ganoderma* presence in the soil at the time of sampling or some technical or human errors during the sampling. However, *Ganoderma* colony forming may not a reliable parameter in evaluating the colonization of *Ganoderma* in soil in future study.

Conclusion:

Combinations of phenolic acids; caffeic acid, syringic acid and 4-hydroxybenzoic acid with the concentrations from 1.0 g to 4.0 g are capable to suppress the colonization of *Ganoderma* in infected oil palm. However, the most effective concentration in suppressing this pathogen is combination with 4 g of each of the phenolic acids where the *Ganoderma* ergosterol in infected tissues was reduced the most (-18.6888 µg g^{-1}).

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REFERENCES

[1] Ahmed, E.Y. and C. Carlstrom, 2003. Food Microbiology: A Laboratory Manual. John Wiley & Sons, Inc. Hoboken, New Jersey

[2] Arif, M.S., A. Roslan, A.S. Idris and M. Ramle, 2011. Economics of oil palm pests and *Ganoderma* disease and yield losses. Proceedings of the 3rd MPOB-IOPRI International Seminar: Integrated Oil Palm Pests and Diseases Management. 14 November 2011. Kuala Lumpur Covention Centre, Kuala Lumpur, Malaysia

[3] Ariffin, D. and A.S. Idris, 1992. The *Ganoderma* Selective Medium (GSM). PORIM Information Series. ISSN 0128-5726.

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

[5] Chong, K.P., M. Atong and S. Rossall, 2012. 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. Doi: 10.1111/j.1365-3059.2011.02577x

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

[7] Chong, K. P., S. Rossall and M. Atong, 2009a. *In vitro* antimicrobial activity and fungitoxicity of syringic acid, caffeic acid and 4-hydroxybenzoic acid against Ganoderma boninense. Journal of Agricultural Science. 1(2): 15-20.

[8] Chong, K.P., S. Rossall and M. Atong, 2009b. *In vitro* synergy effect of syringic acid, caffeic acid, 4-hydroxybenzoic acid against Ganoderma boninense. International Journal of Engineering and Technology, 1(4): 282-284.
[9] Chong, K.P., S. Rossall and M. Atong, 2011. HPC Fingerprints and *in vitro* antimicrobial activity of syringic acid, caffeic acid and 4-hydroxybenzoic acid against Ganoderma boninense. Journal of Applied Science, 11(13): 2284-2291
[10] Clé, C., L.M. Hill, R. Niggeweg, C.R. Martin, Y. Guisez, E. Prinsen and M.A.K. Jansen, 2008. Modulation of chlorogenic acid biosynthesis in *Solanum lycopersicum*; consequences for phenolic accumulation and UV-tolerance. Phytochemistry, 69: 2149-2156.
[11] Idris, A.S., D. Ariffin, T.R. Swinburne and T.A. Watt, 2000. The identity of *Ganoderma* species responsible for BSR disease of oil palm in Malaysia-Morphological characteristics MPOB Information Series. MPOB TT No. 77a
[12] Idris, A.S., S. Ismail, D. Ariffin and H. Ahmad, 2004. Prolonging the productive life of *Ganoderma*-infected palms with Hexaconazole MPOB Information Series. MPOB TT No. 214.
[13] Lynn, D.G. and M. Chang, 1990. Phenolic signals in cohabitation: implications for plant development. Annual Review of Plant Physiology and Plant Molecular Biology, 41: 497-526.
[14] Ozyigit, I.I., M.V. Kahraman and O. Ercan, 2007. Relation between explant age, total phenols and regeneration response of tissue cultured cotton *(Gossypium hirsutum L). African Journal of Biotechnology*, 6: 3-8.
[15] Randhir, Lin and Shetty, 2004. Phenolics, their antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors. Asia Pacific Journal of Clinical Nutrition, 13: 295-307.
[16] Schmitz-Hoerner, R. and G. Weissenbock, 2003. Contribution of phenolic compounds to the UV-B screening capacity of developing barley primary leaves in relation to DNA damage and repair under elevated UV-B levels. Phytochemistry, 64: 243-255.
[17] Thomas, P. and M.B. Ravindra, 1999. Shoot tip culture in mango: influence of medium, genotype, explant factors, season and decontamination treatments on phenolic exudation, explant survival and axenic culture establishment. Journal of Horticultural Science, 72: 713-722.