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Antifungal Activity of Phenolic Acids against *Ganoderma boninense* and Possible Development of Resistance

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ABSTRACT

This paper presents results on the antifungal activity of phenolic acids; caffeic acid (CA), syringic acid (SA) and 4-hydroxybenzoic acid (4-HBA) against *Ganoderma boninense* and possible resistance developed in this pathogen after treated with combination of the three phenolic acids at the ratio of 1:1:1 (w/w) with different concentrations. Lower concentrations of phenolic acids such as 0.1 to 0.5 mg mL^{-1} failed to inhibit the growth of *G. boninense* completely, while, higher concentrations of phenolic acids (0.4 and 0.5 mg mL^{-1}) gave significant slower growth rate of *G. boninense* in comparison to control (without phenolic acids). However, the highest concentration of phenolic acids tested in this experiment (2.5 mg mL^{-1}) inhibits the growth of *G. boninense* completely. From the *in vitro* study, although, *G. boninense* was exposed and trained with lower concentrations such as 0.1 to 0.5 mg mL^{-1} before re-introduced to 2.5 mg mL^{-1}, no resistance was recorded.

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INTRODUCTION

Malaysia currently accounts for 39% of world palm oil production and 44% of world exports [13]. In 2013, the oil palm plantation areas in Malaysia reached 5.2 Million hectares [12]. However, the oil palm industry is hampered by the devastating disease Basal Stem Rot (BSR). This incurable disease is caused by *Ganoderma boninense*. The oil palm industry is seriously damaged by BSR for more than 50 years and eventually causing large amount of losses in revenue [8,15]. Many researches have been conducted to search for potential control of this disease but to date no conclusive solution is reported. The more recent approach is investigating the role of phenolic acids in oil palm defense against *Ganoderma* infection [1,3,4,5,6]. Plants does have its own defense system which acts upon the infection. Phenolics are components of secondary metabolite which is a preformed antifungal produced in plant and activated in response to infection. Caffeic acid, syringic acid and 4-hydroxybenzoic acid (4-HBA) are phenolic acids which reported to have high antifungal activity against *Ganoderma* [3,5]. Although phenolic acids are produced in plant, the amount being produced might not be sufficient to encounter the infection if the accumulation of those phenolic acids is low. The information on the sufficient amount of phenolic acids to contribute to resistance of oil palm against *Ganoderma* is very little. Therefore, this experiment is designed to investigate the response of *Ganoderma* to the different concentration of caffeic acid, syringic acid and 4-HBA which was reported to be effective against *G. boninense* and the possible development of resistance of this pathogen to these phenolic acids.

MATERIALS AND METHODS

Preparation of Cell Assay Plates:

Cell assay plates with concentrations of 0.0, 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg and 2.5 mg mL^{-1} of each caffeic acid (CA), syringic acid (SA) and 4-hydroxybenzoic acid (4-HBA) were prepared by incorporating the respective phenolic acids into Potato Dextrose Agar (PDA), with the phenolic acids being first dissolved in acetone: water (50: 50; v/v) before incorporated into the medium. PDA without phenolic acids served as control.

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Antifungal Activity of Phenolic Acids against G. boninense:

The synergy effect of CA, SA and 4-HBA on the antimicrobial activity against *G. boninense* was investigated by measuring the diameter growth of mycelium in centimeter (cm) of *G. boninense* on cell assay plates incorporated with different concentrations of phenolic acids. Plugs (8 mm) of *G. boninense* were taken from the edge of seven (7) to eight (8) days old cultures, using a sterile micropipette tip and placed to the middle of the cell assay plates. The growth of *G. boninense* was observed daily for 14 days.

Possible Resistance of G. boninense to Phenolic Acids:

Plugs (8 mm) of *G. boninense* were placed to the middle of the cell assay plates containing 0.1 mg mL^{-1} of individual phenolic acids. The growth of the pathogen was monitored for 14 days. Surviving mycelia of *G. boninense* were transferred to cell assay plates with higher concentration; 0.2 mg mL^{-1} of each phenolic acids. The growth of the pathogen was further monitored for 14 days. The transferring and monitoring processes were repeated to other higher concentration such as 0.3 mg, 0.4 mg, 0.5 mg and 2.5 mg mL^{-1} of cell assay plates with respective phenolic acids. The diameter growth (cm) of *G. boninense* on cell assay plates was measured throughout the experiment. The assays were repeated three times to closely monitor if any possible resistance of *G. boninense* derived from this incubation.

Results:

Antimicrobial Activity of Phenolic Acids against G. boninense:

Lower concentrations of phenolic acids (0.1 to 0.5 mg mL^{-1}) failed to inhibit the growth of *G. boninense* completely (Figure 1). However, phenolics with concentrations of 0.4 and 0.5 mg mL^{-1} gave a significant slower growth rate of this pathogen compared to control. However, the highest concentration of phenolic acids (2.5 mg mL^{-1}) tested in this experiment had inhibited the growth of *G. boninense* completely.

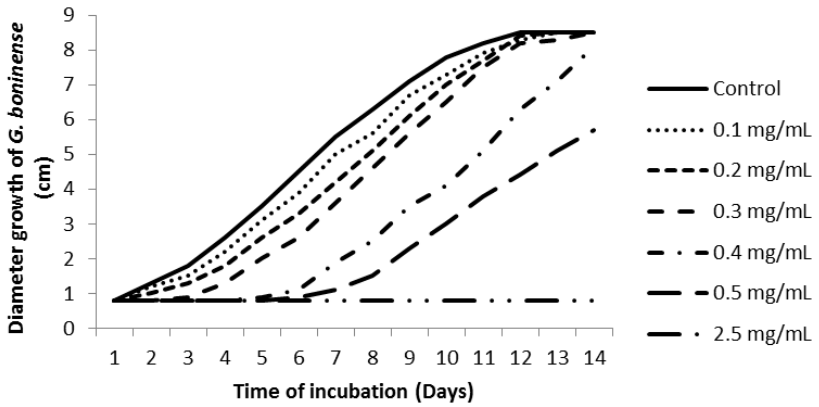

Fig. 1: Diameter growth of *G. boninense* on cell assay plates containing PDA incorporated with combination of three phenolic acids; SA, CA and 4-HBA at the ratio of 1:1:1 (w/w) with different concentrations (0.0, 0.1, 0.2, 0.3, 0.4, 0.5 and 2.5 mg mL^{-1}).

*PDA denotes for Potato Dextrose Agar, SA: Syringic acid, CA: Caffeic acid, 4-HBA: 4-hydroxybenzoic acid.

Possible Resistance of G. boninense to Phenolic Acids:

Although the *G. boninense* was exposed and trained under different concentrations of phenolic acids (0.1 mg mL^{-1} to 0.4 mg mL^{-1}) in cell assay plates, *G. boninense* was incapable to develop resistance to higher concentrations of phenolic acids (0.5 mg mL^{-1} and 2.5 mg mL^{-1}) (Figure 2 to 5).

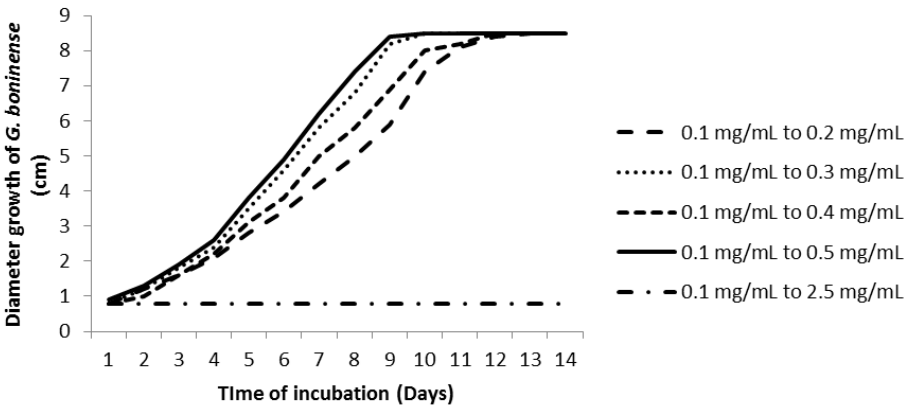

Fig. 2: Diameter growth of *G. boninense* after being transferred from cell assay plates (0.1 mg mL^{-1}) to cell assay plates (0.2, 0.3, 0.4, 0.5 and 2.5 mg mL^{-1}) respectively. The concentrations tested and shown in graph were with equal amount of each syringic acid, caffeic acid and 4-hydroxybenzoic acid.

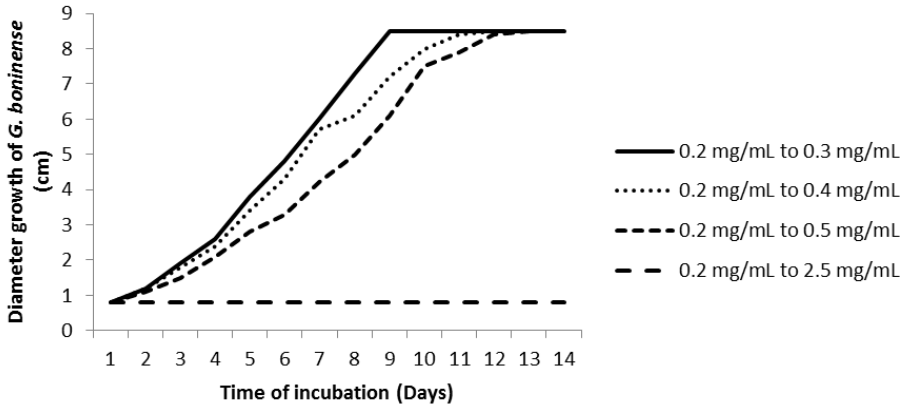

Fig. 3: Diameter growth of *G. boninense* after being transferred from cell assay plates (0.2 mg mL^{-1}) to cell assay plates (0.3, 0.4, 0.5 and 2.5 mg mL^{-1}) respectively. The concentrations tested and shown in graph were with equal amount of each syringic acid, caffeic acid and 4-hydroxybenzoic acid.

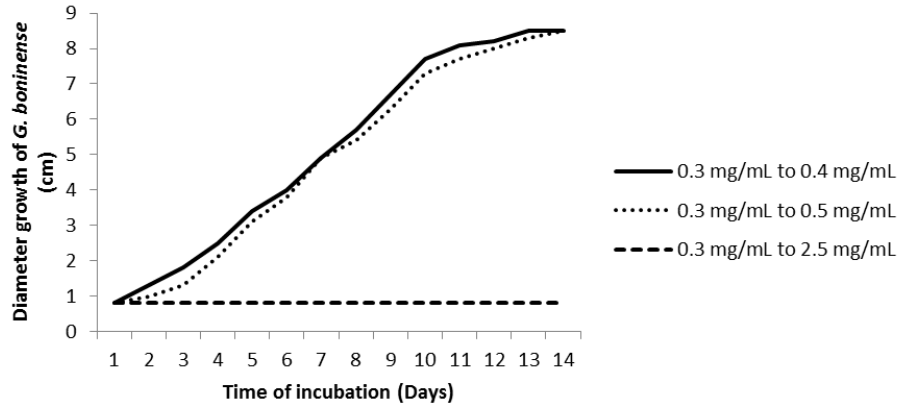

Fig. 4: Diameter growth of *G. boninense* after being transferred from cell assay plates (0.3 mg mL^{-1}) to cell assay plates (0.4, 0.5 and 2.5 mg mL^{-1}) respectively. The concentrations tested and shown in graph were with equal amount of each syringic acid, caffeic acid and 4-hydroxybenzoic acid.

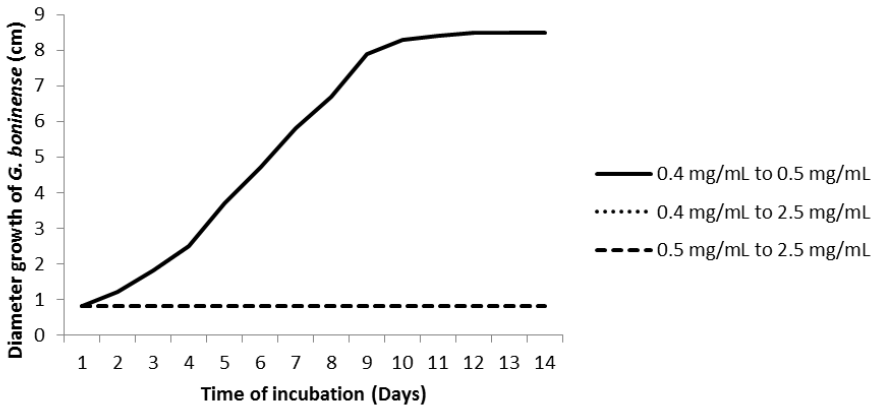

Fig. 5: Diameter growth of *G. boninense* after being transferred from cell assay plates (0.4 mg mL^{-1}) to cell assay plates (0.5 and 2.5 mg mL^{-1}) respectively and from cell assay plates (0.5 mg mL^{-1}) to cell assay plates (2.5 mg mL^{-1}). The concentrations tested and shown in graph were with equal amount of each syringic acid, caffeic acid and 4-hydroxybenzoic acid.

Discussion:

Antimicrobial Activity of Phenolic Acids against G. boninense:

The lag phase of *G. boninense* was affected by different concentrations of phenolic acids in cell assay plates. Lag phase is the condition where the fungi are trying to adapt the new environment when their cells had depleted various essential constituents and required time to restart the biosynthesis. The growth of *G. boninense* was slowly inhibited as the concentration of phenolic acids was increased. Extended of lag phase usually occurred when microbes were transferred from a rich cultured medium to a poorer one [11]. Rich culture medium possesses all nutrients needed by the subject of culture. In this case, *G. boninense* which was first cultured on PDA without phenolic acids (rich medium), was struggling when transferred to the media containing phenolic acids with different concentrations within the 14 days of incubation. Besides the extension period of lag phase, *G. boninense* in phenolic acids amended media was also unable grow to maximum (full plate) after 14 days. Combination of these three phenolic acids at the concentration of 2.5 mg mL^{-1} was found to have the ability to inhibit *G. boninense* growth and hence gave a constant diameter size throughout the period of incubation. This is as accordance to the findings reported by Chong *et al*. 2009b [7] which 2.5 mg mL^{-1} of these three phenolic acids were very fungitoxic to *G. boninense*.

Possible Resistance of G. boninense to Phenolic Acids:

Antifungal resistance is defined as a stable, inheritable adjustment by a fungal cell to an antifungal agent, which resulted in a less than normal sensitivity to that antifungal [2]. In addition, mutation in the regulatory gene can cause resistance of fungi towards the phenolic acids [16]. Caffeic acid and 4-hydroxybenzoic acid required a higher concentration (up to 2.0 mg mL^{-1}) to inhibit *G. boninense* but only 0.5 mg mL^{-1} for syringic acid [7]. Phenolics have the probability to be degraded in several ways. *Fusarium flocciferum* shows its capability to degrade some phenolic compounds namely, syringic, caffeic, ferulic acids, syringic aldehyde, gallic, and vanillic. After 24 hours of incubation, the fungus was found capable to reduce the phenolic concentration from 200 mg L^{-1} to below detection limits. However, syringic acid and its aldehyde have the lowest degradation rates [14].

The capability of *G. boninense* to develop resistance against phenolic acids was also related to the enzymes secreted by the white rot fungi. *G. neo-japonicum* is a white rot fungi which was reported to have the ability to produce extracellular enzymes, including β-glucosidase, ligninase, cellulase, avicelase, pectinase, xylanase, protease, and amylase [9]. Among the enzymes, β-glucosidase and ligninase showed the highest activity in *G. neo-japonicum*. These enzymes were capable to catalyse the oxidation of phenols and their related compounds. Secretion of enzymes has led to the colour changes of the media surrounding *Ganoderma* culture. A formation of deeply coloured zone around the mycelium has also been reported as the reaction of extracellular oxidase [10]. The brown colour formation occurred as early as first day of the growth. In this experiment, *G. boninense* was trained in lower concentration of phenolic acids, during the transferring of *G. boninense* from cell assay plates with lower phenolic acids to higher, it was noticed that *G. boninense* which was cultured on cell assay plates (0.5 mg mL^{-1} and 2.5 mg mL^{-1}) started to form brown colour spot on the media few hours soon after transferred, which suggesting the degradation of phenolic acids (Figure 6). However, the mycelia of the pathogen turned to brown and dead indicating it failed to develop resistance and further degrade the high concentration of phenolic acids.

G. boninense was not successful to be re-isolated on fresh PDA suggesting this combination has fungicidal effect instead of fungistatic.

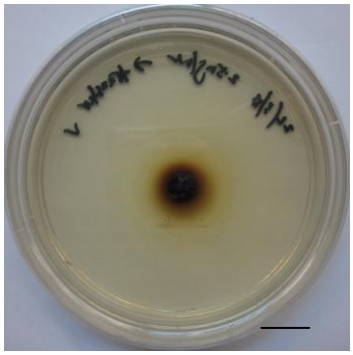

Fig. 6: Brown colour spot formation on the media surrounding the *G. boninense* plug on PDA amended with 2.5 mg ml^{-1} of syringic acid, caffeic acid and 4-hydroxybenzoic acid respectively. Bar: 6c

Conclusion:

The growth of *G. boninense* was inhibited as the concentration of phenolics acids (syringic acid, caffeic acid and 4-hydroxybenzoic acid) increased. Highest concentration tested in this experiment (2.5 mg ml^{-1}) of the combination of these three phenolic acids successfully killed the pathogen. *G. boninense* failed to develop resistance against these phenolic acids.

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REFERENCES

[1] Arif, M.A.M., O. Abrizah, B.Y. Zetty Norhana, S. Syahanim, A.S. Idris, A. Mohd Din and S. Ravigadevi. 2007. Molecular and biochemical approaches to understanding oil palm-*Ganoderma* Interactions. pp. 228-247. In: *Proceedings of Agricultural Biotechnology Sustainability Conference Vol. 1*. Malaysian Palm Oil Board (MPOB), Malaysia

[2] Bosshe, H.V., 1997. Mechanisms of antifungal resistance. Revista Iberoamericana de Micologia., 14: 44-49

[3] 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

[4] Chong, K.P., M. Atong and S. Rossall, 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.

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

[6] 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.

[7] 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.

[8] Idris, A.S., 2009. Basal stem rot in Malaysia-biology, economic importance, epidemiology, detection and control. In: *International Workshop on Awareness, Detection and Control of Oil Palm Devastating Diseases*. 6 November 2009. Kuala Lumpur Convention Centre, Malaysia.

[9] Jo, W.S., H.N. Park, D.H. Cho, Y.B. Yoo and S.C. Park, 2011. Detection of extracellular enzyme activities in Ganoderma neo-japonicum. Mycobiology, 39(2): 118-120.

[10] Käärik, A., 1965. The identification of the mycelia of wood-decay fungi by their oxidation reactions with phenolic compounds. Studia Forestalia Suecica., 31: 1-75.

[11] Madigan, M.T., J.M. Martinko, P.V. Dunlap and D.P. Clark, 2009. Brock biology of microorganisms. 12th ed. Pearson Benjamin Cummings. Sans Francisco

[12] Malaysian Palm Oil Board, 2013. Overview of the Malaysian Oil Palm Industry 2010. Available online at http://bepi.mpob.gov.my/index.php/statistics/area/114-area-2013/639-oil-palm-planted-area-dec-2013.html
[13] Malaysian Palm Oil Council, 2014. Overview of the Malaysian Oil Palm Industry. (Available online at http://www.mpoc.org.my/Malaysian_Palm_Oil_Industry.aspx)
[14] Mendonca, E., A. Martins and A.M. Anselmo, 2004. Biodegradation of natural phenolic compounds as single and mixed substrate by Fusarium flocciferum. Electronic Journal of Biotechnology, 17(1): 30-37.
[15] Miller, R.N.G., M. Holderness, P.D. Bridge, R.R.M. Paterson, M. Sariah, M.Z. Hussin and E.J. Hilsley, 1995. A multidisciplinary approach to the characterization of *Ganoderma* in oil palm cropping systems. In: Ganoderma, Systematicsm Phytopathology and Pharmacology. Proceedings of the Fifth International Mycological Congress, 1994, pp: 57-66. P.K. Buchanan, R.S. Hseu and J.M. Moncalvo (eds.). Vancouver, Canada.
[16] Triyana, S.Y., 2009. Antibiotic resistance of pathogenic bacteria. Majalah Kesehatan Pharma Medika., 1(2): 92-94.