In Vitro Synergy Effect of Syringic Acid, Caffeic Acid and 4-hydroxybenzoic Acid against Ganoderma boninense

Chong Khim Phin, Rossall S. and Markus Atong

***Abstract* - This paper discusses the in vitro synergy effect of syringic acid, caffeic acid and 4-hydroxybenzoic acid which found in oil palm root. Experiments were observed for fourteen days, repeated at least three times and data were recorded daily. The synergy effect of the phenolics against *Ganoderma boninense* were expressed in inhibition of radial growth of** G. boninense **on PDA ameliorated with the combination of either two or three different phenolics with concentration of 0.5 to 2.5 mg/ml. Several combinations showed strong fungitoxicity effect to** G. boninense. **Combinations of 4-hydroxybenzoic acid, syringic acid and caffeic acid up to 2.5 mg/ml strongly inhibited the growth of** G. boninense **in comparison to the control.**

***Index Terms* - *Ganoderma boninense*, Syringic acid, Caffeic acid, 4-hydroxybenzoic acid**

I. INTRODUCTION

The Malaysian palm oil industry recorded a satisfactory performance in 2008. Export earnings of oil palm products rose to record RM 65.2 billion. The total oil palm planted area in the country increased by 4.3% to 4.48 million hectares in 2008. The expansion in planted area occurred mainly in Sabah and Sarawak with a combined growth of 7% compared to 2% in Peninsular Malaysia. Sabah remained the largest oil palm planted state, accounting for 1.33 million hectares or 30% of the total planted area in the country [7]. Optimal yield production of oil palm in Malaysia and other countries of South East Asia is hampered by the presence of devastating Basal Stem Rot (BSR) disease caused by Ganoderma boninense. Oil palm has an economic life span of 25-30 years. Basal stem rot can kill more than 80 percent of stands by the time they are half-way through normal economic life [1]. In the late 1960s and early 1970s in Sumatra, there was little decline in the yield of oil palm until the surviving stand had fallen to about 115 palm/ha, but in more recent plantings, any loss of palm was associated with a loss of yield [4]. Yield of infected palms was also reduced by 20-40% compared to the year before infection was detected [8].

Palms with Ganoderma yielded between 13 and 21% less than healthy palms at the same age [12]. Heavily infected field yielded 26% less at 11 years after planting, and 46% less at 15 years by which time incidence was 67% [6]. There is currently no effective cure for G. boninense infection in an existing stand. Preventive and ameliorative treatments which are commonly carried out show various degrees of effectiveness [13]. Determination of total phenolic content in G. boninense infected and healthy oil palm roots showed susceptible palm roots at week four had low phenolic content, whereas week one had high phenolic content. Gallic acids concentrations decreased in the four weeks old roots of infected susceptible palms compared to healthy roots. Determination of total phenolic content in infected palm seedlings root (D X P) also showed low phenolic content compared to the non infected palm seedlings root. This indicate phenolic compounds are involved in oil palm resistance against Ganoderma [11].To identify the possibility of oil palm resistance against G. boninense in certain circumstances need further investigation. However, if resistance in oil palm against G. boninense is possible, it may contribute to tackling the problem. In a collaborative experiment to this research, we found syringic acid, caffeic acid and 4-hydroxybenzoic acid present in oil palm roots in natural condition or after elicitation. In this paper, we present the works on in vitro synergy effect of syringic acid, caffeic acid and 4-hydroxybenzoic acid to G. boninense.

II. MATERIALS AND METHODS

A. Ganoderma boninense

Cultures were provided by Borneo Samudera Sdn Bhd, Sabah, Malaysia, maintained at 25oC on Potato Dextrose Agar (PDA).

B. In vitro bioassay

A series of 0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml of syringic acid, caffeic acid and 4-hydroxybenzoic acid from Sigma® were ameliorated into the PDA with the combination as table 1. The phenolics were first dissolved in Acetone: Water (50:50; v/v). Solvent was served as positive control. The growth of the pathogen was expressed in centimeter of radial growth.

Table 1: Combination of different phenolics in different concentration for *in vitro* synergy effect experiments.

Combination	Phenolics & Concentration
1	Sy0.5mg/ml + Caff0.5mg/ml
2	Sy0.5mg/ml + Caff2.5mg/ml
3	Sy1.0mg/ml + Caff0.5mg/ml
4	Sy1.0mg/ml + Caff2.5mg/ml
5	Sy2.5mg/ml + Caff2.5mg/ml
6	Sy0.5mg/ml + Hy2.5mg/ml

Manuscript received September 10, 2009.
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7	Sy1.0mg/ml + Hy2.5mg/ml
8	Sy2.5mg/ml + Hy2.5mg/ml
9	Caff0.5mg/ml + Hy0.5mg/ml
10	Caff1.0mg/ml + Hy1.0mg/ml
11	Caff1.5mg/ml +Hy1.5mg/ml
12	Caff2.0mg/ml + Hy2.0mg/ml
13	Caff2.5mg/ml + Hy2.5mg/ml
14	Sy0.5mg/ml + Caff0.5mg/ml +Hy0.5mg/ml
15	Sy0.5mg/ml + Caff2.5mg/ml +Hy2.5mg/ml
16	Sy1.0mg/ml + Caff1.0mg/ml +Hy0.5mg/ml
17	Sy1.0mg/ml + Caff1.0mg/ml +Hy1.0mg/ml
18	Sy1.0mg/ml + Caff2.5mg/ml +Hy2.5mg/ml
19	Sy2.5mg/ml + Caff2.5mg/ml +Hy2.5mg/ml

* Sy= Syringic acid, Caff= Caffeic acid,
Hy= 4-hydroxybenzoic acid

III. RESULTS AND DISCUSSION

A. In Vitro bioassays

In vitro bioassays were conducted to test the synergy effect of syringic acid, caffeic acid and 4-hydroxybenzoic acid to G. boninense. 2.5 mg/ml of each phenolic, either syringic with 4-hydroxybenzoic acid or caffeic with 4-hydroxybenzoic acid or syringic with caffeic acid or the combination of all the three phenolics totally inhibited the growth of G. boninense compare to control (Fig 1, 2, 3 & 4). But a better performance were shown in combination of caffeic acid and 4-hydroxybenzoic acid which required only 2.0 mg/ml of respective phenolics to stop the growth of this pathogen up to day-14 (Fig 3). On another hand, G. boninense also failed to grow in the combination of 0.5 or 1.0 mg/ml of syringic acid + 2.5 mg/ml of caffeic acid + 2.5 mg/ml of 4-hydroxybenzoic acid (Fig 4). Most of the other combinations were also inhibiting the growth of this pathogen up to day-14 which the pathogen failed to reach the maximum size of 9 cm (maximum size of the petri dish) in comparison to control. Other combinations which poorly inhibiting the growth of this pathogen include combinations of lower concentration (0.5 mg/ml or 1.0 mg/ml) of the phenolics (Fig 1, 2, 3 & 4). Surprisingly, the combination of 0.5 mg/ml of syringic acid with high concentration (2.5 mg/ml) of caffeic acid also failed to stop the growth of G. boninense (Fig 1). This was also found in the synergy effect of 1.0 mg/ml of syringic acid and 2.5mg/ ml of 4-hydroxybenzoic acid.

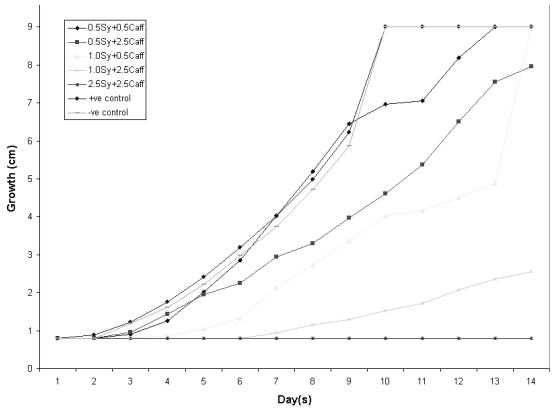

Fig. 1: Radial growth of Ganoderma boninense on PDA ameliorated with combination of syringic and caffeic acid

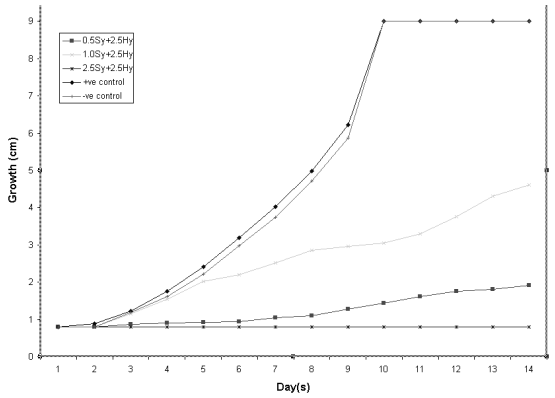

Fig. 2: Radial growth of Ganoderma boninense on PDA ameliorated with combination of syringic and 4-hydroxybenzoic acid

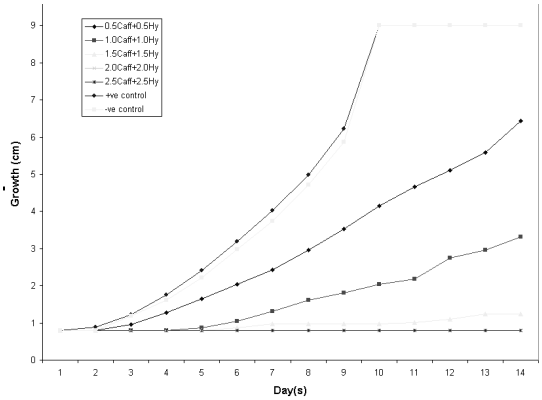

Fig. 3: Radial growth of Ganoderma boninense on PDA ameliorated with combination of caffeic and 4-hydroxybenzoic acid

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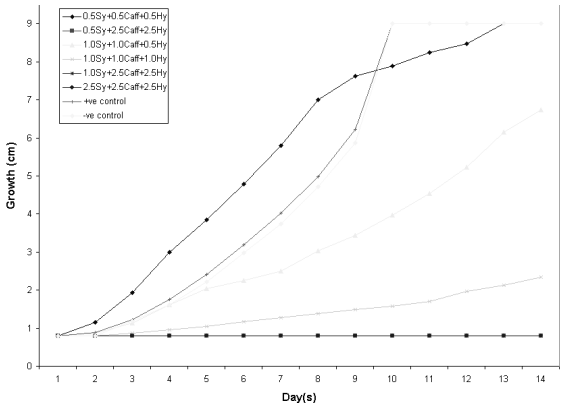

Fig. 4: Radial growth of Ganoderma boninense on PDA ameliorated with combination of syringic, caffeic and 4-hydroxybenzoic acid

In general, many works on plants have demonstrated the fungitoxicity effect of syringic acid. In resistance raspberry to fungus Didymella, syringic acid was found accumulated in the bordering zone of lesion forming a barrier to the fungus. The in vitro fungitoxic of syringic acid was later confirmed to be very toxic at low concentration [10]. In sugar cane, cultivar Mayarí 55–14, which is highly resistant to smut disease showed a major accumulation pattern of syringic acid when interact with the pathogen [5]. In a relevant in vitro experiment conducted by Chong et. al., 2009, syringic acid was found to be very fungitoxic to G. boninense even at concentration of 0.5 mg/ml. When the concentration was increase to 1.0 mg/ml, the pathogen was fully inhibited.

The role of 4-hydroxybenzoic acid has been demonstrated in rice hull against various microorganisms. An evaluation of 50% inhibition of growth (IC50) revealed that most of the gram-positive and some gram-negative bacteria were sensitive to 4-hydroxybenzoic acid at IC50 concentrations of 100-170 µg/ml [2]. Meanwhile many research showed caffeic acid is ubiquitously present in plants and a potent phytotoxin affecting plant growth and their physiology [14]. In another report, caffeic acid was also found inhibiting the growth of four sweet potato pathogenic fungi and germination of proso millet seeds in bioassays. Inhibitory activity in the bioassays reported also suggests high periderm caffeic acid levels contribute to the storage root defense chemistry of some sweet potato genotypes [9]. In vitro antimicrobial activity and fungitoxicity of these phenolics also showed caffeic acid and 4-hydroxybenzoic acid were having inhibitory effect with the highest concentration tested; 2.5mg/ml strongly inhibited the growth of G. boninense in comparison to the control [3].

IV. Conclusion

In this paper, we presented the result of in vitro synergy effect of syringic acid, caffeic acid and 4-hydroxybenzoic acid to G. boninense. We found combination of phenolics with higher concentration such as 2.5 mg/ml can fully inhibit the growth of G. boninense in comparison to lower concentrations such as 0.5 or 1.0 mg/ml

ACKNOWLEDGEMENTS

The authors wish to extend their highest appreciation to Prof. Dr. Ridzwan Abd Rahman, the Dean, School of Sustainable Agriculture, Universiti Malaysia Sabah for his continous supports and advices throughout the study. We acknowledged the support from Ministry of Science, Technology and Innovation, Malaysia for funding the research through e-Science Fund 0026 and Borneo Samudera Sdn Bhd for supply of seedlings.

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