

RESEARCH PAPER

## Effect of combination of organosulfur and polyphenols organic fungicide on *Ganoderma*-infected oil palm seedlings

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### ABSTRACT

The curative potential of an organic fungicide against *Ganoderma boninense* was previously assessed *in vitro* and yielded promising results. This organic fungicide was formulated using a combination of organosulfur compounds and polyphenols (OSC-P) as active ingredients. This study aimed to evaluate the effectiveness of OSC-P application in controlling basal stem rot (BSR) disease in infected oil palm seedlings of different varieties and to determine the optimum application dose. Two oil palm varieties were used: a *G. boninense*-susceptible variety and a moderately tolerant variety. The seedlings were artificially infected with *G. boninense* using inoculated rubber wood blocks (RWB). OSC-P was applied to 16-week-old seedlings by soil drenching at a volume of 200 mL per plant at 2-week intervals for 24 weeks. A total of six treatments, including controls, were arranged with four replications and observed at 4-week intervals. Each replication consisted of 10 seedlings. Observed parameters included disease incidence, disease severity, and vegetative growth parameters. The results showed that OSC-P application significantly reduced the incidence and severity of BSR disease in infected oil palm seedlings. BSR symptoms were less severe in the moderately tolerant variety than in the susceptible variety; however, disease incidence did not differ significantly between the two varieties. The optimum application dose of OSC-P was 0.8% (v/v), and variations in application dose did not significantly affect disease incidence or severity at 24 weeks after application. These findings are expected to complement existing preventive strategies for BSR management.

**Keywords:** Basal stem rot, efficacy, garlic extract, *in vivo*, liquid smoke

### INTRODUCTION

Oil palm plantations in Indonesia continue to face a serious threat from basal stem rot (BSR) disease caused by *Ganoderma boninense* (Corley & Tinker, 2016). BSR is considered one of the most destructive diseases of oil palm, as it can infect plants at all growth stages and often leads to plant death (Flood et al., 2022; Jazuli et al., 2022; Priwiratama et al., 2022; Priwiratama & Susanto, 2020). Early symptoms of BSR are difficult to detect, once visible symptoms appear, the infection of *G. boninense* has usually spread extensively within the basal stem tissue (Jazuli et al., 2022; Priwiratama et al., 2022; Rees et al., 2009). The incidence and severity of BSR are influenced by plant generation and age, with disease incidence increasing as oil palm plants mature (Flood et al., 2022; Lisnawita et al., 2016). *Ganoderma boninense* is a soil-borne fungus capable of surviving saprophytically in the soil

for more than four years (Corley & Tinker, 2016).

To date, no method has been found that can completely control BSR disease (Supramani et al., 2022). Current management strategies mainly focus on preventive measures (Flood et al., 2022; Supramani et al., 2022), which are generally ineffective once plants become infected. BSR is a complex disease that requires an integrated control strategy combining both preventive and suppressive approaches (Priwiratama et al., 2020). Therefore, curative methods targeting *G. boninense* are needed to complement existing preventive strategies. The curative control of *Ganoderma* spp. using hexaconazole has been reported to reduce plant mortality caused by upper stem rot (USR) disease by up to 48.76% (Nur-Rashyeda et al., 2023). Although these results indicate potential, there is still no conclusive evidence demonstrating effective control of BSR disease in oil palm. Furthermore, the use of synthetic pesticides is known to pose risks to the environment and to applicators' health (Decourtye et al., 2013; Mahmood et al., 2016; Martínez et al., 2013; Tudi et al., 2021). Extensive pesticide use in oil palm plantations has been associated with health problems among workers, ranging from skin irritation to lung

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cancer (Sulaiman et al., 2019).

Antifungal compounds derived from organic sources are generally more environmentally friendly and safer for applicators (Hazra & Purkait, 2019; Nagy et al., 2020), although their stability is often inferior to that of synthetic compounds (Ngegba et al., 2022; Smith & Perfetti, 2020). Organosulfur compounds, commonly found in garlic extracts, are known for their strong antimicrobial activity (Bhatwalkar et al., 2021; Kutawa et al., 2018) and are effective against a wide range of pathogenic fungi (Aala et al., 2014; Daniel et al., 2015; Kutawa et al., 2018; Wallock-Richards et al., 2014; Yusup et al., 2024a). The antifungal mode of action of organosulfur involves inhibition of fungal cell membrane growth (Borlinghaus et al., 2014; Sarfraz et al., 2020). Another compound with strong antifungal activity is polyphenol, which is present in liquid smoke and has been shown to inhibit the growth of several fungal species (Ennacerie et al., 2019; Yusup et al., 2024a) as well as insects (Diptaningsari et al., 2022; Prabowo et al., 2016). Polyphenols disrupt the synthesis of ergosterol, glucan, chitin, protein, and glucosamine in fungi (Ennacerie et al., 2019).

Our previous studies demonstrated that organosulfur and polyphenol exhibit antifungal activity against *G. boninense*, along with other compounds such as hesperidin and rutin (Yusup et al., 2024a). Combinations of these compounds resulted in reduced effective concentrations while maintaining similar antifungal activity against *G. boninense* (Yusup et al., 2023). An organic fungicide formulated from organosulfur and polyphenol showed synergistic interactions and completely inhibited *G. boninense* colony growth in vitro at a minimum concentration of 0.4% (Yusup et al., 2024b). Scanning electron microscope (SEM) observations revealed that contact application caused mycelial lysis at a minimum concentration of 1.6%. Therefore, this study aimed to evaluate the effectiveness of a formulated organic fungicide containing a combination of organosulfur and polyphenol against BSR disease in infected oil palm seedlings of different varieties, to assess its effects on vegetative growth parameters, and to determine the optimum application dose.

## MATERIALS AND METHODS

**Research Site.** This study was conducted at an oil palm nursery and the Plant Protection Laboratory of the Indonesian Oil Palm Research Institute (IOPRI), Marihat Unit, Simalungun, North Sumatra, Indonesia.

**Materials.** Two oil palm varieties with different levels of resistance to *G. boninense* were used in this study: the Simalungun variety, which is considered susceptible, and the 540-NG variety, which is classified as moderately tolerant. Rubber wood blocks (RWB) inoculated with *G. boninense* were also used. The pathogen was isolated from infected oil palm plants at Bah Jambi Plantation, Simalungun, North Sumatra, Indonesia.

**Preparation of *G. boninense* Inoculum.** *G. boninense* cultures grown on potato dextrose agar (PDA) were cut into 1 × 1 cm pieces and inoculated onto sterile rubber wood blocks (6 × 6 × 6 cm). The inoculated RWBs were incubated for 8 weeks prior to use.

**Treatment and Experimental Design.** The experiment was arranged in a factorial randomized block design with six treatments, including a control (Table 1). The experimental factors were organic fungicide application dose and oil palm variety. Each treatment was replicated four times, with 10 seedlings per replicate. The application doses of the organic fungicide were determined based on the results of previous in vitro assays (Yusup et al., 2024b).

**Seedling Planting.** The planting medium consisted of a soil and sand mixture (1:1, w/w) placed in 5-kg polybags. Prior to planting, RWBs containing *G. boninense* inoculum were placed into the planting holes. Seedlings were watered twice daily until 16 weeks after planting (WAP). Before the first application of the organic fungicide, a subset of seedlings was sampled to observe root development and to confirm contact between the roots and the RWB. Root contact with the RWB was used as an indicator of seedling infection by *G. boninense*.

### Preparation and Application of Organic Fungicide.

The organic fungicide was prepared following the method described by Yusup et al. (2024b). Fresh garlic cloves were peeled, blended with distilled water at a 1:1 ratio (w/v), and filtered through a 74- $\mu$ m mesh. The concentration of garlic extract in the filtrate was determined to be 50% (w/v) by subtracting the weight of the solid residue from the initial weight of the cloves. Polyphenols were obtained from burned coconut shells through a pyrolysis process. The organic fungicide formulation consisted of 1.6% crude garlic extract and 0.125% polyphenols. Applications were performed every two weeks for a total of eight applications using

a soil drenching method at the base of each seedling, with an application volume of 200 mL per seedling.

**Observation Parameters.** Observations were conducted monthly from the first application until six months thereafter, when the seedlings were 10 months old. The observed parameters included: (1) disease incidence based on the presence of BSR symptoms; (2) disease severity assessed using BSR symptom scoring (Table 2) and stem rot scoring at the end of the observation period (Table 3); (3) calculation of the disease severity index (DSI) using the formula of Townsend & Heuberger (1943) and the area under the disease progress curve (AUDPC) according to Simko & Piepho (2012); and (4) vegetative growth

parameters, including plant height, stem diameter, and number of leaves.

**Data Analysis.** Data were analysed using SPSS Statistics version 26 for Windows. Analysis of variance (ANOVA) was performed, and mean comparisons were conducted using Tukey’s test at a 5% significance level.

## RESULTS AND DISCUSSION

**Development of BSR Disease Symptom on Oil Palm Seedlings.** The development of BSR disease symptoms on oil palm seedlings, from score 1 to 4 (Figure 1), varied over time and was influenced by disease

Table 1. Organic fungicide treatments applied to oil palm seedlings

No	Variety	Treatment	Description
1	Simalungun 540-NG	Control (-)	Seedling + sterile RWB without organic fungicide treatment
2	Simalungun 540-NG	Control (+)	Seedling + RWB inoculated with <i>G. boninense</i> without organic fungicide treatment
3	Simalungun 540-NG	OSC-P 0.8% (v/v)	Seedling + RWB inoculated with <i>G. boninense</i> and treated with organic fungicide at 0.8%
4	Simalungun 540-NG	OSC-P 1.6% (v/v)	Seedling + RWB inoculated with <i>G. boninense</i> and treated with organic fungicide at 1.6%
5	Simalungun 540-NG	OSC-P 2.4% (v/v)	Seedling + RWB inoculated with <i>G. boninense</i> and treated with organic fungicide at 2.4%
6	Simalungun 540-NG	OSC-P 3.2% (v/v)	Seedling + RWB inoculated with <i>G. boninense</i> and treated with organic fungicide at 3.2%

Table 2. Disease severity scoring based on BSR symptoms in oil palm seedlings

Score	Description
0	Healthy plant
1	Chlorotic on lower leaves with the formation of a hyphal mass on the basal stem
2	Necrosis on lower leaves with button-like sporophores formed on the basal stem
3	50% or more of the leaves are necrotic, with sporophores formed on the basal stem
4	Dead plant

Source: Abdullah et al. (2003); Kok et al. (2013).

Table 3. Disease severity scoring based on stem tissue rot

Score	Description
0	Healthy; no rot in the stem tissue
1	<20% of the stem tissue is rotted
2	21–50% of the stem tissue is rotted
3	51–90% of the stem tissue is rotted
4	> 90% of the stem tissue is rotted

Source: Breton et al. (2006).

triangle factors, including a conducive environment, a susceptible host plant, and pathogen virulence (Rakib et al., 2019; Sharma et al., 2023). Based on monthly observations, disease progression in the susceptible Simalungun variety was generally faster than in the 540-NG variety. Simalungun seedlings typically died when *G. boninense* formed fully developed fruiting bodies, whereas the leaves of 540-NG seedlings remained green (Figure 2). These results indicate that the 540-NG variety has a higher level of tolerance to *G. boninense* infection than the Simalungun variety, resulting in slower disease development.

The use of *Ganoderma*-tolerant varieties is considered one of the most effective approaches for controlling BSR disease in oil palm, as biological and chemical control methods remain largely ineffective (Supramani et al., 2022). Seedling mortality was first observed at 16 weeks after application (WAA; 32 weeks after planting, WAP) in all OSC-P treatments and the positive control. This finding suggests that BSR disease required approximately four weeks to progress from the initial visible symptoms observed at 12 WAA (28 WAP) to seedling death.

**Effect of Organic Fungicide Application on BSR Disease.** Observation conducted at 24 WAA showed that the application of OSC-P to *Ganoderma*-infected oil palm seedlings significantly reduced disease incidence, disease severity, and the percentage of basal stem tissue rot compared to the control ( $P < 0.05$ ) (Table

4). The greatest reduction in both disease incidence and severity was observed at the 0.8% OSC-P dose. However, differences among OSC-P doses had no significant effect ( $P \geq 0.05$ ) on all disease parameters measured (Table 5). This result indicates that increasing OSC-P concentration did not proportionally enhance disease suppression, which may be related to the limited stability of organic fungicides. Organic-derived pesticides are generally more susceptible to degradation by environmental factors such as sunlight (BaQais et al., 2024), temperature, and humidity (Mitra et al., 2024).

Varietal differences did not significantly affect disease parameters, nor did they significantly interact with OSC-P treatments.

**Disease Incidence of BSR.** Regular application of OSC-P for 16 weeks at two-week intervals reduced disease incidence by 51–73% compared to the positive control and delayed the appearance of visible BSR symptoms. In the absence of OSC-P application, BSR symptoms appeared eight weeks after application, which was four weeks earlier than in treated seedlings (Figure 3). This finding indicates that OSC-P application inhibited the development of *G. boninense*, resulting in delayed symptom expression.

The progression of BSR disease is influenced by pathogen characteristics, host factors, environmental conditions, and management practices (Jazuli et al., 2022). In this study, OSC-P application functioned



Figure 1. Disease severity scoring based on BSR symptoms. A. Score 0 (healthy); B. Score 1; C. Score 2; D. Score 3; E. Score 4.



Figure 2. Infected oil palm seedlings at 24 weeks after application. A. Simalungun variety; B. 540-NG variety.

as a management practice capable of suppressing disease development. Although OSC-P significantly affected disease incidence, its interaction with varietal differences was not significant, suggesting that varietal tolerance played a more dominant role. Despite the lack of statistical significance, symptom expression occurred later in the tolerant variety than in the susceptible one.

OSC-P application also reduced the infection rate of BSR disease. The infection rate in treated seedlings ranged from 0.01 to 0.03 units per four weeks, compared to 0.06 units per four weeks in the

control. An infection rate below 0.5 units per day is considered low and may be attributed to low pathogen aggressiveness, host tolerance, or unfavorable environmental conditions (Manengkey & Senewe, 2011). Disease development is also influenced by soil pH (Rahman & Othman, 2020). Consistent with this, Rini et al. (2022) reported no visible BSR symptoms in eight-month-old oil palm seedlings. BSR is therefore considered a cryptic disease, as early infections are difficult to detect (Priwiratama et al., 2022). Cryptic diseases are typically asymptomatic or exhibit hidden symptoms, often associated with endophytic pathogens

Table 4. Recapitulation of analysis of variance results

Parameter	Variety (V)	Treatments (T)	V × T
Diseases incidence	ns	*	ns
Diseases severity	ns	*	*
Stem tissue rot	ns	*	*
Plant height	*	ns	ns
Stem diameter	*	ns	ns
Number of leaves	ns	ns	ns

Table 5. Effect of organic fungicide application on BSR disease in oil palm seedlings

Treatments <sup>1)</sup>	Parameters		
	Disease incidence (%)	Disease severity (%)	Stem tissue rot (%)
Control (-)	0 a	0 a	0 a
Control (+)	68.67 ± 18.40 b	48.02 ± 21.76 b	49.68 ± 22.09 c
OSC-P 0.8% (v/v)	18.50 ± 4.61 a	12.63 ± 4.71 a	21.69 ± 5.55 b
OSC-P 1.6% (v/v)	33.33 ± 6.40 a	21.26 ± 3.61 a	29.12 ± 8.35 b
OSC-P 2.4% (v/v)	21.50 ± 9.21 a	13.19 ± 4.98 a	22.31 ± 8.64 b
OSC-P 3.2% (v/v)	22.17 ± 5.73 a	19.24 ± 3.86 a	26.11 ± 9.24 b

<sup>1)</sup>OSC-P = organic fungicide containing organosulfur and polyphenols. Means followed by the same letter within the same column are not significantly different based to Tukey’s test at p = 0.05.

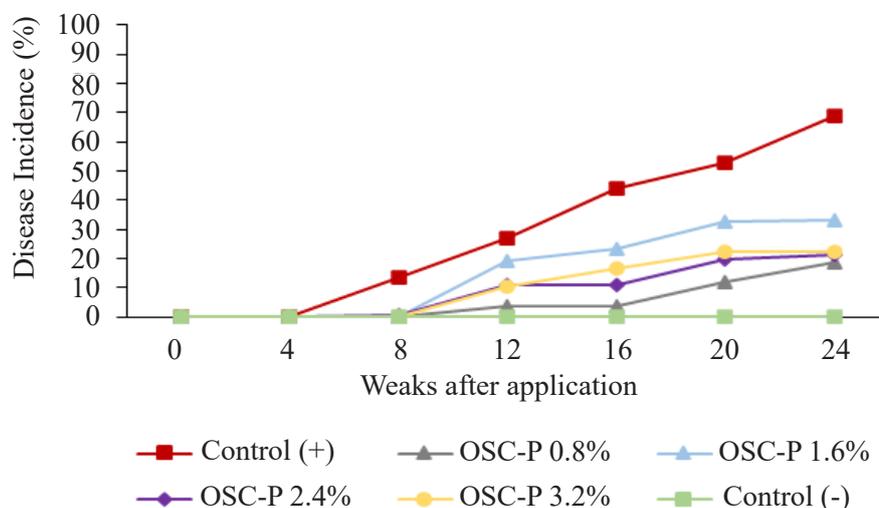


Figure 3. Effect of organic fungicide application on BSR disease incidence in oil palm seedlings.

(Shaw et al., 2016; Stergiopoulos & Gordon, 2014).

These findings were further supported by the area under the disease progress curve (AUDPC) values calculated using the method of Campbell & Madden (1990). AUDPC provides an integrated measure of disease development and reflects quantitative resistance or the inhibitory effects of biotic and abiotic factors (Bocianowski et al., 2020; Jeger & Viljanen-Rollinson, 2001). The control treatment exhibited the highest AUDPC value (6.56 units), whereas OSC-P treatments ranged from 1.83 to 4.19 units. This result indicates that OSC-P inhibited BSR disease development by 36–72%.

**Disease Severity Based on Leaf Symptoms.** The interaction between OSC-P treatment and oil palm variety had a significant effect ( $P < 0.05$ ) on disease severity, as assessed by leaf symptoms and stem rot. OSC-P application reduced disease severity by 56–74% compared to the control. Monthly increases in disease severity were consistently lower in treated seedlings than in the control (Figure 4), indicating slower disease progression.

Similar to disease incidence, no correlation was

observed between increasing OSC-P dose and disease severity. This may be attributed to the mode of action of organosulfur and polyphenols, which require direct contact to exert antifungal effects (Bhatwalkar et al., 2021; Chanjun et al., 2015; Ennacerie et al., 2019; Mendoza et al., 2019). The soil drench method used in this study may have limited compound uptake due to low root absorption. Previous studies have reported that soil drenches and foliar applications are often ineffective or pose environmental risks, whereas trunk injection is a more efficient delivery method (Archer et al., 2022). However, soil drenching was selected in this study due to the small stem diameter of the seedlings. Severe BSR symptoms were first observed in the control at 8 WAA, whereas similar severity levels appeared four weeks later in OSC-P-treated seedlings (Figure 4).

**Disease Severity Based on Basal Stem Tissue.** Disease severity was also evaluated based on basal stem tissue rot and external symptoms. Basal stem rot was assessed at the end of the observation period (24 WAA) by longitudinally cutting the stems and assigning severity scores (Figure 5). OSC-P application

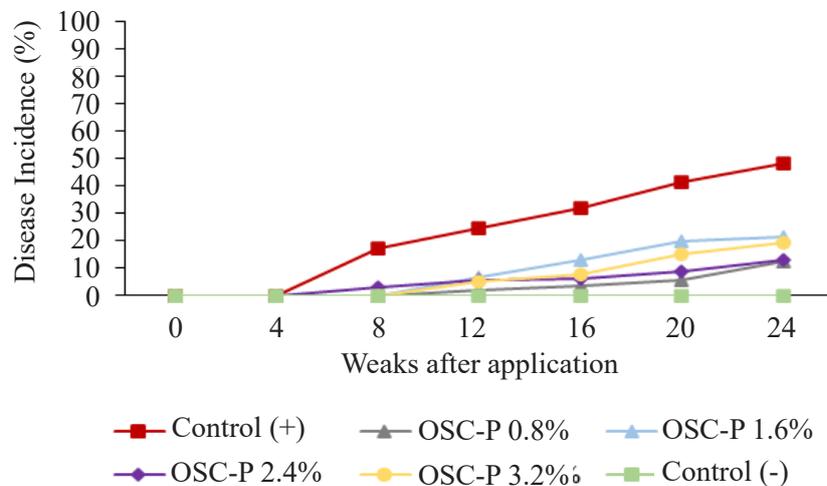


Figure 4. Effect of organic fungicide application on BSR disease severity in oil palm seedlings.



Figure 5. Disease severity scoring based on rotting of basal stem tissue. A. Score 0 (healthy); B. Score 1; C. Score 2; D. Score 3; E. Score 4.

reduced basal stem tissue rot by 41–56% compared to the control. Notably, disease severity based on internal stem tissue was higher than that indicated by external symptoms (Table 5), suggesting that *G. boninense* infection remained asymptomatic during early stages while internal damage progressed.

Early, undetected infection is a major challenge in BSR disease management, as early intervention represents the optimal window for disease control (Assis et al., 2016; Flood et al., 2022; Jazuli et al., 2022; Supramani et al., 2022). Healthy oil palm seedlings exhibit clean, fresh stem tissue and vigorous root systems. *Ganoderma* infection typically initiates at the roots and spreads toward the basal stem (Rees et al., 2009).

**Effect of Organic Fungicide on Vegetative Performance.** Vegetative growth parameters, including stem diameter and leaf number, were not significantly affected by OSC-P application or its interaction with oil palm variety, but were influenced by varietal differences (Table 5). Vegetative growth in oil palm seedlings is primarily determined by genetic factors (Martin et al., 2022) and nursery management practices such as polybag size, growing medium, and fertilization (Akpo et al., 2014a). The Simalungun variety exhibited generally higher vigor than the 540-NG variety (Table 6). These results indicate that OSC-P application had no adverse effects on seedling growth. Similar findings suggest that vegetative performance is more strongly influenced by genetics and nursery management than by disease control treatments (Akpo et al., 2014b; Martin et al., 2022). Additionally, the application of arbuscular mycorrhizal fungi has been reported to enhance vegetative growth and increase resistance to *Ganoderma* in oil palm seedlings (Priwiratama et al., 2022; Rini et al., 2022).

## CONCLUSION

The organic fungicide containing organosulfur and polyphenols as active ingredients significantly

reduced disease incidence in *Ganoderma*-infected oil palm seedlings at 24 weeks after application. Leaf symptoms appeared later in the tolerant variety than in the susceptible variety. In terms of disease severity, symptoms were influenced by organic fungicide application and were more severe in the susceptible variety. The optimum application dose for oil palm seedlings was 0.8% (v/v). No correlation was observed between increasing application dose and decreasing BSR disease incidence or severity. Furthermore, organic fungicide application did not adversely affect the vegetative performance of oil palm seedlings.

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## AUTHORS' CONTRIBUTIONS

CAY and DDE conceived and designed the experiment. DDE, ASR, and MGP collected the data. CAY performed the data analysis and interpreted plant damage symptoms. CAY and DDE prepared the manuscript. HW and DS contributed research ideas and assisted in completing the manuscript. All authors contributed to the discussion of the research design, data analysis, interpretation, and manuscript structure. All authors have read and approved the final manuscript.

Table 6. Vegetative parameters of susceptible and moderately tolerant *Ganoderma* varieties at 40-week-old seedlings

Variety	Vegetative Parameters*		
	Plant height (cm)	Stem diameter (mm)	Number of leaves
Simalungun (Susceptible)	39.78 a	13.49 a	6.78
540-NG (Moderately tolerant)	36.72 b	12.57 b	6.97

\*Means followed by the same letter in the same column are not significantly different based on Tukey's test at  $p = 0.05$ .

## COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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