

Effectiveness of a combination of organosulfur and polyphenols in controlling basal stem rot disease at selected levels of disease severity

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Received 03 Jun 2025/ Revised 17 Sep 2025/ Accepted 20 Oct 2025

Abstract

Basal stem rot (BSR) is a major disease of oil palm. One approach to control the pathogen is by using organic fungicides. This study aims to assess the efficacy of two kinds of fungicides, organosulfur and polyphenol, in suppressing *Ganoderma boninense* and their impact on seedlings. Poisoned agar media were used in the *in vitro* assay with organosulfur at concentrations of 0.8% and 0.125%, polyphenol at 1.6% and 0.125%, and two combinations: 0.8% organosulfur + 0.125% polyphenol and 1.6% organosulfur + 0.125% polyphenol (v/v), with a 3-replication test. In vivo trials were conducted on 4-month-old oil palm seedlings inoculated with *G. boninense*. Two treatments were tested: Combination 1 (0.8% organosulfur + 0.125% polyphenol, v/v) and Combination 2 (1.6% organosulfur + 0.125% polyphenol, v/v). The solution was applied by soil drenching, 200 mL per plant, at the stem base every 14 days, totalling four applications within a 3-month period. The treatments were evaluated under three levels of disease severity: Group 1 (asymptomatic plants with fungal fruiting bodies), Group 2 (leaf necrosis $\leq 50\%$), and Group 3 (leaf necrosis $\geq 50\%$). The first result demonstrated a full inhibition of fungal growth in treatments with 1.6% polyphenol and the combination of 1.6% organosulfur + 0.125% polyphenol (v/v). In vivo assay results showed that an increase in resistance (measured by prolonged survival) was observed in Groups 1 and 2. These findings indicate that combining organosulfur and polyphenol fungicides can improve seedling resistance to BSR, particularly at early and moderate disease stages.

[Keywords: *in vitro*, *in vivo*, *Ganoderma boninense*]

Introduction

Oil palm (*Elaeis guineensis* Jacq.), a member of the Arecaceae family, is a key agricultural commodity in Indonesia (Rahmawati, 2023). According to BPS-Statistics Indonesia (2023), national crude palm oil (CPO) production rose from 45.12 million tons in 2021 to 46.82 million tons in 2022. To sustain this growth, proper crop management is essential for achieving optimal yields. However, one of the main challenges to improving oil palm productivity is Basal Stem Rot (BSR), a disease caused by *Ganoderma* spp. (Panggabean, 2023).

Basal Stem Rot was initially found in older oil palm plantations, particularly in third-generation replantings, due to the persistence of *Ganoderma* inoculum in the soil. Recent reports, however, show that the disease also affects younger palms, even at immature palms (Harefa, 2022). Typical symptoms include basal stem decay followed by leaf necrosis (Sinaga, 2024). BSR severely reduces yield and farmers' income. Harefa (2022) reported that *Ganoderma* infection decreases fresh fruit bunch (FFB) production from 19.02 to 13.95 tons per hectare per year, equivalent to a loss of 5.07 tons or 26.66%.

In oil palm plantations, farmers employ several strategies to manage BSR, such as uprooting infected plants, land sanitation through burning, and the use of synthetic fungicides (Rupaedah et al., 2018). However, excessive fungicide application may result in more virulent pathogen strains, the loss of natural enemies and beneficial organisms, health risks, environmental pollution, and ecological imbalance (Firhalzar & Wisdawati, 2022). As a sustainable alternative, organic pesticides offer an effective and eco-friendly method to control BSR. These utilize antagonistic mechanisms by extracting phytochemicals from plants to suppress the growth of *Ganoderma boninense* (Agustina, 2020).

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Among the organic phytochemicals used to control BSR are organosulfur and polyphenol compounds. Organosulfur compounds, commonly found in garlic extracts, show strong antimicrobial activity with both fungistatic and fungicidal effects (Widiastuti et al., 2017; Yusup et al., 2024). Their activity is largely attributed to allicin, which acts as a natural insecticide and fungicide by inhibiting cell formation, disrupting metabolism, and damaging membranes and organelles, ultimately causing fungal cell death (Moulia et al., 2018; Putra & Sukohar, 2018; Yusup et al., 2023; Basuki et al., 2024). Similarly, polyphenols possess broad-spectrum antimicrobial properties and can be obtained from liquid smoke derived from coconut shells and husks (Fardhyanti et al., 2018; Qomariyah et al., 2019). Their antifungal mechanism involves disrupting the biosynthesis of key fungal cell components such as ergosterol, glucans, chitin, glucosamine, and proteins, thereby impairing cell structure and function (Ennacerie et al., 2019; Mendoza et al., 2019). The combination of organosulfur and polyphenols has been shown to effectively inhibit the growth of *G. boninense*, highlighting their potential as active ingredients in organic fungicide formulations (Yusup et al., 2024). However, in that study, the effect of the applied fungicide on disease severity level has not yet been determined.

The use of organic fungicides not only minimizes ecological impacts but also enhances overall plant health. However, studies investigating the combined effects of organosulfur and polyphenol compounds against *G. boninense* remain limited. Further research is therefore essential to evaluate their effectiveness in controlling the pathogen in oil palm, particularly in identifying the optimal application timing and their role in promoting plant recovery.

Materials and Methods

The experiment was conducted over five months, from November 1, 2024, to March 31, 2025, at the Indonesian Oil Palm Research Institute (IOPRI) Bogor Unit, West Java Province. This study utilized organosulfur compounds extracted from garlic (Widiastuti et al., 2017) and polyphenols derived from the pyrolysis of coconut shells and husks. Oil palm seedlings of the Simalungun variety and a *Ganoderma boninense* isolate (PPKS strain) isolated from the Bah Jambi estate in North Sumatra were used. An artificial inoculum was prepared using rubberwood blocks (RWB, 6 x 6 x 6 cm³) colonized by the *G. boninense* isolate after a two-month incubation period.

A quantitative approach was employed to evaluate the antifungal effectiveness of organosulfur compounds, polyphenols, and their combinations, both in vitro and in vivo. For the in vitro antifungal assay, a Completely Randomized Design (CRD) was applied with seven treatments, each replicated five times. Treatments included a control (no treatment); organosulfur crude extract formulated as Ganor™ at 0.8% and 0.125%; crude polyphenol extract from kopyor coconut fiber pyrolysis at 1.6% and 0.125%; and two combinations: 0.8% organosulfur + 0.125% polyphenol and 1.6% organosulfur + 0.125% polyphenol (all concentrations in v/v). These concentrations were selected based on preliminary studies on *G. boninense* (Yusup et al., 2024).

The most effective combinations from the in vitro assay were chosen for further evaluation in vivo using oil palm seedlings grouped by disease severity: mild (asymptomatic plants with *G. boninense* fruiting bodies), moderate ($\leq 50\%$ leaf necrosis), and severe ($\geq 50\%$ leaf necrosis). Each group included six replications. The in vivo treatments tested were Combination 1 (Ganor™ 0.8% organosulfur + 0.125% crude polyphenol extract) and Combination 2 (Ganor™ 1.6% organosulfur + 0.125% crude polyphenol extract) (v/v). Fungicides were applied by soil drenching at 200 mL per plant at the stem base every 14 days, with a total of four applications over a three-month period.

Efficacy testing of antifungal treatments in vitro

The in vitro antifungal efficacy test involved the preparation of Potato Dextrose Agar (PDA) medium, an isolate of *G. boninense* fungus from Bah Jambi, and the incorporation of organosulfur, polyphenol, and their combinations into the culture media at the respective concentrations. The effectiveness of each treatment was evaluated by measuring the average diameter of fungal colony growth. These measurements were used to calculate the percentage inhibition of fungal growth using the following formula adapted from Purba (2024):

$$\text{Relative inhibition (\%)} = \frac{D1 - D2}{D1}, \text{ where:}$$

DHR = Relative inhibition rate

D1 = Diameter of the fungal colony in the control (cm)

D2 = Diameter of the fungal colony in the treatment (cm)

Efficacy testing of antifungal treatments in vivo

The in vivo test began by classifying artificially inoculated oil palm seedlings based on disease severity: mild (score 1), moderate (score 2), and severe (score 3) (Table 1) (Izzati & Abdullah, 2008). The treatment solution (fungicide combinations) was applied biweekly after the inoculated plant

seeds reached the specified score. Observations of disease severity on the canopy and bulb were recorded weekly using the scoring scale developed by Izzati and Abdullah (2008). Disease severity on root and basal stem tissues was assessed at the end of the treatment period using the scoring method described by Rakib et al. (2015) (Table 2). The disease severity index was calculated using the method from Townsend and Heuberger (1943) with the formula:

$$I = \frac{\sum (n \times V) \times 100 \%}{N \times Z}$$

Explanation:

I = Disease severity index (%)

n = Number of infected tissues in each score category

v = Score value

Z = Highest score value

N = Total number of observed plants

Data analysis

The collected data were analyzed using SPSS Statistics 25 software. Analysis of Variance (ANOVA) was performed at a 5% significance level. If significant differences were found, a post hoc test was conducted using Tukey's Honest Significant Difference (HSD) test at the same significance level.

Results and Discussion

Characteristics of *G. boninense* (in vitro)

The macroscopic and microscopic observations of *G. boninense* fungal colonies were consistent with those described in previous studies by Yulianti et al. (2021), Hamzah et al. (2021), and Elfina et al. (2024). Macroscopically, the observed colonies appeared white to yellowish-white, with cotton-like edges. The colony exhibited lateral spreading growth, while the mycelia appeared as irregular, filamentous strands with a smooth texture, as shown

in Figure 1. Microscopically, the hyphae were aseptate and displayed clamp connections, which are morphological features characteristic of *G. boninense* (Figure 2). These characteristics align with previous reports that describe the mycelia of *G. boninense* as undulating, thick, smooth-textured, and laterally spreading.

The similarity in macroscopic and microscopic characteristics confirms that the fungal sample studied is indeed *G. boninense*. These morphological traits can serve as important reference points for the identification and further study of this pathogen. Thus, the findings of this observation not only corroborate earlier research but also strengthen the validity of the morphological characterization of *G. boninense* as a foundation for developing disease diagnosis and control methods associated with this pathogen.

In vitro assessment of the organic fungicide on *G. boninense* colony growth

Observation of *G. boninense* colony diameter growth was conducted daily from 1 to 14 days after inoculation (DAI), until the fungal colony in the control treatment fully covered the surface of the Petri dish, as shown in Figure 3. Further observations were extended to 30 days to assess the consistency of the fungicidal treatments. Data analysis revealed that fungicide treatment 1.6% polyphenol (Pf) and the combination of 1.6% organosulfur (Or) + 0.125% polyphenol (Pf) (v/v) were the most effective in inhibiting the growth of *G. boninense* colonies. These treatments showed either no visible colony growth or no development in colony diameter compared to other treatments, as illustrated in Figure 3. These findings indicate the high effectiveness of both fungicidal treatments in suppressing the development of *Ganoderma* tested.

Table 1. Disease severity score for the canopy and stem of oil palm seedlings (Izzati & Abdullah, 2008)

Score	Symptoms of <i>G. boninense</i> infection in oil palm seedlings
0	Healthy plant
1	Yellowing (chlorosis) of lower leaves with the formation of a hyphal mass at the base of the stem
2	Necrosis at the lower part of the plant and the appearance of button-like sporophores at the stem base
3	50% or more leaf necrosis and sporophore production at the stem base
4	Plant death

Table 2. Disease severity score for root and basal stem tissues of oil palm seedlings (Rakib et al., 2015)

Score	Symptoms of <i>G. boninense</i> infection
0	Healthy seedling, roots are free from pathogen infection.
1	Roots are infected by the pathogen, but the infection is limited to the root surface.
2	Pathogen colonies are formed, and the infection has spread to the root collar.
3	The basal stem (corm) of the seedling shows rotting due to pathogen attack.
4	The plant is dead.



Figure 1. Macroscopic characteristics of *G. boninense* fungal colony (control) without treatment: (A) top view of the isolate, (B) bottom view of the isolate at 4, 9, and 14 days after inoculation (DAI)

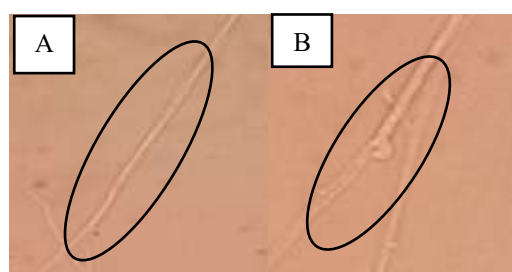


Figure 2. Microscopic characteristics of *G. boninense*: (A) Aseptate hyphae, (B) Presence of clamp connections (1000x magnification)

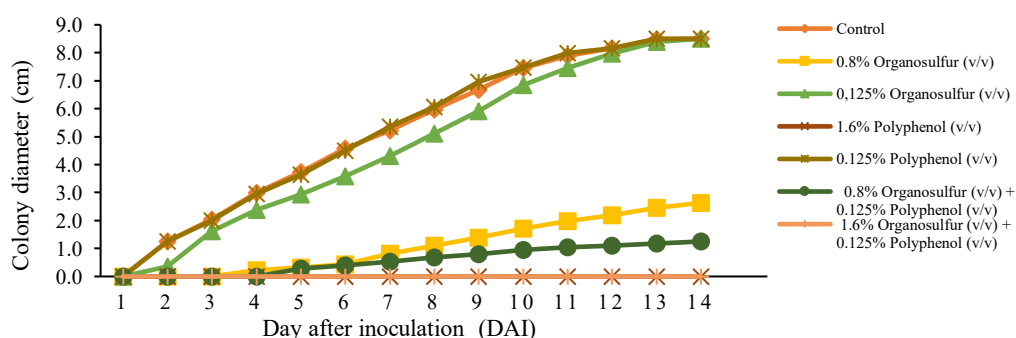


Figure 3. Growth of *G. boninense* colonies under seven treatments from 1 to 14 DAI

The effectiveness of these treatments can be explained by the mode of action of the active compounds. Polyphenols are natural compounds that function as antimicrobial and antioxidant agents (Qomariyah et al., 2019). These polyphenols are derived from liquid smoke produced by the pyrolysis of coconut shells and husks (Yusup et al., 2023). According to Agustina (2020), coconut shell liquid smoke effectively inhibits fungal growth due to its phenolic compounds, which possess

antimicrobial properties. On the other hand, organosulfur compounds, particularly allicin, also exhibit strong antimicrobial activity. Allicin can alter fungal morphology and metabolism, contributing to the inhibition of fungal activity. This compound is capable of penetrating cell membranes and organelle membranes, such as those of mitochondria, causing organelle damage and cell death (Putra & Sukohar, 2018).

Furthermore, organosulfur compounds and polyphenols primarily act as contact fungicides with a multisite mode of action that inhibits fungal growth through toxic effects. These effects disrupt the fungal cell wall structure as well as the function of metabolic enzymes, thereby impeding vital processes necessary for fungal growth and reproduction (Nulhasanah & Sulhaswardi, 2021). In the context of this study, the biochemical mechanisms are suspected to be the main cause of the inhibition of *G. boninense* colony growth observed in the fungicide treatments, as evidenced by the significant suppression of colony expansion compared to the control. Therefore, it can be concluded that the polyphenol fungicide treatment and its combination are effective in inhibiting the growth of *G. boninense*. This effectiveness is supported by the antimicrobial properties of both compounds, which act synergistically through a multisite inhibitory mechanism affecting the structure and metabolism of the fungus.

The results of the analysis of variance presented in Table 3 and Figure 4 indicate that the application of various treatment concentrations had a significant effect on the percentage inhibition of *G. boninense* growth. Treatments that proved effective included 0.8% organosulfur (Os) (v/v), 1.6% polyphenol (Pf) (v/v), the combination of 0.8% organosulfur (Os) + 0.125% polyphenol (Pf) (v/v), and the combination of 1.6% organosulfur (Os) + 0.125% polyphenol (Pf) (v/v). Based on the test results shown in Table 3, treatment with 0.8% organosulfur (Os) (v/v) showed very thin hyphal growth (malformation) (picture not shown) that was distinctly different compared to the control. The 1.6% polyphenol (Pf) (v/v) treatment and the combination of 1.6% organosulfur (Os) (v/v) + 0.125% polyphenol (Pf) (v/v) exhibited the highest inhibition

percentages, reaching 100%, thus categorizing them as the most effective treatments in suppressing fungal colony growth. However, in general, a decline in the inhibition percentage was observed across all treatments from day one to day fourteen after inoculation. This decrease in effectiveness is likely attributable to several factors. First, the concentration of antimicrobial compounds such as organosulfur and polyphenol may decrease over time, reducing their ability to maintain optimal microbial growth inhibition. This aligns with the statement of Hafizah et al. (2024), who reported that the antimicrobial effect is directly proportional to the maximum concentration of the active compounds, which correlates with the inhibition and killing of microorganisms.

Unfavorable environmental conditions could contribute to the decline in the effectiveness of antimicrobial compounds during the observation period. Research has demonstrated that phenolic compounds possess a strong inhibitory potential against pathogenic fungi, particularly by suppressing the production of both hydrolytic and ligninolytic enzymes. Exposure to these compounds can induce notable alterations in the morphology and structural integrity of *G. boninense* mycelia, including changes in branching patterns, hyphal elongation, and cell wall stiffness, ultimately impairing enzymatic secretion (Sidiqqi & Ganapathy, 2024). The observed mortality of *Ganoderma* mycelial tissues is primarily attributed to the cytotoxic effects of the tested phenolic compounds. However, the persistent survival of fungal hyphae under certain treatments suggests that *G. boninense* may possess inherent tolerance mechanisms when the concentration of these phenolic compounds remains within its physiological threshold.

Table 3. Percentage inhibition of treatments on *G. boninense* colony growth (in vitro)

Treatment (%) (v/v)	Percentage inhibition (%)*	
	7 DAI**	14 DAI
Control	0 ^a	0 ^a
0.8% Organosulfur (Os)	91.0 ^c	74.9 ^b
0.125% Organosulfur (Os)	23.4 ^b	5.7 ^a
1.6% Polifenol (Pf)	100 ^c	100 ^b
0.125% Polifenol (Pf)	0.5 ^a	0.0 ^a
0.8% Organosulfur (Os) + 0.125% Polifenol (Pf)	94.1 ^c	87.0 ^b
1.6% Organosulfur (Os) + 0.125% Polifenol (Pf)	100 ^c	100 ^b

Note: *Numbers within the same column followed by different letters indicate significant differences based on Tukey's test at the 5% significance level.

** DAI = Days After Inoculation.

Fourth, the type of antimicrobial substance as active ingredients in the agar medium also influences the growth of *G. boninense* fungus by the mode of action (MoA) of the active compounds. Arastehfar et al. (2020) stated that the fungal response variability to antimicrobial compounds is affected by the MoA of the active substances and the genetic differences of the fungi. Allicin, the main compound in organosulfur, has a MoA that inhibits the growth of fungal membranes and cell walls, causing cytoplasmic damage, membrane and cell wall rupture, and inhibiting mycelial growth (Aala et al., 2014). In contrast, polyphenols act by disrupting the synthesis of ergosterol, glucan, chitin, proteins, and glucosamine in fungi (Ennacerie et al., 2019; Mendoza et al., 2019). Therefore, the varying percentage of inhibition observed in treatments with organosulfur, polyphenol, and their combinations between 7 days and 14 days of observation is caused by a complex interaction between the stability of the compounds under changing environmental conditions compared to the development of hyphae of the control treatment. In the 14-day observation over time, the fungus produces more inactivator enzymes, so that at certain concentrations, there is a decrease in the percentage of inhibition of hyphal growth (El-Gendi et al., 2021). A thorough understanding of these factors is crucial for optimizing the use of antimicrobial compounds for effective and sustainable control of *G. boninense*.

Effect of treatments on the resistance of oil palm seedlings infected with *G. boninense* (in vivo)

Observations presented in Table 4 indicate that Combination 1 applied to Groups 1 and 2, and Combination 2 applied to Groups 1, 2, and 3, exhibit a longer time to first mortality compared to the positive control. Combination 2 in Group 1 demonstrated the best performance, as evidenced by the absence of initial plant mortality due to *G. boninense* inoculation throughout the study, as well as in the non-inoculated plant in the negative control treatment. Several factors could explain how the application of combinations 1 and 2 influenced plant resistance. Group 1 consisted of healthy plants, although fruiting bodies were observed in the planting medium. This suggests that, although no external symptoms were visible, the plant itself had not yet been infected by *G. boninense*. Therefore, the application of fungicidal combinations (Combination 1 and 2) in Group 1 likely enhanced the resistance of the plants and inhibited the progression of *Ganoderma* infection.

In Group 2, where plants exhibited necrosis of $\leq 50\%$, the application of both fungicide combination treatments also showed a positive influence on prolonging the life of the plants. Meanwhile, in Group 3 (necrosis $\geq 50\%$), different resistance responses were observed: Combination 1 extended plant survival by 41 days, while Combination 2

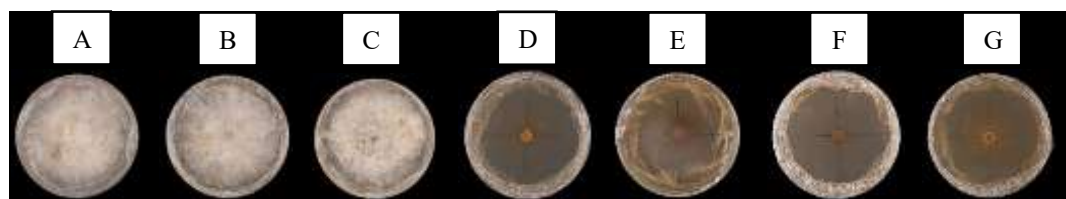


Figure 4. Growth of *G. boninense* colonies on PDA medium: (A) Control, (B) 0.125% Organosulfur (Os), (C) 0.125% Polifenol (Pf), (D) 0.8 % Organosulfur (Os), (E) 1.6% Polifenol (Pf), (F) Combination of 0.8% Organosulfur (Os) + 0.125% Polifenol (Pf), (G) 1.6% Organosulfur (Os) + 0.125% Polifenol (Pf) after 14 days incubation

Table 4. Time to first plant mortality of oil palm seedlings after the symptom (within observation period)

Treatment (v/v)	Time to first plant death (Days)		
	Group 1**	Group 2***	Group 3****
0.8% Organosulfur (Os) + 0.125% Polifenol (Pf) (1)	61	33	19
1.6% Organosulfur (Os) + 0.125% Polifenol (Pf) (2)	ps*	54	33
Positive control	42	28	14
Negative control	ps*		

Note: *ps: plant survives till the end of research

**Group 1: Mild severity (healthy plants with visible *G. boninense* fruiting bodies) (score 1 in Table 1)

***Group 2: Moderate severity disease (plants showing $\leq 50\%$ necrosis) (score 2 in Table 1)

****Group 3: Severe severity disease (plants showing $\geq 50\%$ necrosis) (score 3 in Table 1)

prolonged it to 56 days. This difference in effectiveness is likely due to the concentration of antimicrobial compounds applied. When concentrations are too low, organosulfur and polyphenol compounds may be insufficient to suppress microbial growth more effectively. Hafizah et al. (2024) noted that higher concentrations of antimicrobial compounds correlate positively with inhibitory effects, which may even lead to microbial death. Organosulfur compounds, particularly those containing allicin, exhibit strong antimicrobial properties that can combat various pathogens, including bacteria and fungi, thereby enhancing plant resistance. Hasrianda & Setiarto (2022) also reported that allicin helps prevent tissue damage caused by microbial attacks, contributing to overall plant health and extending productive lifespan. Antimicrobial compounds such as phenols also enhance the systemic resistance of plants. Azzahra et al. (2024) stated that phenolic and alkaloid compounds not only eliminate pathogens but also stimulate plant immune responses. For instance, lignin derived from phenols reinforces plant cell walls, thereby hindering fungal penetration. Similarly, allicin, a key organosulfur compound, demonstrates strong antimicrobial activity. Polyphenols, including flavonoids and tannins, exhibit the ability to disrupt the membranes of pathogen cells and inhibit the function of essential enzymes (Azzahra et al., 2024).

The combination of organosulfur and polyphenol compounds is believed to produce a synergistic effect in controlling various pathogens, including fungi and bacteria. The physiological condition of the plant and the biochemical mechanisms described above are likely responsible for the increased resistance observed in oil palm seedlings treated with the organosulfur–polyphenol fungicide combination against *G. boninense* infection.

Effect of treatment on disease severity in crown and basal stem (in vivo)

The results indicate that disease severity in the crown and basal stem of plants treated with Combination 1 and 2 was less than that observed in the positive control (Table 5). Disease severity in the crown and stem of Group 1 plants treated with treatment 2 showed a response similar to the negative control. These results suggest the effectiveness of the treatments in suppressing the development of *G. boninense* disease at mild disease severity. Group 1 consists of plants with mild symptoms, where the plants still appear visually healthy despite the presence of *G. boninense* fruiting bodies. This condition indicates that pathogen colonization within the plant tissues has not occurred extensively, or because of the ‘escape’ event, where the fruiting bodies developed directly from the RWB. Therefore, the application of treatments to plants in this condition is suspected to significantly strengthen the plant’s defense system. This aligns with the statement by Azzahra et al. (2024), who reported that the combination of allicin and polyphenols can induce systemic resistance in plants, providing long-term protection against pathogen infection. Consequently, the disease severity in the crown and basal stem of Group 1 treated with combination 2 did not differ significantly from the negative control. Hafizah et al. (2024) explained that the effectiveness of microbial inhibition increases with the concentration of active compounds used. The insignificant increase in disease severity in the crown and basal stem in groups 2 and 3 compared to the positive control indicates that the application of the combination of allicin and polyphenols provides a relatively similar time of death. Yusup et al. (2023) also stated that allicin, the active compound

Table 5. Severity of disease in the crown and basal stem of oil palm seedlings

Treatment (v/v)	Disease severity index (%)					
	Group 1**		Group 2***		Group 3****	
	bf	af	bf	af	bf	af
0.8% Organosulfur (Os) + 0.125% Polifenol (Pf) (combination 1)	8.3 ^a	58.3 ^b	50 ^b	75 ^b	75 ^b	95.8 ^b
1.6% Organosulfur (Os) + 0.125% Polifenol (Pf) (combination 2)	4.1 ^a	29.1 ^{ab}	50 ^b	79.1 ^b	66.6 ^b	87.5 ^b
Positive control	25 ^a	100 ^c	50 ^b	100 ^b	75 ^b	100 ^b
Negative control	0 ^a					

Note: *Values in the same column followed by different letters indicate significant differences based on Tukey’s test at the 5% level.

**Group 1: Mild disease severity (roots are infected by the pathogen, but the infection is limited to the root surface)(score 1 in Table 2)

***Group 2: Moderate disease severity (pathogen colonies are formed, and the infection has spread to the root collar)(score 2 in Table 2)

****Group 3: Severe disease severity (the basal stem (corm) of the seedling shows rotting due to pathogen attack)(score 3 in Table 2)

*bf: before treatment

*af: 75 days after first treatment

from garlic, has higher antifungal activity than polyphenols. Allicin, the main compound in organosulfur, has a MoA that inhibits the growth of fungal membranes and cell walls, causing cytoplasmic damage, membrane and cell wall rupture, and inhibiting mycelial growth (Aala et al., 2014). In contrast, polyphenols act by disrupting the synthesis of ergosterol, glucan, chitin, proteins, and glucosamine in fungi (Ennacerie et al., 2019; Mendoza et al., 2019). With this mode of action effect, it is synergistically able to increase the defense time of plants against *Ganoderma* attacks. This combination of compounds has been proven to have a synergistic effect in suppressing pathogen growth. This is supported by Yusup et al. (2023), who stated that the combination of allicin and polyphenols can inhibit pathogen growth by up to 100% in vitro. This effectiveness results in a low rate of disease severity increase in the crown and basal stems, and in some cases, no symptom progression occurs at all. Prihatiningsih et al. (2020) also noted that inhibiting pathogen growth results in a reduction in the rate of infection and the severity of disease symptoms. Overall, the physiological condition of the plant and the biochemical mechanisms induced by the application of the combination of organosulfur and polyphenols are suspected to be the main factors in reducing the severity of disease in the crown and basal stems.

Effect of treatment on root and stump tissue death (in vivo)

The symptoms of tissue death in roots and stumps differed significantly between treatment groups and the negative control (Table 6). In Group 1, the plants treated with combinations 1 and 2 had low disease severity and differed significantly from the positive control. This suggests that these two combinations have the potential to suppress disease development, even though the infection process occurred in mild disease severity. The primary cause

of root and stump death is the pathogenesis of *G. boninense* inoculum on rubber wood blocks. This inoculation enables the pathogen to directly infect plant tissues by root contact.

In contrast, roots in the negative control remained healthy because they had no contact with the *G. boninense* isolate. Nurhayati (2013) stated that soil-borne pathogen infection occurs through direct interaction between roots and inoculum sources present in the growing media. Visually, plants in Group 1 appeared healthy, although *G. boninense* fruiting bodies were found in the growing media. This indicates that, despite the absence of visible symptoms such as wilting or chlorosis, the pathogen infection has progressed slowly. This finding supports Rahmana et al. (2024), who noted that early symptoms of *G. boninense* infection are often undetected visually and only appear when the infection advances, characterized by wilting, yellowing leaves (chlorosis), and basal stem rot. Therefore, at this early stage, the treatment has a curative effect.

The effects of organosulfur and polyphenol compound treatments are strengthening the stump and root tissue, increasing resistance to *G. boninense* infection. Sarilla (2022) stated that allicin, an active component of the organosulfur group, has strong antimicrobial properties and can inhibit the growth of various pathogens, including fungi. Hasrianda & Setiarto (2022) further reported that allicin can reduce oxidative stress in plant roots, thus supporting healthier root growth and increased resistance to infection. In addition, polyphenols also play a role in enhancing plant resistance. Apriyelita (2023) explained that polyphenols can stimulate plants to produce secondary defense compounds such as flavonoids and tannins, which possess antimicrobial activity. The combination of organosulfur and polyphenol compounds is believed to work synergistically, enhancing the plant's immune response against pathogen attacks. Therefore, the biochemical mechanisms induced by

Table 6. Severity of Disease on the Basal Bulb of Oil Palm Seedlings at the end (75 DAI) of observation

Treatment (v/v)	Severity of disease (%)		
	Group 1**	Group 2***	Group 3****
0.8% Organosulfur (Os) + 0.125% Polifenol (Pf)	62.5 ^b	83.3 ^b	91.6 ^b
1.6% Organosulfur (Os) + 0.125% Polifenol (Pf)	50 ^b	83.3 ^b	100 ^b
Positive control	100 ^c	100 ^b	100 ^b
Negative control		0 ^a	

Note: *Values in the same column followed by different letters indicate significant differences based on Tukey's test at the 5% level.

**Group 1: Mild disease severity (healthy plants with visible *G. boninense* fruiting bodies)

***Group 2: Moderate disease severity (plants showing $\leq 50\%$ necrosis)

****Group 3: Severe disease severity (plants showing $> 50\%$ necrosis)

the treatment with organosulfur and polyphenols contribute to reducing the severity of root and stump tissue death symptoms caused by *G. boninense* infection. These results demonstrate the potential of such treatments as a curative strategy for controlling basal stem rot disease in oil palm.

Overall, the findings suggest that the interaction between the plant's physiological state and its biochemical defense mechanisms, particularly organosulfur and polyphenol compounds, plays a crucial role in determining the response of oil palm seedlings to *G. boninense* infection. These compounds, when combined, have proven effective in suppressing pathogen development at selected severity levels on oil palm seedlings.

Conclusion

The treatment with 1.6% polyphenol and the combination of 1.6% organosulfur + 0.125% polyphenol effectively inhibited 100% of *G. boninense* growth in vitro up to 30 days after inoculation. The application of a combination fungicide comprising organosulfur and polyphenol in in vivo tests was able to enhance resistance, reducing disease severity on crown and basal stem tissues by 31.25%, and on roots and basal bulb tissues by 21.55%. The combined organosulfur and polyphenol fungicide treatment effectively suppressed the severity of basal stem rot in oil palm seedlings with mild symptoms; however, it was not effective at advanced stages of infection. Further replicate experiments are necessary to verify the consistency and reproducibility of the treatment effects.

Acknowledgements

The authors sincerely thank the technical staff of the Plant Protection Research Group at the Indonesian Oil Palm Research Institute (IOPRI) for their dedication and invaluable assistance during this research. We also gratefully acknowledge the financial support provided by the Palm Oil Plantation Fund Management Agency (BPDPKS) through the 2024 Ganor Organic Fungicide research program.

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