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In vitro and *in vivo* Evaluations of the Antifungal Activity of Salicylic Acid and Silicon against *Ganoderma boninense*

(Penilaian *in vitro* dan *in vivo* Aktiviti Antikulat Asid Salisilik dan Silikon terhadap *Ganoderma boninense*)

AINNUR ATIRA MOHAMMAD SERI[1], DZARIFAH MOHAMED ZULPERI[1] & SITI IZERA ISMAIL[1,2,*]

[1]*Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[2]*Laboratory of Climate-Smart Food Crop Production, Institute of Tropical Agriculture and Food Security (ITAFoS), Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

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ABSTRACT

Basal stem rot disease (BSR) in oil palms is one of the primary diseases that has led to the wide use of fungicides. This increased the development of fungal isolates resistant to fungicides and led to the search for alternative strategies to replace the use of fungicides. This study aimed to evaluate *in vitro* antifungal activity of salicylic acid (SA) and silicon (Si) in inhibiting mycelial growth of *Ganoderma boninense* using Poison Food Technique and to evaluate the in vivo efficacy of Si treatment on oil palm seedlings growth and resistance towards G. boninense. Percentage inhibition of radial mycelial growth (PIRG) was assessed, and Si treatment significantly reduced mycelial radial growth of *G. boninense* up to 100% inhibition at concentrations of 200 and 250 mg/L. The half-maximal effective concentration (EC_{50}) for Si was 68.57 mg/L, while for SiO_2, it was 273.95 mg/L. The EC_{50} for salicylic acid was 381.33 mg/L. For *in vivo evaluation,* oil palm seedlings treated with Si at 150, 200, and 250 mg/L showed the lowest severity of leaf chlorosis and necrotic symptoms, which were 7.36%, 6.49%, and 4.05%, respectively. In contrast, the seedlings without Si showed the highest severity of leaf symptoms. Examination of internal bole tissues of oil palm seedlings treated with Si at a concentration of 250 mg/L also recorded a 3.0% mean percentage of disease severity compared to the untreated infected seedlings, which showed 35.0% disease severity. Our findings demonstrated the potential of Si application in controlling the BSR disease caused by *Ganoderma boninense*.

Keywords: Basal stem rot disease; *Ganoderma boninense;* oil palm; salicylic acid; silicon

ABSTRAK

Penyakit reput pangkal batang (BSR) ialah salah satu penyakit kelapa sawit yang paling serius sehingga menyumbang kepada penggunaan racun kulat secara berleluasa. Ini meningkatkan ketahanan kulat terhadap racun tersebut dan telah mendorong kepada pencarian strategi alternatif bagi menggantikan penggunaan racun kulat. Tujuan penyelidikan ini adalah untuk menilai aktiviti antikulat asid salisilik (SA) dan silikon (Si) secara *in vitro* dalam menyekat pertumbuhan miselium *G. boninense* menggunakan teknik *Poison Food*, serta menilai kesan silikon terhadap pertumbuhan dan ketahanan anak pokok kelapa sawit terhadap *G. boninense* secara *in vivo*. Peratusan perencatan pertumbuhan miselium (PIRG) telah dicatatkan dan Si telah merencatkan pertumbuhan miselium *G. boninense* sehingga 100% pada kepekatan 200 dan 250 mg/L. Kepekatan berkesan maksimum separuh (EC_{50}) silikon yang boleh menghalang pertumbuhan miselium ialah 68.57 mg/L manakala bagi silikon dioksida ialah 273.95 mg/L. EC_{50} untuk salisilik asid pula ialah 381.33 mg/L. Bagi penilaian *in vivo* pula, anak pokok yang menerima Si pada kepekatan 150, 200 dan 250 mg/L menunjukkan keterukan simptom daun paling rendah, iaitu masing-masing sejumlah 7.36%, 6.49% dan 4.05% manakala anak pokok yang tidak menerima Si menunjukkan keterukan simptom klorosis dan nekrosis paling tinggi pada daun. Pemeriksaan terhadap tisu pangkal batang anak pokok menunjukkan bahawa anak pokok yang menerima Si pada kepekatan 250 mg/L juga mencatatkan purata 3.0% keterukan pangkal batang berbanding kumpulan anak pokok yang tidak dirawat yang mempunyai 35.0% purata peratusan keterukan pangkal batang. Penyelidikan ini telah menunjukkan potensi Si dalam mengurangkan keterukan penyakit BSR.

Kata kunci: Asid salisilik; *Ganoderma boninense;* kelapa sawit; penyakit reput pangkal batang; silikon

INTRODUCTION

Oil palm (*Elaeis guineensis*) has become one of the most valuable oil-producing crops in the world. Therefore, oil palm trees play an important role as Malaysia's most valuable agricultural commodity, essential to the country's economic growth due to their suitability for the Malaysian climate (Awalludin et al. *2015). Given the substantial production and export*s, any yield loss due to oil palm diseases would significantly impact the country's annual yield. However, the spreading of some diseases is unavoidable, and immediate action is required to control them.

Fungal infections can significantly affect the productivity of palm oil production. When pathogenic fungi infect oil palm trees, they often lead to a disease known as wood rot. This disease can be further categorized based on the site of infection. According to Rees et al. (2009), one of the most prevalent diseases afflicting oil palms is basal stem rot (BSR). Additionally, diseases such as upper stem rot, caused by Ganoderma spp. (common in Asia), and vascular wilt, commonly induced by the Fusarium oxysporum pathogen, pose growth and oil yield reduction challenges for oil palm growers *(*Riyadi 2021).

Ganoderma boninense, a white rot fungus that destroys hardwoods, is the causal agent of BSR and poses a danger to oil palm plantations. The *G. boninense* inoculum that causes root diseases in grown oil palms often survives longer in the soil. This disease gradually deteriorates the oil palm's root system before ultimately causing rot in the basal stem of the tree. The incidence of BSR disease can be reduced if the mycelial growth or the spread of *G. boninense* spores can be inhibited. Plants usually produce salicylic acid during plant growth, plant development and cell signaling. Salicylic acid is crucial for plant resistance to pathogen attacks and is vital in modulating plant responses to many abiotic stresses (Chen et al. 2007; Wu et al. 2008). It can enhance the plant›s defence mechanism against *G. boninense* attacks, thereby improving its tolerance to the disease.

Silicon (Si) is among the essential trace elements in plants that play a significant role in the immune system's defence against pathogen attacks. Si enhanced plant growth and increased the yield and stress tolerance of rice, sugarcane, and wheat (Kim et al. *2002)*, as well as sugarcane and date palms (Bokor et al. 2019; Frazão et al. 2020). High levels of Si have been shown to enhance the resistance of rice plants to brown plant hoppers and reduce insecticide application (He et al*. 2015).* Si increases rice resistance to lodging and drought in rice and cucumber. Applying Si fertilizers reduced symptoms› expression and delayed the progression of BSR (Najihah et al. 2015*).* As Song et al. (2021) highlighted, Si deficiency in plants increases their susceptibility to abiotic factors such as fungi, bacteria, fungal infections, and insect feeding, which can negatively impact crop yield and quality. Thus, searching for a compelling product that has fungicidal activity is needed.

The repeated use of fungicides to manage the severity of the disease leads to the development of fungal resistance, resulting in both high costs and negative environmental consequences. Currently, limited information is available regarding the use of Si and salicylic acid to control BSR pathogens in Malaysia. Considering that Si can control several rice diseases compared to fungicides, Si might reduce the number of fungicide applications to control *G. boninense in oil palm plantations.* More information is needed on its effectiveness against Basidiomycetes fungi, such as the BSR pathogen. Therefore, effective management strategies are required to achieve integrated management against BSR.

MATERIALS AND METHODS

SOURCE OF *G. BONINENSE* ISOLATE

This study was conducted in Mycology Laboratory 2, Faculty of Agriculture, Universiti Putra Malaysia. A pure fungal culture of *G. boninense*, designated as strain PER71, was obtained from the microbial culture collection of the Department of Plant Protection, Faculty of Agriculture, UPM. The isolate was grown and maintained on potato dextrose agar (PDA) medium (Difco, USA) at 28 °C for seven days. Molecular identification using PCR was performed to ensure the correct identity of the isolate used for in vitro and in vivo experiments. The genomic DNA of the isolate was extracted using the DNeasy Plant Mini Kit (Qiagen, USA) following the manufacturer's instructions. The primer sets ITS5 (5' GGA AGT AAA AGT CGT AAC AAG G 3') and ITS4 (5' TCC TCC GCT TAT TGA TAT GC 3') for PCR amplification targeting the internal transcribed spacer (ITS) region (Yunita et al. 2016). The DNA sequencing was outsourced to Apical Scientific Sdn. Bhd. The retrieved sequence was compared to the GenBank database using the NCBI BLAST nucleotide program.

IN VITRO ANTIFUNGAL ACTIVITY OF SA, SIO_2, AND SI AGAINST *G. BONINENSE* USING POISON AGAR TECHNIQUE

Based on preliminary tests for all treatments, six different concentrations of salicylic acid (SA), silicon dioxide (SiO_2), and pure silicon (Si) were prepared following the guidelines of Bivi et al. (2012). These concentrations were then used to evaluate *in vitro* antifungal activity on the mycelial growth of *G. boninense*. Six concentrations of SA, SiO_2 and Si (0, 50, 100, 150, 200, and 250 mg/L) were individually added to sterilized melted PDA (potato dextrose agar). Petri dishes containing approximately 15 mL PDA media and 0.5 mL of each SA, SiO_2, and Si concentration were added to the PDA medium, agitated gently to become homogenized. Seven-day-old culture agar discs (6 mm) of *G. boninense* were placed at the center of each PDA media, and the plates

were incubated at 28 °C *until the control plates were fully colonized with G. boninense*. Plates treated with SA, SiO_2, and Si were labeled as treatment plates, while negative controls contained only PDA media. Mycelial growth on the culture plates was observed, and after seven days of inoculation, data were collected on the radial growth of *G. boninense*. Colony radial measurements from treatment plates and control plates were compared to assess the effect of treatments. This comparison was made using the formula provided by Royse and Ries (1978). The inhibition percentage of *G. boninense* growth was calculated using the following equation:

$$\text{PIRG\% (Inhibition percentage)} = (R1-R2)/R1*100 \quad (1)$$

where R1 is the average radial measurement in the control plates; and R2 is the average radial measurement in the treatment plates.

TREATMENTS AND EXPERIMENTAL DESIGN

This study was conducted at Experimental Field 15 of Universiti Putra Malaysia in a semi-open glasshouse area. A total of 60 three-month-old oil palm seedlings (including control) were tested with six different treatments. Treatments were applied to oil palm seedlings to evaluate the effect of Si application on reducing basal stem rot symptoms caused by *G. boninense* on oil palm seedlings. Untreated seedlings were used as controls. A randomised complete block design (RCBD) of 6 treatments with 5 oil palm seedlings for each treatment was used as the experimental design for the *in vivo* glasshouse experiment.

The 30 oil palm seedlings in the polythene bags were arranged in 5 rows in a randomized manner. Therefore, each row would be a block, and all treatments were randomly assigned to every block to eliminate the biassed environmental factors, particularly the light factor. Therefore, the current study managed to solely observed differences between treatments due to the application of silicon between treatments. Table 1 shows the treatment groups applied for the *in vivo* experiment. Healthy seedling groups that did not receive artificial inoculation of *G. boninense* and Si were also observed to ensure that the disease symptoms observed were caused by pathogen introduction to the seedlings. The seedlings were watered manually twice daily at 8:00 a.m. and 5:00 p.m. and received standard NPK (15:15:15) fertilizer every two weeks throughout the experiment.

PREPARATION OF *G. BONINENSE* CULTURE

The fungal culture of *G. boninense* was sub-cultured on potato dextrose agar (PDA) at 28 °C for eight days until the mycelium grew on the PDA plate. The hyphal tip transfer was used and was preserved at -80 °C for further analysis. The *G. boninense* culture was examined daily to observe the morphology and growth of the mycelia. These cultures were then used as inocula for oil palm seedling infection using the Ganoderma artificial inoculation with Dip, Place, and Drench (DPD) technique (Nusaibah, Saad & Tan 2017) two weeks before application of the treatments to the seedlings.

GANODERMA ARTIFICIAL INOCULATION WITH DIP, PLACE AND DRENCH (DPD) TECHNIQUE

This experiment was conducted according to the method described by Nusaibah, Saad and Tan (2017). In 250 mL of potato dextrose broth (PDB), *G. boninense* mycelia was grown without shaking for ten days before grinding using a kitchen electric grinder (MX-800S, Panasonic Malaysia). The 250 mL media containing the mycelia of *G. boninense* mentioned earlier was prepared for the artificial inoculation of one oil palm seedling. The inoculum suspension was then delivered to the nursery once it was ready for the inoculation process. The oil palm seedling was carefully uprooted after removing half of the soil from the polybag. After that, the uprooted roots were submerged or dipped into the freshly prepared suspension of *G. boninense* with approximately 250 mL of the inoculum per seedling Two plates of fully-grown *G. boninense* on PDA were added to the remaining soil in the polybag. The inoculum-dipped oil palm seedling roots were placed in the polybag on top of the G. boninense cultures. The residual suspension from the dip phase was then drenched onto the roots before being covered with the same soil mixture removed earlier.

TABLE 1. The six treatment groups were applied for the *in vivo* experiment

Treatments	Concentration of Si (mg/L)
T1	0 (Control)
T2	50
T3	100
T4	150
T5	200
T6	250

ASSESSMENT OF DISEASE SEVERITY

The severity of leaf chlorosis and necrosis development during the experiment was assessed monthly for five consecutive months. The symptoms observed include yellowing, desiccation, browning of the fronds, and the death of oil palms with or without *G. boninense* fruiting bodies. The disease severity for leaf symptoms was assessed as described by Sariah and Zakaria (2000) as follows:

$$\mathrm{DS(leaf)} = \frac{(a \times 1) + (b \times 0.5)}{c} \times 100 \qquad (2)$$

where a is the number of desiccated (browned/wilted) leaves; b is the number of yellowing leaves; c is the total number of leaves; 1 is the constant for desiccated leaves; and 0.5 is the constant for yellowing leaves.

After five months of the inoculation process with *G. boninense*, the oil palms were reaped, and the stem was cut open to observe the internal disease symptoms of the pathogen. The oil palm roots were also cut using a cutter, and root samples were collected. Then, the sampled roots were washed with tap water. The stem bulb tissue was noted, and the severity of the internal symptoms based on the proportion of root and bole tissue damage by *G. boninense* was assessed as described by Breton et al. (2006) (Figure 1 & Table 2). Disease Severity (DS) for internal symptoms of bole tissues was measured using the following formula, as described by Liu et al. (1995)*:*

$$\mathrm{DS(leaf)} = \frac{\text{Number of seedlings in the rating} \times \text{Rating number}}{\text{Total number of seedlings assessed} \times \text{Highest Rating}} \times 100 \qquad (3)$$

STATISTICAL ANALYSIS

The experiment was conducted using a completely randomized design (CRD) for *in vitro* experiment and randomized complete block design (RCBD) for *in vivo* greenhouse experiment. It was repeated twice, with three replicates each for *in vitro and five* replicates each for *in vivo*. The oil palm seedlings were arranged in rows in a randomized manner, and each row was used as a block to minimize environmental variability, such as temperature and sunlight, from affecting the data obtained. The data were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS) version 9.4 (SAS et al. USA). The means were compared using Duncan's Multiple Range Test (DMRT) at a significance level of 0.05 to indicate statistically significant differences.

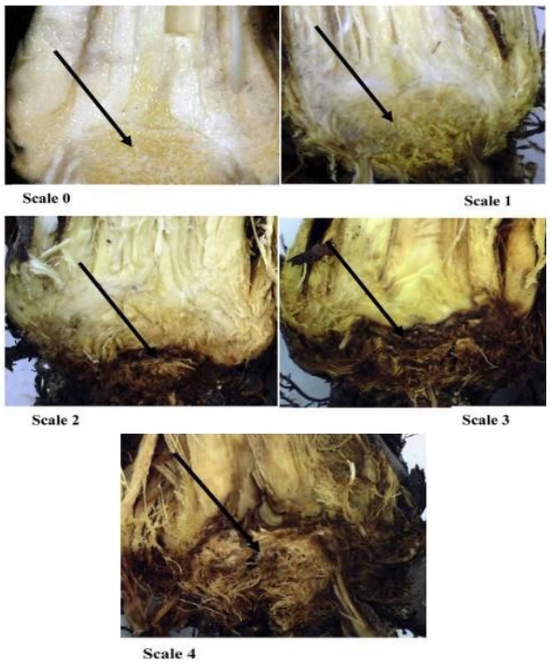

FIGURE 1. Disease rating to assess disease severity from 0 to 4 based on internal symptoms (arrows) in the oil palm bole tissues (Breton et al. 2006)

TABLE 2. The scale was used to score the disease severity index of internal rot on oil palm seedlings inoculated with *G. boninense*

Scale	Symptoms
0	Healthy, no internal rot
1	<20% rotting of root tissues
2	20-50% rotting of root tissues
3	>50% rotting of root tissues
4	>90% rotting of root tissues

RESULTS AND DISCUSSION

IN VITRO ANTIFUNGAL ACTIVITY OF SA, SIO_2, AND SI AGAINST *G. BONINENSE* USING POISON AGAR TECHNIQUE

The 600 bp 18S ITS rDNA gene sequence of PER71 *G. boninense* isolate was identified as *G. boninense* with 100% identity to *G. boninense* GenBank accession no. KM015454. According to the results, the inhibitory effect of SA, SiO_2, and Si increased with increased concentrations against the mycelial growth of *G. boninense*. The control plate was fully colonized seven days after inoculation, while slower growth of *G. boninense* was observed on Si-treated plates, followed by SiO_2 and SA. Results showed that inhibition percentages of SA at concentrations 0, 50, 100, 150, 200, and 250 mg/L were 0%, 29.2%, 30.3%, 33.5%, 3.5% and 41.7%, respectively. The percentage of *G. boninense* radial growth at different concentrations on treated and control plates is illustrated in Table 3.

A lower measurement of *G. boninense* radial growth and a higher percentage of mycelial growth inhibition were recorded as Si concentration increased. There was a significant ($p<0.05$) difference in the radial growth inhibition in the plates treated with SA, SiO_2, and Si. Pure Si inhibited the mycelial growth of *G. boninense* at Si concentrations of 200 and 250 mg/L, the PIRG was 100%, and there was no growth of *G. boninense* in the treated plates with concentrations of 200 mg/L of Si. The mycelial growth of *G. boninense* for different treatments and concentrations is shown in Figure 2.

Si effectively prevented the mycelial growth on PDA and showed promising antifungal activity against *G. boninense*. The antifungal activities were assessed based on the growth inhibition rate and the EC_{50} value, where the higher the rate of mycelial growth inhibition, the higher the antifungal activity against *G. boninense*. This was supported by the results of the *in vitro* screening, where the EC_{50} value of Si that can inhibit mycelial growth was only 68.57 mg/L, while the EC_{50} values for SiO_2 and SA were 273.95 mg/L and 381.33 mg/L, respectively.

In the observation, there were significant differences in almost all concentration levels for both Si and SiO_2 except for the PIRG at 200 and 250 mg/L, which showed no significant differences. Comparing this data to the PIRG of SA, which showed no significant difference between 50 and 100 mg/L, between 100 and 150 mg/L, and between 200 and 250 mg/L, it is proven that both Si and SiO_2 treatments have the potential to inhibit the growth of *G. boninense.* Si treatment had the highest antifungal efficacy in inhibiting the mycelial growth of *G. boninense.*

Si efficiently suppressed the mycelial growth of *G. boninense* on PDA, and the suppression was concentration-dependent. The results support the reports that claimed Si had the strongest *in vitro* inhibitory effect on other fungal pathogens, for example, *Sclerotinia sclerotiorum* and *Phytophthora cinnamomi* (Carneiro-Carvalho et al. 2017; Elsherbiny & Taher 2018). Kaiser et al. (2005) also reported that Si inhibited the *in vitro* mycelial growth of phytopathogenic fungi such as *Fusarium oxysporum, Fusarium solani*, and *Colletotrichum coccodes*. Nasser and Bhai (2021) reported a study on the Si antifungal effect on *Macrophomina phaseolina,* which caused dry rot on ginger, and it was reported that Si successfully restricted the growth of the pathogen *in vitro* as well as reducing the disease incidence *in vivo*.

In terms of *in vitro* dosage responses against many other fungal pathogens, Bekker, Kaiser and Labuschagne (2009) reported that Si may operate as the structural protective barrier in Si-treated plants and may prevent colonization and subsequent infection by reducing the growth of the fungi on the plant surface, according to the observation that Si has an immediate inhibiting impact on fungal mycelial growth. The *in vitro* screening results thus prove that pure Si works most effectively against *G. boninense.*

GANODERMA ARTIFICIAL INOCULATION WITH DIP, PLACE AND DRENCH (DPD) TECHNIQUE

The experimental design used was a randomized complete block design (RCBD) to minimize environmental errors, such as the inadequate amount of sunlight received by each seedling, from affecting data analysis. The *in vivo* experiment was conducted using the Dip, Place, and Drench technique proposed by Nusaibah, Saad and Tan (2017). It was repeated twice with five replications each in the glasshouse. The disease severity for leaf symptoms observed includes yellowing, desiccation, and browning

of the fronds (Rakib et al. 2015). The data collected was calculated using the formula described by Sariah and Zakaria (2000), and Table 4 shows the results after five months of treatments on the oil palm seedlings. The monthly progress of disease severity for five consecutive months is further illustrated in Figure 3.

ASSESSMENT OF DISEASE SEVERITY

Based on the disease severity of leaf symptoms described in Table 4, the results showed that the disease severity of leaf symptoms reduced significantly after the seedlings were treated with the Si treatments. The seedlings infected with *G. boninense* that did not receive any Si treatments, T1 (UTC), exhibited a faster progress of leaf symptoms as compared to Si-treated seedlings throughout five months of *G. boninense* inoculation, as the group showed no external symptoms of BSR only for the first month. The seedlings later showed leaf symptoms as early as the second month after inoculation. Treatment with Si delayed the initiation of leaf symptoms and suppressed disease progression during the first and second months of inoculation. Not only that, the leaf symptoms for the T6 group of seedlings that were treated with 250 mg/L of Si treatments were only visible during the fourth and fifth months after inoculation, in contrast with the control group, T1, which was inoculated with *G. boninense* but did not receive Si treatments and showed relatively severe degree of disease severity of *G. boninense* infection (2.5%) at two months after planting. Five months after inoculation, further penetration and colonization of fungal hyphae caused damage and physiological stress to the root cells. Overall, disease development occurred slowly in the seedlings treated with Si.

TABLE 3. Mean percentage inhibition of radial growth (PIRG) values by poison agar method against mycelial growth of *G. boninense* supplemented with six different concentrations of salicylic acid (SA), silicon (Si), and silicon dioxide (SiO_2)

Concentration (mg/L)	PIRG (%)		
	Salicylic acid, SA	Silicon hydroxide, SiO_2	Silicon, Si
0	0 (±0)	0 (±0)	0 (±0)
50	29.2 (±1.66)^{a}	23.5 (±1.45)^{a}	34.8 (±1.33)^{a}
100	30.3 (±1.99)^{a,b}	29.7 (±0.95)^{b}	66.2 (±1.62)^{b}
150	33.5 (± 1.10)^{b}	37.2 (±1.71)^{c}	82.7 (±1.53)^{c}
200	38.5 (± 1.33)^{c}	43.5 (±0.6)^{d}	100.0 (±0)^{d}
250	41.7 (± 1.29)^{c}	45.2 (±1.85)^{d}	100.0 (±0)^{d}

Data are the average ± SD for each treatment group. Different letters represent significant differences between treatments, at 5%, when using DMRT

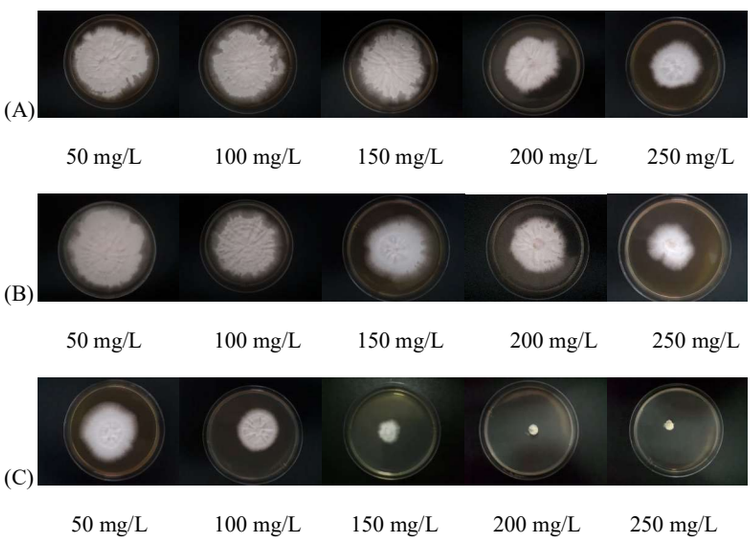

FIGURE 2. Mycelial growth of *G. boninense* supplemented with five different concentrations of (A) Salicylic acid (SA), (B) Silicon dioxide (SiO_2), and (C) Pure silicon (Si) after 7 days of incubation at 28 °*C*

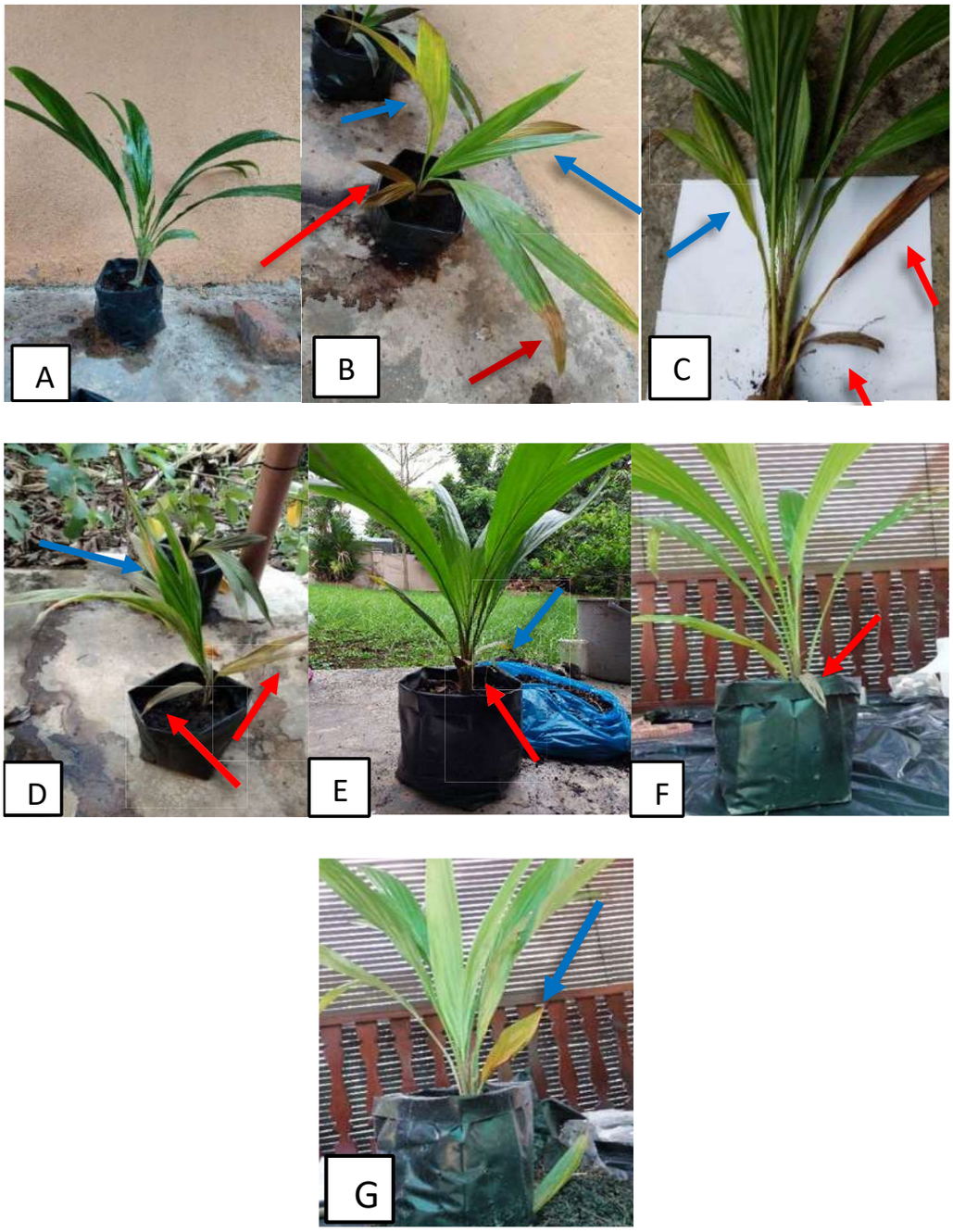

FIGURE 3. Disease severity of oil palm seedlings observed after five months of *G. boninense* inoculation. (A) a healthy seedling with greener leaves and showed no symptoms of yellowing of the leaf or leaf necrosis at all, (B) Seedlings treated with 0 mg/L of Si (UTC) showed many chlorotic and necrotic leaves, (C) Seedlings treated with 50 mg/L of Si with multiple dead leaves, (D) Seedlings treated with 100 mg/L of Si showed fewer dead leaves and some yellowing leaves, (E) Seedlings treated with 150 mg/L of Si showed healthier leaves and much less yellowing on leaves, (F) Seedlings treated with 200 mg/L of Si had healthier leaves and only one visibly necrotic leaf, (G) Seedlings treated with 250 mg/L showed very few chlorotic leaves. Blue arrows indicate the chlorotic leaves, while red arrows indicate the necrotic leaves observed

The severity of the leaf symptoms in inoculated seedlings varied significantly ($p<0.05$) according to the different treatments after five months of Si application. The severity of the control group that received no Si treatment, T1, was 24.28%, with many necrotic leaves observed and multiple yellowing and browning leaves by the end of five months of inoculation. Meanwhile, the leaf severity on the seedlings for T2 was 13.89%, suggesting good disease suppression at a concentration of 50 mg/L. This was further supported by the fewer yellowing leaves observed on the seedlings compared to the observations made on seedlings in T1. The disease severity for seedlings that received 200 mg/L and 250 mg/L of Si, T5, and T6 was significantly reduced compared to the control group, T1. Meanwhile, it is worth noting that in Figure 3, the leaves of the healthy seedling group that received no *G. boninense* and no Si treatment appeared to be greener and showed no symptoms of yellowing of the leaf or leaf necrosis at all. This thus confirms that the infected seedlings suffer from the disease caused by *G. boninense* infection.

In contrast, the Si treatment at the concentration of 150 mg/L significantly reduced the severity of leaf symptoms compared to the untreated seedlings and the seedlings that received 50 and 100 mg/L, respectively. During the final month of the experiment, there was a significant difference in leaf symptoms between seedlings that received no Si (T1), 50 mg/L of Si (T2), and 250 mg/L Si (T6). However, there were no apparent significant differences between the treatment groups T2, which received 50 mg/L Si; T3, which received 100 mg/L Si; and T5 and T6, which received 200 and 250 mg/L, respectively. The data and observation suggested that the T4, T5, and T6 seedlings appeared healthier, with slightly greener leaves than the rest of the treatment groups (Figure 3). The results also showed that the seedlings' disease severity of leaf symptoms was 4.05%. No visible necrotic (dead) leaves were observed on the seedlings treated with Si at 250 mg/l in T6, thus proving that Si helped reduce the disease severity significantly compared to untreated seedlings in T1 (Figure 3).

In addition, the results shown in Table 4 also showed a decreasing trend in disease severity as the concentration of Si treatment increased. This result aligns with the trend shown in previous reports by Najihah et al. (2015), where more excellent disease suppression was achieved by applying a treatment with a higher Si content. In the report, Najihah et al. (2015) also stated that fewer disease incidences indicated that the disease was being suppressed by the Si-treated plants, which agreed with the results obtained in this study. Plants with higher Si content in parts of the shoot or root that are usually very prone to being attacked by pests exhibit increased stress resistance, as reported by Song et al. (2021), and this explains the cause of the disease suppression by Si-treated seedlings in this study.

It was previously reported by Najihah et al. (2015) and Rakib et al. (2015) that the symptoms of BSR infection on oil palm seedlings after months of inoculation with *G. boninense* include gradual yellowing of the lower and older leaves, followed by desiccation of the yellowing leaves, and it would move progressively to the youngest leaves. In this study, the same observation was obtained, with the first symptoms appearing on the lower and older leaves, followed by the upper and younger ones. In conclusion, from this set of data, we can conclude that among the concentrations of Si treatment used in this experiment, the concentration of 200 mg/L can significantly reduce the disease severity of leaf symptoms in comparison to untreated seedlings infected with *G. boninense* due to the lowest disease severity observed for the seedlings in that treatment group.

Aside from observing the leaf symptoms on infected seedlings, the internal rotting of bole tissues and the root system of the oil palm seedlings were also observed. The disease severity for internal rot of internal tissues was calculated using the formula described by Liu et al. (1995) and compared to the scale proposed by Breton et al. (2006) (Figure 1). Figure 4 shows the mean percentage of internal disease severity for infected oil palm seedlings after five months of inoculation.

Based on Figure 4, the internal disease severity of oil palm seedlings five months after inoculation was found to decrease as the concentration of the Si treatment increased. Upon the introduction of 50 mg/L of Si to the inoculated seedlings, it was observed that the internal damage caused by *G. boninense* was reduced from 35% to 22% of disease severity, thus supporting that Si treatment helped in suppressing and reducing the disease severity caused by *G. boninense* infection. This decreasing trend applied to all Si-treated seedling groups as the concentration of Si introduced to the infected seedlings increased. The seedlings treated with 200 and 250 mg/L of Si showed a significant reduction from 35% to only 15% and 9%, respectively, thus, proving the correlation between the increased concentration of Si treatment and the reduction of disease severity. The visible rotting of bole and primary root tissues is further shown in Figure 5.

In Figure 5, the symptoms of internal damage caused by *G. boninense* inoculation on the bole tissues and oil palm root system of all infected seedlings were further discussed. Any Si treatments at all did not treat the infected seedlings; T1 showed a most severe disease severity rating of scale 2 out of all infected seedling groups, where 20-50% of its bole tissue and root tissues were rotting after five months of *G. boninense* inoculation (A). The T1 treatment was considered the untreated control group (UTC) in this study. From the observation, there was an apparent rotting of the bole tissues at the basal stem of the seedlings, and the primary roots of the seedlings were also rotting and

TABLE 4. Effect of Si treatment on the severity of leaf symptoms of oil palm seedlings within five months of inoculation

Treatment	Concentration of Si (mg/L)	Disease severity of leaf symptoms at month (%)				
		1	2	3	4	5
T1	0 (Control)	0.00 a*	2.50 a	6.83 a	15.86 a	24.28 a
T2	50	0.00 a	0.00 b	3.82 b	9.78 b	13.89 b
T3	100	0.00 a	0.00 b	1.82 bc	7.08 bc	11.52 b
T4	150	0.00 a	0.00 b	2.02 bc	5.22 cd	7.36 c
T5	200	0.00 a	0.00 b	1.91 bc	3.44 d	6.49 cd
T6	250	0.00 a	0.00 b	0.00 c	1.60 d	4.05 d

*Means followed by the same letter in the same column are not significantly different at 5% using DMRT

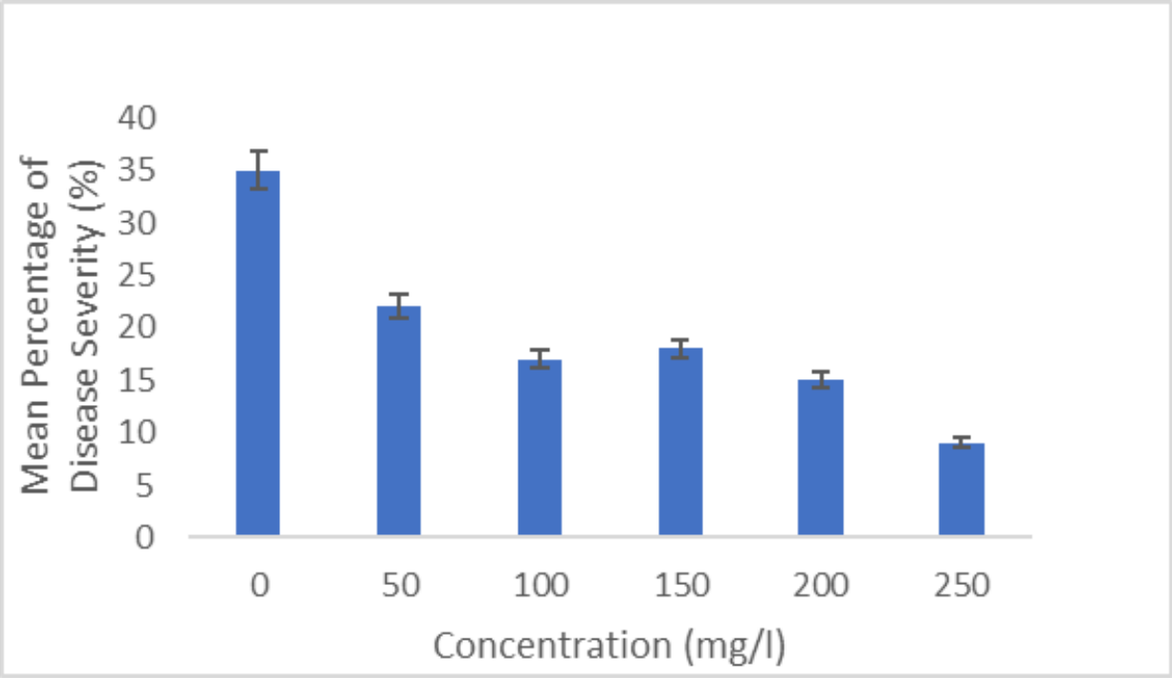

FIGURE 4. Mean percentage disease severity of internal symptoms for the oil palm seedlings 5 months after inoculation. The error bars represent the standard deviation (SD) of the sample

turning into necrotic lesions. The same symptoms of bole tissue damage were observed in the T2 group treatment (B), where the seedlings were only treated with 50 mg/L of Si treatment. This is in contrast to the observation made on the T3, T4, T5, and T6 group of seedlings (C, D, E, and F, respectively), where the bole tissues appeared to be slightly healthier than the seedlings treated with a lower concentration of Si treatment. As observed, the disease severity of the internal symptoms was reduced, and the primary roots appeared healthier as the concentration of Si treatment increased. This trend was consistent with the results in *in vitro evaluation*, where the higher the Si treatments inhibited the growth of *G. boninense better compared to the untreated control,* thus proving that the higher the concentration of Si introduced to the seedlings inoculated with *G. boninense*, the lower the disease severity rating of bole tissue damage in the inoculated oil palm seedlings. In addition to that, it was also observed that the bole tissues of the seedling group that received no *G. boninense* and no Si treatment (G) appeared to be healthy and showed no symptoms of rotting at all, confirming that the infected seedlings are suffering from the disease caused by *G. boninense* infection.

Successful penetration and colonization of pathogens require the pathogens to enter the host plant by penetrating through physical barriers, including wax, cuticles, and

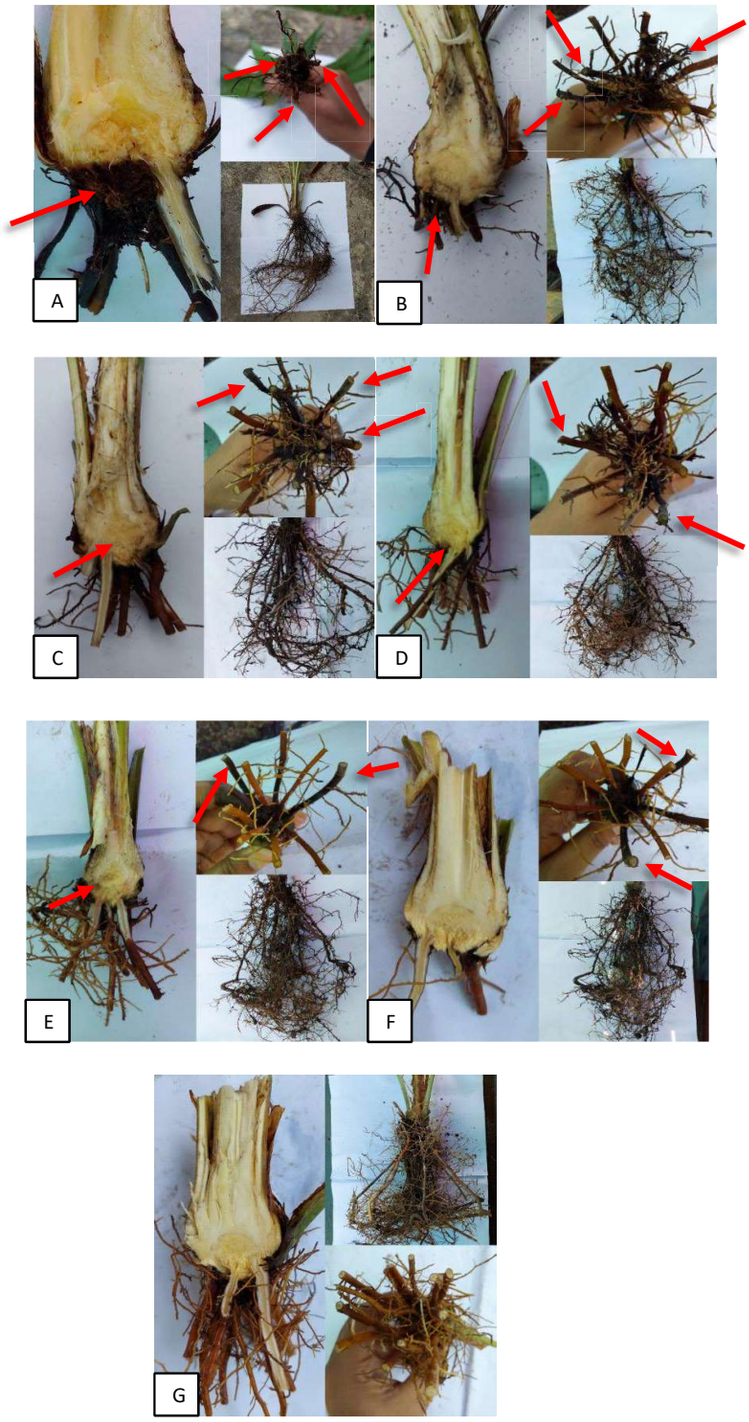

FIGURE 5. Symptoms of *G. boninense* infection on oil palm seedlings. Disease severity of internal bole tissue for different treatments applied to the oil palm seedlings after 5 months of inoculation in the glasshouse. (A) Inoculated oil palm seedling received 0 mg/L of Si treatment with rotting bole tissue and primary roots (arrow). (B), (C), (D), (E), and (F): Inoculated oil palm seedlings that received 50 mg/L, 100 mg/L, 150 mg/L, 200 mg/L, and 250 mg/L of Si treatments, respectively, for 5 consecutive months. (G) Healthy oil palm seedling that did not received *G. boninense* inoculation and Si treatment. Arrows indicate the internal symptoms of rotting, which include rotting of either bole tissues, primary root tissues, or both

cell walls (*Łaźniewska*, Macioszek & Kononowicz 2012). Previous studies have proposed that Si from the soil solution has been translocated into plants as mono-silicic acid and soon polymerized to form phytoliths inside the plant cells. The accumulation of these phytoliths strengthens the plant defense mechanism and physical toughness and acts as a barrier to the penetration of fungi (Song et al. *2021).* This was further proven by a study by Sousa et al. (2013). Si administration limited hyphal penetration to the first-invaded epidermal cell for infected wheat leaves with *Pyricularia oryzae*; at the same time, hyphae successfully invaded numerous neighboring leaf cells without Si treatment. A similar result was observed in rice infected with *Pyricularia grisea and Rhizoctonia solani.* Fewer lesions were on the leaf blade as the incubation period of Si deposition on leaf tissues increased (Rodrigues et al. 2001). Henriet et al. (2008) demonstrated a direct correlation between the concentration of silicon in several shoot organs of banana plants and their transpiration rate. Euliss et al. (2005) showed a clear correlation between the transpiration rate and the Si concentration of several grasses and wetland species, while Cornélis et al. (2011) attributed the higher Si uptake by Douglas fir leaves compared to Black pine leaves to a higher transpiration rate.

In addition to that, according to Shetty et al. (2012), Si application increased the number of papillae in leaf cells in response to Podosphaera pannosa infection in rose plants. The papillae formation was stimulated by Si intake during pathogen infection, and it was reported to happen in the haustorial neck and collar region of the fungus, which helped to keep pathogens from invading the plants (Wang et al. 2017). Cai et al. (2008) also stated that papillae's prevalence after Si treatment could increase rice resistance to blast and wheat and barley resistance to powdery mildew, as Bélanger, Benhamou and Menzies (2003) reported. This explained the role of Si in enhancing the plant cells' physical barriers against the pathogen. In this study, the oil palm root's cell walls thus inhibited fungal pathogen invasion.

Aside from enhancing the cell wall of oil palm roots, Si might also have aided in the stress-related signaling systems of the oil palm seedlings that further improve the plants' response against the infection of the pathogen (Wang et al. 2017). Si helped in inducing phenolic compounds, phytoalexin, glucanase, and peroxidase production in plants, as well as limiting the expression of genes related to pathogenicity or stress to prevent the invasion and colonization of pathogens (Sakr 2016; Wang et al. 2017). A study by Liang et al. (2005) also reported that Si induced systemic resistance in cucumber roots that inhibited the spread of powdery mildew. Other than that, it was previously mentioned by Waewthongrak, Pisuchpen and Leelasuphakul (2015) that Si plays a role in disease resistance by activating defense-related enzyme activities such as chitinase, peroxidases, polyphenol oxidases, β-1,3-glucanase, phenylalanine ammonia-lyase (PAL), superoxide dismutase, ascorbate peroxidase, glutathione reductase, catalase, lipoxygenase, and glucanase. PAL, which is involved in synthesizing plant secondary antimicrobial substances, is essential for plant disease resistance responses. These studies explained that Si enhanced the plant resistance against pathogen attacks in mechanical and biochemical ways, aiding the oil palm resistance and reducing the severity of the internal rot caused by *G. boninense infection.*

CONCLUSIONS

In vitro experiments showed that a pure Si treatment at 200 mg/L significantly reduced mycelial radial growth of *G. boninense*, with the highest potential of controlling the causal agent of basal stem rot, compared to SiO_2 and SA treatments. It may be a candidate for managing basal stem rot on oil palm. Based on the results obtained, Si also showed the highest potential in controlling the causal agent of basal stem rot, *G. boninense*, in an *in vivo* trial due to its ability to reduce disease severity in leaf as well as the internal symptoms of BSR in comparison to the untreated infected seedlings. Si concentrations of 200 mg/L and 250 mg/L were found to be best at suppressing both leaf and internal symptoms of basal stem rot (BSR) disease on oil palm seedlings tested in this study. These findings suggest that Si could add to the current control measures practiced by the industry, especially if it is introduced during the early stages of oil palm planting to utilize Si's potential in enhancing the plant's defence against BSR attack.

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*Corresponding author; email: izera@upm.edu.my