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The potential of soluble silicon for managing white root disease in rubber (*Hevea brasiliensis*)

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Abstract

Rubber growers in Malaysia depend on soil drenching with propiconazole fungicide to control white root disease (WRD) caused by *Rigidoporus microporus*. The fungal infection affected the environmental ecosystem, giving rise to fungicide resistance. Recently, silicon (Si) has become an alternative to reduce and delay pathogenic fungal invasion. Therefore, the present study investigates the antifungal property of soluble silicon against *R. microporus* in rubber trees (*Hevea brasiliensis*). *In vitro* dose-response towards soluble silicon types, i.e., silicic acid, sodium meta-silicate, sodium silicate, and calcium silicate with different concentrations (10, 100, 500, 1000, 1500, 3000, 5000, and 8000 ppm) were determined on the Ayer Molek strain of *R. microporus* using the Poisoned Food Technique. Results showed that sodium meta-silicate inhibited mycelial growth (100%) at 5000 and 8000 ppm concentrations compared to other types of soluble silicon. However, silicic acid inhibited more than 50% *R. microporus* at a minimal concentration of 500 ppm, which could be considered the most effective antifungal from the soluble silicon group. Moreover, the higher pH values did not solely affect the inhibition rate of *R. microporus*. Microscopic observation showed the changes of *R. microporus* hyphae width grown on soluble silicon medium agar compared to the control (without Si). The Dipped Stick Inhibition Assay revealed that a higher concentration and more frequent soluble silicon application effectively inhibited *R. microporus* growth. Thus, this study proved that soluble silicon, especially silicic acid and sodium meta-silicate, showed promising results as antifungal agents and fungicidal in controlling white root disease.

Keywords: *Hevea brasiliensis*, white root disease, propiconazole, soluble silicon, inhibition.
Abbreviations: DMRT_ Duncan's multiple range test, PDA_Potato dextrose agar, PIRG_Percentage inhibition of radial growth, ppm Part per million, SPSS_Statistical package for the social sciences.

Introduction

Malaysia is categorised as one of the important natural rubber producing countries in the world. A considerable effort has been made to increase the production volume of natural rubber by expanding rubber planting areas, inventing new high-yielding rubber clones, developing technologies, and creating value-added materials from natural rubber such as green technology tyres, seismic bearing, and many else. However, the infection of *Rigidoporus microporus* that causes the white root disease (WRD) of rubber has become a problem, particularly for rubber smallholders. The incidence of WRD, one of the major diseases of *Hevea brasiliensis*, has negatively impacted the rubber industries. For instance, it caused significant reductions in economic returns due to the disease since the infection kills rubber trees irrespective of age (Chaiharn et al., 2019). Fatin Farhana et al. (2017) proved that WRD could infect *H. brasiliensis*, regardless of clone and age, and it occurs in any planting areas such as young rubber plantations, virgin jungles, and replanted areas. In 2012, a survey revealed the incidence of WRD in 10%–15% of rubber plantations in Peninsular Malaysia, 20%–30% in Sabah, and 9%–20% in Sarawak, covering 1,065,630 hectares of total area (Atan, 2015). It has been determined that the incidence of WRD is more severe than the red and brown root disease of rubber, particularly in Malaysia (Hashim, 2014). The occurrence of WRD was found more commonly from the first to the fourth year after planting. Sulphur was used previously as one of the control methods for the disease, and in the 1990s, the most common fungicides used to control the disease were bayfidan and bayleton (Chan et al., 1991; Gohet et al., 1991; Satchuthananthavale and Halangoda, 1971).

Soil drenching with fungicides, e.g., propiconazole, was introduced to overcome fungal invasion, and until now, it is the most effective method to reduce the incidence of WRD (Hashim and Chew, 1997; Lam and Chiu, 1993; Shabbir et al., 2021). It has been applied intensively throughout rubber plantations as a quick, simple, and easy treatment. However,

these fungicides resulted in several issues affecting environmental ecosystems, such as the effect on soil microbial diversity (Brent and Hollomon, 2007; Go et al., 2013). The adverse impacts of continuous fungicide application on the environment rendered the requirement for alternative methods that are more environmentally friendly in controlling WRD caused by *R. microporus.* Silicon (Si) application has been identified as an essential element that offers a greener technology approach in managing the issues caused by biotic and abiotic factors. Several *in vitro* studies had been carried out to observe the direct effect of soluble silicon against several plant pathogens. A study on silicon and antifungal of other fungal pathogens such as *Rhizoctonia solani* revealed that silicon completely inhibited the pathogen at 30, 200, and 500 ppm (Fayadh and Aledani, 2011). The potential of soluble silicon as an alternative fungicide was also observed with the effective *in vitro* potassium silicate application to control *Pythium cinnamomi* that caused the disease of chestnut plants. Inhibition rates of 50%, 80%, and 90% were noted in the 5, 7.5, and 10 mM SiK® treatments, respectively (Carneiro-Carvalho et al., 2017). In addition, Shabbir et al. (2020b) claimed the effectiveness of silicate-solubilising bacteria UPMSSB7 as a potential biocontrol agent against white root rot disease pathogen of the rubber tree, where it inhibited 57.24% of *R. microporus* radial growth *in vitro*.

Interestingly, a single application of insoluble calcium silicate as a silicon source in the field was not effective to control the incidence of WRD in rubber seedlings compared to co-inoculation of silicate-solubilising bacterial strain *Enterobacter sp*. UPMSSB7 and AMF (*Glomus mosseae*) in the presence of silicon that could effectively suppress white root rot disease and enhance the plant growth of rubber seedlings (Shabbir et al., 2020a). Recently, Ahammed and Yang (2021) revealed that silicon treatments exhibited a significant impact in controlling plant diseases. However, there was no specific study on the effects of different types of soluble silicon on the growth of *R. microporus in vitro*. Therefore, this study had been designed to examine the ability of a single application or direct effect of different types of soluble silicon known as silicic acid, sodium silicate, sodium meta-silicate, and calcium silicate in suppressing WRD of rubber before applying it to the field in planta experiment.

Results

Evaluation of the effect of soluble silicon on the radial growth of R. microporus

The effect of different concentrations of various types of soluble silicon on the growth of *R. microporus* is shown in Table 3. The maximum inhibition capacity (100%) is recorded in the potato dextrose agar (PDA) Petri plates enriched with sodium meta-silicate at the concentrations of 5000 and 8000 ppm, while the negative control (PDA) did not present any inhibition (percentage inhibition of radial growth, PIRG). A maximum capacity PIRG (100%) was recorded on the positive control PDA Petri plates enriched with propiconazole, where the 100% inhibition started to occur at the concentration of 100 ppm. Interestingly, although not giving the maximum inhibition capacity (100%), silicic acid is observed as the most potent soluble silicon. It inhibited 36.08% of *R. microporus* mycelial growth even at the low concentration of 100 ppm. Silicic acid showed a consistent inhibition trend starting from 500 ppm to 5000 ppm, with the PIRG recorded around 68.63% to 75.00%, and reached the highest PIRG of 80.39% at the concentration of 8000 ppm.

On the contrary, sodium silicate only started to inhibit 24.71% of *R. microporus* growth at the concentration of 5000 ppm and further showed highly significant inhibition of 86.18% at 8000 ppm. Among the soluble silicon tested in this study, calcium silicate is the least effective in inhibiting the growth of *R. microporus*, where no inhibition of *R. microporus* is recorded at any of the concentrations (ppm) tested. Fig. 1 indicates that silicic acid achieved the highest total means of PIRG against *R. microporus* (52.49%), followed by sodium meta-silicate (43.55%), sodium silicate (12.32%), and calcium silicate (0.00%). The observation of culture and hyphae of *R. microporus* under an Olympus CX41 light compound microscope had confirmed morphological changes of the hyphae after exposure to the different concentrations of silicic acid and sodium meta-silicate (Fig. 2). The hyphae of *R. microporus* showed a greater width (µm) in the PDA plate enriched with silicic acid, whereas thinner hyphae are observed in the enriched PDA with sodium meta-silicate. The observation is compared to the control plates that showed longer, even, and tubular hyphae with a smooth surface. The differences in hyphae width (µm) between silicic acid and sodium meta-silicate are highly significant at the concentration of 5000 ppm compared to the control, with the width measurement of 30.30, 2.73, and 7.96 µm, respectively (Fig. 3).

The effect of pH values on the growth of R. microporus

The effect of pH values on the growth of different *R. microporus* strains was evaluated by comparing the changed pH values after the incorporation of propiconazole and soluble silicon into PDA (Table 4) with the amended PDA adjusted to get the pH values of 2, 4, 8, and 10 using 1.0 M hydrochloric acid (HCl) and 1.0 M sodium hydroxide (NaOH, Table 5). Table 3 shows fluctuated but not dose-dependent pH values in the PDA plates incorporated with propiconazole. In comparison, silicic acid shows significant pH value differences compared to the control, albeit insignificant among the concentrations tested. Both propiconazole and silicic acid changed the pH values of the amended PDA to slightly acidic between 5.35 to 5.91 across the concentrations tested. On the contrary, the incorporation of sodium meta-silicate, sodium silicate, and calcium silicate showed a significant inclining trend of pH values towards alkaline following the increase of concentrations. The highest pH value recorded is 10.34 on sodium meta-silicate at the concentration of 8000 ppm. Table 5 shows that all *R. microporus* strains are entirely inhibited at pH 2, whereas various PIRG of *R. microporus* strains are recorded at pH 4. It is also noted that the higher pH values promoted *R. microporus* growth, where no PIRG is recorded on all *R. microporus* strains at pH 8 and pH 10.

The effect of different soaking times of rubber sticks in soluble silicon

Fig. 4 shows that no inhibition of *R. microporus* growth is found in all the control treatments. However, significant differences are observed in the inhibition percentage (PIRG) of *R. microporus* growth for both silicic acid and sodium meta-silicate treatments tested. The more significant inhibition of fungal growth is observed at a higher concentration for all of the treatments. After 24 hours of soaking in silicic acid at the concentration of 500 and 5000 ppm, the growth of *R. microporus* on rubber sticks are inhibited at 15.00% and 38.33%, respectively. In addition,

the growth of *R. microporus* is inhibited at 30.00%, 52.50%, 30.83%, and 55.83% after 48 and 78 hours of soaking in silicic acid. The inhibition percentage of *R. microporus* growth did not significantly differ for both silicic acid concentrations used at 48 and 72 hours but showed significant differences in the 24 hours soaking time. The results revealed that the optimal soaking time of rubber sticks in silicic acid is 48 hours, which effectively reduced the growth of *R. microporus*. On the contrary, sodium meta-silicate is less effective in suppressing the growth of *R. microporus* on rubberwood sticks compared to silicic acid. The results showed no significant differences observed in all treatments tested for sodium meta-silicate. The inhibition percentages of *R. microporus* growth are 23.33% and 41.67% after 24 hours, 18.33% and 33.33% after 48 hours, and 21.67% and 33.33 after 72 hours soaking in both concentrations of sodium meta-silicate at 500 and 5000 ppm. Generally, it is observed that higher concentrations of silicic acid and sodium meta-silicate are more effective to inhibit fungal growth.

Discussion

The Poisoned Food Technique showed that the PIRG of *R. microporus* on commercial fungicide, propiconazole, and different types of soluble silicon varied depending on the concentrations (ppm) applied. In general, propiconazole exhibited the most significant inhibition rate of *R. microporus* at 100%, even at a low concentration rate of 100 ppm (Table 3). Rubber growers have realised the effectiveness of propiconazole in controlling *R. microporus* infection on rubber trees. However, the higher price of chemical fungicides renders the low adoption level among rubber growers, negatively impacting the users and environment (Jayasuriya and Thennakoon, 2007; Komárek et al., 2010). Besides propiconazole, sodium meta-silicate could inhibit mycelial growth up to 100% at higher concentrations (5000 and 8000 ppm). On the contrary, silicic acid had only inhibited 75.0% and 80.4% of mycelial growth at similar concentrations (5000 and 8000 ppm). Meanwhile, sodium silicate showed a slightly lower inhibition rate of 24.71% at 5000 ppm and a higher inhibition percentage of 86.18% at 8000 ppm than silicic acid.

Li et al. (2009) demonstrated the dose-dependent effect of sodium silicate against *Fusarium sulphureum*, where the incorporation of 100 and 200 mM sodium silicate into PDA effectively inhibited fungal growth at 52% and 90%, respectively, after incubation for seven days at 25°C. Meanwhile, Abd-El-Kareem et al. (2019) revealed the complete inhibition of strawberry black root rot fungi linear growth at 4 and 6 g/L of sodium silicate for *R. solani* and *Fusarium solani*. On the other hand, calcium silicate has been classified as the least effective type of soluble silicon to inhibit *R. microporus*. It did not show any significant inhibition in all concentrations (ppm) used in this study. The results contradicted Biggs et al. (1997), who found the efficacy of calcium silicate incorporated into PDA that reduced the growth of *Monilinia fructicola* (G. Wint.) Honey that caused brown rot of peach fruit compared to control. However, Oliveira et al. (2012) reported a contradictory observation in their *in vitro* experiment where no inhibition of *Xanthomonas citri* subsp. Malvacearum growth was recorded with the application of calcium silicate at any of the tested doses. This finding was consistent with Shabbir et al. (2020a), who suggested that the single application of calcium silicate did not effectively suppress the growth of *R. microporus* on rubber seedlings.

Interestingly, silicic acid was effective on the Ayer Molek (AM) strain of *R. microporus* even at the minimal concentration (ppm) applied. The AM strain of *R. microporus* had been classified as the most virulent strain due to its aggressiveness, where it stimulates the production of different protein expressions in AM infected samples compared to the other strains (Siddiqui et al., 2017). Silicic acid inhibited more than 50% of mycelial growth at the lowest concentration of 500 ppm, and it showed significant differences compared to other soluble silicon at the concentration tested (Table 3; Fig. 1). The observations are also in line with the results by da Silva et al. (2020), who revealed the efficacy of orthosilicic acid that can completely inhibit (100%) the growth of *Cercospora arachidicola* at the concentration of 1000 ppm. The effectiveness of soluble silicon depends on the difference in silicon formulation, concentrations applied, and the inhibition percentage of the fungal species.

The incorporation of propiconazole and soluble silicon into PDA caused changes to the pH values of the substrate (Table 1). Generally, the pH values increased concurrently with the increased concentration (ppm) of sodium meta-silicate, sodium silicate, and calcium silicate, except for propiconazole and silicic acid. In a separate test, enhanced mycelial growth of *R. microporus* was observed with the increase of pH values, whereas it was fully inhibited at the extreme lower pH (Table 5). In a similar study, Kaiser et al. (2005) found that the incorporation of PDA with a minimal concentration of potassium silicate at 5000 ppm (pH 10.3) inhibited (100%) the growth of *Phytophthora cinnamomi* entirely. However, in the absence of potassium silicate with the same pH value of PDA amended with potassium hydroxide (pH 10.3), the inhibition of *P. cinnamomi* was recorded at 12.6%. The research contradicted Bekker et al. (2009), who claimed that the increase of pH values after incorporating potassium silicate could not be concluded as the main effect of fungal growth inhibition. Instead, they found that the effect of pH on mycelial growth was inconsistent because, in certain conditions, it was observed that at higher pH values, the absence of a soluble silicon medium had also promoted fungal growth. Thus, it can be concluded that soluble silicon could have a direct inhibitory effect on mycelial growth, and it is not primarily the result of soluble silicon changing the pH of the medium.

Further observation was carried out to see the microscopic structure of *R. microporus* hyphae under the light microscope. A significant difference in hyphal changes between *R. microporus* treated with silicic acid and sodium meta-silicate is observed under 500 and 5000 ppm concentrations at 20× magnification (Fig. 2; Fig. 3). According to Kaewchai et al. (2009), the average width of *R. microporous* hyphae was between 2.8 µm to 7.2 µm, while Fatin Farhana et al. (2017) mentioned that the width of the hyphae ranged from 3.0 µm to 5.7 µm. These results showed that the hyphal morphology of *R. microporus* was affected by the incorporation of soluble silicon in PDA. A study on the effects of silicon on cell disruption and deformation of the hyphae demonstrated that the newly developed hyphae had rough surfaces with a thin and distorted appearance (Rachniyom and Jaenaksorn, 2008). Moreover, soluble silicon caused a reduction in the cell turgor pressure of *Aspergillus alternata*, *Fusarium semitectum*, and *Trichoderma roseum*, which resulted in the collapse and shrunken hyphae and spores (Yang et al., 2006). Similarly, da

Table 1. List of *R. microporus* strains used in this study.

Strain	Locality of Collection
RL 18	Sungai Buloh, Selangor
RL 19	Seremban, Negeri Sembilan
RL 26	Kota Tinggi, Johor
SEG	Segamat, Johor
AM	Ayer Molek, Melaka

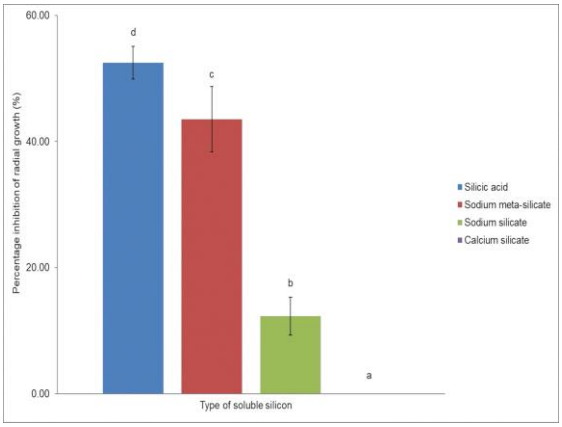

Fig 1. Total means of percentage inhibition of radial growth (PIRG) against *R. microporus* development on poison plate with different soluble silicon types. The PIRG (%) was measured at 7 days after incubation (n=6).

Table 2. Description of treatments for dipped stick inhibition assay.

Treatments	Explanation	Soaking time in soluble silicon (Hours)
T1	No Si added with *Rigidoporus microporus* inoculation	24
T2	Silicic acid added (500 ppm) with *Rigidoporus microporus* inoculation	
T3	Silicic acid added (5000 ppm) with *Rigidoporus microporus* inoculation	
T4	No Si added and with *Rigidoporus microporus* inoculation	
T5	Silicic acid added (500 ppm) with *Rigidoporus microporus* inoculation	
T6	Silicic acid added (5000 ppm) with *Rigidoporus microporus* inoculation	48
T7	No Si added with *Rigidoporus microporus* inoculation	72
T8	Silicic acid added (500 ppm) with *Rigidoporus microporus* inoculation	
T9	Silicic acid added (5000 ppm) with *Rigidoporus microporus* inoculation	
T10	No Si added with *Rigidoporus microporus* inoculation	24
T11	Sodium meta-silicate added (500 ppm) with *Rigidoporus microporus* inoculation	
T12	Sodium meta-silicate added (5000 ppm) with *Rigidoporus microporus* inoculation	
T13	No Si added with *Rigidoporus microporus* inoculation	48
T14	Sodium meta-silicate added (500 ppm) with *Rigidoporus microporus* inoculation	
T15	Sodium meta-silicate added (5000 ppm) with *Rigidoporus microporus* inoculation	
T16	No Si added with *Rigidoporus microporus* inoculation	72
T17	Sodium meta-silicate added (500 ppm) with *Rigidoporus microporus* inoculation	
T18	Sodium meta-silicate added (5000 ppm) with *Rigidoporus microporus* inoculation	

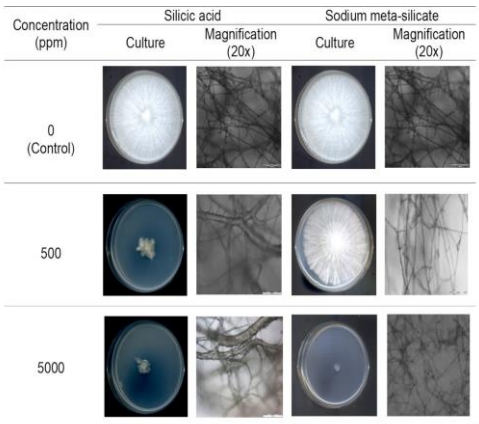

Fig. 2 Cultures and hyphae of *R. microporus* in control and different concentrations of silicic acid and sodium meta-silicate incorporated into the PDA after 7 days incubation showed greater width.

Table 3. Percentage inhibition of radial growth (%) of *R. microporus* at different soluble silicon concentrations of propiconazole, silicic acid, sodium meta-silicate, sodium silicate and calcium silicate in PDA poison plate.

Concentration (ppm)	Percentage Inhibition of Radial Growth (%)				
	Propiconazole	Silicic acid	Sodium meta-silicate	Sodium silicate	Calcium silicate
0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
10	73.63 ± 1.71^{b}	0.59 ± 0.59^{a}	2.35 ± 0.90^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
100	100 ± 0.00^{c}	36.08 ± 1.60^{b}	18.14 ± 3.47^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
500	100 ± 0.00^{c}	68.63 ± 1.79^{c}	15.09 ± 4.48^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
1000	100 ± 0.00^{c}	68.63 ± 1.60^{c}	19.80 ± 2.56^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
1500	100 ± 0.00^{c}	71.18 ± 1.29^{c}	39.02 ± 4.48^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
3000	100 ± 0.00^{c}	71.95 ± 0.99^{cd}	80.09 ± 1.48^{d}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
5000	100 ± 0.00^{c}	75.00 ± 0.98^{d}	100.00 ± 0.00^{e}	24.71 ± 6.87^{b}	0.00 ± 0.00^{a}
8000	100 ± 0.00^{c}	80.39 ± 0.64^{e}	100.00 ± 0.00^{e}	86.18 ± 4.18^{c}	0.00 ± 0.00^{a}

Data are means of six replicates ± S.E. Different letters within each column indicate significantly different values at $p \leq 0.05$ according to Duncan's multiple range test (DMRT).

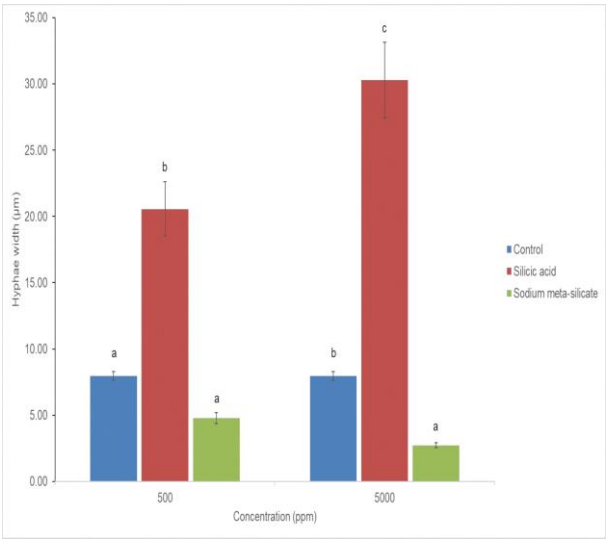

Fig. 3 Hyphae width (µm) of *R. microporus* recorded in control and different silicic acid and sodium meta-silicate concentrations incorporated into the PDA after 7 days incubation.

Table 4. pH values recorded in amended PDA with propiconazole and soluble silicon.

Concentration (ppm)	pH				
	Propiconazole	Silicic acid	Sodium meta-silicate	Sodium silicate	Calcium silicate
0	6.00^{f}	6.00^{b}	6.00^{b}	6.00^{b}	6.00^{ab}
10	5.75^{c}	5.46^{a}	5.68^{a}	5.57^{a}	5.94^{a}
100	5.68^{b}	5.53^{a}	6.33^{c}	5.95^{b}	6.05^{b}
500	5.91^{d}	5.52^{a}	7.21^{d}	6.81^{c}	6.50^{c}
1000	5.73^{bc}	5.48^{a}	7.67^{e}	7.06^{d}	6.59^{d}
1500	5.74^{c}	5.48^{a}	8.08^{f}	7.58^{e}	7.15^{e}
3000	5.71^{bc}	5.50^{a}	8.67^{g}	8.11^{f}	7.31^{f}
5000	5.45^{a}	5.54^{a}	9.01^{h}	8.60^{g}	7.77^{g}
8000	5.91^{e}	5.57^{a}	10.33^{i}	9.68^{h}	8.06^{h}

Data are means of three replicates ± S.E. Different letters within each column indicate significantly different values at $p \leq 0.05$ according to Duncan's multiple range test (DMRT).

Table 5. Percentage inhibition of radial growth (%) as compared to control, pH 6 (colony diameter: 85.00 mm) of different *R. microporus* on amended PDA at different pH values.

pH	Percentage Inhibition of Radial Growth (%)				
	RL 18	RL 19	RL 26	SEG	AM
2	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{b}	100.0 ± 0.00^{b}	100.00 ± 0.00^{c}
4	20.78 ± 5.37^{b}	23.14 ± 1.30^{b}	0.00 ± 0.00^{a}	1.76 ± 1.50^{a}	22.75 ± 2.09^{b}
8	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
10	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00a	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Data are means of three replicates ± S.E. Different letters within each column indicate significantly different values at $p \leq 0.05$ according to Duncan's multiple range test (DMRT).

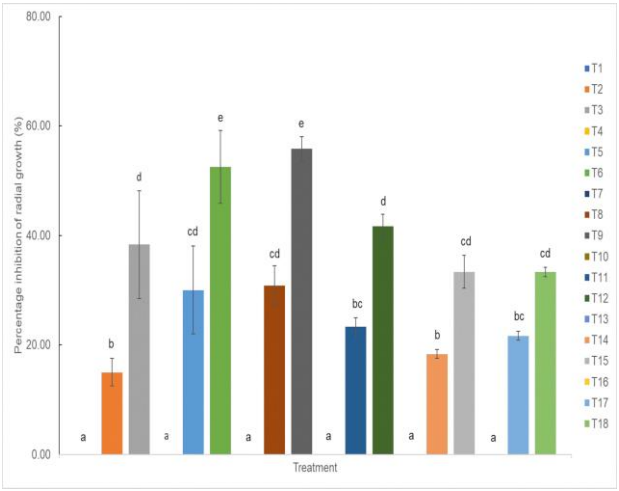

Fig. 4 Percentage inhibition of radial growth of *R. microporus* recorded in control and different silicic acid and sodium meta-silicate concentrations for dipped sticks inhibition assay after 14 days incubation (n=3).

Silva et al. (2020) observed remarkable changes in the morphology of mycelium raised on PDA drenched with orthosilicic acid. Changes such as excessive mycelial branching, abnormal shapes, swelling, and reduction of hyphae sizes were observed in all test pathogens, i.e., *Alternaria solani*, *Corynespora cassiicola*, *Curvularia lunata*, and *Mycosphaerella fragariae*.

The dipped stick inhibition assay was used to confirm the efficacy of soluble silicon as an alternative to control WRD in rubber caused by *R. micoporus* in the field experiment. Silicic acid and sodium silicate were selected for an in-depth understanding of the effect of different application times of soluble silicon on the growth of *R. microporous* on rubberwood sticks. Fig. 4 depicts the absence of *R. microporus* growth inhibition in all control treatments. However, significant differences are noted in the inhibition of *R. microporus* growth for all treatments tested. The greater inhibition of fungal growth was observed at a higher concentration for all the treatments.

According to Omorusi et al. (2014), *R. microporus* typically attacks the taproot of the rubber trees with three stages of disease infection, including penetration, colonisation, and degradation. In addition, *R. microporus* is classified as "typical white rot basidiomycete with saprotrophic abilities degrading the major components of wood, including lignin"(p. 2) (Oghenekaro et al., 2015). The ability of soluble silicon to inhibit the growth of *R. microporus* could reduce the incidence of WRD in rubber by preventing the penetration of *R. microporus* into the root system. The study of sodium silicate on *F. sulphureum* by Li et al. (2009) revealed the hyphae structural changes compared to the control treatment. The scanning electron microscope (SEM) observation of hyphae treated with sodium silicate indicated the potential of silicon inhibition of the fungal by changing the hyphae structure with the appearance of mycelial sparsity, asymmetry, curling, and twisting.

The higher concentration of silicon was found more effective in inhibiting the mycelial growth of *Phytophthora infestans* compared to the lower concentration applied *in vitro* (Kedarnath et al., 2016). This statement was supported by Carneiro-Carvalho et al. (2017), who claimed the positive correlation between the increase of silicon concentration and a higher percentage inhibition of radial fungal growth phytotoxic effect of silicon. Bekker (2007) suggested the reapplication of silicon to ensure silicon effectiveness in protecting the plants from being infected by plant pathogens. Moreover, the frequent application of silicon fertilisers on powdery mildew development in greenhouse cucumber has improved the fungal inhibitive effect (Wolff et al., 2012). The dipped sticks inhibition assay results can be used as a guideline for the field experiment study. It revealed that frequent application of soluble silicon might help the plant absorb more silicon than less soluble silicon application. The absorption of silicon could stimulate the diseased plants' defence mechanisms by strengthening the physical barrier and enhancing the formation of organic defence compounds by altering gene expression (Epstein, 2009; Lux et al., 2020).

Materials and methods

Siliceous materials and commercial fungicide

The three types of soluble silicon used in this study, i.e., sodium silicate nonahydrate ($Na_2SiO_{39}H_2O$: mean weight = 284.22 g/mol), sodium meta-silicate (Na_2O_3Si: mean weight = 122.06 g/mol), and calcium silicate (CaO: 12%–122%, SiO_2: ≥ 87%) were obtained from Chemolab Supplies Sdn Bhd. Silicic acid (H_4O_4Si: 72%) or Sika was obtained from Siben Agriculture Company, Taiwan. Meanwhile, the commercial fungicide, propiconazole (trade name: Tilt) purchased from Hup Huat Agrotech, Malaysia was used as a positive control.

Agar preparation

The Poisoned Food Technique (Balamurugan, 2014) with slight modification was executed to evaluate the antifungal activity of different sources of soluble silicon in inhibiting the mycelial growth of *R. microporus* from Ayer Molek strain. Four types of soluble silicon (silicic acid, sodium silicate, sodium meta-silicate, and calcium silicate) were used in this study. The stocks (10000 ppm) for each soluble silicon and propiconazole (positive control) were prepared and autoclaved at 121°C, 1.05 kg/cm^2 for 21 minutes. The specific amount of propiconazole and soluble silicon stock was incorporated into autoclaved PDA to achieve the desired concentrations of 10, 100, 500, 1000, 1500, 3000, 5000, and 8000 ppm. The Petri plates were shaken gently and laterally to allow propiconazole and soluble silicon distribution on the PDA medium. PDA without propiconazole

and soluble silicon served as the negative control, while PDA incorporated with propiconazole served as a positive control. Three pH values of the prepared medium were recorded using Delta 320 pH meter. Subsequently, the agar solutions were decanted into Petri dishes and left to set before being incubated for two days to ensure no contamination.

Antifungal activity assay

A 5-mm diametric mycelial plug of the 7-day-old culture of *R. microporus* from the Ayer Molek strain was placed at the centre of the plate. The Petri plates were then sealed with Parafilm® (Pechiney, Chicago, IL) and incubated at room temperature. Data on the radial colony diameter were recorded after seven days of incubation by measuring two colony diameters at right angles to each other. A colony radius on PDA for each propiconazole and soluble silicon concentration was measured from the bottom side of the Petri plates. The mycelium was observed *in situ* under an Olympus CX41 light compound microscope with a magnification of 20× and images recorded using an Xcam-a camera. Percentage inhibition of radial growth (PIRG) was calculated using the following formula:

$$\text{PIRG (\%)} = \left(\frac{fungal\ growth - control\ growth}{control\ growth}\right) \times 100\%$$

Six replicates of PDA plates were used for each negative control, positive control and soluble silicon types. The mycelial growth of *R. microporus* from Ayer Molek strain was recorded after seven days of incubation at 25°C ± 2°C.

pH determination

Different pH values could influence fungal growth suppression. The effects of different pH values in the amended PDA plates with propiconazole and soluble silicon were investigated compared to the control PDA plates (pH 6). In this experiment, the pH values of PDA plates were adjusted to pH 2, 4, 8, and 10 using 1.0 M hydrochloric acid (HCl) and 1.0 M sodium hydroxide (NaOH). Three replicates of PDA plates were used per treatment, and mycelial growths of five *R. microporus* strains (Table 1) were recorded after eight days of incubation at 25°C ± 2°C.

Dipped stick inhibition assay

The dipped stick inhibition assay was used to test the inhibition of soluble silicon against *R. microporus* on rubber sticks (Maiden et al., 2017). Two types of soluble silicon (silicic acid and sodium meta-silicate), which showed the optimal inhibition rate against *R. microporus* on amended PDA, were chosen for this experiment (Table 2). Silicic acid and sodium meta-silicate with two different concentrations (500 and 5000 ppm) were used to test the growth of *R. microporus*, Ayer Molek strain on rubber sticks. The Ayer Molek strain was grown on 5 mL sterile PDA in test tubes for three days at 25°C ± 2°C. Sterile rubberwood sticks (4.0 cm long and 1.5 cm in diameter) were soaked in different sterile soluble silicon solution concentrations at three different times, 24, 48, and 72 hours. In comparison, sterile rubberwood sticks soaked into sterile distilled water served as the control. The rubberwood sticks were then transferred into test tubes containing *R. microporus* cultures. Data were collected after 14 days of incubation when the *R. microporus* in the control set had completely covered the whole length of the rubber sticks. The PIRG of *R. microporus* growth was observed and calculated using the following formula:

$$\text{PIRG (\%)} = \left(\frac{RT - RC}{RC}\right) \times 100\%$$

where RC is the length (cm) of *R. microporus* growth in control tubes and RT is the length (cm) of *R. microporus* growth in the treatment tubes. Three replicates of rubberwood sticks were used per treatment. PIRG (%) were recorded after 14 days at 25°C ± 2°C.

Statistical analysis

The experiments were conducted in a completely randomised design (CRD). An analysis of variance was performed using SPSS® 24.0 for the Windows program. One-way ANOVA analyses and Duncan's multiple range test (DMRT) were used to detect significant differences between treatments, and differences were considered significant when $p \leq 0.05$.

Conclusion

We conclude that soluble silicon has the potential as an antifungal agent against *R. microporus* due to its ability to interfere with fungal sporulation *in vitro*. The outcome renders the possibility of soluble silicon application as a fungicide. Furthermore, results obtained from the Poisoned Food Technique identified two types of soluble silicon, silicic acid and sodium meta-silicate, as the most potent element to control the infection of *R. microporus* at the rubber field plantation. However, pot and field trials are suggested to be conducted in the near future to confirm the efficacy of soluble silicon against *R. microporus* in planta. Besides, soluble silicon is expected to be more economical and environmentally friendly than the current commercial fungicide. It also acts as a biostimulant, i.e., providing potential benefits to plant growth and development and stress responses. Further understanding of the interactions among biostimulant components such as silicon and other systemic elements (e.g., *R. microporus*, soil type, and crop variety) will provide sufficient reliable results in rubber research.

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Compliance with ethical standards

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflicts of interest

All authors certify that they have no affiliations with or involvement in any organisation or entity with any financial or non-financial interest in the subject matter or materials discussed in this manuscript.

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