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Optimizing Silicon Application for Enhancing Growth and Chlorophyll Concentration in Pepper Plants (*Piper nigrum* L.) Cultivar Kuching

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ABSTRACT

Silicon is known to play a central role in regulating various aspects of plant growth and development, including nutrient uptake, root formation, and growth. Silicon is the second most abundant element found in soil primarily as neutral, monomeric silicic acid, which is the biologically available form for plant uptake. Although silicon is not considered an essential nutrient for the basic life cycle of most plants, its availability can significantly benefits to plant health, growth, and stress tolerance. However, previous research has mainly focused on plants grown in silicon, and silicon occurs naturally as silicon dioxide (SiO_2), and is not in a form that is easily absorbed by plants. Therefore, this study investigates the effects of silicon (Si) in silicic acid form (H_4O_4Si) on the growth and chlorophyll concentration of pepper (*Piper nigrum* L.) seedlings, particularly the Kuching variety. The Si application had been applied once a week with five different concentrations via root applications; T1 [0.5% Si (v/v)], T2 [1.5% Si (v/v)], T3 [2.0% Si (v/v)], T4 [1.5% potassium silicate (v/v)] as positive control and T5 [negative control (without silicon)] on pepper cutting-grown plants. Growth parameters such as plant height, stem diameter and chlorophyll concentration were observed and collected. Our results showed that the treatment with Si nutrients is promising, as the Si-treated pepper clones showed faster and more robust growth compared to the control plants in the early growth stages. The results indicate that a 0.5% Si concentration (v/v) effectively maintains the high chlorophyll content over four weeks, in contrast to the decreasing trend observed in the control group. This study thus presents the first report on the application of Si in *P. nigrum* L., demonstrating the feasibility of Si uptake and growth enhancement in pepper plants. The results suggest a stepwise application of Si, starting with low concentrations (0.5% Si v/v) via the root in the early growth stages to strengthen young plants before transplanting to the field. However, foliar spraying could also be considered in future studies as the silicon is absorbed faster compared to root application. Further studies on the passive defence structure (physical barriers such as cuticle, wax, and trichomes) are needed to prove that it can repel pathogens and insects.

Keywords: Chlorophyll concentration, *Piper nigrum*, growth enhancement, silicon, silicic acid, Terengganu

INTRODUCTION

The pepper plant, scientifically known as *Piper nigrum* L. and often referred to as the "king of spices," because of its active ingredient piperine, which has various pharmacological effects and offers numerous health benefits (Srinivasan, 2007). The cultivation of black pepper is widespread in various Asian countries, including Malaysia, where the pepper plant is one of the four most economically important agricultural crops after palm, rubber and cocoa (Joy *et al*., 2007; Damanhouri & Ahmad, 2014; Abdulla *et al*., 2015). In Malaysia, there are seven commonly cultivated varieties: "Semongok Aman", "Semongok Emas", "Kuching", “Sarikei”, "Semongok Perak", "Uthirancotta", "Nyerigai", and "PN129" (Paulus, 2011). In Sarawak, the most common varieties are Sarikei and Kuching (Ravindran *et al*., 2000). Kuching grows faster and higher yielding than Sarikei (Paulus & Sim, 2005), making it the predominant variety in Sarawak and Johor (Paulus, 2011). However, Kuching is highly susceptible to diseases such as Phytophtora foot rot, blackberry, Fusarium wilt, root-knot, and wrinkle leaf (Paulus, 2011). Despite other widely grown varieties such as "Semongok Perak”," "Semongok Emas" and "Semongok Aman", Kuching remains the most widely grown variety in Malaysia.

Malaysia considered the fifth largest pepper producer in the world (Malaysia Pepper Board, 2022), relies mainly on Sarawak, which contributes 98% of the country's annual production (Adam *et al*., 2018). The average domestic consumption of pepper in 2017-2021 was 10, 891 tonnes (Malaysia's Open Data Portal, 2022). Domestic consumption of pepper increased from 12,000 tonnes in 2014 to 13,500 tonnes in 2015 (Adam *et al*., 2018). Black pepper production has declined since the early 1980s, primarily due to pest infestations and disease outbreaks in field plantations (Chen *et al*., 2010). Moreover, due to biotic and abiotic stress factors, farmers have difficulty in procuring healthy planting materials, resulting in minimised pepper yields (Sharma & Kalloo, 2004). Consequently, this has resulted in an annual reduction of approximately 2% in the total pepper cultivation area (Adam *et al*., 2018).

There is therefore a need to promote the growth and development of plants, e.g. by administering silicon (Si) application, which can reportedly help to improve plants' resistance to disease. For example, rock melon and strawberry growers have added Si to the irrigation system to increase plant defenses against diseases such as powdery mildew (Samuels *et al*., 1991; Menzies *et al*., 1992; Belanger *et al*., 2003; Ouellette *et al.,* 2017). Numerous studies have reported the promotion of root development by the combined application of salicylic acid (SA) and Si fertilization. SA, a phenolic compound, is known to regulate plant growth and development in several areas, including nutrient uptake and root initiation and growth (Wani *et al.,* 2016; Khan *et al.,* 2017; Pasternak *et al.,* 2019). SA exposure has been shown to stimulate the development of adventitious, primary, and lateral roots in numerous plant species (Yang *et al.,* 2013; Pasternak *et al.,* 2019). Similarly, the beneficial effects of Si fertilization on plants have been extensively documented (Laane, 2017), including its regulatory role in enhancing the uptake of other essential plant nutrients (Ma & Yamaji, 2006; Liang *et al.,* 2007; Al-Wasfy, 2013; Deshmukh *et al.,* 2017). It is known that Si strengthens the cell walls of plants, and thus potentially increases resistance to biotic and abiotic stress factors such as pests, diseases, and drought, while at the same time increasing plant quality and yield.

However, conventional Si sources, especially silicon dioxide, can pose problems for pepper plants in terms of uptake. To solve this problem, a technology called Silicic Acid Agro Technology (SAAT) has been introduced for the application of Si in agricultural crops (Laane, 2017). SAAT has shown promising efficacy in facilitating Si uptake, even in crops that were previously unable to absorb Si efficiently, including non-accumulating Si plants (Epstein, 1994; Laane, 2017). The application of Si can improve plant growth parameters by increasing root growth, root mass, stem length and diameter, plant height, number of shoots and leaf area (Laane, 2017), which would indirectly increase plant yield potential. Plant height is an important indicator of plant growth, biomass production and yield potential in cereals and wheat (Martin *et al.,* 2017). In addition, the Si content of wheat grown under salt stress was determined.

Si can be applied to plants either by foliar spraying or by watering into the soil. However, Si in the form of silicic acid can be applied via foliar spraying in lower concentrations during the vegetative stage and act as a growth promoter (Lanne, 2017). To date, Si has not been recognized as an essential plant nutrient, although it can promote plant growth and agricultural production (Zellener *et al.,* 2021). However, the Association of American Plant Food Control Officials (AAPFCO) recognizes Si and classifies it as a beneficial substance. Si products are also approved by the Organic Materials Review Institute (OMRI) for use in organic farming. Nevertheless, there are only few studies on the use of Si in the pepper plant. The aim of this study was therefore to determine the effect of Si applications on the growth of pepper plant in the early growth phase. This is the first report on the application of silicon (in the form of silicic acid) to pepper plants, *P. nigrum* L.

MATERIALS AND METHODS

Experimental Plot

The experimental plot is located at pepper plantation in Setiu, Terengganu, Malaysia (5°32'1N, 102° 57' 15E). The experimental plot was set up in a randomized complete block design (RCBD). The duration for this study was two months starting on August 2018 until October 2018.

Pepper plants, variety Kuching, had been selected in this study due to the most widely grown cultivar and it is readily accessible. It also has vigorous growth and high yield production. Each treatment was conducted with three replicates. For irrigation and fertilization, pepper plants were irrigated using a drip irrigation system. Irrigation was scheduled twice a day automatically by drip tape. Additionally, this study followed the Standard Operating Procedures (SOP) for planting procedures, irrigation, and fertilization in black pepper plantations. These procedures were based on the guidelines provided by the Agriculture Department of Lembah Bidong, ensuring that all practices were aligned with established agricultural standards.

Silicon Nutrient Treatments

Five different silicon (Si) concentration were applied to cutting-grown pepper plants: T1 (0.5% Si v/v), T2 (1.5% Si v/v), T3 (2.0% Si v/v), T4 (1.5% potassium silicate) as positive control and T5 (control without silicon) as negative control. The dilution of Si nutrients was calculated using the standard dilution formula, $M_1V_1= M_2V_2$; where M1= initial percentage of solution, V1= volume needed for dilution, M2= final percentage of solution, V2= final volume needed for the experiment. A total of fifty pepper plants were given Si in the form of monosilicic acid (72% of SIKA, Taiwan) once a week as root treatments. The Si was prepared on the day of application and all seedlings had been given 40 ml of the Si solution.

Plant Growth Assessments

Throughout the study, the plant growth of the seedlings was measured twice a month. Plant growth was assessed by stem diameter, plant height and leaf chlorophyll concentration. Plant height was measured using tape measure (cm), while stem diameter was measured using a digital vernier caliper and chlorophyll content was measured using a Konica Minolta chlorophyll meter. Chlorophyll content data were measured three times per plant and 10 leaves per treatment.

Statistical Analysis

All data were analyzed using analysis of variance (ANOVA) in SPSS Statistics 20. Comparison with the mean values of the control and the treatment was performed using Tukey's HSD test at a significance level of $p< 0.05$ (95% confidence level). A Pearson correlation coefficient was performed to evaluate the relationship between treatment and pepper plant height, stem diameter and chlorophyll concentration (SPAD).

RESULTS AND DISCUSSION

The effect of Si application on pepper plants showed a promising result for stem cutting seedling growth and development.

Plant Height Response to Silicon Treatments

The results showed a significant difference ($p<0.05$) in increment of plant height between the Si treatments over a four-week period (Figure 1). The plant height increment showed that T1 (0.5% Si v/v) consistently had the highest plant height across all weeks and thus showed the most significant growth development. From week 1 until week 4; 13.2 cm, 29.4 cm, 38.9 cm and 51.7 cm, respectively. Plants without Si (T5) showed the slowest growth from week 1 to week 4 (4.4 cm, 8.9 cm, 11.7 cm and 12.7 cm, respectively) (Figure 1). In week 1, treatments T2 (1.5% Si v/v), T3 (2.0% Si v/v), and T4 (1.5% Psi v/v) had similar plant heights, ranging from 5 to 10 cm, with no significant differences between them.

All Si-treated pepper plants were growing faster than control plants and the range from the highest to the lowest (on week-4); T1 (51.7 cm) > T2 (39.5 cm) > T3 (30.9 cm) > T4 (27.9 cm) > T5 (12.7 cm) (Figure 1). The pepper seedlings treated with Si showed a positive result and had enhanced plant growth. Control treatment show no significant correlation between Si and plant height over the four weeks. The correlation coefficients range in Week 3 (r [8] = [-0.58], p= [.077] and in Week 4 r [8] = [0.19], p= [0.60], indicating weak and negative relationships for most weeks (Table 1). In T1 (0.5% Si), weak negative correlations were observed throughout all weeks, with r values ranging from -0.539 to 0.130. (Table 1). The application of 1.5% Si shows a transition from negative correlations in Weeks 1 and 2 (r = -0.495 and -0.552, respectively) to a statistically significant positive correlation in Week 3 r [8] = [0.67], p = [0.05] (Table 1). This suggests that by Week 3, the pepper plants responded positively to Si application.

Higher Si concentrations may bring fewer positive effects on plant height for several reasons, despite its overall benefits to plant growth. One possible explanation is that excessive Si application can alter the balance of other essential nutrients and water uptake, potentially limiting growth. At high concentrations, Si can interfere with normal physiological processes, including the regulation of plant hormones like gibberellins, which play a key role in promoting stem elongation and overall plant height (Sah *et al*., 2022). Therefore, while Si promotes growth at optimal levels, overapplication can shift the plant's resources more towards stress resistance and structural reinforcement rather than height growth (Shivaraj *et al*., 2022)

Similar findings from previous studies by Jufri *et al*. (2016) and Suhaizan *et al*. (2017) that have been confirmed that Si-treated chili plants exhibit greater leaf length and showed significant improvements in vegetative growth compared to control plants. In addition, similar growth-promoting effects of Si have been observed in other crops such as rice, tomatoes, cucumbers, coffee and strawberry (Ma & Yamaji, 2006; Silva *et al.*, 2010; Ouellette *et al.*, 2017). Suhaizan *et al*. (2023) reported that the

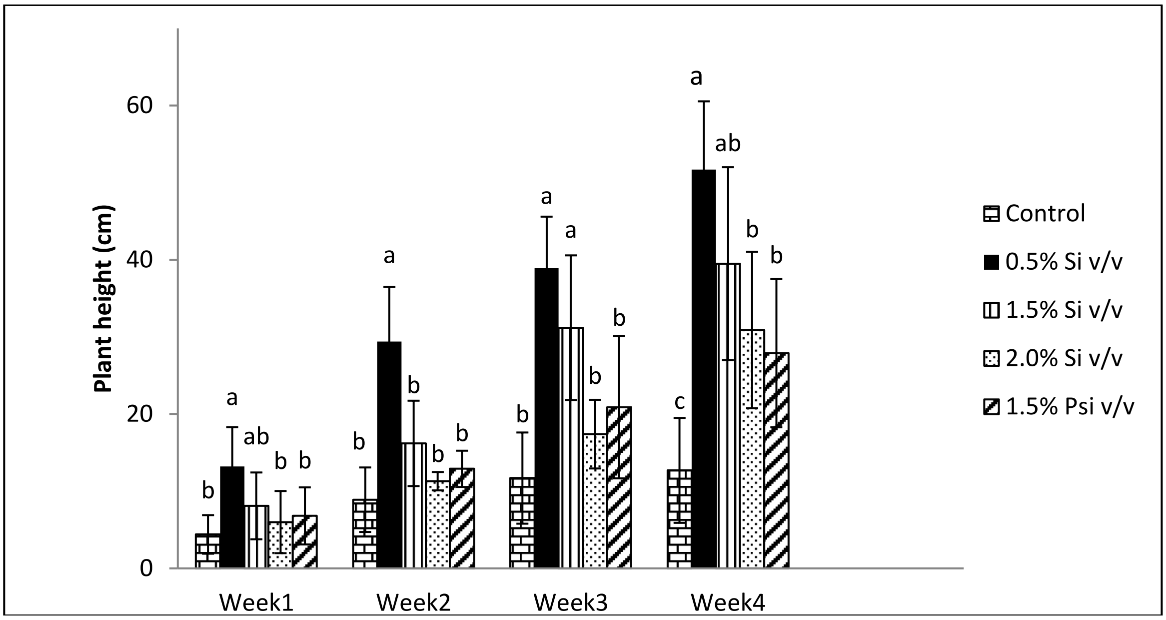

Figure 1. Pepper plant height increment (cm) from week-1 until week-4 grown from stem cutting seedlings. Silicon was applied once a week by root application. The treatments: T1 (0.5% Si v/v), T2 (1.5% Si v/v), T3 (2.0% Si v/v), T4 (1.5% potassium silicate) as positive control and T5 (control without silicon) as negative control

Table 1. Pearson correlation coefficients between treatments and pepper plant height

		W1	W2	W3	W4
Plant Height	Controls	-0.321	-0.316	-0.584	0.190
	0.5% Si	-0.390	-0.452	-0.539	0.130
	1.5% Si	-0.495	-0.552	0.665*	0.190
	2.0% Si	-0.662*	-0.234	0.808**	-0.62
	1.5% PSi	-0.702*	0.858**	-0.668*	0.156

*Correlation is significant at the 0.05 level (2-tailed) **Correlation is significant at the 0.01 level (2-tailed)

impact of Si nutrient application on the growth of chili plants grown using a fertigation system. The study tested different Si concentrations (0 ppm, 108 ppm, 180 ppm, and 360 ppm) and evaluated their effects on various growth parameters, including stem diameter and plant height. The findings showed that Si significantly enhanced the growth performance of chili plants. The highest concentration (360 ppm) resulted in the greatest increase in stem diameter, plant biomass, and Si accumulation in stems and fruits. From this study, it suggests that Si application improves structural growth and helps chili plants better withstand abiotic stress, enhancing overall plant development. Other study by Gong *et al.* (2003) reported that wheat plants grown in pots with Si application showed greater plant height and leaf area compared to control plants without Si. While peppers were previously considered low silicon accumulators (Epstein, 1994), the application of Si in the form of silicic acid has now been shown to enable pepper plants to uptake Si, providing significant benefits. In this study, Si-treated pepper seedlings grown faster and healthier compared to control plants during the early stages of development.

Si plays a crucial role in regulating plant growth and development, affecting various aspects such as nutrient uptake, root formation, and growth (Ma & Yamaji, 2006; Liang *et al*., 2007; Al-Wasfy, 2013; Wani *et al*., 2016; Deshmukh *et al*., 2017; Khan *et al*., 2017; Pasternak *et al*., 2019). However, studies back then were mostly focusing on Si effects on plants cultivated in solution culture (with controlled microclimate or lab condition) while there were limited studies on plants cultivated in soil medium and large scale-field (with natural microclimate).

Stem Diameter Response to Silicon Treatments

There were no significant differences (p>0.05) in stem diameter for all treatments. The T2 treatments showed the widest in increment of stem diameter on the first week (0.5 mm) compared to other Si treatment and control. But by week 3 and week 4, the T1 (0.5% Si v/v) treatment showed a significant increase in stem diameter compared to the control and other treatments. T1 was 2.8 mm whereas control was only 1.5 mm (Figure 2). Other Si treatments had intermediate stem diameters, while the 1.5% Psi v/v treatment consistently showed the lowest stem diameter by week 3 and week 4 (Figure 2). Unexpectedly, the lowest Si concentration (T1) had increased (stem diameter) faster than other treatments [0.4 mm (week 1), 1.2 mm (week 2), 2.1 mm (week 3) and 2.8 mm (week 4)] (Figure 2). The increment rank of stem diameter on week-4; T1 [2.8mm]> T2 [2.1 mm]> T3 [1.9 mm]> T4 (1.7 mm) > T5 (1.5 mm) (Figure 2). Results from Table 2 show that T2 shows a gradual increase in correlation strength from Week 1 to Week 4, transitioning from a negligible correlation in Week 1 r [8] = [-0.05], p = [0.08]) to a significant positive correlation in Week 3 r [8] = [0.477], p = [0.49] (Table 2). This indicates that 1.5% Si had a delayed but beneficial effect on stem diameter, which became more pronounced as the study progressed. The increase in stem diameter by Week 3 might suggest that 1.5% Si concentration optimally supports structural growth at this stage.

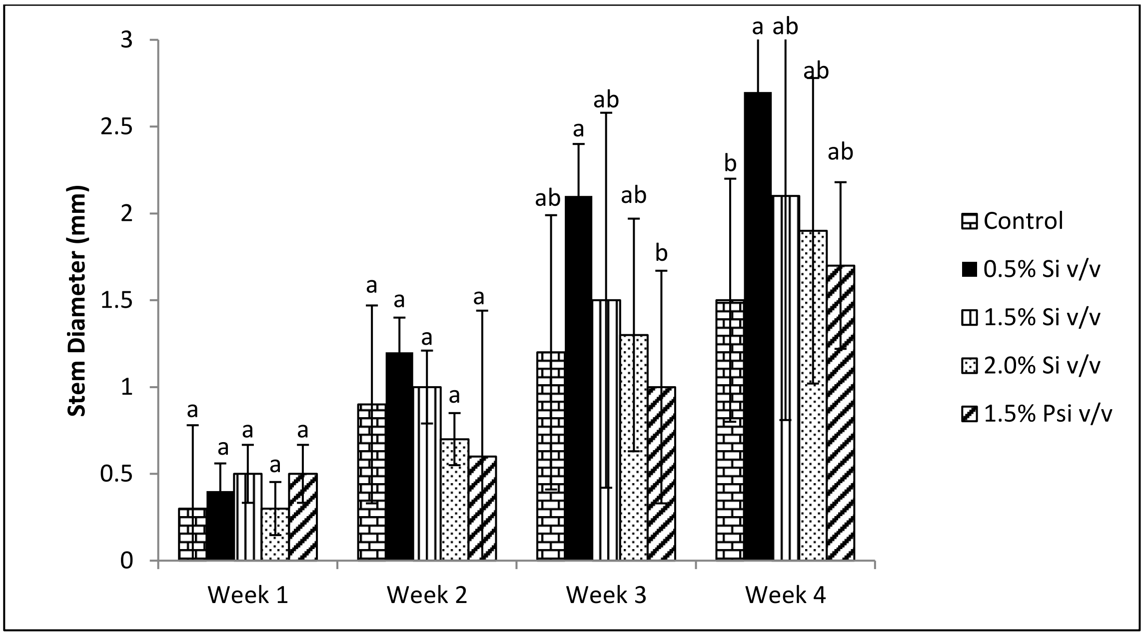

Figure 2. Pepper plant stem diameter increment (mm) from week-1 until week-4 grown from stem cutting seedlings. Silicon was applied once a week by root applications. The treatments: T1 (0.5% Si v/v), T2 (1.5% Si v/v), T3 (2.0% Si v/v), T4 (1.5% potassium silicate) as positive control and T5 (control without silicon) as negative control

Table 2. Pearson correlation coefficients between treatments and pepper plant stem diameter

		W1	W2	W3	W4
Stem-diameter	Controls	-0.419	-0.584	-0.628	0.723*
	0.5% Si	-0.141	0.050*	0.060**	0.013**
	1.5% Si	-0.048	0.054*	0.477*	0.492
	2.0% Si	0.109	0.098	0.144	0.416
	1.5% PSi	-0.244	-0.205	-0.164	-0.454

*Correlation is significant at the 0.05 level (2-tailed) **Correlation is significant at the 0.01 level (2-tailed)

These results indicate that the T1 concentration is most effective in increasing stem diameter over the course of four weeks. The control and the other Si treatments had moderate effects, while the potassium silicate treatment was the least effective in promoting stem diameter growth.

In rice plants, Si has been shown to increase resistance to fungal attack by strengthening the stem base and promoting cell elongation (Wakabayashi *et al*., 2002; Liang *et al.*, 2013). In addition, Si depots such as silica gel, contribute to the strengthening of cereal stems and leaves by increasing the number of silicate cells and Si content in the stem, even at elevated nitrogen levels (Fallah, 2012). The observed improvement in stem strength following Si supplementation is consistent with previous studies demonstrating the positive effects of Si on plant biomechanics and structure. Consequently, the presence of Si can increase stem strength and reduce stem lodging, especially during periods of increased peppercorn production.

Chlorophyll Concentration (SPAD) Response to Silicon Treatments

There were no significant differences (p>0.05) in chlorophyll concentration were observed between all Si treatments (Figure 3). At week 1, all treatments, including the control, had similar chlorophyll concentrations, approximately 60 SPAD units. Nevertheless, pepper plants treated with Si generally had higher chlorophyll content (SPAD) compared to the control plants (Figure 3). Chlorophyll content was higher in T1 (65.7 SPAD) at week 3 (Figure 3), whereas pepper plant treated with T2 has the highest chlorophyll concentration on week 2 (54.9 SPAD) (Figure 3). The T5 had the lowest chlorophyll content (38.1 SPAD) on week 3 (Figure 3). Results indicated that there was no relationship between control treatment and chlorophyll concentration (SPAD in week 1 r [7] = [-0.39], p = [.18] and week 3 r [7] = [-0.63], p = [.037] (Table 3). For T1, correlation coefficients fluctuate over the study period, showing a positive correlation in Week 1 r [7] = 0.445, p = [.024] and weak negative correlations in subsequent weeks (Weeks 2, 3, and 4) (Table 3). None of the correlations are statistically significant (p > 0.05), suggesting that T3 had little effect on chlorophyll concentration throughout the study. This finding aligns with other studies indicating that high Si concentrations may not significantly impact chlorophyll levels unless applied under specific conditions or during stress conditions. The T4 shows a strong positive correlation in Week 3 r [7] = [0.91], p= [.029], where a strong positive correlation is observed though it is not statistically significant (Table 3). This high positive correlation suggests a strong association between the T4 and chlorophyll concentration.

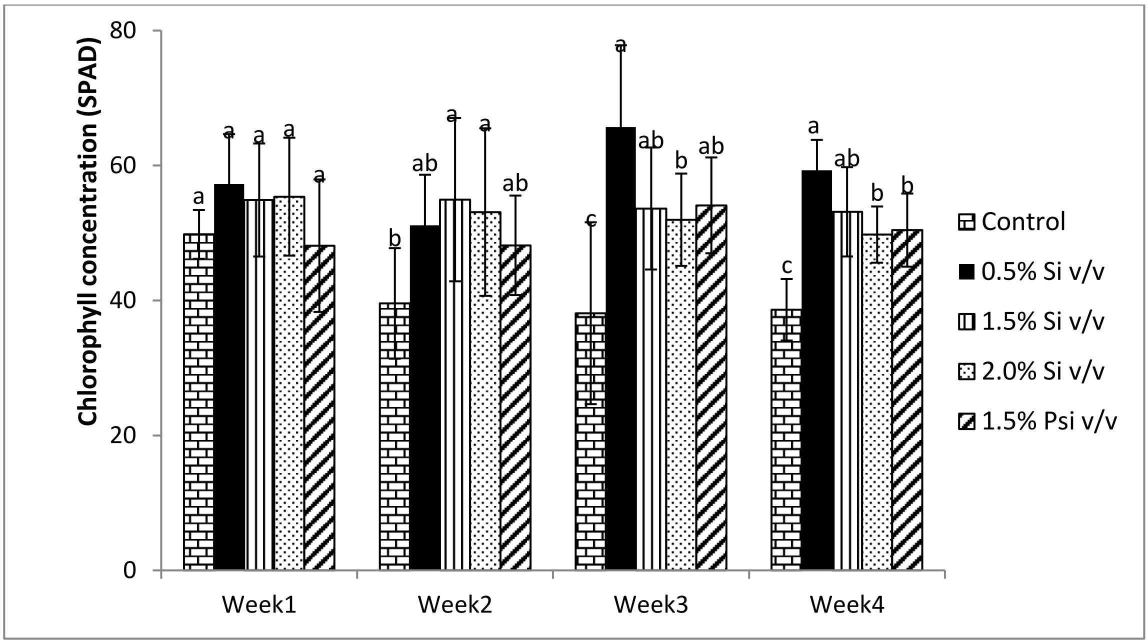

Figure 3. Chlorophyll concentration (SPAD) of pepper plant from week-1 until week-4 grown from stem cutting seedlings. Silicon was applied once a week by root applications. The treatments: T1 (0.5% Si v/v), T2 (1.5% Si v/v), T3 (2.0% Si v/v), T4 (1.5% potassium silicate) as positive control and T5 (control without silicon) as negative control

Table 3. Pearson correlation coefficients between treatments and pepper plant chlorophyll concentration

		W1	W2	W3	W4
	Controls	-0.394	0.007	-0.627	0.735*
	0.5% Si	0.445	-0.432	-0.387	0.064
Chlorophyll	1.5% Si	-0.200	-0.709*	0.442	-0.070
	2.0% Si	-0.232	0.290	-0.071	0.121
	1.5% PSi	-0.468	0.502	0.919	0.573

*Correlation is significant at the 0.05 level (2-tailed)

These results indicate that the 0.5% Si v/v concentration is most effective in maintaining a high chlorophyll concentration a four weeks period. The control group consistently showed a decrease in chlorophyll concentration, indicating the potential benefits of Si and Psi treatments in maintaining chlorophyll concentration.

It has been found that nutrient Si has a significant influence on the chlorophyll content and consequently on the photosynthetic activity of plants. Si has a significant influence on chlorophyll content. It also appears to improve various other plant growth parameters. Chlorophyll content is subject to the influence of various environmental factors such as light intensity, temperature and water content. In a previous study, it was reported that salt-treated barley, enriched with Si, showed enhanced growth due to improved chlorophyll content and increased photosynthetic activity in the leaf cell organelles (Al-aghabary *et al.*, 2005). Productive changes in plants include improved in leaf epidermal development, increased leaf chlorophyll content (Yao *et al*., 2011; Teixeira *et al*., 2020) and increased photosynthetic rate (Asmar *et al*., 2013; Lavinsky *et al*., 2016). During a two-month observation period, it was observed that T1 [0.5% Si (v/v)] grew healthier and faster compared to T4 (positive control) and T5 (negative control) in the nursery (Figure 4).

Figure 4. Comparison of pepper plants treated with 0.5% Si (v/v) [T1], positive control (1.5% Potassium Silicate v/v) [T4] and negative control (without Si) [T5]

CONCLUSION

Si in the form of silicic acid has the potential to improve growth and increase the chlorophyll concentration of pepper plants cultivar Kuching. This is the first report on the application of Si to pepper plants in Malaysia and the results show that pepper plant could uptake Si and grow healthier. These results suggest that the 0.5% Si v/v concentration is the most effective in influencing plant growth over the course of four weeks. The control and other Si treatments had moderate effects, while the potassium silicate (1.5% Psi v/v) treatment was the least effective in plant growth. From this result, we suggest that Si could be applied gradually starting from the lowest concentration [0.5% Si (v/v)] to the highest concentration [1.5% Si (v/v)] by root application for pepper plant from stem cutting seedling. During early-stage application, it will strengthen the young plant before being transplanted to field. By strengthening the stem basis and promoting cell elongation, it reduces the risk of lodging, particularly during periods of increased peppercorn abundance. However, foliar spraying should be considered in the future since silicic acid absorption is quicker on leaves than via root.

Further research should investigate Si with other agronomic practices, such as fertilization, fertigation (fertilization by drip irrigation), irrigation management, and pest control, to develop integrated management strategies for optimizing pepper production while minimizing environmental impacts. We encourage further research on Si to investigate the mechanisms behind these observations and to determine the long-term effects of these treatments on overall plant health and productivity. By addressing this topic, could provide valuable insights into the sustainability and durability of silicon-based management practices in pepper plants production.

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