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Silicon Sources on Biochemical Responses and *Corynespora cassiicola* Control in Cucumber Plants

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Abstract

Target spot, caused by *Corynespora cassiicola*, is an important leaf disease affecting cucumber plants, and alternative management studies are essential for the economic viability of this crop. Silicates were evaluated for the control of target spot and its pathogen, and the biochemical responses of plants were characterized. Preventive spraying was performed three times, at weekly intervals, with K_2SiO_3 + Cu and K_2SiO_3, at the concentrations 0, 28, 56, 84 and 112 mg L^{-1} silicon (Si). Assessments included mycelial growth and germination *in vitro*; disease severity; number and diameter of lesions; pathogen sporulation on leaves; activities of the enzymes peroxidase, polyphenol oxidase and β-1,3-glucanase, and leaf levels of phenolic compounds. The application of Si-based sources had no effect *in vitro*, nor did it affect lesion diameter and sporulation. Disease severity was lowest after five days of inoculation for treatments with 56 and 84 mg L^{-1} Si, but there was no disease control after three and seven days. The number of lesions was smallest from 28 to 112 mg L^{-1} Si, after five days of inoculation, and at 56 mg L^{-1} Si after seven days of inoculation for the treatment K_2SiO_3 + Cu, as well as at 56 and 112 mg L^{-1} Si after five days and at 84 and 112 mg L^{-1} Si after seven days of inoculation for K_2SiO_3. In general, there were no differences between the tested silicates. Enzyme activities and phenolic compound levels were not influenced by Si. The reduction in the disease severity and in the number of lesions, even if dependent on Si concentration or day of evaluation, demonstrated the possible viability of Si in controlling cucumber target spot, especially after further studies.

Keywords *Cucumis sativus* · Target spot · Alternative management · Resistance induction

Introduction

Target spot, caused by *Corynespora cassiicola* (Berk. & M. A. Curtis) C.T. Wei, has a great importance for cucumber crops (*Cucumis sativus* L.). Epidemics of this disease causing frequent losses to cucumber producers have been reported both in the field and under controlled environment in several states of Brazil (Teramoto et al. 2011; Bezerra and Bentes 2015; Fischer et al. 2022) and other countries like China (Liu et al. 2019) and the United States (Sumabat et al. 2018).

The first symptoms are small angular spots of light coloration, showing a pale center and a small light yellow halo. Then, the spots grow becoming round with soaked margins of olive coloration. Coalescence of spots causes shriveling of leaf limbs and consequent plant defoliation (Pavan et al. 2016).

One of the recommended control measures is leaf spraying with fungicides that are used for the same crop but for different diseases, such as *Alternaria* (*Alternaria cucumerina* (Ellis & Everh.) J.A. Elliott) and *Cercospora* spots (*Cercospora citrullina* Cooke) (Pavan et al. 2016), since there is no product registered by the Brazilian Ministry of

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Agriculture, Livestock and Supply (MAPA) to control target spot in cucumber plants in Brazil (Agrofit 2023).

Alternative control of target spot could involve the use of products different from fungicides. Silicon-based fertilizers have become a viable alternative in disease control. There is scientific evidence of the beneficial effect of Si, especially for plants under abiotic or biotic stress (Van Bockhaven et al. 2013; Zhu and Gong 2014). Silicon assists in disease control through the biochemical mechanism, accumulating natural defense compounds like phenolic compounds, and through the physical mechanism, promoting silica deposition on the surface and forming a cuticle-silica double layer, which increases the cell wall stiffness and provides the plant with greater resistance to the attack of insects and pathogens (Vivancos et al. 2015; Reynolds et al. 2016; Wang et al. 2017). In addition, silica deposition results in better leaf architecture, favoring the photosynthetic capacity of plants (Liang et al. 2015). Cucumber exhibits a relatively high capacity for Si accumulation (Sun et al. 2017), and has shown efficient reduction in powdery mildew and anthracnose due to potassium silicate (Gama 2003; Schuerger and Hammer 2003). In a review article, Ma (2004) mentioned that the frequency of target spot in cucumber decreased as Si supply increased; however, there is a lack of experimentation to prove the effect of silicon on the aforementioned pathosystem, either on the pathogen, on the disease or as a resistance inductor. Leaf fertilizer containing Si and potassium improved the efficiency of target spot (*C. cassiicola*) and anthracnose (*Colletotrichum truncatum*) control in soybean plants when associated with fungicides; it also substituted one fungicide application in a total of three applications, besides promoting gains in productivity when employed either alternately or associated with fungicides (Andrade Júnior 2009). Greater accumulation of phenolic compounds and lignin, due to treatment with Si, improved the resistance to damping-off (*Pythium ultimum*) and target spot in cucumber (Cherif et al. 1994) and soybean plants (Fortunato et al. 2015), respectively. Beneficial effects of Si in suppressing infections by *Fusarium oxysporum* f. sp. *cucumerinum, Podosphaera xanthii* and *Pythium* spp. in cucumber were linked to increased activity of the enzymes peroxidases and/or polyphenol oxidases (Cherif et al. 1994; Liang et al. 2005; Sun et al. 2022).

Studies investigating foliar application of Si have predominantly used potassium silicate, which is a soluble source (Prado 2021). It is important to consider new sources that include other nutrients capable of inducing synergy with Si, as is the case for Cu. However, such a combination can induce Si polymerization, which in turn can reduce both absorption and beneficial effects during the disease suppression. Therefore, if the performance of this alternative source of potassium silicate including Cu is similar to that of the conventional source in mitigating diseases, even after subsequent application to balance Cu, the former may emerge as a new alternative Si source for use in agriculture to reduce the occurrence of diseases.

Research on the alternative management of target spot in cucumber plants is scarce, while studies aiming at disease management strategies are needed and important for the economic viability of the crop. The hypothesis in the present study is that silicon application may constitute an effective alternative to reduce the effects of target spot, changing the plant's biochemical activities. Thus, the present study aimed to evaluate Si doses using two silicates on *C. cassiicola in vitro* and target spot control under controlled growth conditions, as well as to verify the relationship between Si and the plant's biochemical changes.

Material and Methods

In Vitro Sensitivity of *Corynespora cassiicola* to Silicates

Corynespora cassiicola was isolated from a diseased cucumber crop, located in the municipality of Avaí, São Paulo State (SP), Brazil, coordinates 22°030 12.100 S, 49°150 31.500 W. The isolate is preserved (MMBF 01/20) at the Fungal Collection "Mário Barreto Figueiredo" of the Agency for Agribusiness Technology (APTA), São Paulo Biological Institute, Brazil.

Germination and mycelial growth of the pathogen were evaluated for two silicates, one of which is in experimental phase and contains 13.4 g L^{-1} Cu, 14.1 g L^{-1} K_2O and 120.9 g L^{-1} Si, while the other one is the commercial potassium silicate, containing 137 g L^{-1} Si and 164.4 g L^{-1} K_2O; both were tested at five Si concentrations (0, 28, 56, 84 and 112 mg L^{-1}). The other chemical elements, such as potassium and copper, present in the tested products, had their concentration adjusted to 0.223 g L^{-1} K_2O (KCl: 60% K_2O) and 0.0355 g L^{-1} Cu ($CuSO_4$: 35% Cu) for all treatments, so that the only variation was the applied Si.

Assessments were carried out in potato-dextrose-agar (PDA) medium for mycelial growth and agar-water (AW) medium for germination. The products were added to AW or melting PDA, at 45–50ºC, and pH was adjusted to 7.0 with acetic acid. Then, 20 mL of each medium were poured into a 90 mm-diameter Petri dish (Fischer et al. 2022).

For mycelial growth evaluation, after ten-day growth in PDA, at 25 °C, a 5-mm mycelium disc was transferred to the center of the plate containing the tested products. The radial perpendicular diameter of colonies was measured when the pathogen growth in the control treatment almost reached the whole plate (Fischer et al. 2022). The inhibition percentage

(I %) of treatments, compared to the control, was calculated by the formula:

$$I\ \% = (\text{colony diameter in the control treatment} - \text{colony diameter in the treatment with products}) \times 100/(\text{colony diameter in the control treatment})$$

To evaluate conidial germination, 100 µL spore suspension (10^4 conidia mL^{-1}) was spread onto the surface of the AW medium. Germination was assessed after 12 h incubation of plates in the dark at 25 °C. A conidium showing germ tube length equal or superior to that of the conidium was considered germinated (Teramoto et al. 2013). For each plate, 100 conidia were evaluated to determine the I% of treatments, compared to control.

A completely randomized factorial design was adopted for two Si-based products, five Si concentrations, and three replicates for each plot, which was represented by one Petri dish. Experiments were performed twice, followed by a joint analysis. For statistical analysis, germination and mycelial growth data were compared between products and concentrations, according to Tukey's test at 5% probability.

Target Spot Control Under Greenhouse

Experiments were conducted under greenhouse conditions and controlled maximum temperature of 30 °C from May to August 2020. Cucumber plants of Soldier variety were grown in 5 L plastic pots (one plant per pot) containing substrate composed of Sphagnum peat moss, expanded vermiculite and dolomite limestone (pH: 5.5, electrical conductivity: 0.2 mS cm^{-1}, density: 130 Kg m^{-3} and water retention capacity: 60%).

From the stage of two true leaves (17 days after sowing), plants were sprayed until runoff using the same products and doses evaluated *in vitro* plus one inoculated control treatment. Since the composition of products includes other chemical elements, such as copper and potassium, fertilization was carried out with 0.223 g L^{-1} K_2O (KCl: 60% K_2O) and 0.0355 g L^{-1} Cu ($CuSO_4$: 35% Cu) in a second spraying, right after the leaves have dried from the first application, so that the only variation was the applied Si. The pH of the solution was adjusted to 7.0 with acetic acid.

Foliar application was performed at above 60% relative humidity and temperature below 28 °C. During spraying, the substrate in the pots was covered with paper to prevent root absorption and ensure Si supply was only through the leaves. Treatments were weekly applied as a preventive measure, totaling three applications.

Irrigation was daily and manually with 100 mL water per pot, while fertilization was carried out twice a week via fertigation with the application of 1 g pot^{-1} formulation containing (%): 15 N, 2.2 P, 8.3 K, 1 Ca, 1 Mg, 13 S, 0.2 Fe, 0.2 Zn, 0.06 B, 0.1 Mn, 0.05 Cu and 0.005 Mo.

The inoculum of *C. cassiicola* was multiplied in Petri plates containing tomato juice medium (Fischer et al. 2022) and incubated for 15 days, at 25 °C, under fluorescent light. The conidial suspension in distilled water had its concentration adjusted to 10^4 conidia mL^{-1} in a Neubauer chamber. Plants were inoculated with the conidial suspension, which was sprayed until runoff on both surfaces of the second and third fully expanded definitive leaves, on the fourth day after the third spraying of treatments. Then, the plants were kept wrapped in a transparent high-density polyethylene bag for 24 h to allow the formation of a humid chamber.

Severity, and number and diameter of lesions were evaluated at three, five and seven days after the pathogen inoculation. Disease severity, expressed as percentage of injured area, and number of lesions were evaluated based on a photographed area of 36 cm^2 (6 × 6 cm) in the central region of the leaf, using ImajeJ software®. The diameter of lesions was measured with a ruler in orthogonal directions; ten individual lesions per leaf were evaluated, considering the largest lesions. The number of spores per leaf was estimated at seven days after the pathogen inoculation. A brush was used to collect the spores into 5 mL water and the concentration was estimated in a Neubauer chamber.

Factorial randomized block design was adopted for two products, five Si concentrations, four replicates and one plant per plot, which consisted of the average of the two inoculated leaves. Since the studied variables showed slight variability, data from both experiments were grouped for statistical analysis. Mean results of the different evaluated parameters were compared by analysis of variance (ANOVA) using the software Systems for Analysis of Variance—SISVAR (Ferreira 2019), and differences among treatments were examined according to Tukey's Range test at 5% probability. To meet residue normality criteria, data on severity and number of spores per leaf were transformed into $\sqrt{X}$.

Biochemical Responses of Silicate-treated Plants Inoculated with *Corynespora cassiicola*

Plants were treated with K_2SiO_3 + Cu and inoculated with *C. cassiicola*, as described in the previous item, from June to August 2020.

Biochemical responses were assessed at 1, 3 and 5 days after inoculation. Proportional samples of the second and third definitive leaves were ground in a mortar and pestle with liquid nitrogen. Then, they were homogenized in potassium phosphate buffer 0.01 M (pH 6.6), at 1:4 ratio (weight/volume), plus polyvinylpyrrolidone at 0.5% (w/v)

(Fischer et al. 2022). Subsequently, the homogenate was centrifuged at 20,000 g for 20 min, at 2 °C, and the supernatant (protein extract) was used to determine both the total protein content, according to the Bradford method (1976), and the activities of the enzymes peroxidases, polyphenol oxidases and β-1,3-glucanases.

Peroxidase activity was determined by converting guaiacol to tetraguaiacol, at 30 °C, according to the direct spectrophotometric method (Lusso and Pascholati 1999). The reaction mixture consisted of 100 µL diluted protein extract and 2.9 mL reaction solution (250 µL guaiacol, 306 µL hydrogen peroxide, and 100 mL potassium phosphate buffer 0.01 M, pH 6.6). The protein extract was diluted twice in the extraction buffer (50 µL extract in 100 µL buffer) to reduce the activity (Fischer et al. 2022). For the reference cuvette, 2.9 mL reaction solution and 100 µL phosphate extraction buffer 0.01 M (pH 6.6) were used. Peroxidase activity was expressed as absorbance unit min^{-1} mg $protein^{-1}$.

Polyphenol oxidase activity was based on the measurement of the oxidation of catechol converted into quinone (Duangmal and Apenten 1999). The enzyme substrate was prepared with 110.1 mg catechol dissolved in 50 mL sodium phosphate buffer 0.01 M (pH 6.0), forming a catechol solution 0.02 M. After 30 min the reaction was carried out by mixing 450 µL substrate and 50 µL protein extract (Fischer et al. 2022). Results were expressed as absorbance units min^{-1} mg $protein^{-1}$.

β-1,3-glucanase activity was determined by colorimetric quantification of glucose released from the laminarin substrate (Kombrink and Hahlbrock 1986). The reaction was prepared with 150 µL laminarin (4 mg mL^{-1}) dissolved in potassium phosphate buffer 0.01 M (pH 6.6) and 100 µL protein extract. After incubation at 40 °C for 2 h, the samples received 125 µL of 3,5-dinitrosalicylic acid (DNS) for the quantification of reducing sugars and were incubated in water bath, at 95 °C, for 5 min, followed by cooling on ice. The volume of samples was completed with water to 1.5 mL (Fischer et al. 2022). Results were expressed as mg glucose h^{-1} mg $protein^{-1}$.

To quantify phenolic compounds, 0.5 g plant tissue was ground in liquid nitrogen; the obtained powder was resuspended in 4 mL methanol 50% and allowed to rest in water bath for 1.5 h at 80 °C. The extract was cooled and centrifuged at 20,000 rpm, for 15 min, at 2 °C. The supernatant was collected to determine the level of free phenols. NaOH 0.5 M (2 mL) was added to the pellet and incubated during approximately 24 h for saponification of phenols bound to the cell wall. The reaction was neutralized with 9.5 mL HCl 2M and the extract was centrifuged at 20,000 rpm, for 15 min, at 2 °C. Supernatant (150 µL, free and wall-bound phenols) received 3 mL Na_2CO_3 2% (m/v) and 150 µL Folin-Ciocalteu reagent diluted in water (1:1 v/v) (Kofalvi and Nassuth 1995; Fischer et al. 2022). Reading was carried out in a spectrophotometer at 750 nm. Phenol concentration was expressed as chlorogenic acid equivalents (mg) per g fresh tissue.

A completely randomized factorial design was adopted for five Si concentrations, three sampling periods and three replicates. Each experimental unit consisted of one plant and each plot was represented by the two true leaves. Mean results of each variable per plot were tested according to analysis of variance (ANOVA) using the software Systems for Analysis of Variance—SISVAR (Ferreira 2019), and the means of treatments were compared according to Tukey's test at 5% significance.

Results and Discussion

In Vitro Control of *Corynespora cassiicola* with Silicates

A significant interaction ($p < 0.01$) was noted between the two silicates and the five tested Si doses for both germination and mycelial growth of *C. cassiicola*. Differences were found between Si doses of K_2SiO_3 + Cu , which promoted greater mycelial growth inhibition at 28 and 56 mg L^{-1}, compared to 112 mg L^{-1}, but did not significantly differ from the silicon-free treatment.

The tested products had a negative ($p < 0.05$) effect on spore germination, causing 24.5–35.8% inhibition; however, there was no significant effect of Si doses for the studied sources. Differences between Si sources were observed for 28 mg L^{-1} and 112 mg L^{-1}, at which

Table 1 Effect of different silicon concentrations using K_2SiO_3 (137.0 g L^{-1} Si + 164.4 g L^{-1} K_2O) and K_2SiO_3 + Cu (120.9 g L^{-1} Si + 14.1 g L^{-1} K_2O + 13.4 g L^{-1} Cu) on *Corynespora* germination and mycelial growth inhibition (%) in cucumber, tested in agar-water and potato-dextrose-agar media, at pH 7.0, respectively

Silicon concentration (mg L^{-1}) [a]	Germination inibition (%)		Mycelial growth inibition (%)	
	K_2SiO_3 + Cu	K_2SiO_3	K_2SiO_3 + Cu	K_2SiO_3
0	29.6 ± 3.0 aA	29.6 ± 3.0 aA	–1.5 ± 0.1 aA	–1.5 ± 0.1 aA[b]
28	35.8 ± 3.4 aA	24.5 ± 3.3 aB	–0.6 ± 0.3 aA	–3.4 ± 0.3 aA
56	26.2 ± 4.7 aA	31.2 ± 5.9 aA	–1.5 ± 0.1 aA	–5.2 ± 0.3 aA
84	26.6 ± 6.1 aA	26.6 ± 3.7 aA	–4.3 ± 0.3 abB	–0.3 ± 0.2 aA
112	34.6 ± 2.8 aA	24.7 ± 4.8 aB	–8.1 ± 0.1 bB	–1.9 ± 0.2 aA

[a]The concentrations of 0.223 g L^{-1} of K_2O and 0.0355 g L^{-1} of Cu were standardized for all treatments
[b]Mean values ± standard deviations. Data followed by the same letter, lowercase in the column and uppercase in the line, did not differ from each other (Tukey, $p < 0.05$)

K_2SiO_3 + Cu led to greater germination inhibition (Table 1). Since Si had no influence on germination, inhibition was possibly due to the addition of copper to the culture medium. Some studies have already shown the effect of Cu on mycelial growth. Copper oxychloride inhibited *C. cassiicola* mycelial growth in soybean from 28.9% at 1500 mg L^{-1} to 41.1% at 2500 mg L^{-1} (Ishwari et al. 2020). In rubber trees, *C. cassiicola* mycelial growth inhibition was 71.2% with 250 mg L^{-1} copper oxychloride and 84.2% with 500 mg L^{-1} fungicide, evidencing the fungicidal effect of Cu on the pathogen (Manju et al. 2019).

Potassium silicate (50 and 100 mg Fertisil®/L), equivalent to 6.21 and 12.43 mg L^{-1} Si, was previously reported to cause 100% *C. cassiicola* mycelial growth inhibition *in vitro*, which showed that the pathogen was highly sensitive to potassium silicate. However, there is no mention of pH adjustment for the adopted culture medium, which could have influenced the results, as already verified in studies with phosphites (Fischer et al. 2022). When not corrected to 7, the pH of treatments containing Si varied between 9 and 10 (data not shown), and pH >9 causes *C. cassiicola* growth inhibition. According to Madhavi and Murthy (2001), *C. cassiicola* spore germination and germ tube growth occurred at pH varying between 3 and 9 and peaked at pH 6. Thus, the best results for mycelial growth and sporulation were obtained within the pH range 6.5–7.5 (Almeida 1977). Stabilized ortho silicic acid (Silixol™ OSA), a bio available form of silicon, inhibited 91% mycelial growth of *C. cassiicola* from tomato, at a concentration of 1 ml L^{-1}, and may be a control alternative (Sharma et al. 2021). Silicon, as sodium silicate (83.3 mg L^{-1} Si), showed 23% *in vitro* inhibition of the mycelial growth of *Fusarium oxysporum* f. sp. *cucumerinum*, the causal agent of cucumber fusarium wilt (Sun et al. 2022).

Target Spot Control

There were no differences in disease severity due to the used sources. However, when compared to control, Si application at 56 and 84 mg L^{-1} reduced the severity at five days after inoculation (Table 2), indicating that employing Si could be an efficient and economic strategy in the management of the studied disease.

The conventional source of potassium silicate and its alternative source containing Cu had similar performance in mitigating the disease severity, even after subsequent application to balance Cu. This suggests that the combined use of Si/Cu in the same mixture could benefit the crop and consequently the farmer, who can save product since there is no need for concentrations higher than 56 mg L^{-1} Si.

Both Si sources reduced the number of target spot lesions at five and seven days after the pathogen inoculation (Table 3): K_2SiO_3 + Cu at the four tested doses at five days after inoculation, compared to the inoculated control, and at 56 mg L^{-1} Si at seven days after inoculation, and K_2SiO_3: at 56 and 112 mg L^{-1} Si at five days and at 84 and 112 mg L^{-1} Si at seven days after inoculation (Table 3).

In general, no differences were observed between the tested Si sources, except for K_2SiO_3 + Cu at 28 mg L^{-1} after five days of inoculation and K_2SiO_3 at 84 mg L^{-1} after seven days of inoculation , both of which showed smaller number of lesions. For the silicon-free treatment, the number of lesions was smaller after seven days of inoculation, compared to the inoculated control, which suggests that the fertilizer KCl + $CuSO_4$ can also affect the occurrence of the studied disease (Table 3). Regarding the diameter of target spot lesions, no differences were observed among treatments or between Si sources (Table 4).

Application of copper and potassium silicate at different doses reduced *C. cassiicola* spore production per cm^2 cucumber leaf, compared to the inoculated control. This

Table 2 Severity (%) of target spot on cucumber leaves at three, five and seven days after inoculation (dai) in plants preventively subjected to three weekly applications of different silicon concentrations using K_2SiO_3 (137.0 g L^{-1} Si + 164.4 g L^{-1} de K_2O) and K_2SiO_3 + Cu (120.9 g L^{-1} Si + 14.1 g L^{-1} de K_2O + 13.4 g L^{-1} Cu), under greenhouse conditions

Silicon Concentration (mg L^{-1}) [a]	Severity (%) of target spot					
	3 dai		5 dai		7 dai	
	K_2SiO_3 + Cu	K_2SiO_3	K_2SiO_3 + Cu	K_2SiO_3	K_2SiO_3 + Cu	K_2SiO_3
Inoculated control	2.7 ± 0.4 a	2.7 ± 0.4 a	36.8 ± 8.3 a	36.8 ± 8.3 a	55.8 ± 2.8 a	55.8 ± 2.8 a[b]
0	3.0 ± 0.3 a	3.0 ± 0.3 a	30.9 ± 7.6 ab	30.9 ± 7.6 ab	45.5 ± 8.9 a	45.5 ± 8.9 a
28	3.1 ± 0.3 aA	2.6 ± 0.4 aA	25.3 ± 4.8 abA	29.7 ± 2.3 abA	49.3 ± 7.0 aA	50.0 ± 3.9 aA
56	3.0 ± 0.4 aA	2.4 ± 0.3 aA	20.5 ± 7.5 bA	22.0 ± 5.5 bA	46.8 ± 7.3 aA	48.4 ± 5.3 aA
84	2.8 ± 0.1 aA	2.6 ± 0.2 aA	17.4 ± 8.5 bA	22.6 5.5 bA	44.4 ± 8.6 aA	44.8 ± 5.5 aA
112	2.5 ± 0.3 aA	2.8 ± 0.3 aA	25.8 ± 5.6 abA	27.8 ± 9.1 abA	51.1 ± 3.2 aA	47.0 ± 7.0 aA

[a]The concentrations of 0.223 g L^{-1} of K_2O and 0.0355 g L^{-1} of Cu were standardized for all treatments, with the exception of the inoculated control

[b]Mean values ± standard deviations. Data followed by the same letter at each inoculation time, lowercase in the column and uppercase in the row, do not differ from each other (Tukey, $p < 0.05$). Statistical analysis with data transformed into root x

Table 3 Number of target spot lesions on cucumber leaves at three, five and seven days after inoculation (dai) in plants preventively subjected to three weekly applications of different silicon concentrations using K_2SiO_3 (137.0 g L^{-1} Si + 164.4 g L^{-1} de K_2O) and K_2SiO_3 + Cu (120.9 g L^{-1} Si + 14.1 g L^{-1} de K_2O + 13.4 g L^{-1} Cu), under greenhouse conditions

Silicon Concentration (mg L^{-1}) [a]	Number of target spot lesions					
	3 dai		5 dai		7 dai	
	K_2SiO_3 + Cu	K_2SiO_3	K_2SiO_3 + Cu	K_2SiO_3	K_2SiO_3 + Cu	K_2SiO_3
Inoculated control	28.3 ± 11.3 a	28.3 ± 11.3 a	127.0 ± 7.0 a	127.0 ± 7.0 c	232.7 ± 43.4 a	232.7 ± 43.4 a[b]
0	38.8 ± 10.0 a	38.8 ± 10.0 a	102.8 ± 31.8 ab	102.8 ± 31.8 abc	131.7 ± 17.2 c	131.7 ± 17.2 b
28	30.5 ± 4.1 aA	27.8 ± 7.5 aA	76.3 ± 25.4 bB	106.5 ± 21.4 bcA	181.3 ± 32.0 abcA	205.7 ± 27.5 abA
56	37.3 ± 6.0 aA	28.0 ± 9.4 aA	75.5 ± 7.9 bA	61.8 ± 9.0 abA	148.3 ± 31.7 bcA	175.7 ± 40.5 abA
84	32.3 ± 1.3 aA	24.3 ± 6.1 aA	59.8 ± 3.8 bA	81.0 ± 23.0 abcA	216.3 ± 31.7 abA	145.7 ± 10.3 bB
112	26.3 ± 12.9 aA	33.3 ± 5.7 aA	66.3 ± 11.4 bA	57.5 ± 13.5 aA	158.0 ± 42.9 abcA	141.7 ± 38.6 bA

[a]The concentrations of 0.223 g L^{-1} of K_2O and 0.0355 g L^{-1} of Cu were standardized for all treatments, with the exception of the inoculated control

[b]Mean values ± standard deviations. Data followed by the same letter at each inoculation time, lowercase in the column and uppercase in the row, do not differ from each other (Tukey, $p < 0.05$)

Table 4 Diameter (cm) of target spot lesion on cucumber leaves at three, five and seven days after inoculation (dai) in plants preventively subjected to three weekly applications of different silicon concentrations using K_2SiO_3 (137.0 g L^{-1} Si + 164.4 g L^{-1} de K_2O) and K_2SiO_3 + Cu (120.9 g L^{-1} Si + 14.1 g L^{-1} de K_2O + 13.4 g L^{-1} Cu), under greenhouse conditions

Silicon Concentration (mg L^{-1}) [a]	Diameter (cm) of target spot lesion					
	3 dai		5 dai		7 dai	
	K_2SiO_3 + Cu	K_2SiO_3	K_2SiO_3 + Cu	K_2SiO_3	K_2SiO_3 + Cu	K_2SiO_3
Inoculated control	0.16 ± 0.01 a	0.16 ± 0.01 a	0.34 ± 0.01 a	0.34 ± 0.01 a	0.42 ± 0.02a	0.42 ± 0.02 a[b]
0	0.16 ± 0.02 a	0.16 ± 0.02 a	0.32 ± 0.02 a	0.32 ± 0.02 a	0.41 ± 0.02 a	0.41 ± 0.02 a
28	0.17 ± 0.02 aA	0.15 ± 0.01 aA	0.34 ± 0.02 aA	0.33 ± 0.01 aA	0.40 ± 0.01 aA	0.40 ± 0.03 aA
56	0.15 ± 0.02 aA	0.16 ± 0.02 aA	0.33 ± 0.01 aA	0.34 ± 0.01 aA	0.40 ± 0.02 aA	0.40 ± 0.03 aA
84	0.15 ± 0.01 aA	0.14 ± 0.01 aA	0.33 ± 0.02 aA	0.33 ± 0.02 aA	0.38 ± 0.01 aA	0.39 ± 0.02 aA
112	0.15 ± 0.00 aA	0.15 ± 0.00 aA	0.33 ± 0.02 aA	0.33 ± 0.03 aA	0.40 ± 0.04 aA	0.40 ± 0.02 aA

[a]The concentrations of 0.223 g L^{-1} of K_2O and 0.0355 g L^{-1} of Cu were standardized for all treatments, with the exception of the inoculated control

[b]Mean values ± standard deviations. Data followed by the same letter at each inoculation time, lowercase in the column and uppercase in the row, do not differ from each other (Tukey, $p < 0.05$)

indicates that there was no significant difference between Si sources or concentrations. However, considering that the silicon-free treatment decreased the number of spores in relation to the inoculated control, KCl + $CuSO_4$ may also have affected spore production (Table 5).

Besides affecting sporulation, the silicon-free treatment containing KCl + $CuSO_4$ also influenced pathogen germination (Table 1), number of lesions (Table 3) and, less evidently, severity (Table 2). Five applications of copper oxychloride reduced the diseased leaf area by 50% in cucumber plants inoculated with *C. cassiicola* (Castro 1979). For soybean target spot, preventive application of copper oxychloride was efficient in reducing severity at the end of evaluations, while copper oxychloride + azoxystrobin + cyproconazole was the treatment that most suppressed the disease development over the experiment, suggesting that the association between protective and systemic fungicides can be a viable practice to control this disease (Souza and Vidal 2018).

Even though the reduction in target spot symptoms due to Si applications was not very evident, differences in severity and number of lesions were significant on the fifth day after inoculation. However, the treatments with KCl + $CuSO_4$ also contributed to decreasing sporulation in cucumber leaves, thus decreasing the pathogen inoculum, which is important for the disease progress. The concentration of *C. cassiicola* aerospores was positively associated with cucumber target spot severity (Zhao et al. 2021). Moreover, the sources potassium silicate plus Cu and potassium silicate showed similar behavior in reducing sporulation, which suggests that using Si/Cu in the same mixture does not affect the performance, compared to Si alone. Silicon accumulation was found in the leaves of cucumber plants sprayed with 1 g L^{-1} Si , which constituted a physical barrier preventing the growth of *S. fuliginea* hyphae (Samuels et al. 1991). In other studies, Si reduced powdery mildew severity by decreasing the number and size of colonies of the pathogen, as well as the conidial germination frequency,

Table 5 *Corynespora cassiicola* spores produced per cm^2 in cucumber leaves at seven days after inoculation of the pathogen in plants preventively subjected to three weekly applications of different silicon concentrations using K_2SiO_3 (137.0 g L^{-1} Si + 164.4 g L^{-1} de K_2O) and K_2SiO_3 + Cu (120.9 g L^{-1} Si + 14.1 g L^{-1} de K_2O + 13.4 g L^{-1} Cu), under greenhouse conditions

Silicon concentration (mg L^{-1}) [a]	*C. cassiicola* spores per cm^2 cucumber leaf	
	K_2SiO_3+Cu	K_2SiO_3
Inoculated control	937.5 ± 241.9 a	937.5 ± 241.9 a[b]
0	279.4 ± 117.1 b	279.4 ± 117.1 b
28	135.0 ± 20.4 bA	211.9 ± 68.0 bA
56	312.5 ± 101.4 bA	244.7 ± 72.4 bA
84	131.2 ± 77.1 bA	365.9 ± 129.8 bA
112	391.6 ± 132.1 bA	279.4 ± 116.6 bA

[a]The concentrations of 0.223 g L^{-1} of K_2O and 0.0355 g L^{-1} of Cu were standardized for all treatments, with the exception of the inoculated control

[b]Mean values ± standard deviations. Data followed by the same letter, lowercase in the column and uppercase in the row, do not differ from each other (Tukey, $p < 0.05$). Statistical analysis with data transformed into root x

and the number of haustoria per colony (Menzies et al. 1991a, b). In addition, greater disease control was observed at 20 °C, compared to 25–30 °C; according to Schuerger and Hammer (2003), temperature was found to act in a synergistic manner with silicon. Besides physical barriers, osmotic effects due to Si application via leaves are also linked to the greater resistance of cucumber plants to powdery mildew, while Si application via the roots can induce systemic resistance (Liang et al. 2005).

Si-induced resistance in cucumber plants was also reported against root rot, caused by *Pythium ultimum* (Cherif et al. 1994), and fusarium wilt (Safari et al. 2012; Zhou et al. 2018), which evidences that Si can become a useful alternative for disease management in this crop. In addition to such a direct effect on the pathogen, resistance to fusarium wilt after application of silicon was associated with improved antioxidant system, photosynthetic capacity, and stomatal movement in cucumber leaves (Sun et al. 2022). Soybean leaf fertilization with potassium silicate, alternated with fungicide application, was efficient in controlling target spot, Asian rust and anthracnose; it also provided productivity gains in relation to control (Andrade Júnior 2009).

Biochemical Responses of Silicon-treated Plants

Differences ($p < 0.01$) were found among seven treatments and among three evaluation times for enzymes and free phenolic compounds in cucumber plants inoculated or not with *C. cassiicola* and preventively receiving three weekly applications of different Si doses (Figs. 1 and 2). Interaction ($p < 0.01$) between treatments and times were noted for the enzymes peroxidase and polyphenol oxidase (Fig. 1a, b).

Peroxidase had increased activity at three and five days after the pathogen inoculation (Fig. 1a); polyphenol oxidase had lower activity after three days of inoculation (Fig. 1b), while β-1,3-glucanase and free and bound phenolic compounds showed no differences (Fig. 1c and 2), compared to the inoculated and non-inoculated controls. Higher peroxidase activity after pathogen inoculation was already found in a previous study using phosphites and chitosan in cucumber plants (Fischer et al. 2022); however, increased bound phenolic compounds, as reported by Fischer et al. (2022), was not detected in the present study (Fig. 1a).

A plant affected by target spot may present significantly increased peroxidase activity as a natural response to infection. This can lead to accumulation of oxidizing compounds like hydrogen peroxide, which are capable of eliminating pathogens or indicating other plant defense disorders (Fischer et al. 2022). Peroxidase is involved in diverse biological processes like polysaccharide bonding reactions, indole-3-acetic acid oxidation, monomer bonds, lignification, lesion healing, phenol oxidation, pathogen defense, and cell elongation regulation (Gaspar et al. 1982; Kao 2003).

Phenolic compounds can be involved in biochemical and structural mechanisms of plant resistance to pathogens (Nicholson and Hammerschmidt 1992). According to Vidhyasekaran (1988), resistance to diseases is associated with several phenolic substances such as chlorogenic acid, protocatechuic acid and catechol, phloridzin and arbutin (phenolic glycosides).

For inoculated plants, the different Si doses had no influence on peroxidase activity, which progressively increased with the inoculation time: it was higher at three and five days than at one day after inoculation for silicon-free treatment, whereas the inoculated control only showed superior peroxidase activity at five days after inoculation (Fig. 1a). According to Schneider and Ullrich (1994) and Carlos et al. (2010), some resistance inducers in cucumbers do not raise peroxidase level at the magnitude of the induced protection; this was noted for plants treated with potassium silicate and the phosphites Mn, Zn and K (Fischer et al. 2022).

Di Piero and Pascholati (2004) observed that several biotic and abiotic inducers increased the activity of peroxidases and β-1,3-glucanases in cucumber plants, but no relationship was found between the increased activity of a particular enzyme and the induced resistance to anthracnose

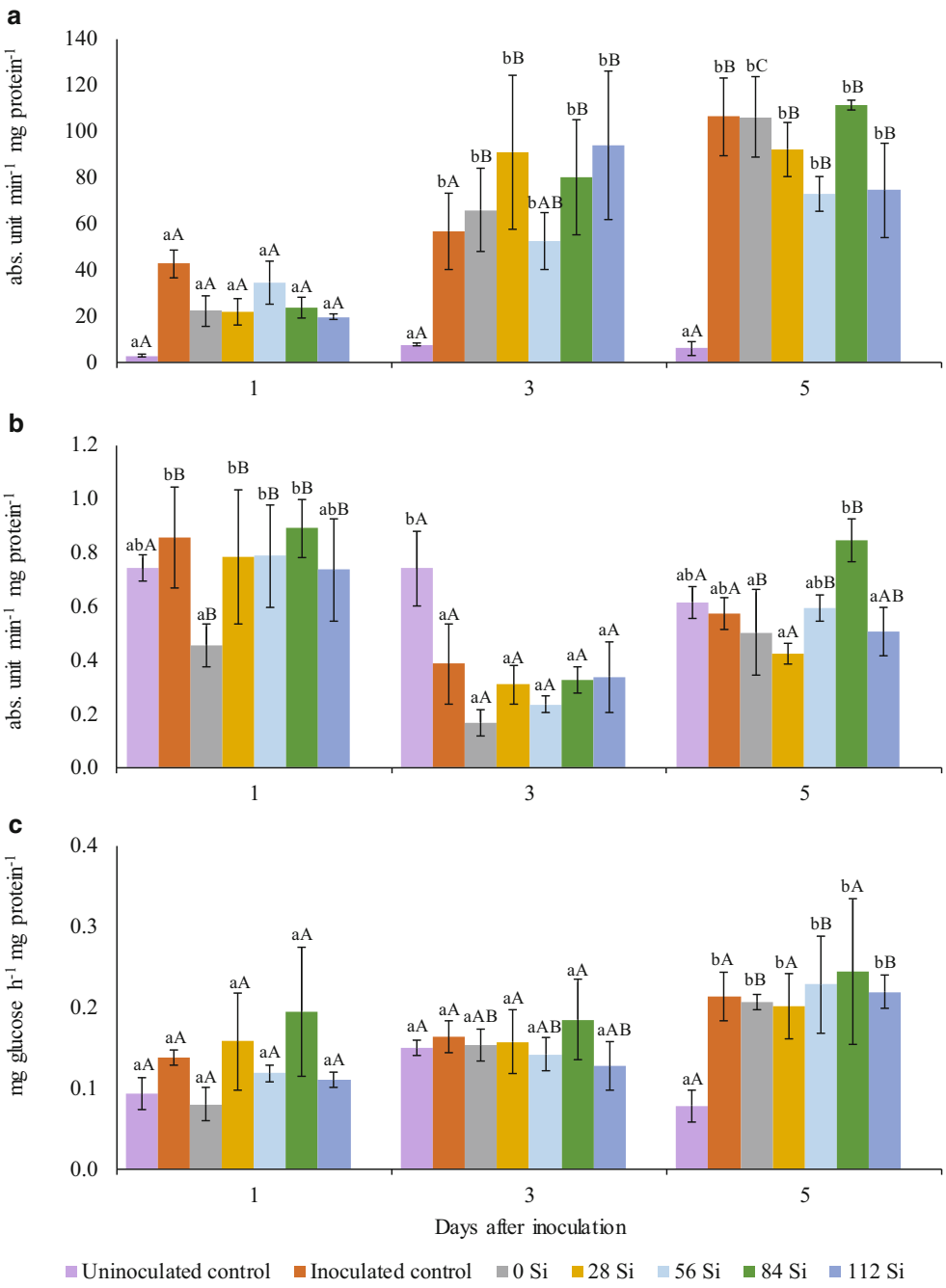

Fig. 1 Activity of peroxidase (**a**), polyphenol oxidase (**b**) and β-1,3-glucanase (**c**) enzymes in cucumber plants preventively submitted to three weekly applications of different silicon concentrations (mg L^{-1}) using K_2SiO_3 + Cu (120.9 g L^{-1} Si + 14.1 g L^{-1} de K_2O + 13.4 g L^{-1} Cu), under greenhouse conditions, at one, three and five days after inoculation or not with *Corynespora cassiicola*. Columns with the same letter, *lower* case comparing the silicon concentrations on each day and *upper* case comparing the days for each silicon concentration, do not differ from each other (Tukey, $p < 0.05$). *Bars* correspond to the standard deviations

(*Colletotrichum lagenarium*), which reinforces the hypothesis that resistance is a multicomponent process. Comparable to the present results, a marked increase was found for peroxidase activity in cucumber leaves at six days after *C. lagenarium* inoculation, while such an increase occurred already on the third day with the resistance inducer *Lentinula edoles*, regardless of pathogen inoculation (Di Piero and Pascholati 2004).

Most treatments had no effect on polyphenol oxidase activity, which was only lower for the silicon-free treatment at one day after inoculation, compared to the inoculated control. Considering the inoculation time, in general, polyphenol oxidase activity was lower at three days after inoculation, regardless of the applied treatments (Fig. 1b).

β-1,3-glucanase was not affected by the Si doses relative to the inoculated control, since increased activity was only observed at 84 mg L^{-1}, compared to the non-inoculated control. In general, all treatments had higher β-1,3-glucanase activity at five days after inoculation (Fig. 1c).

The activity of free (Fig. 2a) and bound (Fig. 2b) phenolic compounds was not significantly influenced by the tested Si doses, compared to the inoculated control, but the

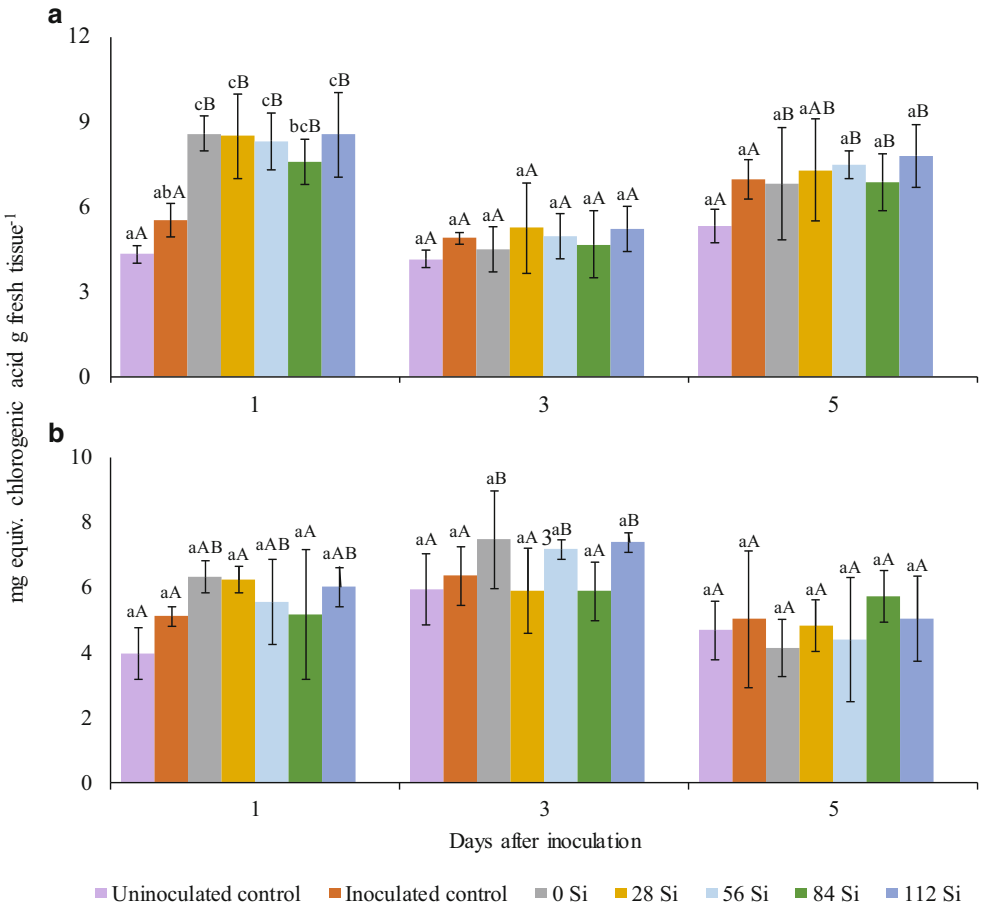

Fig. 2 Activity of free (**a**) and bound (**b**) phenolic compounds in cucumber plants preventively subjected to three weekly applications of different silicon concentrations (mg L^{-1}) using K_2SiO_3 + Cu (120.9 g L^{-1} Si + 14.1 g L^{-1} de K_2O + 13.4 g L^{-1} Cu), under greenhouse conditions, at one, three and five days after inoculation or not with *Corynespora cassiicola.* Columns with the same letter, *lower* case comparing the silicon concentrations on each day and *upper* case comparing the days for each silicon concentration, do not differ from each other (Tukey, $p < 0.05$). *Bars* correspond to the standard deviations

activity of free phenolic compounds was superior to that of the non-inoculated control. Presence and synthesis of phenolic compounds in the plants in response to infection is associated with resistance (Safari et al. 2012). In general, at three days after the pathogen inoculation, free phenolic compounds had lower activity and bound phenolic compounds had higher activity (Fig. 2).

Silicon supply can increase the total concentration of soluble phenolic compounds in host plants and improve the plant resistance by delaying pathogen growth (Dallagnol et al. 2011; Fortunato et al. 2015). Similarly, flavonoids are Si-induced phenolic compounds that have enhanced the resistance of cucumber to *S. fuliginea* (Fawe et al. 1998), of rose hips to *Podosphaera pannosa* (Shetty et al. 2012) and of wheat to *Pyricularia oryzae* (Silva et al. 2015).

Recently, there has been an increasing interest in using Si to induce plant defense mechanisms in response to attacks by fungal pathogens, since it influences the degree of plant resistance to biotic or abiotic stress (Ma 2004; Ranjan et al. 2021). Silicon has long been known to play an important role in plant resistance against pathogens (Datnoff et al. 2002; Cai et al. 2008, 2009). The beneficial effects of Si to plant resistance are attributed to: its accumulation on epidermal tissue as silica; formation of complexes with organic compounds in cell walls; induction of phenolic compounds; reduction in phytoalexin/glucanase/peroxidase, and regulation of stress-related pathogenicity or gene expression to limit pathogen invasion and colonization (Brunings et al. 2009; Chain et al. 2009; Vivancos et al. 2015; Sakr 2016; Wang et al. 2017; Ahammed and Yang 2021; Ruonan et al. 2022).

Spraying silicates on *C. cassiicola* inoculated plants had little influence on enzymatic activities or phenolic compound levels in the present study; however, there were increases in β-1.3-glucanase, at 84 mg L^{-1} Si, and free phenolic compounds in relation to the non-inoculated control. For the pathosystem cucumber x *Pythium ultimum*, Si application via fertigation resulted in rapid activation of peroxidases and polyphenol oxidases and in greater accumulation of phenol derivatives after infection , while β-1,3-glucanase activity did not differ (Cherif et al. 1994). β-1,3-glucanase and peroxidase activity, as well as phenolic compound levels, increased in plants inoculated with *F. oxysporum* and treated with sodium silicate , evidencing that the disease control was a consequence of resistance induction against the pathogen (Safari et al. 2012; Zhou et al. 2018; Sun et al. 2022).

Conclusion

Silicon had no direct effect on *C. cassiicola*, not inhibiting its mycelial growth and germination. It did not influence the biochemical responses of plants either, expressed by enzymatic activities and phenolic compound levels, and, in general, there were no differences between the two tested silicates. Although silicon did not reduce the diameter of target spot lesions and pathogen sporulation on cucumber leaves preventively sprayed with $K_2SiO_3 + Cu$ or K_2SiO_3, at concentrations ranging from 28 to 112 mg L^{-1} Si, some punctual reduction in disease severity or number of lesions was verified, depending on the Si concentration or the day of disease assessment. Such results demonstrate the possible viability of using Si to control cucumber target spot; nevertheless, additional studies are needed to evaluate crop productivity and disease management, including soil silicon applications and other silicate formulations, such as ortho silicic acid and silica nanoparticles, as well as product mixtures, with the aim of finding a possible synergistic effect in controlling the disease.

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Conflict of interest I.H. Fischer, J.V.P. Morales, L.M. da Silva, R.M. de Almeida Bertani, A.C.F. Deus, R. de Mello Prado and S.F. Pascholati declare that they have no competing interests.

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