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Impact of silicon application on downy mildew severity in melon plants during the rainy season

Impacto da aplicação de silício na severidade de míldio em meloeiro durante período chuvoso

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ABSTRACT - Downy mildew is one of the main foliar diseases affecting melon plants during the rainy season in the Northeast region of Brazil. Silicon (Si) application has emerged as an alternative method for pathogen control, forming physical barriers and activating defense mechanisms in plants. The objective of this study was to reduce the severity of downy mildew in melon plants during the rainy season through foliar application of Si. The treatments consisted of five rates (0, 0.25, 0.5, 1, and 2 L ha⁻¹) of potassium silicate (12% Si and 15% potassium) applied from May to July 2022, with four replications. Disease severity was assessed using rating scales, chlorophyll contents, and transient chlorophyll *a* fluorescence (OJIP). The rate of 2 L ha⁻¹ resulted in the best results by delaying pathogen development in leaves with disease incidence. Disease severity decreased to 68.27% and chlorophyll a, b, and total increased by 8.21%, 13.86%, and 9.72%, respectively. Si application resulted in beneficial changes in the following OJIP test parameters: ABS/RC, TR0/RC, ET0/RC, ABS/CS0, and TR0/CS0. During periods of high rainfall intensity and mild temperatures, Si application to melon plants reduces downy mildew severity and protects chlorophylls, enhancing the absorption flux (ABS) and electron storage (TR0) and transport (ET0).

RESUMO - O míldio é umas das principais doenças foliares que acomete o meloeiro durante o período chuvoso na região Nordeste do Brasil. O silício (Si) tem se destacado como um método alternativo para o controle de patógenos tanto por constituir barreira físicas, como na ativação de mecanismos de defesa da planta. Assim, objetivou-se com este trabalho avaliar a severidade de míldio em meloeiro durante período chuvoso com aplicações foliares de Si. Durante as chuvas, nos meses de maio a julho de 2022 realizaram-se duas pulverizações de silicato de potássio (12% de Si e 15% de potássio) nas doses de 0; 0,25; 0,50; 1,0 e 2,0 L ha⁻¹, com quatro repetições por dose. Realizaram-se avaliações da severidade do míldio em escalas de notas, índices de clorofila e fluorescência transiente da clorofila a (OJIP). A dose de 2,0 L ha⁻¹ proporcionou melhores resultados retardando o desenvolvimento do patógeno em folhas com incidência da doenca. Houve diminuição na severidade da doença de até 68,27%, além do aumento nos índices de clorofila a, b e total, em 8,21%, 13,86% e 9,72%, respectivamente. Além disso, o Si promoveu alterações benéficas nos parâmetros do teste OJIP: ABS/RC; TR0/RC; ET0/RC; ABS/CS0 e TR0/CS0. Durante períodos de alta intensidade de chuvas e temperaturas amenas, a aplicação de Si em meloeiro reduz a severidade do míldio e protege as clorofilas, resultando em maior absorção (ABS), armazenamento (TR0) e transporte de elétrons (ET0).

Keywords: Cucumis melo L. Pseudoperonospora cubensis. Biotic stress. Phytosanitary. Potassium silicate.

Palavras-chave: *Cucumis melo* L. *Pseudoperonospora cubensis*. Estresse biótico. Fitossanidade. Silicato de potássio.

Conflict of interest: The authors declare no conflict of interest related to the publication of this manuscript.



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INTRODUCTION

Melon (*Cucumis melo* L.) crops have significant economic and social importance in Brazil, with a prominent position among global producers (MOREIRA et al., 2022). Melon is produced in all regions of Brazil, with a production value exceeding R\$ 877 million; however, over 97% of the production is concentrated in the Northeast region, particularly in the states of Rio Grande do Norte and Ceara (IBGE, 2022).

Moreover, phytosanitary issues have limited melon production in this region during the rainy season (CÂMARA et al., 2007). Downy mildew is a disease caused by the fungus *Pseudoperonospora cubensis* [(Berkeley & Curtis) Rostovzev, 1903], which stands out in the Northeast region of Brazil as one of the main foliar diseases affecting melon crops during the rainy season (ALBUQUERQUE et al., 2015). This is primarily due to high relative air humidity and free moisture on the leaves, which reduce crop viability during this season (RHOUMA et al., 2022). High downy mildew severity results in decreased fruit quality, with a reduction of up to 49% in total soluble solids (CARDOSO;



SANTOS; VIDAL, 2002). Several agricultural pesticides have been used to control this pathogen, including metalaxyl and mancozeb, but populations of *P. cubensis* resistant to these fungicides have emerged (SAVORY et al., 2011). Thus, the use of alternative disease control methods has significantly increased to manage pathogen resistance and reduce environmental contamination (ADHIKARI et al., 2020).

Silicon (Si) application has emerged as an alternative for pathogen control, as it has reduced the severity of fungal diseases in various crops, including corn, common bean, tomato, and cucumber (SAKR, 2016). In this context, two hypotheses have been raised regarding the mode of action of Si in reducing the severity of fungal diseases. The first is that the Si deposition on leaves forms a physical barrier that prevents pathogen penetration, and the second is that it promotes the expression of natural defense mechanisms in plants, including increases in the activity of defense-related enzymes in leaves (SOURI et al., 2021).

Si application provides protection for chlorophyll (POZZA; POZZA; BOTELHO, 2015), as it results in beneficial changes in chlorophyll *a* fluorescence parameters in infected plants (BEREZOVSKA et al., 2021). Chlorophyll *a* fluorescence has been an effective and well-established tool for assessing disease effects on cultivated plants, allowing qualitative and quantitative descriptions of light energy absorption and utilization by the photosynthetic apparatus (PÉREZ-BUENO; PINEDA; BARÓN, 2019). Furthermore, protection of the photosynthetic apparatus in melon plants under stress conditions has been well-documented (FAN et al., 2019).

Considering the economic importance of melon crops in Brazil, the approaches to phytosanitary issues, and the benefits of Si application, the objective of this study was to reduce downy mildew severity in melon plants during the rainy season through foliar application of Si.

MATERIALS AND METHODS

The study was conducted from May to July 2022 at the Experimental Farm of the Rural Campus of the Universidade Federal de Sergipe (SE), in Sao Cristovao, SE, Brazil (10° 55'27"S, 37°12'01"W, and altitude of 46 meters). The region's climate was classified as tropical rainy, with an average annual air temperature of 25.2 °C (SANTOS et al., 2009). The soil of the region was classified as Typic Hapludult (Argissolo Vermelho-Amarelo; SANTOS et a., 2018), typical of plains in Brazil.

Seeds of a hybrid melon cultivar (Pingo de Mel), naturally infected with downy mildew, were sown in the field, using three seeds per hole, at a depth of 2 cm. After seedling emergence, less vigorous plants were thinned, leaving only one plant per hole. Soil fertilizers were applied according to recommendations for the region (SOBRAL et al, 2007), using 100 kg ha⁻¹ of urea, 600 kg ha⁻¹ of simple superphosphate, and 275 kg ha⁻¹ of potassium chloride at planting, and 112 kg ha⁻¹ of urea as topdressing. The irrigation water depth applied was determined by the reference evapotranspiration method (ETo) and based on the crop coefficient (Kc) (ALLEN et al., 1998) and 100% of the crop evapotranspiration (ETc), using a drip irrigation system. Meteorological data were recorded using an automatic weather station (A-409; National Institute of Meteorology - INMET).

The planting spacing was 0.5 m between plants and 2.0 m between rows. Each experimental plot was 2 m wide and 3 m long, with 6 plants per plot. A randomized complete block experimental design with four replications was used, totaling 20 experimental plots. The treatments consisted of foliar applications of five Si rates, using a commercial product based on potassium silicate (K_2SiO_3 ; SIFOL[®]) composed of 12% Si and 15% potassium, with a pH of 10.96. The product was diluted in 300 L of water and applied at the rates of 0 (control), 0.25, 0.5, 1 (recommended rate), and 2 L ha⁻¹. The solution was mixed with a spreader-sticker (WIL FIX[®]), according to the manufacturer's recommendations, to increase the contact area on the leaf. These Si rates were applied twice, at 30 and 40 days after planting, according to the treatments.

The evaluations were carried out on the four central plants of each experimental plot seven days after the second application of Si rates. Six leaves from the middle third of each plant were randomly selected and analyzed using the diagrammatic scale proposed by Michereff et al. (2009), with modifications. This scale ranged from zero to five, where zero = no symptoms; 1 = fewer than 10 isolated spots on the leaf area; 2 = 11 to 20 isolated spots; 3 = spots and 30% of the leaf area affected; 4 = necrotic spots and 50% of the leaf area affected.

Chlorophyll contents (Chl *a* and Chl *b*) were measured non-destructively using a chlorophyll meter (CFL1030; Falker, Brazil). Total chlorophyll (Chl *a* + Chl *b*) and the Chl *a/b* ratio were calculated. These measurements are based on the light absorbance emitted by diodes at three wavelengths (λ): 635 and 660 nm (red) and 880 nm (infrared); after passing through the leaf, the light is captured by silicon photodiodes, which transmit signals in analog form. The device provides absorbance readings that enable the estimation of chlorophyll *a* and *b* pools.

Information on structural and functional parameters of Photosystems I and II and the electron transport chain was obtained through the analysis of transient chlorophyll *a* fluorescence. The analyses were carried out on leaves after dark adaptation using clips for 30 minutes. A portable fluorescence meter (OS-30p; Opti-Sciences, USA) was used. Transient states were induced by a maximum illumination of 3,000 µmol (photons) $m^{-2} s^{-1}$ with actinic light (60 nm) for 1 second. The irradiation was uniformly applied over a leaf area with diameter of 4 mm.

The fast fluorescence kinetics from initial (F0) to maximum (Fm) fluorescence was measured by the fluorescence emissions of the OJIP curve: O \Box F0 (50µs), K (300µs), J (2ms), I (30ms), and P \Box Fm (maximum fluorescence intensity). The time to maximum fluorescence emission (Fm) and the area above the OJIP curve (A) were also measured. The results of the OJIP curve were used to calculate the following biophysical parameters using the test equations: absorption flux by the reaction centers of photosystem II (PSII) (ABS/RC); electron storage by the PSII reaction centers (TR0/RC); absorption flux per cross-section of PSII (ABS/CS0); and electron storage per cross-section of



PSII (TR0/CS0) (STIRBET et al., 2018).

The obtained data were subjected to analysis of variance using the F-test (p<0.05), and when significant, the means of downy mildew severity and chlorophyll contents were subjected to regression analysis and Tukey' test (p<0.05), using the SISVAR 5.6 program (FERREIRA, 2019). Graphs were plotted using the SigmaPlot 12.5 program (Systat Software, San Jose, EUA).

RESULTS AND DISCUSSION

The accumulated rainfall depth during the experimental period was 339.4 mm, and the maximum and minimum temperatures were 29.9 and 21.3 °C, respectively (Figure 1), favoring the occurrence of downy mildew (RHOUMA et al., 2022).

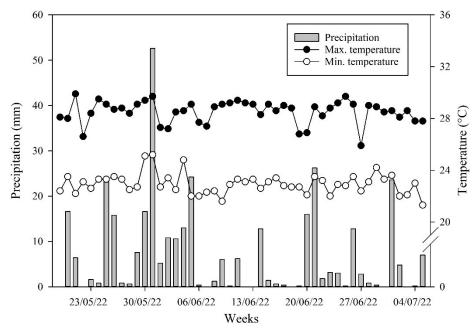


Figure 1. Data of daily rainfall depths and daily maximum and minimum temperatures during the experimental period (May 19, 2022 to July 07, 2022).

Silicon (Si) application reduced disease development, showing a decreasing linear trend as the rate was increased. Rates of 1 and 2 L ha⁻¹ were considered effective in reducing downy mildew severity, differing from the control, with reductions of 37.08% and 68.27%, respectively (Figure 2). Similarly, there was a direct correlation between disease incidence and Si rate. This disease-mitigating effect of Si has been observed in several fungal infections in plants (POZZA; POZZA; BOTELHO, 2015).

The reduction in severity may have occurred due to the presence of a physical barrier formed by Si accumulation, especially in the cell wall, which prevented the mycelium's attempt to colonize the leaf (SAKR, 2016; KIM et al., 2002). Grapevine seedlings subjected to potassium silicate application showed a reduction in penetration of the fungus Uncinula necator: this was also found for the fungus Sphaerotheca fuliginea in pumpkin, cucumber, and melon plants (POZZA; POZZA; BOTELHO, 2015). Si stimulates the action of enzymes related to defense mechanisms against phytopathogenic fungi (AHAMMED; YANG, 2021). An increase in the activity of these enzymes was has been reported as one of the factors that reduced downy mildew severity by up to 60% in cucumber plants (RAMEZANI et al., 2017). Decreases in downy mildew severity due to potassium silicate application probably occur through Si deposition on leaves-forming a physical barrier that prevents pathogen penetration and the expression of natural defense mechanisms (SAKR, 2016).

The main effect of foliar diseases is a reduction in leaf and, consequently, active photosynthetic area area (OLIVEIRA; AUCIQUE-PÉREZ; RODRIGUES, 2019). Thus, the higher downy mildew severity, the lower the photosynthesis, which is related to chlorophyll contents CHATTOPADHYAY; MANDAL, (CHAKRABORTY; 2022) Si application increased or preserved the contents of chlorophyll *a* and *b* and total chlorophyll (Figure 3); however, the chlorophyll a/b ratio was not affected. The chlorophyll acontent showed an increasing linear trend; Si rates of 1 and 2 L ha⁻¹ differed from the control, resulting in increases of 6.78% and 8.21%, respectively. A similar trend was found for the chlorophyll b content, with Si rates of 1 and 2 L ha⁻¹ resulting in increases of 14.49% and 13.86%, respectively, compared to the control. The total chlorophyll content showed an increasing linear trend: Si rates of 1 and 2 L ha⁻¹ differed from the control, resulting in increases of 8.81% and 9.72%, respectively (Figure 3).

Severe downy mildew attacks in cucumbers can decrease chlorophyll *a* and *b* contents by up to 72% and 68%, respectively (RAMEZANI et al., 2017). Si application on garlic and onion plants infected with *Stromatinia cepivora* also resulted in the protection of chlorophyll *a* and *b* (ELSHAHAWY; OSMAN; ABD-EL-KAREEM, 2021). Diseases can induce the overproduction of free radicals, such as hydroxyl radicals, hydrogen peroxide, and superoxide, causing oxidative stress and resulting in chlorophyll degradation (AHAMMED; YANG, 2021).



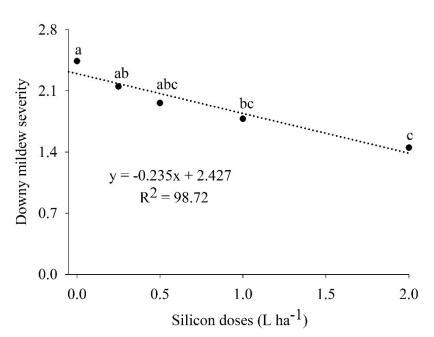


Figure 2. Effects of Si applications on downy mildew severity in melon plants during the rainy season. Means followed by the same letter are not significantly different from each other by the Tukey's test (p < 0.05).

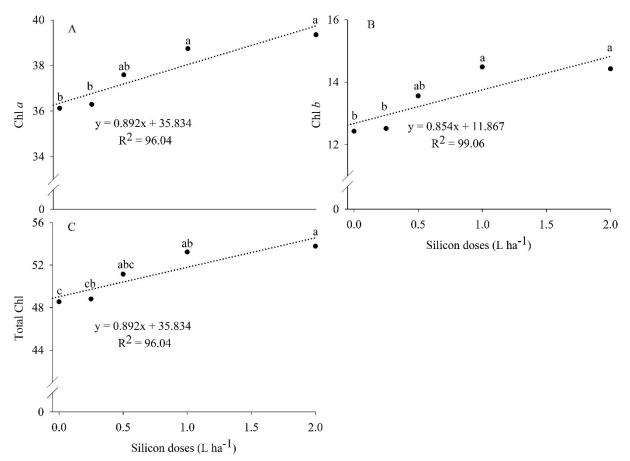


Figure 3. Effects of Si application rates on chlorophyll *a* (Chl *a*) (A), chlorophyll *b* (Chl *b*) (B) and total chlorophyll (total Chl) (C) contents in melon plants during the rainy season. Means followed by the same letter are not significantly different from each other by the Tukey's test (p<0.05).



One of the first impacts of pathogen attacks is observed through the analysis of chlorophyll *a* fluorescence, which is a non-invasive and non-destructive method to estimate the effect of fungal pathogens on the photosynthetic metabolism of host plants (PEREZ-BUENO; PINEDA; BARÓN, 2019). Parameters of transient chlorophyll *a* fluorescence (OJIP) were altered by Si applications to melon plants infected with *P. cubensis* (Figure 4). The Si rate of 2 L ha⁻¹ resulted in the highest values for all parameters, with increases of 95.66%, 51.04%, 37.58%, 81.09%, 20.20%, and 18.73% in ABS/RC, TR0/RC, ET0/RC, ABS/CS0, and TR0/ CS0, respectively, compared to the control.

Thus, the highest Si concentration showed greater

preservation of the photosynthetic apparatus due to lower disease severity, which also resulted in greater light energy absorption by the reaction centers and per cross-section of PSII (ABS/RC and ABS/CS0). Increases in ABS/RC represent increases in the total number of photons absorbed by chlorophyll molecules in all PSII reaction centers (KUMAR et al., 2015), whereas ABS/CS0 represents the flow of photons absorbed by the excited cross-section of PSII (KOVAČEVIĆ et al., 2017). The control treatment (without Si application), resulted in high downy mildew severity with chlorotic spots on the leaf surface, progressing to necrosis (RHOUMA et al., 2022), resulting in a smaller active photosynthetic leaf area and lower ABS/RC and ABS/CS.

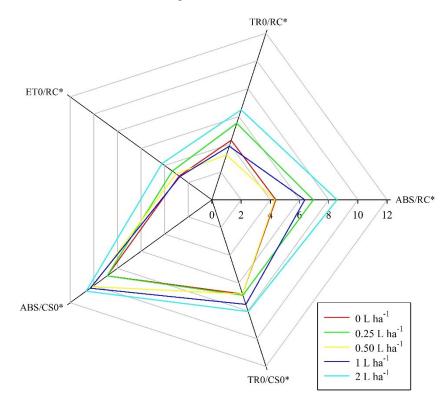


Figure 4. Effects of Si application rates on parameters of the OJIP test in melon plants during the rainy season. * = significant by the F test (p<0.05).

The reductions in light energy absorption due to chlorosis and necrosis in control plants resulted in a lower amount of energy stored by the reaction centers and crosssection of PSII (TR0/RC and TR0/CS0). TR0 represents the maximum rate at which an exciton is captured by PSII reaction centers, resulting in the reduction of quinone A (QA). Katanić et al. (2021) also found reductions in these variables in wheat plants infected with Fusarium spp., with the application of high Si rates resulting in a more intact photosynthetic apparatus, presenting higher storage values. The flow or transfer of electrons from QA to plastoquinone (PQ) through PSII reaction center activity (ET0/RC) (STIRBET et al., 2018) was higher when using the highest Si rates were applied, as higher light energy absorption (ABS) and greater storage (TR0) increase electron transport (ET0). Reductions in electron transport (ET0/RC) were also observed in Betula pendula infected with Phytophthora plurivora (BEREZOVSKA et al., 2021).

The pathogen attack is easily perceived through the set of parameters used to asses chlorophyll *a* fluorescence, which are the phenomenological flows to the cross-section of PSII, representing areas of photon capture by light-absorbing pigments in the PSII antennae (STIRBET et al., 2018). The greater protection of the photosynthetic apparatus in plants treated with Si affected the ABS/CS0 and TR0/CS0, as well as variables related to reaction centers. The reduction in parameter values in plants without Si application may also be attributed to high ROS production, leading to deleterious effects on electron transport (MOUSTAKAS, 2022).

CONCLUSION

The application of 1 to 2 L ha⁻¹ of potassium silicate (12% Si and 15% potassium) to melon plants during periods of high rainfall intensity and mild air temperatures effectively



reduces downy mildew severity and protect leaf chlorophylls from the effects of downy mildew attack, resulting in enhanced absorption flux (ABS) and electron storage (TR0) and transport (ET0).

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