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Assessment of Downy Mildew (*Pseudoperonospora cubensis*) Control in Cucumber (*Cucumis sativus*) Using Image Processing under Silicon (SiO2) Application

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Article History	Abstract
Article History Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 13 Oct 2023	Abstract The objective of this research was to evaluate the effect of the application of silicon $(SiO2)$ on the control of downy mildew (Pseudoperonospora cubensis) in cucumber (Cucumis sativus) crops. The location of the research was carried out at the "La María" Experimental Farm belonging to the State Technical University of Quevedo (UTEQ), located on the Quevedo-El Empalme road at km 7 of the Province of Los Ríos for two years. The fertilizer that was applied was silicon oxide (SiO2 at 32%), in different doses applied at 8, 23 and 40 days after sowing. A completely randomized block design (DBCA) was used, with six treatments and three repetitions per treatment. The variables that were evaluated were the severity of the downy mildew disease in different treatments and leaves of different heights using a visual evaluation scale and image processing. Some variables of agronomic interest and a benefit/cost type economic analysis were evaluated. To validate the image processing, a linear correlation with a visual scale was used and the rest of the variables were subjected to analysis of variance (Tukey p<0.05) to determine statistical differences. The results obtained show a positive correlation (R2: 0.98) of the Leaf Doctor program with the visual evaluation. The severity of downy mildew did not present differences between the treatments, but the greatest impact was on the lower leaves. Silicon presented positive effects on the different agronomic variables, but only the yield, diameter, length, and average weight of the fruit were higher at doses
CC License CC-BY-NC-SA 4.0	of 150 ppm of silicon. However, the 125-ppm dose is more profitable as it has no statistical differences with the 150-ppm dose. Keywords: Leaf Doctor, Pseudoperonospora cubensis, evaluation.

1. Introduction

Cucumber (*Cucumis sativus*) is an annual herbaceous plant with robust roots, a sturdy climbing stem, and green fruits. The fruits are marketed both in the fresh and processing markets. Currently, cucumber is a widely cultivated vegetable in Europe and the Americas, ranking fourth in global vegetable production (Cormilot, 2014). Being a member of the Cucurbitaceae family, cucumber is susceptible to various pathogens, with downy mildew being a prominent one.

Downy mildew is a cosmopolitan disease caused by the oomycete *Pseudoperonospora cubensis*. This disease has been reported in several countries across the Americas, Africa, Europe, and Asia, including Australia (González *et al.*, 2010). The infection by *P. cubensis* is temperature-dependent, occurring in two hours at 20°C and in ten hours at 15°C, provided there is a film of water on the tissue. The pathogen spreads through rainwater or irrigation, wind, utensil contact, or worker movement. Some insects have also been reported to transmit fungal structures. The presence of downy mildew in cucumber is of great concern to

producers. Once infection starts, strong efforts are required to combat the fungus because it has a high capacity for dissemination and infection of new crop tissues (Fernández & Guerrero, 2015).

The fungus's mycelium is hyaline, and the sporangia are gray, often visible on the undersides of leaves. In the presence of free water, sporangia release spores that swim in the water, thanks to their flagella. When they find a suitable area, they germinate and infect plant tissues, with the optimal temperature for infection being between 16 to 22°C. Once a leaf is infected, the fungus's growth is favored by alternating temperatures and high relative humidity, ranging from 80% to 90%. Temperatures below 5°C or above 35°C halt its development. Depending on temperature conditions, sporangia generations can be produced within 5-13 days (Quesada-Ocampo *et al.*, 2012).

The infection time and lesion formation depend on weather conditions. Depositing a film of water on the leaf surface triggers sporangia germination, resulting in zoospores that briefly swim before adhering and producing germination tubes that penetrate the leaves. In cucumber, leaf infection begins following periods of 2 hours of mist at 20°C, 6 hours at 15°C to 20°C, or 12 hours at 10°C to 15°C. Sporangia's effectiveness decreases as temperatures rise because they must remain moist until germination; otherwise, they die. Once infection occurs, a new generation of sporangia is produced within 4 to 12 days (Babados, 2004). All these conditions require precise disease evaluation when conducting experiments.

The Horsfall-Barratt scale is a system used in plant pathology to evaluate plant diseases. Each plant is assigned a numerical value based on the percentage of leaf area showing disease symptoms. The assessment of downy mildew severity on the Horsfall-Barratt scale (1=0%, 2=0-3%, 3=3-6%, 4=6-12%, 5=12-25%, 6=25-50%, 7=50-75%, 8=75-88%, 9=88-94%, 10=94-97%, 11=97-100%, 12=100%) is done in relation to the percentage of affected leaf tissue (Carreño *et al.*, 2019). This evaluation method is straightforward but requires expertise to avoid biases. These methods have been optimized using programs that reduce evaluator errors.

Leaf Doctor is a mobile application designed for smartphones. The program uses color images to distinguish between healthy and diseased plant tissues, calculating disease severity percentages (Pethybridge & Nelson, 2015). Users can capture real-time images using the phone's camera or select previously saved images. Leaves or plant organs are photographed against a non-reflective background or black background (Pethybridge & Nelson, 2015). The application allows users to select up to eight colors to represent "healthy" tissue, which enhances result accuracy and precision (Barbedo, 2014). This program enables the evaluation of different control methods mentioned by various authors.

Managing downy mildew in cucurbits involves various strategies. Cultural practices such as crop rotation, sanitation, and irrigation management help reduce disease incidence and severity by breaking the disease cycle and reducing inoculum levels (Lebeda & Cohen, 2010; Lebeda *et al.*, 2006a). Chemical control using fungicides is common, but their excessive use can lead to resistance and environmental contamination, so their prudent use and rotation with different modes of action are recommended (Lebeda & Cohen, 2010; Lebeda *et al.*, 2006a). Novel approaches, such as nanotechnology-based fungicides and plant-derived compounds, are also being explored (Lebeda *et al.*, 2006a). Biological control shows potential with agents like Trichoderma spp., Bacillus spp., and Pseudomonas spp., although their effectiveness under field conditions still requires further evaluation (Lebeda & Cohen, 2010). Lastly, host resistance through the selection of resistant plants is considered one of the most effective and sustainable methods, including classical and transgenic breeding

approaches (Lebeda & Cohen, 2010; Lebeda *et al.*, 2006a). One of the methods to reduce the use of agrochemicals is the use of micronutrients, and silicon has been evaluated in various trials.

Silicon has benefits for plants under stressful conditions. These effects include improving drought tolerance, delaying premature defoliation in non-irrigated crops, increasing plant resistance to micronutrient toxicities and other metals, as well as fungal pathogen attacks (López, 2003). Silicon is classified as a beneficial element and has shown positive effects in crops such as rice, wheat, and barley under stress conditions (McCray *et al.*, 2001). Silicon can affect the dynamics of other elements in the soil in crops like grasses, legumes, and cucurbits (Landell, 2016). In the case of cucumber, silicon reduces soluble sugar levels in leaves and increases starch content in roots. This can alleviate the negative effects of photosynthesis and provide greater energy storage capacity under salt stress conditions (Quezada-Ocampo *et al.*, 2012).

In the present study conducted over two years, the effect of silicon (SiO_2) application on the control of downy mildew (*P. cubensis*) in cucumber (*C. sativus*) cultivation under field and greenhouse conditions was evaluated. Downy mildew severity was assessed using visual assessment scales and image processing with the Leaf Doctor software. Additionally, some agronomically relevant variables related to different silicon applications were analyzed. Lastly, an economic analysis of the studied treatments was conducted based on crop yield.

Methodology

Location

The present research was conducted in both field and greenhouse conditions during the years 2021 and 2022 at the Experimental Farm "La María" of the Technical State University of Quevedo (UTEQ). The farm is situated on Quevedo-El Empalme Road, at kilometer 7 in the Los Ríos Province, with geographical coordinates of $79^{\circ}27$ " west longitude and $01^{\circ}32$ " south latitude, at an altitude of 67 meters above sea level. Prior to implementing the trial, soil analyses were conducted to calculate fertilization requirements, and laboratory tests confirmed the presence of the pathogen (Sun *et al.*, 2022).

Experimental Design

In the present research work, the effect of the SILICAMAG fertilizer, which contains SiO_2 (32%), was evaluated in cucumber cultivation. A reference fertilization dose of 130-120-130 kg/ha (N-P-K) and a silicon dose of 100 ppm were used (Samuel *et al.*, 1990). The experiment consisted of six treatments (Control, 150 ppm Si, 125 ppm Si, 100 ppm Si, 75 ppm Si, 50 ppm Si). Three fertilizer applications were made (8 days after planting (dap), 23 dap, and 40 dap). Experimental units were used in a completely randomized block design of 0.5 x 1.5 m with three replicates both in the field and greenhouse, resulting in a plant density of 13,333 plants/ha.

Agronomic Management

For the experiment, cucumber seeds of the Marketmore variety were used. Seedlings were grown in plastic cups using soil from the 'La María' farm. The seeds were watered daily until germination. After 21 days, and once the plants reached their third true leaf, they were transplanted into 2 L bags (18 cm x 29 cm) to prevent waterlogging and control watering at field capacity.

For the field trial, the land was prepared using agricultural machinery with two passes of harrowing and an application of Glyphosate (1 L/ha) to control pre-emergent weeds. Subsequently, plots (0.5 m x 1.5 m) were delimited, with each experimental unit consisting of 10 plants. For the greenhouse trial, the plants were transplanted into black plastic bags with a capacity of 5 L (40 cm x 45 cm) and spaced at the same distance as the field plants.

To promote the vertical growth of cucumber plants and prevent damage, a staking system was implemented using stakes, wires, and twine when the plants reached a height of 25 to 30 cm. Irrigation was carried out to maintain the substrate at field capacity, providing the appropriate amount of water. Fertilization was performed following the previously described treatments, with split doses: 40% in the first application, 30% in the second, and 30% in the third, applied at 8, 23, and 40 days after transplanting. For phytosanitary control, a manual approach with a machete was used, as cucumber plants are susceptible to herbicides. Additionally, a contact insecticide with translaminar action (Furadan) was applied at a rate of 1.2 L/ha in 200 L of water. Harvesting was conducted when the plants completed their maturity cycle, typically between 70 and 75 days after transplanting.

Evaluated Variables

Downy mildew leaf severity

The evaluated variables included disease severity using the Horsfall and Barratt visual scale (1945) and the Leaf Doctor software, as well as fruit weight at the end of the vegetative cycle. Three leaves at different heights on nodes 2, 4, and 6 from the base of the plant were assessed using both methods. A correlation analysis was conducted to determine if the software could be used to evaluate plant foliar diseases. Data were recorded in a field notebook and through photographic records. Analysis of variance and Tukey's test at the 0.5% level were applied to determine statistical differences in the doses of silicon used.

Agronomic Variables

The plant height was measured from the base of the stem to the tip of the last leaf using a tape measure 40 days after planting. An average of 10 plants per experimental group was considered. As for stem diameter, it was evaluated using a vernier caliper at 20 cm above the ground, also at 40 days after planting. The number of true leaves was counted on 10 cucumber plants 40 days after planting. To determine the number of flowers per plant, a count was made when the plants reached 50% flowering stage, between 40 and 45 days after transplanting. To determine the number of fruits from each experimental group were counted, and the data from the two harvests conducted were recorded. The length of the fruits was evaluated by measuring the distance from the top to the bottom in 10 randomly selected fruits. The diameter of the fruits was examined in 10 randomly selected fruits from each experimental plot were weighed in the two harvests using an electronic balance to obtain the production value. Finally, the total yield in the usable plot area for the two harvests was calculated and expressed in kilograms per hectare using a simple rule of three.

Cost-Benefit Analysis

The costs of each treatment were developed, and both variable and total costs were determined. Subsequently, the gross income (yield multiplied by the selling price) was established, and then the net benefit and profitability per treatment were calculated. It is important to mention that the selling price of cucumber at the time of the trial was \$0.45/kg (Ecuadorian Agricultural Public Information System, 2021).

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Results

Through the correlation analysis (Figure 1), it is evident that the Leaf Doctor image processing program is useful for assessing downy mildew severity in relation to the visual evaluation of Horsfall and Barratt, showing a positive correlation among the data (R^2 of 0.98)."

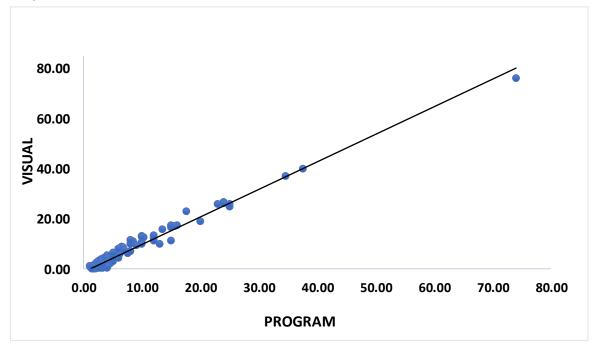


Figure 1. Correlation analysis between visual assessment and the Leaf Doctor program for the evaluation of downy mildew (*Pseudoperonospora cubensis*) severity in cucumber (*Cucumis sativus*) leaves. R^2 of 0.98.

According to the fieldwork conducted during the research project, it was determined that the lower leaves exhibit higher disease severity, reaching up to the 6^{th} scale (foliar tissue damage of 25-50%), while the upper leaves have lower severity with a scale close to 2 (foliar tissue damage of 0-3%) compared to the others (Figure 2). The results did not vary regardless of the evaluation method used.

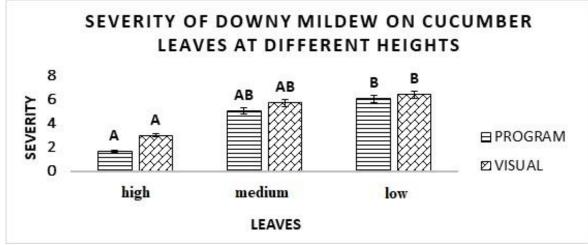


Figure 2. Analysis of downy mildew severity (*Pseudoperonospora cubensis*) in cucumber (*Cucumis sativus*) leaves at different heights. Different letters indicate statistical differences (Tukey: p<0.05). The bars represent the standard error.

When evaluating the different silicon doses, the statistical analysis did not show significant differences in any of the treatments used. However, significant differences were observed when evaluating the different agronomic variables in the treatments (Table 1).

Table 1. Analysis of agronomic variables in cucumber (*Cucumis sativus*) cultivation under the influence of different silicon doses. Different letters indicate statistical differences (Tukey: p<0.05).

(1000 gr, p<0.05).									
Variable	T1 (Control)		T2 (Si 150 ppm)		T3 (125 ppm)		Т	T4	
(100ppm)	T5 (75 ppm)	T6 (50) ppm)						
Yield(kg/ha)	7527.96 cd		9754.32 a		9686.95 a				
9046.31 b	8256.	51 c	7894.67 c						
Vine length	1.75 a	1.67 a	1.59 a	ı	1.77 a		1.53		a
1.48 a									
Steam diameter	6.50 a	6.48 a	6.66 a	ı	6.33 a		6.	54	a
6.75 a									
Number of leaves	21.46 b		23.31 a	23.22	a	23.25 a	a		
23.68 a23.34	a								
Number of flowers/p	lant 20.53	a	21.23 a	22.53	a	23.48	a		
22.67 a23.49 a									
Fruits/plant	3.17 a	3.23 a	3.33 a	ı	3.63 a		3.58		a
3.44 a									
Fruit length	20.01 b		24.96 a	24.66	a	24.67	a		
24.55 a	24.56 a								
Fruit diameter	5.55 b	6.02 a	6.00 a	ı	6.10 a		6.03		а
6.05 a									
Average fruit weight	(g) 1527.	90 cd	1979.	77 a		1966.2	23 a		
1836.07 b	1675.	77 с	1602.33 c						

The economic analysis reveals that all treatments with the addition of silicon show higher profitability compared to the control group. During the trial, it was observed that the selling price of the product was \$0.45 per kilogram, according to data from the Ecuadorian Agricultural Public Information System for the year 2021. Treatment 2, which involved the application of 150 ppm of silicon, resulted in a significant increase in yield, although this increase did not offset the additional cost associated with the higher silicon dose compared to Treatment 3, which used 125 ppm of silicon. The silicon-treated treatments showed yield improvements ranging from 5% to 30% compared to the control group, depending on the dose used.

Discussion

The results of the correlation analysis revealed that the Leaf Doctor program exhibits comparable validity to the visual evaluation conducted using the Horsfall-Barratt scale (Horsfall, J., & Barratt, R., 1945). This tool is used to quantify the severity of downy mildew (*Pseudoperonospora cubensis*) on cucumber leaves at various heights in the crop. These findings contrast with those obtained by González (2021) in their study, where statistically significant differences were identified between the methods used, specifically regarding the different scales employed. During the trial, a higher level of practicality in the evaluation was observed when the scale was applied compared to the values obtained without its use. This suggests that the use of scales to assess the disease can yield variable results and levels of reliability depending on the expertise of the evaluator (Nutter, 1993).

The study reveals that the lower leaves in the cucumber crop exhibited a more pronounced level of damage due to downy mildew (*P. cubensis*) compared to the middle and upper leaves. These results are in line with the conclusions drawn by Calixtro (2017). Downy mildew tends to primarily affect the lower foliage of the plant, although it occasionally appears on stems, peduncles, calyxes, and flower petals. The leaf evaluation showed that these are considered moderately or highly susceptible, depending on the scale used. Since there is a clear preference for investigating this pathogen on leaves, it is of utmost importance to conduct comprehensive monitoring of leaves at different heights to effectively implement disease control (Acevedo, 2021).

No statistically significant differences were found when applying visual evaluation scales and the Leaf Doctor software in the context of various silicon treatments. These results are consistent with previous research documented by Cruz and Centeno (2017). Several disease management strategies, such as early planting, prevention of prolonged leaf moisture periods, regular application of fungicides, and the selection of cucumber varieties with low susceptibility or resistance to *P. cubensis*, have been identified in the scientific literature (Sun *et al.*, 2022). The results of this study suggest that silicon can be a useful tool, although it does not constitute a complete solution to mitigate the disease.

In the context of cucumber cultivation, Treatment 2 and 3 (150 and 125 ppm of silicon, respectively) recorded more favorable yields. This result highlights the relevance of silicon in cucumber production, as supported by previous studies (Liang *et al.*, 2005). In that study, the effects of potassium phosphite and silicon as resistance inducers against downy mildew were examined. These treatments exhibited intermediate behavior in terms of disease severity, with increased plant yields and reduced disease incidence compared to conventional crop plots.

In the analysis of various agronomic variables, no statistically significant differences were observed in aspects such as vine length, stem diameter, number of flowers per plant, and number of fruits per plant. However, it is essential to emphasize that silicon application has evident positive effects on yield and the morphological characteristics of the fruits. Numerous scientific studies have consistently supported the notion that the incorporation of silicon significantly contributes to increased plant yields (Yan *et al.*, 2018; Amin *et al.*, 2016), as well as the increased length, diameter, and weight of harvested products (Savvas & Ntatsi, 2015; Seo *et al.*, 2004). These results translate into more vigorous and productive crops, with the potential for a positive economic impact in agriculture. Silicon, by strengthening plant structure and enhancing its resistance to abiotic stress factors and pathogens, promotes healthy growth and robust development, resulting in higher quality and quantity of agricultural production.

The most economically outstanding treatment was Treatment 3 (Si: 125 ppm of silicon), which showed a 29% increase compared to the control group. In a similar context, Miranda's study (2020) conducted a cost-benefit analysis regarding fungicides used to prevent downy mildew in cucumber cultivation. By evaluating agricultural yield, production costs, and benefits obtained, it was evident that the treatment based on benzamides with plant extract showed the highest profitability, amounting to \$0.98, in contrast to the control group, whose profitability was limited to \$0.30. These findings underline the importance of employing various strategies in controlling downy mildew in cucumber cultivation, leveraging different disease management approaches.

This study supports the comparability between visual evaluation with the Horsfall-Barratt scale and the Leaf Doctor software for measuring downy mildew severity in cucumber cultivation. It also confirms the positive effects of silicon on yield and fruit characteristics.

Furthermore, it highlights the profitability of certain treatments. These findings provide valuable information for improving downy mildew management in cucumber cultivation and optimizing agricultural productivity and profitability.

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