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# Assessment of the severity of anthracnose (*Colletotrichum lindemuthianum*) using image processing software in beans (*Phaseolus vulgaris* L.) var. cuarentón under the influence of silicon application.

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**Abstract**

The objective of the study was to evaluate the severity of anthracnose (*Colletotrichum lindemuthianum*) in common bean (*Phaseolus vulgaris* L.) of the "cuarentón" variety under different silicon doses. The research was conducted over two years at the Experimental Farm "La María" of the Technical State University of Quevedo-Ecuador. The "cuarentón" bean variety from the area was used, and a complete randomized block design was applied with seven treatments and three replications. The silicon source used as a fertilizer was silicon dioxide ($SiO_2$/silicon at 32%), which was applied in two fractions at the base of the plant. One was applied 15 days after sowing and the second 45 days after sowing. Anthracnose severity was evaluated using visual scales and the Leaf Doctor image processor. The correlation analysis to validate the program showed a correlation of 0.75-0.97 (in leaves and pods) compared to the visual evaluation, indicating its validity for evaluating this disease. The separation of means (Tukey $p < 0.05$) indicates that anthracnose evaluation can be performed on any leaf at a different height of the plant, as no statistical differences were found. However, no treatment showed less severity compared to the control. The only agronomic variables of interest where silicon-fertilized plants demonstrated significant differences was the plant height. The benefit/cost economic analysis of each treatment does not recommend the use of the product due to the elevated cost of the input during the trial and the low selling price of the product.

**Keywords:** Leaf Doctor, fertilization, sampling

## Introduction

The common bean (*Phaseolus vulgaris*) belongs to the group of edible legumes and is considered one of the most important plants due to its wide distribution across all five continents. It is highly nutritious and essential in the human diet. It holds particular significance in Latin America, especially in regions with low food availability (IICA, 2009: Damm *et al*., 2010; Valladares, 2010). This plant is vulnerable to cold, moisture, rapid climate changes, and highly susceptible to diseases that can impact its productivity (Hernández, 2009; Córdova, 2015). In the Quevedo region, for example, it is affected by a disease called anthracnose (Treviño & Rosas, 2013; Tutiven, 2016).

Anthracnose is caused by the fungus *Colletotrichum lindemuthianum*, which is present in most common bean-producing areas worldwide (Mena & Velásquez, 2010; Mycobank, 2017; Uniprot, 2002). This fungus causes a disease known as "anthracnose" in common bean and other legumes. Under favorable environmental conditions, the disease can lead to yield losses of up to 95% in susceptible common bean cultivars. Conditions that promote the disease include high relative humidity, frequent rainfall, and temperatures between 18 and 22°C (Melotto *et al.*, 2000). Colletotrichum comprises the asexual state (anamorph) of Glomerella (teleomorph), which belongs to the phylum Ascomycota, class Hypocreomycetidae, order Glomerellales, family Glomerellaceae (Réblová *et al.*, 2011). Glomerellaceae is a monotypic family characterized by non-stromatic dark perithecia, well-developed periphysate ostioles, and abundant thin-walled paraphyses. Initially, Glomerella was placed in the order Phyllacorales, but some of its characteristics are clearly distinct from other members, such as its lack of stromatic tissue and its exclusive anamorphs of Colletotrichum (Zhang *et al.*, 2006; Zhang *et al*., 2013).

Initial lesions of anthracnose may appear on leaves and stems as angular or linear dark red or brick-red lesions, or as small sunken cankers. The development of affected plants is compromised, as they can die prematurely, or exhibit slowed growth. If favorable environmental conditions for the fungus persist for extended periods, lesions may also appear on the leaf veins (Mena & Velásquez, 2010). The disease is more pronounced on pods, where circular lesions of light brown to reddish color, surrounded by a margin or ring, can be observed. In the center of these lesions, orange to pink fungal growth may occur during very humid periods. Severely affected young pods can become shriveled or wilted if the fungal attack is severe (ASPROMOR, 2012; Pérez *et al*., 2010).

Various methods are employed to control the disease. Cultural control includes seed sterilization with hot water (50-60°C), which helps eliminate the fungus from contaminated seeds. However, this method significantly reduces seed viability. Crop rotation every two to three years with cereals like oats, wheat, or maize and the removal of volunteer bean plants in the following cycle are other cultural control practices (Mena & Velásquez, 2010). Chemical control involves the use of protective or systemic fungicides like benomyl, chlorothalonil, carbendazim, or captan. These have limited effectiveness as they must be applied at the beginning of the epidemic, and the foliage must be completely covered. Infected seeds can also be treated with fungicides like benomyl or methyl thiophanate (Mena

& Velásquez, 2010). Another control method involves nutrient management, where silicon has been used in multiple crops (Álvarez & Osorio, 2014).

Silicon is not naturally free in nature, and due to its strong affinity with oxygen, it forms various forms of $SiO_2$ or other silicates combined with various metals (Al, Fe, Mn, Mg, among others) (Álvarez & Osorio, 2014; Castellanos *et al*., 2015). Silicon is absorbed by the roots in the form of orthosilicic acid ($H_4SiO_4$) and is transported through the apoplast to the xylem and aerial parts of plants, accumulating in epidermal cells (Mitani & Ma, 2005). In field conditions, silicon can stimulate growth and productivity by increasing the availability of elements like P, Ca, Mg, K, and B, counteracting antagonistic effects in soils with high Al and Fe saturation (Epstein & Bloom, 2005; Epstein, 2009).

SEPHU (2009) indicates that recent studies have identified silicon as a beneficial element in agriculture, especially in crops like rice, sugarcane, and other grasses, where it has shown positive results. One of the most significant effects of silicate fertilizers is to improve soil phosphorus availability and enhance its absorption by cultivated plants, ultimately increasing crop yields. This is particularly valuable in tropical soils where phosphorus fixation limits the effectiveness of fertilizers (Álvarez & Osorio, 2014; Pinzón *et al*., 2017).

It is known that silica gel is deposited between plant cell walls and contributes to the beneficial effects of silicon (Álvarez & Osorio, 2014), forming a double cuticular layer to protect and mechanically reinforce all plants. Silicon is the only element that does not cause severe damage in excessive amounts, as it could form a silicon cuticle with "silicified cells" and "silicon bodies," which are formed in high quantities of the element (Snyder *et al.*, 2001). When a disease begins, the plant directs all available silicon to the site of attack to reinforce the surrounding cells. However, silicon is immobile once it is incorporated into the cell wall, and a constant supply of assimilable Si is necessary. Most soils contain less than half of the required silicon, so there can be significant benefits from foliar silicon application, especially at the first sign of disease (Mejisulfatos, 2010).

To assess the effectiveness of disease control, visual scales are often used. These scales are subjective and depend on the expertise of the evaluator. When evaluating the disease, it is important to consider the crop stage since anthracnose development varies with environmental conditions, variety, and cultural practices. Data collection frequency is determined based on the developmental stages of common bean plants (Ibagón & Perafán, 2009). According to the scale proposed by Tamayo (1995), disease evaluation is carried out at stages V2, V4, R6, R7, and R8. In stages V2, V4, and R6, the leaf scale is used, while in stages R7 and R8, the pod scale is applied. Image processing reduces bias from the researcher during evaluations (Pethybridge & Nelson, 2015). These programs allow for customization and are cost-effective (Wijekoon *et al*., 2008).

The objective of this research was to determine whether image processing (Leaf Doctor) can be used as a tool for assessing the severity of anthracnose. Image processing was compared to visual scales using silicon as a biological stressor during two planting periods. Variables assessed included the determination of the optimal soil silicon dose, the evaluation of leaves and pods compared to the visual scale, and a cost-benefit analysis. The results aim to promote the program as an alternative for evaluating different anthracnose control methods in common bean cultivation.

## Materials and Methods

### Location

The research was conducted at the facilities of the Experimental Farm "La María" of the Technical State University of Quevedo, located at Km 7 on the Quevedo - El Empalme road. The geographical coordinates of this location are as follows: 79° 27" West longitude and 01° 06" South latitude, at an altitude of 67 meters above sea level (m.a.s.l.). The trials were conducted during the rainy and dry seasons of the years 2020 and 2021, respectively.

### Experimental Design

For the statistical design, a randomized complete block design (RCBD) was employed, consisting of seven treatments with three replications each. Each experimental plot measured 4.0 x 2.5 meters. The common bean variety "cuarentón" was planted at a density of 1,680,000 plants per hectare. The treatments included a control with fertilizer application based on soil analysis recommendations, using a dose of 70-60-40 kg/ha (N-P-K), and a silicon dose of 90 kg/ha (FAO, 2007; Quintana, 2016; Khadri *et al.*, 2000). The remaining treatments varied in silicon doses at 150%, 125%, 75%, and 50% of the dose used in the Quintana (2016) study. Fertilizers were applied in two soil-based fractions at 15 and 45 days after planting. The fertilizers used included urea (46-0-0), potassium chloride (0-0-60), diammonium phosphate (18-46-0), and silicon oxide (32% silicon). Data were processed using the statistical software InfoStat (Di Rienzo *et al.*, 2018). Each variable was evaluated using analysis of variance (Tukey $p < 0.05$), and linear correlation analysis was performed to assess the program's effectiveness.

### Experiment Management

The land preparation was mechanized with one plowing pass and two harrowing passes to loosen the soil for seed placement. Prior to the installation of the research project, post-emergence chemical control was carried out using Glyphosate at a dose of 2 L/ha in 200 L of water. Before planting, the seeds were disinfected with Vitavax 300 at a rate of 1 g/kg of seed to prevent fungal diseases. Manual seeding was performed by placing two seeds per site or hole, with a spacing of 0.20 m between plants x 0.50 m between rows and a depth of 3 cm. (INIAP, 2002). Thinning was done 10 days after planting to leave one plant per site. Manual weeding with a machete was conducted to keep the crop weed-free between 10 and 25 days after planting. To maintain pest insect populations at manageable levels, control measures were implemented using the broad-spectrum insecticide Benfurol at a dose of 4 ml/L. The first application was made 15 days after planting (dap), and the second at 45 dap. Irrigation was performed once a week by sprinkling when necessary. Data collection for the variables began when the crop reached the harvest period (between 60 to 70 dap). Harvesting was done manually when the pods of the plants reached physiological maturity, marking the end of their vegetative cycle (Ríos & Quiroz, 2002; Ríos *et al*., 2003).

## Evaluated Variables

To determine the level of anthracnose incidence in bean plants, both in leaves and pods, Leaf Doctor application (Pethybridge & Nelson, 2015) and the visual scale used by Tamayo (1995) were employed. Photographs of bean leaves and pods were evaluated in duplicate to reduce experimental error. In both methods, the assessments were conducted on standing plants. Other agronomically relevant variables included: survival percentage (30 days after planting), plant height in centimeters (90 days after planting), days to flowering (at least 50% of plants), number of pods per plant, pod length (cm), weight of 100 seeds (g), and yield (kg/ha). Finally, a cost-benefit analysis was conducted to determine the feasibility of using silicon in the crop.

## Results

To determine the efficiency of the Leaf Doctor program compared to the visual assessment scale proposed by Tamayo (1995), leaves and pods of diseased plants were evaluated in duplicate. The pods were assessed by taking photographs from both sides of the pods. The correlation analysis indicates that the program has a high correlation ($R^2 = 0.97$) (Figure 1).

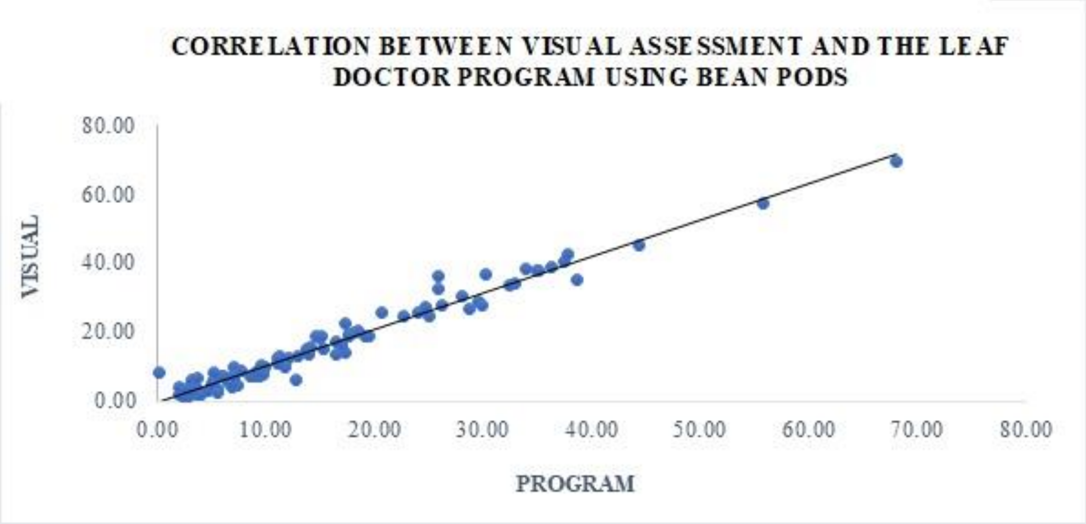

**Figure 1**: Correlation analysis between visual assessment and the Leaf Doctor program to evaluate the severity of anthracnose (*Colletotrichum lindemuthianum*) in bean pods (*Phaseolus vulgaris*). $R^2$= 0.97.

Similarly, leaf samples from different heights were taken and evaluated in duplicate, with a correlation analysis conducted between the Leaf Doctor program and visual assessment. The correlation analysis indicates a correlation of $R^2$= 0.75. This suggests that the program can be used to measure or assess the severity of anthracnose in bean leaves (Figure 2).

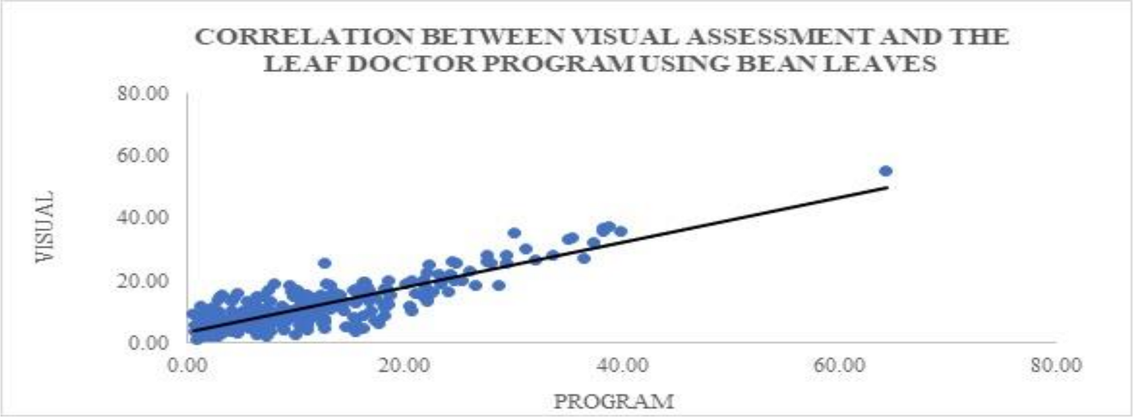

**Figure 2:** Correlation analysis between visual assessment and the Leaf Doctor program to evaluate the severity of anthracnose (*Colletotrichum lindemuthianum*) in bean leaves (*Phaseolus vulgaris*). $R^2= 0.75$.

The cuarentón bean exhibits determinate bush growth, so it is important to determine if there are differences when taking leaf samples to assess disease. When analyzing leaves from different heights in the bean crop, the analysis of variance (Tukey, P<0.05) indicated that there are no statistical differences in any of the evaluated leaves.

When analyzing the silicon dose that provides better disease control in bean leaves, the results differed between the methods used. Figure 3 shows that among the 7 treatments with silicon doses applied to control anthracnose in bean leaves, there is no statistical difference when visual assessment was used. In contrast, when plants are evaluated using the program, treatment 7 (Silicon 135 kg/ha) exhibited greater severity.

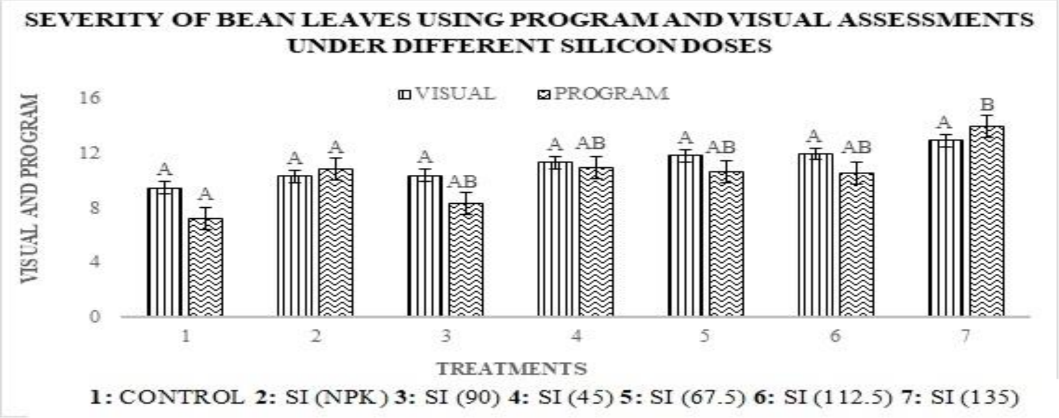

**Figure 3:** Analysis of the severity of anthracnose disease (*Colletotrichum lindemuthianum*) in bean leaves (*Phaseolus vulgaris*). Different letters indicate statistical differences (Tukey, $p < 0.05$). Bars represent the standard deviation.

When analyzing the silicon dose that provides better disease control in bean pods, the results differed between the methods used. Figure 4 shows that among the seven treatments with silicon doses applied to control anthracnose in bean pods, there are no statistical differences when visual assessment was used. In contrast, when plants are evaluated using the program, all treatments where silicon was used exhibited lower severity.

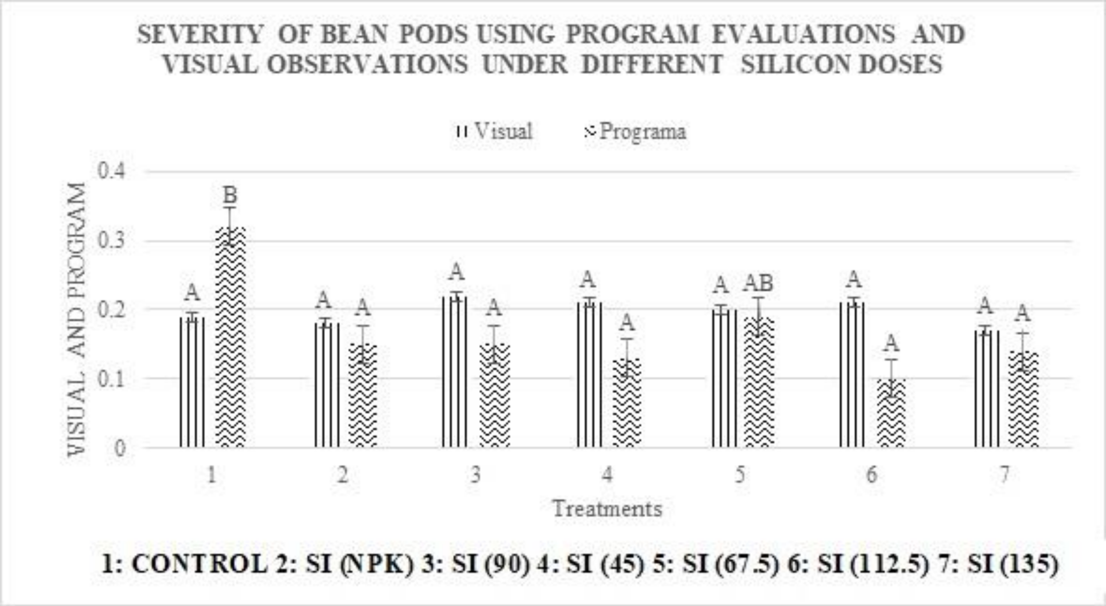

**Figure 4:** Analysis of the severity of anthracnose disease (*Colletotrichum lindemuthianum*) in bean pods (*Phaseolus vulgaris*). Different letters indicate statistical differences (Tukey, $p < 0.05$). Bars represent the standard deviation.

Table 1 shows that there is no statistical difference in the total yield of the treatments in any of the trial periods. In terms of the cost-benefit ratio (b/c) compared to the control, none of the treatments is more profitable because the increase in yield does not justify the cost of silicon. The price of beans was $1.03 per kilogram at the time of the research (Agricultural Public Information System of Ecuador, 2021).

**Table 1:** Analysis of yields of common bean (*Phaseolus vulgaris*) with different silicon doses. Different letters indicate statistical differences (Tukey, $p < 0.05$). The cost-benefit ratio (b/c) is compared to the control.

| TREATMENT (Si: Kg/ha) | TOTAL YIELD (KG) 2020 | B/C 2020 | TOTAL YIELD (KG) 2021 | B/C 2021 |
|---|---|---|---|---|
| 1 (control) | 0.66 a | 1.00 | 1.17 a | 1.00 |
| 2 (70-60-40) | 0.49 a | 0.66 | 0.73 a | 0.55 |
| 3 (Si: 90) | 0.91 a | 0.77 | 1.10 a | 0.52 |
| 4 (Si: 45) | 0.76 a | 0.68 | 1.37 a | 0.69 |
| 5 (Si: 67.5) | 0.70 a | 0.65 | 1.13 a | 0.59 |
| 6 (Si: 112.5) | 0.52 a | 0.53 | 1.37 a | 0.77 |
| 7 (Si: 135) | 0.90 a | 0.76 | 1.40 a | 0.66 |

The agronomic variables in the bean crop did not show significant differences in most of the evaluated variables. Treatment 2 (70-60-40) exhibited greater plant height and number of pods per plant (Table 2).

**Table 2.** Analysis of agronomic variables of interest in the bean crop (*Phaseolus vulgaris*) under silicon application. Treatments indicate the nutrients applied in kg/ha. Different letters indicate statistical differences (Tukey, $p < 0.05$).

| **EVALUATED VARIABLE** | **T1 Control** | **T2 70-60-40** | **T3 Si:90** | **T4 Si:45** | **T5 Si: 67.5** | **T6 Si: 112.5** | **T7 Si: 135** |
|---|---|---|---|---|---|---|---|
| Survival percentage | 75.33a | 76.33a | 79.67a | 76.33a | 74.33a | 73.33a | 73.37a |
| Plant height (cm) | 28.83ab | 30.90b | 29.80ab | 27.47ab | 26.40a | 29.73ab | 29.78ab |
| Days to flowering | 69.33a | 67.67a | 64.67a | 66.67a | 63.67a | 71.33a | 71.32a |
| Number of pods per plant | 12.6a | 16.43b | 14.67ab | 14.63ab | 13.9ab | 14.17ab | 14.18ab |
| Pod height (cm) | 12.18a | 12.63a | 12.73a | 12.75a | 13.04a | 12.70a | 12.79a |
| Weight of 100 seeds (g) | 66.00a | 64.67a | 67.33a | 69.33a | 68.00a | 65.33a | 65.31a |

## Discussion

To determine the incidence level of anthracnose in the cultivation of *P. vulgaris*, visual assessment was employed using the scale established by Tamayo (1995) and the Leaf Doctor program. The former proved to be less straightforward, somewhat subjective, allowing for the evaluation of alterations caused by anthracnose. The program enables the capture of photographs of bean leaves and pods, directly yielding percentages based on the disease-induced damage levels. Both methods are considered suitable for such assessments. These findings contrast with those of Ivancovich & Lavilla (2016), who, in their research project on "Proposals for field and laboratory scales for the assessment of 'leaf blight' and 'purple seed stain' caused by *Cercopora kikuchii* in soybeans," assert that visual evaluation has a disadvantage as it relies solely on less precise visual assessment compared to other programs, making it subjective.

In studies evaluating the severity of damage to *P. vulgaris* leaves at different heights, no statistical differences were found in either the visual assessment scale or the Leaf Doctor program. In contrast, Ilaquiche's (2018) research, titled "Quantitative description of rust (*Uromyces appendiculatus*) and anthracnose (*Colletotrichum lindemuthianum*) in two bean varieties with different resistance," indicates significant differences in assessment models. It states that the middle stratum exhibited the highest disease occurrence with a maximum growth rate of 1.00%, while the high stratum had a higher growth rate of 0.56%.

Similarly, in different silicon treatments to control *C. lindemuthianum*, the control group showed lower severity when evaluated with the Leaf Doctor program, but no statistical difference was observed among treatments using visual scales. Likewise, Unigarro's (2013) study determined that the cultivation of *P. vulgaris* exhibited acceptable agronomic behavior due to the application of chemical fungicides. Among the six fungicides used, the best control of anthracnose was achieved with the Bellis WG fungicide at a dose of 1 g/L. Additionally, Antracol 70 PM and Alto 100 SL fungicides demonstrated high efficiency in controlling the *C. lindenuthianum* fungus. This suggests that different control methods may yield varied results, which can be implemented in future trials.

In this experiment, there is no economically superior treatment for control, making it unbeneficial to apply treatments in any case. This is attributed to the high cost of silicon relative to production levels, resulting in adverse yields in some cases, although not statistically significant. Treatment T6 (Si: 112.5 kg/ha) achieved the lowest cost-benefit ratio at 0.53. This aligns with Arévalo's (2014) project, "Evaluation of the agronomic performance of three soybean (Glycine max) varieties at two planting densities in the Nueva Loja parish, Sucumbíos province," which determined through economic analysis that none of the treatments used in soybean cultivation could counteract diseases like anthracnose, likely due to the aggressiveness of the pathogen. Treatment T5 had the lowest cost/benefit ratio at 0.87, attributed to its low yield (1358.33 kg/ha) and income of $443.17, emphasizing the need for alternative, more cost-effective sources of silicon or adjusting the official product price, which is currently low.

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