

Effect of the application of silicon in the control of *Phytophthora capsici* in bell pepper (*Capsicum annuum*) using image processing software

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Abstract

Phytophthora blight caused by *Phytophthora capsici* is one of the most important diseases of pepper (*Capsicum annuum* L.). It infects pepper plants at all growth stages. It can cause seedling death, root rot, crown rot, stem blight, leaf spot, and fruit rot. Assessment of the disease is usually made by visual scale diseases which do not include bell pepper fruits. This evaluation tends to be biased when different control methods are investigated. One method appears to be the utilization of silicon. Silicon thickens plant cell wall reducing disease progression. The experiment was conducted under field and greenhouse conditions for two years at the Universidad Técnica Estatal de Quevedo/Ecuador. Different doses of silicon were evaluated to determine which one had the greatest control of the disease. The evaluation of the disease included visual scales and image processing software. Leaves and fruits were evaluated. The program appears to be suitable for its implementation by linear correlation analysis (R^2 : 0.90-0.97) compared to the visual disease scales. None of the silicon doses reduced the severity of *P. capsici*. Other agronomic variables like yield, plant height, number of flowers showed statistical differences through the analysis of variance (Tukey $p < 0.05$) compared to the control. The economic analysis did not recommend the use of silicon in field conditions. The dose of 200 kg/ha of silicon had an increase of 29% of benefit compared to the control under greenhouse conditions.

Keywords: *Leaf doctor, damage, bell pepper.*

INTRODUCTION

Plant and disease

The bell pepper (*Capsicum annuum* L.) is a horticultural crop native to America. It is the fifth horticultural crop in total worldwide cultivated area. This vegetable has experienced a considerable increase in its production and export level (Castillo and Chiluisa, 2011). Bell pepper major disease is *Phytophthora* blight. *Phytophthora* blight is caused by the oomycete *Phytophthora capsici*. The incidence and severity of the disease has increased in recent decades. These problems are due to its wide host range and long-lived spores in soil. The pathogen attacks all cucurbits like pepper, tomato, and eggplant. The pathogen also infects pepper plants at all growth stages. It can cause seedling death, root rot, crown rot, stem blight, leaf spot, and fruit rot (Lamour et al., 2012).

In adult plants, the symptoms appear at the base of the stem as cankers or elongated dark green bands with a moist appearance. These lesions then turn dark brown and surround the base of the main stem. The branches may show wilting from the point of infection with large and irregular brown spots on the leaves. Severe infected plants dry and then die fast. Sometimes the fungus produces a white mycelium in the affected organs. The habitat of this fungus is the soil, where it can survive for several years. Warm temperatures, high soil moisture, and poor drainage favor disease development. This fungus can be transmitted by infected seed and contaminated irrigation water (Rosas, 2015).

Disease evaluation methods and controls

Phytophthora blight is evaluated by researchers using visual scales ratings. The scale proposed by (Reyes et al., 2015) utilizes six levels. The

level zero implies a healthy plant with no symptoms. Levels one to four represents an increase in the wilting, leaf spots and stem necrosis of the plant. The level five is assigned to a destroyed plant that will die. This scale does not assign any value to bell pepper fruits since they are discarded.

Other methods to evaluate resistance utilize formulas (Shaner & Finney, 1977). The formulas need data such as converted logits, regression lines, or the calculation of the area under the disease progress curve. The input data for the formulas requires visual criteria of severity. The formula could be more accurate, but it is not friendly for the evaluation of commercial producers. It does not reduce the bias of the gathered data.

The management of the disease needs different approaches. The use of resistant varieties and well-drained soils are the most effective. The use of fungicides and soil fumigants are the most used. Fungicides have a negative impact in soil and water and they could induce resistance (Cantliffe et al., 1995; Chellemi et al., 1997). Cultural practices such as mulching and raised beds can cut the spread in field (Sanogo & Ji, 2013). Rainfall has been the most influential environmental factor for disease incidence under field conditions (Gevens et al., 2007).

Silicon

The beneficial effects of silicon have been documented in several crops (Datnoff et al., 2007). Most of them report an increase in yield and a decrease in disease severity (French-Monar et al., 2010). Silicon does not exist in a free state in soils. It is generally found in the form of silicon dioxide and in complex silicates. Silicon is absorbed by plants as monosilicic acid (H_4SiO_4) (Epstein, 1999). It is transported by the xylem to the rest

of the plant. Silicon distribution within the plant depends on the speed of transpiration (Aguirre et al., 2007). Silicon accumulation in different plant organs have demonstrated to reduce disease severity (French-Monar et al., 2010).

Silicon has a synergistic action with calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn) and molybdenum (Mo). These six elements present a synergistic action with different effects. For example, these elements optimize the development of the crop and yield (Datnoff et al., 2007). They improve the average shelf-life of perishable crops (Quiroga, 2016). The uptake of heavy metals is reduced through the precipitation of compounds formed by silicon (Bent, 2008). In the case of plant pathogens, the role of Si has been attributed in part to its accumulation and polymerization in cell walls (Batista et al., 2005).

The objective of the study was to implement the evaluation of *Phytophthora* blight using image processing software. Leaves from different heights were used and fruits were also evaluated. The silicon was tested as a disease control method to determine the best dose in field and greenhouse conditions for two years. Several agronomic variables of interest for the producer were evaluated such as yield expressed as weight (kg/ha), plant height, number of flowers, number of diseased and healthy fruits and weight of healthy and diseased fruits. An economic analysis was calculated to determine the viability of silicon as an option to control *Phytophthora* blight.

MATERIAL AND METHODS

Location

The research was carried out at the facilities of the Experimental Farm "La María" of the

Universidad Técnica Estatal de Quevedo. The farm is located at Km 7 via Quevedo - El Empalme. Geographical coordinates are 79° 27' and 01° 06' at an altitude of 67 meters above sea level. Two-year experiments (2021-2022) were conducted under greenhouse and field conditions.

Experimental design

Two experiments were performed under greenhouse and field conditions for two years (2021-2022). The experiment utilized a complete randomized block design (CRBD). Each experimental unit consisted of ten plants of bell pepper hybrid "Martha". The experiment had six treatments and three repetitions for each treatment. Treatments were different doses of silicon (Control, 200, 300, 400, 500 and 600 kg/ha).

Seedlings were planted on plastic bags of 14 x 16 cm. The soil consisted of 33:33:33 soil-sand-peat moss. The soil was infected with *Phytophthora capsici* which is ubiquitous to the location of the research. To corroborate that the evaluated plants were infected by the pathogen, the pathogen was recovered using soil and plant samples at the end of the experiment (Roberts et al., 2005). The bags were taken to greenhouse or field conditions depending on the experiment. The irrigation was maintained at field capacity to induce symptoms. Fertilization was calculated based on soil analysis of the bag's mixture and collected field samples. Fertilization was divided in three doses of 294-73-491 kg/ha (N-P-K). The doses were applied at 15, 30 and, 45 days after transplanting (Berrios et al., 2007). Weeds were controlled manually. Chlorpyrifos was used to control insects at a dose of 300 ml/ha. Plants were harvested 110 to 130 days after transplant.

Disease evaluation

The evaluation of *P. capsici* was done using Reyes et al., 2015 disease scale. To check the efficiency of the program infected bell pepper fruits were collected for a month. Infected leaves were also analyzed using both methods. Collected data included yield expressed as weight (kg/ha), plant height, number of flowers, number of diseased and healthy fruits and weight of healthy and diseased fruits. To determine which dose of silicon is recommended, a benefit/cost economic analysis was done based on the obtained marketable yield at a price of \$0.70/kg (CIPA, 2021).

RESULTS

Correlation analysis of infected leaves and fruits

To determine the severity caused by *Phytophthora capsici* two methods were utilized. The scale proposed by Reyes et al., (2015) was utilized first by the evaluator. The samples consisted of leaves from 1st, 2nd, and 3rd plant node (lower, medium, and high leaves) starting from the root. Infected bell pepper fruits were also evaluated. The same samples were evaluated using Leaf Doctor software (Pethybridge and Scot, 2015).

A correlation analysis reported a R^2 of 0.90 in leave samples and 0.97 in infected fruits sample (Figures 1 and 2).

Figure 1. Correlation analysis between two evaluation methods for pepper leaves (*Capsicum annuum*), using visual damage evaluation scales and the Leaf Doctor program for *Phytophthora capsici* disease. $R^2=0.90$. Data points are means of three replications for each treatment.

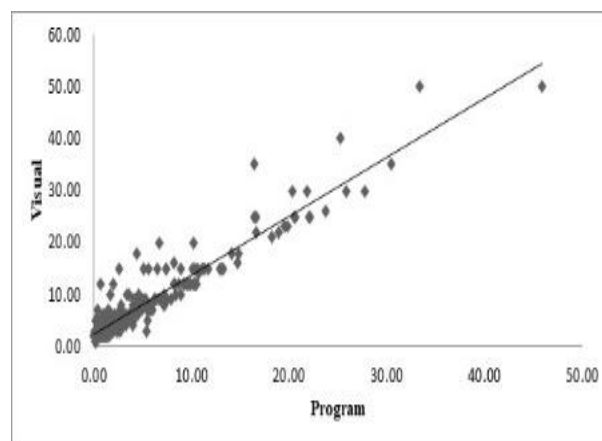
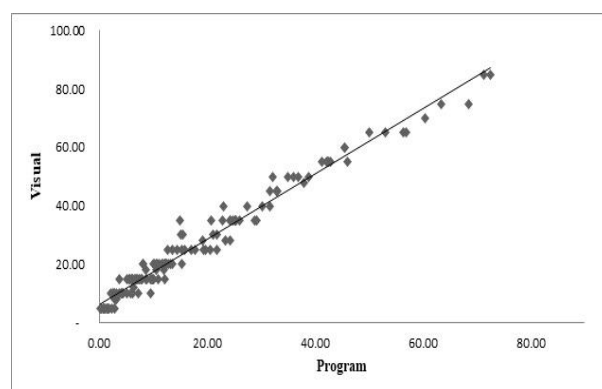


Figure 2. Correlation analysis between two methods of evaluating fruits of the bell pepper crop (*Capsicum annuum*), using visual damage evaluation scales and the Leaf Doctor program for *Phytophthora capsici* disease. $R^2=0.97$. Data points are means of three replications for each treatment.

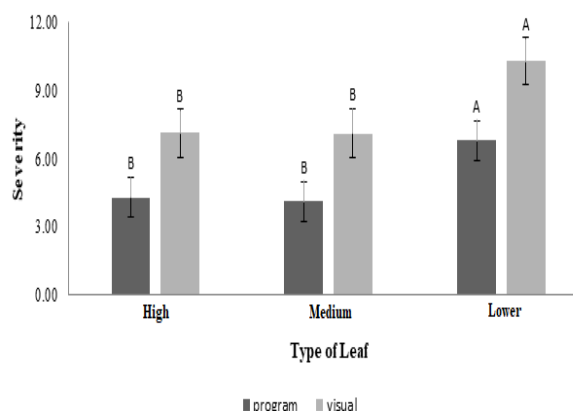


Infected bell pepper leaves and fruits were evaluated 110 to 130 days after the transplant. A visual scale was used to evaluate the leaves and fruits. The same leaves and fruits were evaluated by the program. There are no

statistical differences in the severity of the disease in any of the treatments (Varas, 2021).

Infected bell pepper leaves from different height were evaluated 110 to 130 days after the transplant. Leaf height was considered as lower, medium, and high (1st, 2nd, and 3rd node from the root). A visual scale was used to evaluate the leaves and the same leaves were evaluated by the program. Leaves from the first node (lower) had more severity evaluated by both methods (Figure 3).

Figure 3. Severity of pepper leaves (*Capsicum annuum*) at different heights caused by the pathogen *Phytophthora capsici*. The leaves were evaluated using visual evaluation scales and image processing software. Error bars indicate standard error. Different letters indicate significant differences in each treatment (Tukey $p < 0.05$).



Different agronomical characteristics were evaluated. Plant height, number of flowers, number of harvested fruits, number of diseased fruits, number of healthy fruits and weight of healthy and diseased fruits were evaluated. Treatments with silicon showed a statistical difference compared to the control (Table 1).

Table 1. Agronomic variables in the pepper crop for *Phytophthora capsici* severity under the different doses of silicon.

Variable	T1 Control	T2 Si 100% (400 kg/ha)	T3 Si 50% (200 kg/ha)	T4 Si 75% (300 kg/ha)	T5 Si 25% (500 kg/ha)	T6 Si 50% (600 kg/ha)
Plant height (cm)	63.74b	57.99a	59.36ab	61.20ab	63.92b	61.39ab
Number of flowers	2.81ab	3.25b	2.88ab	2.66ab	3.25ab	3.42b
Number of harvested fruits	3.80ab	2.46a	2.35a	5.00b	2.56ab	3.19ab
Number of diseased fruits	2.10a	2.33a	1.50a	2.20a	1.86a	2.17a
Number of healthy fruits	2.85b	2.51ab	2.65b	2.12a	2.52ab	2.47b
Healthy fruit weight (kg)	182.50a	113.31a	137.06a	160.22a	135.19a	123.31a
Diseased fruit weight (kg)	56.90a	71.56a	41.50a	57.30a	63.79a	58.00a

The analysis of variance determined that there were no statistical differences in yield per treatment. The bell pepper produced under field conditions was not profitable in any treatment. The bell pepper produced at greenhouse conditions showed that the

treatment 3 (Si at 200 kg/ha) was profitable (Table 2). These differences are due to the high prices of the silicon source utilized for the experiment (\$30 for 23 kg) compared to the official price of bell pepper \$ 0.70/kg (CIPA, 2021).

Table 2. Benefit/Cost (B/C) and yield analysis per plant of the bell pepper (*Capsicum annuum*) harvested for 16 weeks. The price of pepper at the time of the trial was \$0.70/kg. The B/C are compared against the control.

Treatment (kg/ha)	Field (kg)	Field B/C	Greenhouse(kg)	Greenhouse B/C
T1 N-P-K (274-73-491)	0.91 a	1.00	1.60 a	1.00
T2 (+Si 400)	0.49 a	0.95	1.68 a	0.94
T3 (+Si 200)	0.78 a	0.97	1.97 a	1.29
T4 (+Si 300)	0.48 a	0.96	1.53 a	0.84
T5 (+Si 500)	0.72 a	0.94	1.84 a	1.06
T6 (+Si 600)	0.92 a	0.98	1.98 a	1.16

DISCUSSION

Bell pepper belongs to the Solanaceae family which contains more than 3,000 species (Mueller et al., 2005). Several *Capsicum* species are described as edible species (Sanatombi et al., 2010). Several pathogens affect this group of plants where *Phytophthora* blight is the most damaging one (Lee et al., 2008). *Phytophthora* blight is caused by the oomycete *Phytophthora capsici*. *P. capsici* is controlled by fungicides such as mancozeb, metalaxyl, mefenoxam and phenylamides. Other controls include soil treatment and water management (Matheron & Porchas, 2002). There is a need to investigate environmentally friendly ways to control the pathogen (Parra & Ristaino, 2001).

Environmentally friendly methods include biological control, resistance genes and nutrient management (Majid et al., 2016). Bacteria such as *Pseudomonas* or *Bacillus* sp. are described to be efficient to control this disease (Jung et al., 2008; Fester & Hause, 2005). Chromosomal regions such as Phyto4.1, 5.1, 5.2, 6.1, 11.1 and 12.1 are involved in resistance to *P. capsici* (Gurr & Rushton, 2005). Silicon has been described to reduce the incidence to the disease in previous experiments (Seo et al., 2004; French-Monar et al., 2010).

The objective of the study was to implement the evaluation of bell pepper fruits and leaves using image processing software. The silicon was tested as a disease control method to determine the best dose in commercial production systems. This methodology differs

from previous investigations in several ways. The essay was executed in greenhouse and field conditions for two years. Previous studies utilized hydroponic systems or pots (Seo et al., 2004; French-Monar et al., 2010). These experiments are more controlled but differ from either greenhouse or field commercial systems.

The assessment of the disease by the program proved to be effective using leaves or bell pepper fruits. Leaves and fruits under different silicon doses did not show statistical differences. The result differs from previous investigations. To assess the disease some experiments utilized formulas (Shanner and Finney, 1977; French-Monar et al., 2010) or visual criteria (Reyes et al., 2015; Seo et al., 2004). The observed difference could be attributed to the experimental conditions and/or the duration of the experiment. In this experiment, the data was analyzed up to 130 days after planting. Other studies determine the disease severity from 9 to 14 days post pathogen inoculation. The authors explained that most of the control plants died in the established experimental conditions (Seo et al., 2004; French-Monar et al., 2010). These differences allowed to determine other variables such as harvested and damaged fruits which previous experiments did not have.

Lower bell pepper leaves showed a higher disease severity. The result is expected since the pathogen infects roots first and then stems (Esfahani et al., 2014). Medium and higher leaves had a lower disease severity. Since bell pepper is a dicot plant, some authors stated that monocot plants mobilize silicon better than dicot plants (Rodrigues et al., 2001; Liang et al., 2006). This could be the reason to not observe differences in the disease severity at the upper or medium part of the plant. Silicon appears to be assimilable in roots but

not in stems and the mobility appears to be very limited (Datnoff, 2007).

The benefit/cost analysis were different at greenhouse and field conditions. At field conditions, silicon was not profitable. In greenhouse experiments, 200kg/ha was 29% more profitable than the control. Warm and humid weather is conducive to the growth of *P. capsici*. The pathogen spreads faster in the field due to multiple spore production and infection cycles (Esfahani et al., 2014). Temperatures between 25-30 °C and 60-80% relative humidity increase the incidence. Rainfall water is the most influential environmental factor for disease incidence (Geuens et al., 2007, Sanogo & Ji, 2013). These differences in the experimental conditions could explain the results in the silicon doses. Previous experiments were more controlled but different from commercial production systems.

Other methods to manage the disease were discarded due to its costs or practicality. Some examples include UV radiation in water irrigation systems (Stanghellini et al. 1984), grafting with resistant rootstocks (Gilardi et al., 2013) and essential oils (Sanogo & Ji, 2013). The application of silicon needs to be constant from transplanting onward (Samuels et al., 1991). This method of application puts silicon ideal to be considered for fertilization plans.

There are two hypotheses of the effect of silicon in plants. First, silicon inhibits fungal growth and penetration of plant tissues. Second, silicon could change the timing of host natural defense mechanisms (Carver et al. 1987; Menzies et al., 1991). Since silicon applications promotes silica bodies embedded in the epidermis wax of bell pepper root cells

(Seo et al., 2004), the former appears to be the right one.

Different forms of silicon presented different results in different experiments. Monosilicic acid (H_4SiO_4) is processed by plants in three phases (Matichencov and Bocharnikova, 2001). Calcium silicate requires an intensive refining process (Park, 2001; Maxim et al. 2008). Magnesium silicate has poor solubility (Weast et al. 1985). Some sources could unbalance manganese, phosphorous or zinc (Marschner et al., 1990). These differences are necessary to consider when silicon is applied.

CONCLUSION

Three conclusions could be obtained from this experiment. First, image processing as an alternative to test *Phytophthora* blight in bell pepper leaves and fruits. The program appears to be more suitable than visual criteria. The program reduces bias, and it is easy to use. Second, silicon is an alternative to manage *P. capsici* in commercial systems. Finally, silicon application is cost efficient in greenhouse conditions at a dose of 200 kg/ha. Field conditions should consider additional alternatives of management compared to the greenhouse.

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