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Control of *Botrytis cinerea* Pers. in "Huascaran" tomato by foliar fertilizer on the coast of Lima, Peru

Control de *Botrytis cinerea* Pers. en tomate cv. "Huascarán" mediante fertilizantes foliares en la costa de Lima, Perú

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Abstract

Botrytis cinerea Pers. (*B.C.*) is the phytopathogen responsible for a wide range of symptom in crops of great importance, such as tomato. Fungicide application are mainly used to control this pathogen; however, their excessive use leads to the development of resistant strains, environmental pollution, and harmful effects on human health. Thus, the aim of the study was to evaluate the effects of two foliar fertilizers contain copper, zinc, and citric acid to reduce gray mold *B.C.* damage in "Huascarán" tomato plants. In laboratory conditions, the effects of the foliar fertilizers on the inhibition of mycelial growth at 6 DAI (days after inoculation) with poisoned PDA (Potato Dextrose Agar) medium were evaluated; and in the field conditions, the foliar fertilizers were sprayed by eight weeks varying the application frequency in one and two weeks. The results showed that in laboratory condition the citric acid improved the antifungal activity complementing well with the Cu and Zn particles presenting T3 the better percentage of mycelial growth inhibition. Under field conditions, most of the foliar applications had a significant control of *B.C.* on foliage but not on flowers and fruits; especially when foliar fertilizers were applied weekly. These results are important to develop strategies to improve disease control and to decrease excessive use of fungicides.

Keywords: *Botrytis cinerea Pers., foliar fertilizers, copper, zinc, citric acid, tomato*

Resumen

Botrytis cinérea Pers. (*B.C.*) es el fitopatógeno responsable de una amplia gama de síntomas en cultivos de gran importancia como el tomate. Su control principalmente se basa en la aplicación de fungicidas químicos; sin embargo, su uso excesivo conduce al desarrollo de cepas resistentes, contaminación ambiental y efectos perjudiciales para la salud humana. Por ello, el objetivo el estudio fue evaluar el efecto de dos fertilizantes foliares que contienen cobre, zinc y ácido cítrico en el control del moho gris *B.C.* en tomate cv. Huascarán. En condiciones de laboratorio se evaluó el efecto de los fertilizantes foliares en la inhibición del crecimiento micelial a los 6 DDI (días después de inoculación) con el método del medio envenenado en PDA (agar de papa

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y dextrosa); y en condiciones de campo se aplicaron los fertilizantes foliares por ocho semanas variando la frecuencia de aplicación en una y dos semanas. Los resultados mostraron que en condiciones de laboratorio el ácido cítrico incrementó la actividad antifúngica complementándose bien con las partículas de Cu y Zn presentando el T3 el mejor porcentaje de inhibición del crecimiento micelial. En condiciones de campo la mayoría de las aplicaciones tuvieron un control significativo de *B.C.* en el follaje, pero no en flores ni en frutos; destacando aquellos donde se aplicó los fertilizantes foliares semanalmente. Estos resultados son importantes para desarrollar estrategias dirigidas a mejorar el control de la enfermedad y disminuir el uso masivo de fungicidas.

Palabras claves: *Botrytis cinérea Pers., fertilizantes foliares, cobre, zinc, ácido cítrico, tomate.*

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the vegetables with the highest fresh and industrial consumption in the world (Pérez, 2014). Because of its great importance in human consumption, it is essential to reduce the limitations in its cultivation; being one of most important in the phytosanitary aspect the damage caused by *Botrytis cinerea* Pers. that infects leaves, stem, flowers and fruits (Tejada, 2014). *B. cinerea* Pers. is difficult to control because it is capable to affect crops at any phenological stage, infects any plant organ and has asexual and sexual stages to survive in favorable and unfavorable conditions (Espinosa, 2006).

Due to the considerable incidence of *B. cinerea* Pers. and its economic importance that it has in other important crops such as grapes, strawberries, ornamentals, etc.; there are many studies on its possible control methods (Benito et al., 2000). Most of the strategies are focusing on the use of chemical fungicides (Dean et al., 2012). However, their overuse leads to the development of resistant strains, environmental pollution and even human health problems because of the toxic residues (Molina et al., 2006).

In this context, innovative approaches such as the optimization of plant nutrition are required to overcome the problems caused by the intensive use of pesticides; in addition, improve the crop health and yields. Nutrients such as copper and zinc are involved in the plant protection by regulating physiological functions and participating in the activation of defense mechanisms (Chmielowska et al., 2010; Cabot et al., 2019). Also, antimicrobial and bactericidal effects have been reported in copper and zinc formulations (La Torre et al., 2018; Kalia et al., 2020). In addition, a variety of antioxidants have the potential to control diseases, one of them is the citric acid reporting antifungal effects (Elkorany & Mohamed, 2008; Morgunov et al., 2017).

The aim of the study was to evaluate the effects of two foliar fertilizers containing copper, zinc and citric acid in the control of gray mold in tomato, in order to develop strategies to improve the disease control and to reduce excessive use of chemical fungicides.

Material and Methods

Laboratory phase

Location

This phase was carried out in the Plant Pathology laboratory of the Universidad Nacional Agraria La Molina (UNALM) in Lima-Peru.

Mycelial growth inhibition

B.C. pure colony endemic from La Molina previously isolated, recognized and purified by Plant Pathology laboratory of the UNALM was used. The inhibition of mycelial growth of the foliar fertilizers was performed with the poisoned medium method. PDA culture medium was prepared in Erlenmeyer and sterilized in the autoclave; subsequently, when the culture medium was at an adequate temperature of 45 °C, approximately, it was poisoned by the four treatments tested in the study (Table 1).

Table 1. Mycelial growth inhibition (%) of *Botrytis cinerea* by treatments under laboratory conditions.

Treatments	Product	Active ingredients	Dose	Mycelium diameter (mm) at 6 DAI	IMG (%)
0	-	-	-	80.00 a	0.00
1	Zynergy	2.11 % Cu + 3.75 % Zinc	0.25 %	24.88 b	68.90
2	Zynergy	2.11 % Cu + 3.75 % Zinc	0.50 %	4.00 c	95.00
3	Fx-31	1.5 % Cu + 3.6 % Zinc + 15.4 % Citric acid	0.25 %	1.50 c	98.13
C.V: 4.64 %					

Note: DAI: Days after inoculation, IMG: Mycelial growth inhibition, C.V: Coefficient of variability. Means with same letters are not different statistically according to Tukey mean test ($p < 0.05$).

When the poisoned medium solidified, a 0.5 cm diameter portion of the active growth zone of the *B. cinerea* Pers. pure colony was placed at the center of the Petri dish. The same procedure was performed with the check treatment, with the difference that the PDA medium was not poisoned. The Petri dish with the inoculum was sealed with Parafilm and placed in the incubator at a temperature of 25 °C. The distance of the diameter of the mycelial growth was measured up to 6 days after inoculation (DAI); without considering the distance of the mycelium disc placed as inoculum.

Inhibition of Mycelial Growth was determined by the following equation:

$$IMG=[(mgc - mgf)/(mgc)] \times 100$$

IMG (%) = Percentage of inhibition mycelial growth
mgf = Mycelial growth of foliar fertilizer treatment
mgc = Mycelial growth of the witness

The laboratory phase was performed under a Completely Randomized Design (CRD) with 5 treatment and 4 repetitions.

Field phase

The tomato field was located in La Molina, Lima, Lima, Peru. The tomato plants were transplanted at a distance 0.6 cm x 1.5 m. The field was under conventional agronomic management. Tomato cultivar was Huascaran.

Treatments (Table 2) were sprayed in the eighth week after transplantation (8 WAT) and lasted for eight weeks, using a 20 L manual application sprayer. The evaluation lasted 10 weeks, from vegetative growth to fruit set as a critical stage of *B. cinerea* Pers. The field phase was performed under a Randomized Complete Block Design (RBCD) with 5 treatments and 4 blocks, with an evaluation area of 300 m^2.

Area Under Disease Progress Curve (AUDPC)

Foliar severity (%) was quantified of the area affected by *B. cinerea* Pers. throughout the plant (as an assessment unit) at weekly intervals. The area under the disease progress curve (AUDPC) was calculated during the 17 WAT to the 21 WAT with the foliar severity data, using the Campbell & Madden (1990) formula. This value was got

Table 2. Foliar severity (%) of *B.C.* and AUDPC using foliar fertilizers in tomato cv. "Huascaran"

Trt	Product	Application	Code	Foliar severity (%) of *B.C.*					AUDPC
				17 WAT	18 WAT	19 WAT	20 WAT	21 WAT	
0	-	-	T0	0.31	4.50	25.81	64.55	79.46 a	943.24 a*
1	Zynergy	Weekly	Zy-1S	0.19	2.72	14.35	45.40	63.33 c	659.62 b
2	Fx-31	Weekly	Fx-1S	0.13	2.02	7.68	33.35	60.86 c	514.79 b
3	Zynergy	Every 2 weeks	Zy-2S	0.27	3.67	25.97	57.59	76.60 ab	879.70 a
4	Fx-31	Every 2 weeks	Fx-2S	0.20	3.02	13.73	45.00	66.92 bc	667.16 b
C.V.								7.34 %	11.70 %

Note: WAT: weeks after transplant, AUDPC: Area Under Disease Progress Curve, C.V: Coefficient of variability. Means with same letters are not different statistically according to Tukey mean test ($p < 0.05$).

by means of the following equation:

$$AUDPC = \sum_{i=1}^{n} \frac{Y_{i+1} + Y_i}{2} [t_{i+1} - t_i]$$

∑= Summation of n observations
Y_i= Percentage of the leaf area affected by the pathogen on day i after transplantation
n= Total number of evaluations
t_i= Days after transplantation

Flowers and fruits incidence (%)

Flowers incidence (%) was determined by counting healthy and diseased flowers in each flower cluster each week after flowering, using the following equation:

$$FI(\%) = (FD/ FT) \times 100$$

(%) = Percentage of diseased flowers
FD= Number of diseased flowers
FT= Number of total flowers (healthy + diseased)

Fruits incidence (%) was determined by counting healthy and diseased fruits, using the following equation:

$$FrI(\%) = (FrD/ FrT) \times 10$$

(%) = Percentage of diseased fruits
FrD= Number of diseased fruits
FrT= Number of total fruits (healthy + diseased)

Phytotoxicity

Phytotoxicity was evaluated one week after the eight applications, using the scale of Alzate et al. (2009) from 0 to 5: a) Scale 0: Plants equal to the witness plants, b) Scale 1: Plants with mild chlorosis leaves, c) Scale 2: Plants with accentuated chlorosis leaves and slight distinguishable reduction in growth, d) Scale 3: Plants with growth inhibition, marked chlorosis leaves and morphological abnormalities, e) Scale 4: Affected plant with no possibility of recovery, but there is still presence of green tissue and f) Necrotic plants.

Statistical analysis

Tukey's mean comparison test was performed with a 95 % confidence interval (α =0.05) using the statistical software R Studio 4.1.2.

Results and Discussion

Laboratory Phase

Inhibition of mycelial growth

Results at 6 DAI under laboratory conditions are shown in figure 1. All treatments (Table 1) showed mycelium diameter significant differences ($p < 0.05$). According to Ouda (2014) copper particles can cause harmful effects in *B. cinerea* Pers. hyphae. In addition, He et al. (2011) mentioned that zinc particles can inhibit the growth of a gray mold when they affect cellular functions.

Treatment T3 (1.5 % Cu + 3.6 % Zn + 15.4 % Citric Acid) presented the best antifungal effect with a value of 98.13 %, citric acid probably goes well with copper and zinc by reducing fungal development. According to Shokri (2011) citric acid has growth-inhibiting mechanisms, such as

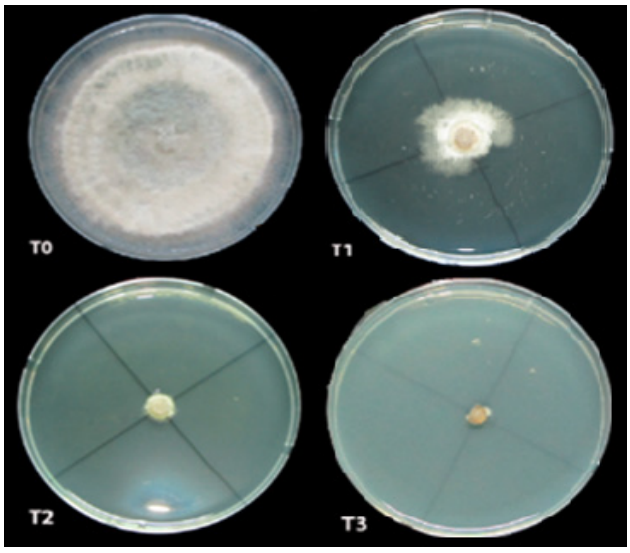

Figure 1. Mycelial growth of *Botrytis cinerea* of each treatment in PDA poisoned medium at 6 DAI

the decrease in the internal pH of the microbial cell by the ionization of unassociated acid molecules and the alteration of cell membrane permeability.

Field phase

Area Under Disease Progress Curve (AUDPC)

Foliar severity (%) of *B. cinerea* Pers. (Table 2) in treatments with foliar fertilizers decreased since 18 WAT. Because of the antifungal activity of the components of foliar fertilizers and also to their essential role in the defense of plants against phytopathogens. Especially in the FX-1S treatment that got at 19 SDT and 20 SDT a third and half of the percentage of severity of T0.

La Torre et al. (2018) and Rai et al. (2019) mentioned that within the pathogen Cu particles can act at multiple sites generating extremely harmful ROS, leading to the denaturation of structural and enzymatic proteins, inhibiting mycelial growth and spore germination. In addition, according to Kalia et al. (2020) Zn particles suppress the spore-forming structures and inhibit their germination because of the multiple mechanism of antimicrobial action; such as ROS generation, destabilization and liquefaction of the cell membrane, organelles and macromolecules

It is essential that Cu and Zn particles are present on leaf surfaces to present antifungal activity (La Torre et al., 2018). In the Zy-2S treatment, the application made each two weeks was not enough to generate a long-term protective effect of the Cu and Zn particles. This protective effect was evidenced in the Zy-1S treatment causing a significantly lower percentage of foliar severity (%) up to 19 WAT compared to T0 and Zy-2S.

The plant absorbs nutrients through the leaf surface, passing through the cuticle, cell wall and cell membrane after foliar application (Fageria et al., 2009). Chmielowska et al. (2010) mentioned that in plant, the stress caused by Cu induces the production of peroxidases that provide resistance against pathogens, creating a highly toxic environment and is involved in the accumulation of lignin and suberin, which strengthens the cell wall. In addition, according to Singh et al. (2018) Zn in the plant participates in protecting the cell membrane against the oxidative damage caused by free radicals.

Also, Cabot et al. (2019) mentioned that high concentration of Zn in the plant and the damage caused by pathogens trigger common signaling pathways, such as salicylic acid and jasmonates (JA), which play fundamental roles in the systemic defense of plants; if the plant's tolerance to Zn increases also increases its tolerance to necrotrophic fungi such as *B. cinerea* Pers. Therefore, despite of decreasing the protective effect of the Cu and Zn particles by their disintegration on the surface of the leaves over the weeks, the increase in the defenses of the plant by the assimilation of these nutrients to the plant tissue, could have favored to reduce the foliar severity (%) at 19, 20 and 21 WAT compared to the check treatment. In the treatments where products were applied weekly, the reduction was greater.

Although the product was applied in the Fx-2S treatment each two weeks, a protective effect contrary to Zy-2S treatment was observed, resulting in values of foliar severity (%) and AUDPC significantly lower than the check and similar to Zy-1S (Table 2). Because the Fx-2S treatment had the citric acid effect that strengthened the protective effect. Citric acid is an antioxidant generated by infected tissues in senescence, which captures free radicals that deteriorate the cell membrane to form innocuous compounds. Thus, the effect of damage by the pathogen and the development of the disease is decreased (Elad, 1992; Elkorany & Mohamed, 2008).

Weekly application of the same product in the Fx-1S treatment had a noticeable improvement in the reduction of foliar severity (%) at 19 WAT, resulting in values of 7.68 % and 13.73 % for Fx-1S and Fx-2S, respectively. The protection was reduced at 20 WAT because of the degradation of the components of foliar fertilizers, presenting in that week a peak of increase in the disease.

The citric acid present in the formulation of Fx-31 produces an effect in plant, Elad (1992)

mentioned that it reduces the production of ethylene in vegetative tissue, because the conversion of 1-aminocyclopropane-1-caboxilic acid (ACC) to ethylene is mediated by the reaction of free radicals. This inhibition of ethylene production effectively controls gray mold; because *B. cinerea* Pers. is a pathogen that colonizes aged tissues, and its infection is associated with the increased of the ethylene production in the host tissue. These effects, in addition to the produced in a plant by the copper and zinc, increased the control of the disease, allowing the Fx-2S and Fx-1S treatments a percentage of foliar severity (%) and AUDPC significantly lower than Zy-2S and Zy-1S.

Flowers and fruits incidence (%)

No significant reduction in flowers and fruit incidence (%) of the treatments regarding the check treatment was observed (Table 3). Because the spraying of the products must fall and cover the entire flower to avoid any infection, since the damage caused by *B. cinerea* Pers. in flower leads to loss of the structure (Mauricio, 2018). Flowers were usually behind the leaves; thus, most of the particles that provided a protective effect were retained in the foliage. In addition, as the flower is of short duration, the increase in the plant's defense was not taking advantage in the long term (Cristescu et al. 2002; Elad & Evensen, 1995).

A low incidence rate in fruits was reported, no greater than 2 % in all treatments until the last week of evaluation (Table 3), because, during the harvest, at the beginning at the 17 WAT, break stage fruits (turning orange) coloration were harvested, this decreased the number of mature fruits in the field susceptible to the *B. C.* infection (Shah et al., 2012).

Phytotoxicity

With the foliar fertilizers, dose and application period used in the experiment, plants with phytotoxicity scale 0 and 1 were observed (Figure 2). Treatments Zy-1S and Fx-1S had the higher percentages (Table 3).

Conclusions

Foliar fertilizers showed a high efficacy in controlling *B. cinerea* Pers. under laboratory conditions; under field conditions most treatments, except for Zy-2S, had some level of control in foliage, but not in flowers or fruits. All treatments reported a lower percentage of phytotoxicity (scale 1).

Author contributions

Elaboration and execution, development of methodology, conception and design; editing of articles and supervision of the study have involved all authors.

Conflicts of interest

The signing authors of this research work declare that they have no potential conflict of

Table 3. Flowers and fruits incidence (%) of *B.C.* and foliar phytotoxicity using foliar fertilizers in tomato cv. 'Huascaran'

Trt	Code	Flowers incidence (5) of *B.C.*			Fruits incidence (%) of *B.C.*			Plants with phyto-toxicity scale 1 (%)
		16 WAT	17 WAT	18 WAT	17 WAT	18 WAT	19 WAT	
0	T0	25.13	29.98	32.33 a*	0.49	1.75	1.67	0
1	Zy-1S	17.29	21.55	26.70 a	0.39	1.18	0.84	75
2	Fx-1S	13.87	15.30	23.02 a	0.30	0.88	0.71	65
3	Zy-2S	23.42	26.26	30.72 a	1.11	1.68	1.26	52
4	Fx-2S	16.70	18.50	27.28 a	0.39	1.26	1.33	30
		C.V.		17.01 %				

Note: WAT: weeks after transplant, C.V: Coefficient of variability. Means with same letters are not different statistically according to Tukey mean test ($p < 0.05$).

Figure 2. Plant with phytotoxicity scale 1 (mild chlorosis) found in the experiment.

personal or economic interest with other people or organizations that could unduly influence this manuscript.

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