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Control of *Botrytis cinerea* Pers. in 'Okitsu' Satsuma mandarin by biological and chemical fungicides in Huaura, Peru

Control de *Botrytis cinerea* Pers. en mandarina Satsuma cv. 'Okitsu' mediante fungicidas biológicos y químicos en Huaura, Perú

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Abstract

The flowers blight caused by *Botrytis cinerea* Pers. is among the most important citrus diseases, especially in cultivars whose flowering coincides with the humid seasons of the year. As a result of the pathogenic features of this fungus and the complex nature of its control, it is necessary to establish a correct plan for the usage of highly efficient fungicides. The study aimed to evaluate the effect of four chemical fungicides, such as Captan (0.25%), Propineb (0.25%), Fludioxonil + Cyprodinil (0.05%), and Iprodione (0.15%); as well as the effect of a biological fungicide, such as *Melaleuca alternifolia* extract (0.1%), on *Botrytis cinerea* Pers. Two phases were established: the first, under laboratory conditions of Department of Plant Pathology of National Agrarian University-La Molina (UNALM), evaluated the effect on mycelial inhibition at 1, 3, and 7 days after inoculation with poisoned potato dextrose agar medium. The second, under field conditions (Sayan - Huaura), evaluated the effect on incidence of the disease in flowers. In the field condition, two applications, incidence, and humid chambers were evaluated. The yield was estimated by counting the fruits. The results showed that, under laboratory conditions, Captan, Fludioxonil + Cyprodinil, and Iprodione exhibited high efficacy in the control of *B. cinerea*. However, under field conditions, Fludioxonil + Cyprodinil and Iprodione exhibited a significant control of *B. cinerea*. A similar trend was obtained for the yield estimates.

Key words: *Flowers blight, Botrytis cinerea, mandarin, Satsuma Okitsu, fungicides.*

Resumen

El tizón de las flores causado por *Botrytis cinerea* Pers. es una de las enfermedades más importantes en el cultivo de cítricos, especialmente en aquellos cultivares cuya floración coincide con épocas húmedas del año. Debido a las características patogénicas de este hongo y a la complejidad de su control, resulta de suma importancia establecer un correcto plan de uso de fungicidas de alta eficacia. El presente trabajo de investigación tuvo como objetivo evaluar el efecto de cuatro fungicidas de origen químico; Captan (0,25%), Propineb (0,25%), Fludioxonil + Cyprodinil (0,05%) e Iprodione (0,15%), y uno de origen biológico; Extracto de *Melaleuca alternifolia* (0,1%). Se establecieron dos fases; la primera bajo condiciones de laboratorio (UNALM) evaluando el efecto en la inhibición micelial a los 1,3 y 7 DDI con la metodología de medio PDA envenenado; y la segunda bajo condiciones de campo (Sayán-Huaura) evaluando el efecto en la incidencia de la enfermedad en flores. Para esta segunda fase se determinó realizar dos aplicaciones y se evaluó la incidencia en campo mismo y en cámaras húmedas. Por último, se estimó la producción bajo la metodología de conteo

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de frutos. Los resultados demostraron que a nivel *in vitro* los fungicidas químicos; Captan, Fludioxonil + Cyprodinil e Iprodione presentaron alta eficacia en el control de *B. cinerea*; sin embargo, bajo condiciones de campo solo los fungicidas; Fludioxonil + Cyprodinil e Iprodione tuvieron un porcentaje de control importante frente a *B. cinerea*. Resultados similares se obtuvieron en el estimado de producción.

Palabras clave: *Tizón de las flores, Botrytis cinerea, mandarina, Satsuma Okitsu, fungicidas.*

Introduction

Citrus fruits is among the main agricultural export products in Peru and the world demand for citrus fruits has increased in recent years. In 2017, the export of citrus fruits in Peru was approximately 143,000 tons, thus positioning Peru as the seventh worldwide exporter of citrus fruits. In addition, we consolidated our position as the leading citrus fruit exporter in America by surpassing countries such as Chile, United States of America, and Argentina. (Ministerio de Comercio Exterior y Turismo [MINCETUR], 2018).

The weather condition of the Peruvian coast is highly temperate and relatively humid. As a consequence of this condition, citrus fruits are affected by pests and diseases throughout their crop cycle. For this reason, in the main citrus production, chemical control method is the most adopted method for better pest control, with less time and high efficiency (Colonia, 2013).

During the flowering season of citrus and many other crops, "Gray mold" or "Flower blight," caused the fungus *Botrytis cinerea* Pers., is the most important disease. This fungus infection causes petals necrosis which are covered with mycelium and gray spores under wet conditions (Benito et al., 2000). Infected flowers often induce fruit dropping and injury to developing fruit which reduces the yield and diminishes the fruit quality (Castro et al., 2000).

For the control of this disease, different fungicides are applied, among which some are no longer effective (Llanos & Apaza, 2018; Pappas and Elena, 1992). In the province of Huaura, fungicides with different active principles are used to control gray mold disease in citrus fruits, among them are Carbendazim, Captan, or Propineb.

The study aimed to determine the effect of different chemical fungicides and one biological fungicide on the development of *B. cinerea* under field and laboratory conditions. Furthermore, profitability was evaluated during a production campaign through the use of these proposed fungicides.

Materials and Methods

The present research was carried out in two phases: (i) the laboratory phase was performed in the Diagnosis Clinic of Agronomy Faculty, Department of Plant Pathology of National Agrarian University - La Molina (UNALM), Lima state of Peru; (ii) the field condition phase was performed in the citrus production fields of "El Chilco" farm located in Santa Rosa town, Sayan district, Huaura province, Lima state, Peru. To evaluate the effect of the treatments on incidence of *B. cinerea*, the products were applied to 'Okitsu' Satsuma mandarin plants.

Tangerine flowers with symptoms *of B. cinerea* were collected and, using a hypodermic syringe, structures of the fungus (mycelium and spores) were collected and placed in sterilized Petri dishes containing potato dextrose agar (PDA) medium. The plates were incubated at 25°C for four days. When the pathogen colony attained a growth greater than 50% of the plate diameter, it was visualized with a microscope to identify its morphology. From the same plates, some portion of PDA medium with structures of the fungus was transferred to another plate containing a sterile culture medium in order to obtain a pure colony of *B. cinerea*.

The fungicide test was performed using the poisoned food technique. In brief, PDA culture medium was prepared and poured into Erlenmeyer flasks in similar quantity (100 mL) and then sterilized in an autoclave.

Six experimental treatments were performed in this study, which correspond to evaluation of four chemical fungicides, one biological fungicide, and one control (control without fungicide) (Table 1).

The culture medium was poisoned before it reached an approximate temperature of 45°C. It was incubated at room temperature until it solidified. Each treatment performed in four replicates (4 plates). A portion of 0.6 cm diameter of PDA medium extracted from the growth zone of the pure colony was placed in the central part of each plate containing solidified poisoned PDA medium. For control, only portions of

Table 1. Treatment with fungicides doses (%), *in vitro* test, and control test for the control of *Botrytis cinerea* in citrus.

Treatment	Active Ingredient	Product	Doses
T0	Control	----	----
T1	Captan	Forsem 80 PM	0.25 %
T2	Propineb	Syl 70 PM	0.25 %
T3	Fludioxonil + Cyrprodinil	Switch 62.5 WG	0.05 %
T4	Iprodione	Fobos 50 PM	0.15 %
T5	Tea Tree Extract	Timorex Gold (20 %)	0.1 %

PDA medium without a colony of the fungus were used following the same procedure above. The seeded plates were conditioned in an incubator at 25°C.

Mycelial growth was measured at 1, 3, and 7 days after inoculation (DAI). Growth of the fungus colony was measured through the distance from end of the initial inoculum disk. Average diameter of mycelium growth (mm) in the Petri dish with poisoned PDA and percentage of growth inhibition (PIC) for each treatment were determined following standard procedures. The experimental design was completely randomized with four replicates. Normality analysis, variance homogeneity, and Tukey's mean comparison test at 95% confidence level ($\alpha = 0.05$) were performed using Minitab statistical program v18.

The second phase, under field condition, was performed in a commercial 'Okitsu' Satsuma mandarin (*Citrus reticulata*) field grafted on Citrumelo rootstock, which is four years old, during the flowering and fruit set season of 2019 campaign. This field was under unconventional agronomic management, with advancement of the phenological stages to obtain early harvests and flowering that coincided with the coldest and wettest times of the year.

Two applications were made at an interval of 10 days (6^{th} and 16^{th} of May, 2019) using a 20 L capacity sprayer backpack. 5 L of water was used for the application of the treatments for each plant. The fungicides used in this phase are shown in Table 1. The treatments, except Timorex Gold and control, were mixed with Aquacid (pH regulator) at a dose of 0.1 mL/L.

The trees were spaced by 6 meters between lines and 4 meters between plants. A tree of 'Okitsu' Satsuma mandarin was considered as a sampling unit. Each treatment consisted of four trees (four replicates). In each tree, 25 inflorescences were evaluated per quadrant of the tree, while five flowers per tree quadrant were evaluated in a humid chamber.

Incidence (%) of *B. cinerea* was measured in citrus fields and humid chambers. With the incidence data, area under the disease progress curve (AUDPC) and relative area under the disease progress curve (AUDPCr) were calculated. AUDPCr was calculated by dividing ABCPE by number of days of the experiment and then multiplied by 100 (Campbell & Madden, 1990). AUDPC was calculated as follows:

$$AUDPC = \sum_{i}^{n-1} (y_i + y_{i+1}/2)/(t_{i+1} - t_i)$$

$\sum$ = summation of n observations

n = I-th observation

y_i = proportion of disease (incidence) affected in the i-th observation

t_i = time (days) after the I-th observation.

For crop yield estimation, the curdled fruit was counted and relationship between the number of fruits per plant and yield per plant was used. For this estimation, the formula proposed by Otero (2004) for Satsuma mandarin was applied.

The experimental design adopted was Randomized Complete Block Design (RBCD), with six treatments and four replicates. For the evaluated variables, normality, analysis of variance homogeneity, and Tukey's mean comparison test were performed at 95% confidence level ($\alpha = 0.05$) using Minitab statistical program v18.

Results and Discussions

The highest PIC values (%) at 7 DAI were obtained by Captan (100%) and Iprodione (100%) treatments, followed by Fludioxonil + Cyprodinil (94.1%) treatment. However, Propineb (0%), tea tree extract (0%), and control (0%) treatments were statistically equal at 1, 3, and 7 DAI (Table 2). The result at 7 DAI under laboratory condition is shown in Figure 1 for each treatment.

Table 2. Percentage growth inhibition (PIC) of *Botrytis cinerea* by treatment under laboratory condition.

	Treatments	Evaluation Dates					
		1 DAI		3 DAI		7 DAI	
T0	Control	0.0%	D	0.0%	D	0.0%	C
T1	Captan	100.0%	A	100.0%	A	100.0%	A
T2	Propineb	59.6%	B	60.0%	B	0.0%	C
T3	Fludioxonil + Cyprodinil	100.0%	A	100.0%	A	94.1%	B
T4	Iprodione	100.0%	A	100.0%	A	100.0%	A
T5	Tea Tree Extract	31.6%	C	36.5%	C	0.0%	C
	α: 0.05	CV: 2.35%		CV: 2.26%		CV: 3.29%	

Note: DAI: Days after inoculation; Equal letters have no statistical difference in Tukey (α: 0.05); CV: coefficient of variability.

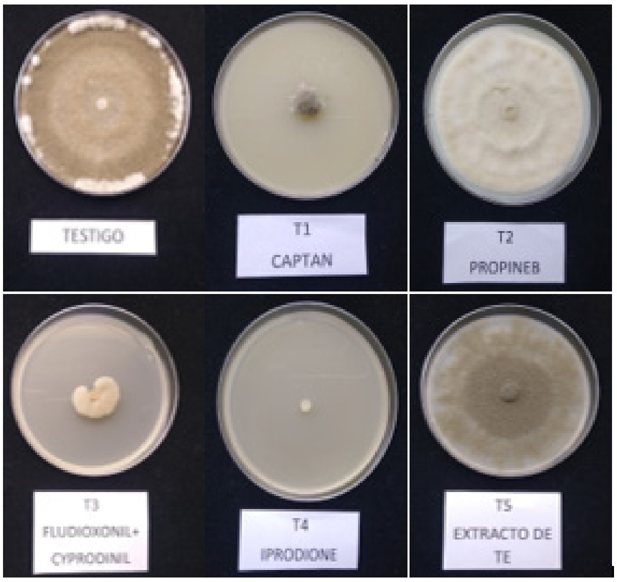

Figure 1. Mycelial growth (mm) of *Botrytis cinerea* from each treatment in poisoned PDA medium at 7 days after inoculation.

The best treatments in the poisoned PDA medium test were Captan and Iprodione treatments, both of which achieved a 100% PIC. However, in Figure 1, it can be seen that *B. cinerea* managed to infect the initial mycelial disc following treatment with Captan. Therefore, it can be concluded that Iprodione treatment was the best, since it completely inhibited the growth of *B. cinerea.* This latter event can be explained by the fact that Iprodione exhibits a local and contact systemic action, unlike Captan which exhibits only a contact action (Williamson et al., 2007).

Ortiz (2009) evaluated (via *in vitro* conditions) different fungicides for the of control *B. cinerea* isolated from artichoke heads and demonstrated that mycelial growth rate was 0 cm in Petri dishes using Iprodione treatment and 0.37 cm using Propineb treatment. This result is similar to the result obtained in this study. However, in the same experiment, a growth rate of 0.58 cm was observed following Captan treatment, which differs from the result of the present study.

Similar results were observed in the *in vitro* efficacy evaluation of fungicides for the control of *B. cinerea* isolated from rose cultivation, in which treatment with Captan allowed mycelial growth of 2.5 cm, while Propineb allowed a mycelial growth of 8.3 cm at 7 DAI in Petri dishes with artificial culture medium (Restrepo, 2010).

Panebianco et al. (2005) conducted a poisoned PDA medium test using 302 isolates of *B. cinerea* from grapes fruits and found 10.3% specific resistance to Iprodione and no isolate resistant to Fludioxonil.

Treatment with *Melaleuca alternifolia* extract achieved a PIC of approximately 35% for the first two evaluations. Antonov et al. (1997) evaluated the inhibition of conidia germination and mycelial growth of *B. cinerea* with natural products and found that, at a concentration of 1%, tea tree oil achieved complete inhibition of conidia germination and growth of germinative tube by 92%. Yu et al. (2015) mentioned that the fungicidal action of tea tree oil against *B. cinerea* is mainly due to two terpenes (terpinen-4-ol and 1,8-cineole) which act by affecting the cell membrane and organelles of the fungus, respectively. For the second phase, under field condition, incidence of 'Okitsu' Satsuma mandarin flowers in field and humid chambers was evaluated.

Figure 2 relates the incidence data obtained during assessments to the relative humidity (RH) data of the area during the assessments. This figure clearly

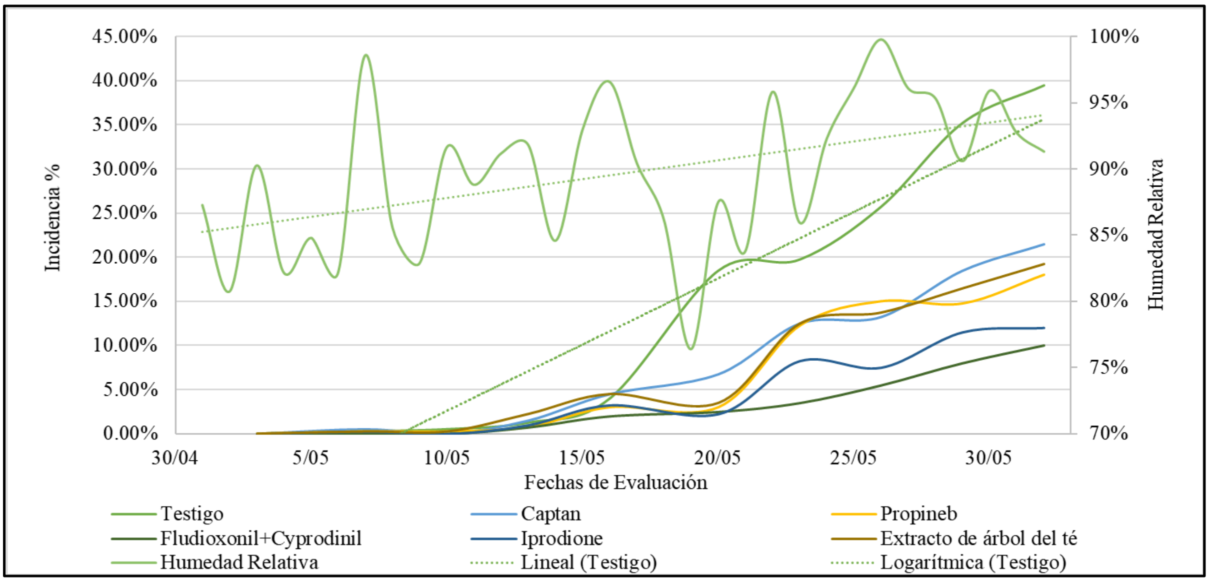

Figure 2. Progress of the incidence of *B. cinerea* in Satsuma var. Okitsu mandarin flowers for each treatment during the evaluation period and its relationship with relative humidity of the area.

shows the difference between the curve drawn by control and other treatments.

In addition, how the trend line of RH increased according to the passage of time was clearly illustrated and this coincides precisely with increase in incidence of the disease in each of the treatments. At very humid climate condition, *B. cinerea* was able to establish itself on citrus flower petals and stamens. This disease can kill single flowers, entire inflorescences, or initiate a regressive death that advances by several centimeters at the peduncle (Mooney, 2001).

In the analysis of the test under field condition, a relationship between RH and incidence of *B. cinerea* in 'Okitsu' Satsuma mandarin flowers was determined. It was observed that the incidence of the pathogen increased considerably when RH is approximately 90%. This coincides with La Torre and Rioja (2002), who report that the optimal conditions for *B. cinerea* infection in many crops is 20°C and RH above 90%. Castro et al. (2000) mentioned that, for citrus cultivation, flower buds or recently curdled fruits are the most susceptible to attack by B. cinerea and that the attack occurs only under very humid condition and cold temperature (18°C).

To determine the effectiveness of each treatment, AUDPC and AUDPCr were determined. The results obtained are shown in Table 3. Based on AUDPCr, the best treatment was Fludioxonil + Cyprodinil, with 2.9% AUDPCr, followed Iprodione, with 4.24% AUDPCr. However, based on the statistical test applied, these results were non-significantly.

The control treatment presented the highest percentage AUDPCr (13.32%), which turned out to be significantly different from the other treatments. Eyzaguirre (1972) compared seven fungicides for the control of *B. cinerea* in open flowers of Valencia orange and found that the disease control is better achieved with systemic/curative action products than with contact/preventive action products. This coincides with our evaluation under field conditions, since Fludioxonil + Cyprodinil had systemic and curative action, unlike the other treatments that presented mainly contact fungicide characteristics.

Treatment with Captan under field condition (Table 3), unlike under laboratory condition, did not achieve such a high percentage of control; however, it maintained a significant difference with control when evaluated in terms of AUDPC. Captan, like most old fungicides, has maintained its effectiveness in spite its intensive usage. This is due to the fact that it has multiple mode of actions and hardly generates resistance. However, the control achieved by Captan is not totally efficient, since it has low biochemical specificity and is limited to a protective action on the applied crop surface (Mondino, 2002).

Propineb, like Captan, is among the first groups of fungicides in the market, which are characterized by multiple sites of action. These two treatments are highly recommended fungicides for the control of gray rot in crops such as grapevines (Trimmer et al., 2003) and are still recommended for this purpose till date, although only for preventive purpose and within a plan of rotating applications.

Table 3. AUDPC, ADPCEr, and incidence of citrus flowers in humid chambers with *Botrytis cinerea* in Satsuma mandarin treated with different fungicides in field condition.

	Treatments	AUDPC		AUDPCr	Incidence of flowers with *Botrytis cinerea* in humid chambers					
					2020, May 13		2020, May 22		2020, May 29	
T0	Control	3.86	A	13.32%	51.25%	B	90.00%	A	56.25%	AB
T1	Captan	2.11	B	7.26%	46.25%	AB	86.25%	A	47.50%	ABC
T2	Propineb	1.78	BC	6.13%	33.75%	BC	80.00%	A	57.50%	A
T3	Fludioxonil + Cyprodinil	0.84	D	0.029	18.75%	C	18.75%	B	21.25%	C
T4	Iprodione	1.23	CD	4.24%	33.75%	BC	37.50%	B	30.00%	BC
T5	Tea tree oil	1.94	BC	0.0667	60.00%	AB	90.00%	A	50.00%	AB
	α: 0.05	CV:17.77%			CV: 24.6%		CV: 16.8%		CV: 27.2%	

Note: Means with different letters have statistical difference (Tukey α: 0.05); AUDPC: Area under disease progress curve; AUDPCr: Area under disease progress curve relative.

Table 4. Economic analysis of Mandarin Satsuma for each treatment according to average yield data.

Treatment	Field (Tn/ha)	Cost (US$)	Doses xha	Application cost (US$/ha)	Profitability (US$/ha)
Testigo	34.65				$34,650.00
Captan	37.14	$14.93	5.0	$74.65	$36,990.70
Propineb	34.8	$10.45	5.0	$52.25	$34,695.50
Fludioxonil + Cyprodinil	40.59	$223.88	1.0	$223.88	$40,142.24
Iprodione	39.99	$41.79	3.0	$125.37	$39,739.26
Extracto de árbol del té	36.08	$73.13	2.0	$146.26	$35,787.48

In this study, tea tree oil treatment showed a deficient control under field condition. In the technical data sheet of products authorized for our country, it does not register a recommended dose for citrus fruits; however, 200 mL/cil was determined due to water used in the area for fungicide application, which is 2000 liters per hectare, as a dose of 2 liters per hectare. The latter would be used if the cost, in comparison with other alternatives, is higher. Based on personal experience, in blueberry crop, the optimum dose for the control of *B. cinerea* is 750 mL / cil, which is remarkably high compared to that of the present study. As at the time, it was determined that the possible cause of the low control was due to the low dose applied.

The incidence of *B. cinerea* in humid chambers was also evaluated for three opportunities during the flowering season, which occurred throughout the month of May 2019. The condition of these humid chambers was of a RH percentage close to 100%. Incidence results of the three evaluations performed are shown in Table 3.

In the three evaluations performed, Fluidoxonil + Cyprodinil is the only treatment that presents a significant difference with control. For the second evaluation performed on May 22nd Iprodione treatment presents a significant difference with control. Finally, estimated average yield per treatment and approximate profitability of each treatment were determined according to the formula proposed by Otero (2004) and number of trees per hectare in the field tested were 416 in total. The results are shown in Table 4.

By analyzing Table 4, it was determined that, for all treatments, it is cost effective to make two applications for the control of *B. cinerea* during the flowering season.

It is important to mention that the present investigation was performed in an advanced field of ‘Okitsu’ Satsuma mandarin, where the beginning of harvest is in the middle of December. Based on this, the flowering coincided with cold and humid climates optimal for the development of *B. cinerea*, for which it is determined that the use of specific fungicides in this particular case is justified to obtain greater profitability.

Conclusions

Products of chemical origin (Iprodione and Fludioxonil + Cyprodinil) are the best for the control of *B. cinerea* in 'Okitsu' Satsuma mandarin based on the parameters evaluated in this study: mycelial PIC, field and wet chamber incidence, and economic analysis. On the other hand, the product of biological origin used in this study does not exhibit a good control of the disease.

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