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Application of Bordeaux mixture for Botrytis control in passion fruit (*Passiflora ligularis* Juss) cultivated under organic farming in the Andean region

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Abstract

The aim of this research was the determination of the best conditions for applying Bordeaux mixture for the control of gray mold or rot (*Botrytis cinerea*) in passion fruit (*Passiflora ligularis* Juss) grown in organic farming in the Andes. This disease is responsible for nearly 30% loss of production in the province of Tungurahua (Ecuador). To do this, three doses and four frequencies of application in two locations were evaluated. The locations were in the sectors El Galpón and Runtun which are cantons of Patate and Baños, respectively, in Ecuador. Therefore, each experimental unit was composed of 12 blocks (3 doses x 4 frequencies) together a control test with three plants each. In total, 78 plants were evaluated. The doses used were 5 g $CuSO_4$/litre of water (D1), 10 g $CuSO_4$/litre of water (D2), and 15 g $CuSO_4$/litre of water (D3). The studied application frequencies were every 7 days (F1), every 14 days (F2), every 21 days (F3) and every 28 days (F4). From the first application of the treatment, the efficacy was evaluated every 15 days up to 90 days, also performing an economic analysis of the treatments. The evaluation of the effectiveness of each treatment was performed using an affection index, defined as the ratio number of affected organs (branches, leaves, flowers or fruits) and the number of total organs. It has been demonstrated that treatment with 0.5 kg of $CuSO_4$ in 100 litres of water applied every 21 days gives good results for the control of gray mold. Complete removal of the Botrytis was achieved at 75 days after starting treatment. Treatments with higher dose level and the same frequency of application did not improve the results.

Key words: Gray mold, Passiflora, Andes, copper (II) sulfate.

Introduction

In Ecuador, passion fruit (*Passiflora ligularis* Juss) production cultivated under organic farming conditions is in expanding in the Andean area because it is a production system that reduces investment costs and increases the subjective value given by consumers [1]. This is because this type of agriculture uses natural inputs maximizes the recycling of nutrients and avoiding the use of products derived from fossil fuel, such as mineral fertilizers and pesticides [2]. Reducing production costs is very important in this area because farmers are generally small producers, whose plots have little area and are on steep slopes [3, 4]. Moreover, this system avoids any toxic waste, not only in the product but also in transport, packaging, packing and labelling. These reasons improve the competitiveness and market consolidation. However, this kind of agriculture has sometimes difficulties to control various diseases, where solutions fully developed do not exist. For this, perfecting natural techniques already known within the framework of organic farming is a scientific challenge.

One of the problems in the cultivation of the passion fruit in the Andean area is gray mold disease (*Botrytis cinerea*) that causes rot, favoured by high humidity of the night and early morning in rainy periods, combined with low insolation in fields located at 2200 m [5, 6]. Gray mold or rot is a disease with high incidence in the production areas of the province of Tungurahua (Ecuador) [7] responsible for losses of up to 30% of production by burnings in inflorescences, fruit rot and cancers in the stem. Therefore, it is an economically important disease.

Conventional treatment of this disease is generally based on the indiscriminate application of pesticides, having abandoned control physical and natural practices as the destruction of weeds, selecting appropriate planting dates, proper management of water and fertilizer, among others Bordeaux mixture is one of the allowed components in organic farming [8, 9]. Bordeaux mixture (also called Bordo Mix) is a mixture of copper (II) sulfate ($CuSO_4$) and slaked lime ($Ca(OH)_2$) used as a fungicide [10]. It is used in vineyards, fruit plants and gardens to control infestations of downy mildew, powdery mildew and other fungi [11, 12]. It is sprayed on plants as a preventative; its mode of action is ineffective after a fungus has become established. Bordeaux mixture has been studied in crops such as tomato [8, 9], grapevine [13], potato [14], garlic [12], citrus [9] or mango [15]. However, its effect is little known in the passion fruit cultivated in Andean areas. The objective of this research was the evaluation of the best conditions for applying Bordeaux mixture for the control of gray mold or rot (*Botrytis cinerea*) in passion fruit grown under organic farming in the Andes. It is intended to determine the dose and frequency of application to maximize the effectiveness of treatment at minimum cost.

Materials and Methods

Study area: The study was conducted in two sectors of the province del Tungurahua (Ecuador) (Fig. 1); El Galpón located at 01°14'42" latitude South and 78°30' 07" longitude West, at 2663 AMSL height; and Runtun located to 01°25'35" latitude South and 78°24' 25" longitude West, at 2173 AMSL height; with average temperatures that oscillate between 15.0 and 19.1°C; average relative humidity from 80% to 86%; monthly precipitation oscillates between 62.8 and 110 mm; and with an wind speed of 20 m/s. These sectors belong to the cantons Patate and Baños, respectively.

Figure 1. Location of Ecuador and Tungurahua Province.

Evaluation of the condition of Botrytis cinerea: The incidence of *Botrytis cinerea* in the passion fruit plots was performed using an index (I) defined by equation 1. From each plant was quantified the total number of organs (B) and the number of organs affected by gray mold (A), such as drying at the tips of young stems, drooping inflorescences, gray spots on the fruit in the first development phase (rot). The average affection indexes were 19.85% in El Gallon and 18.05% in the Runtún, with a standard deviation of 1.87% in both cases. The overall coefficient of variation was 9.8%.

$$I = \frac{A}{B} \cdot 100 \qquad (1)$$

An analysis of variance to determine if differences between the study areas exist in the incidence index of Botrytis. Fig. 2 shows the intervals Least Significant Difference (LSD) obtained from the analysis of variance, which found that there were no significant differences between the municipalities.

Treatment application: Bordeaux mixture was prepared in a tank of 200 l capacity. For this, copper II sulfate, calcium hydroxide and agricultural oil (adherent) were added together and a pH corrector

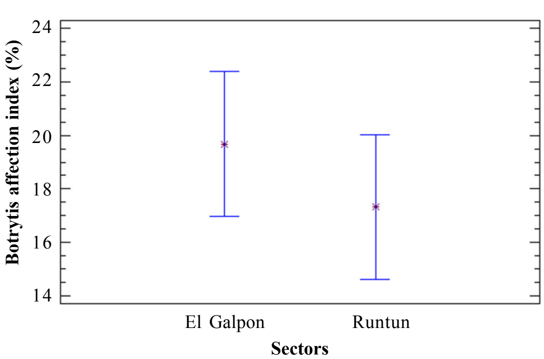

Figure 2. LSD intervals for the analysis of the differences between the Shed and Runtún locations with a confidence level of 95%.

to obtain a pH 7.0 (neutral), stirring continuously. The compositions obtained are shown in Table 1. Before mixing, the copper sulphate was previously dissolved in hot water.

Table 1. Composition of the dose for the treatments.

Components	Dose (kg/100 litres of water)		
	D1	D2	D3
Copper II sulfate	0.5	1.0	1.5
Cal	4.0	4.0	4.0
Ac-Tiv (Adherent)	200 ml	200 ml	200 ml
pH corrector	100 ml	100 ml	100 ml

Four frequency of application were evaluated: every 7 days (F1), every 14 days (F2), every 21 days (F3), and every 28 days (F4). From the first application the efficacy of treatment was evaluated every 15 days, during a period of 90 days, also performing an economic analysis of the treatments. This means that the blocks with F1 frequency had 13 applications, the blocks F2 had 7 applications, the blocks with F3 had 5 applications, and the blocks with F4 had 4 applications.

The evaluation was performed in 13 blocks (3 doses x 4 frequencies plus a control test), with three plants each. This was done in 2 locations, therefore, in total 78 plants were assessed. The experimental design is shown in Table 2. Economic analysis test was conducted.

Table 2. Experimental design.

Dose	Frequency of application	Blocks	Number of evaluated plants
D1	F1	D1F1	6
D1	F2	D1F2	6
D1	F3	D1F3	6
D1	F4	D1F4	6
D2	F1	D2F1	6
D2	F2	D2F2	6
D2	F3	D2F3	6
D2	F4	D2F4	6
D3	F1	D3F1	6
D3	F2	D3F2	6
D3	F3	D3F3	6
D3	F4	D3F4	6

Results and Discussion

Table 3 shows an analysis of variance to determine the effect of following factors: municipality, dose, frequency of application, and their respective interactions, in efficiency of the treatments, which was evaluated every 15 days. As it can be seen, at the beginning no significant difference exists between the treatment blocks (DxF) or locations. This is important because it demonstrates the homogeneity of the test plots. However, it is observed that, from 15 days up to 90 days of treatment, differences in efficacy exist in the blocks with different frequency of application. It is important to point out that the effects associated with different doses or locality were not significant in any of the check times. On the other hand, Frequency x Dose interaction was significant in the evaluations conducted at 60, 75 and 90 days.

To determine the nature of the observed differences in the blocks with different frequency of application, the LSD intervals of evaluations carried out at 15 days (Fig. 3a), at 30 days (Fig. 3b) and at 45 days (Fig. 3c) were represented. They analyze the frequency factor in the affection index of the disease. It can be observed that the affection index of Botrytis in blocks with

Table 3. Variance analysis of the effect of each factor at a confidence level of 95%.

	Initial	After 15 days	After 30 days	After 45 days	After 60 days	After 75 days	After 90 days
Municipality (M)	Ns	Ns	Ns	Ns	Ns	Ns	Ns
Dose (D)	Ns	Ns	Ns	Ns	Ns	Ns	Ns
Frequency (F)	Ns	S	S	S	S	S	S
Municipality x Dose	Ns	Ns	Ns	Ns	Ns	Ns	Ns
Dose x Frequency	Ns	Ns	Ns	Ns	S	S	S
Municipality x Frequency	Ns	Ns	Ns	Ns	S	Ns	Ns

S: significant; Ns: no significant.

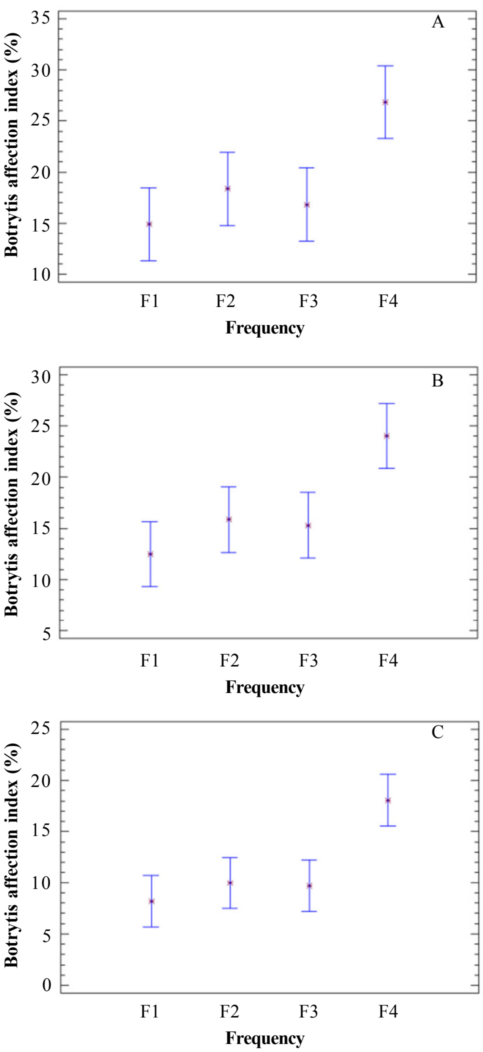

Figure 3. LSD intervals for the analysis of the frequency of application effect in the affection index of botrytis at a confidence level of 95%. (a) evaluated 15 days after first treatment, (b) evaluated at 30 days, (c) evaluated at 45 days.

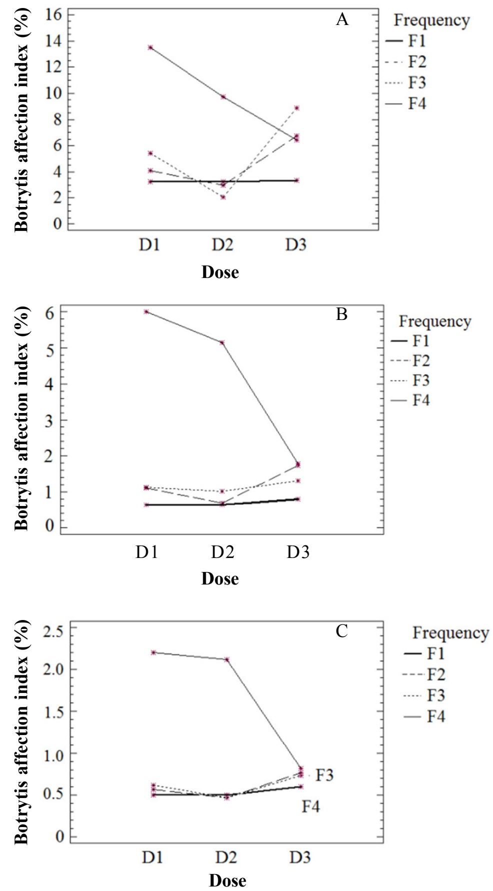

Figure 4. Analysis of the interaction doses of Bordeaux mixture applied x frequency of application in the affection index of botrytis at confidence level of 95%. (a) 60 days after first treatment, (b) evaluated at 75 days, (c) evaluated at 90 days.

applications every 28 days (F4) is greater than in the other treatments, and therefore, they were less effective. Furthermore, no differences in the treatment efficacy were observed for intervals of application every 7 days (F1), every 14 days (F2) and every 21 days (F3). This means 28 days for separation between applications is excessive.

The dose x frequency interaction was significant in the evaluations carried out at 60, 75 and 90 days. To examine the nature of this interaction, Figs 4a-c are shown. In them, it can be observed that the affection values in the F4 treatments are above the other frequencies, which confirms that a period of 28 days between applications reduces significantly the effectiveness of the treatment when the dose is low (D1) or medium (D2). However, when the dose is high (D3) the break between applications does not influence in the achieved affection index. In other words, the applications can be more separated in the time if the dose is increased.

The different treatments evaluated at 90 days after the first application are depicted in Fig. 5. Treatments with dose D1 and D2 are less effective than others when the period between applications is long (28 days), namely D1F4 and D2F4. The other treatments are not significantly different.

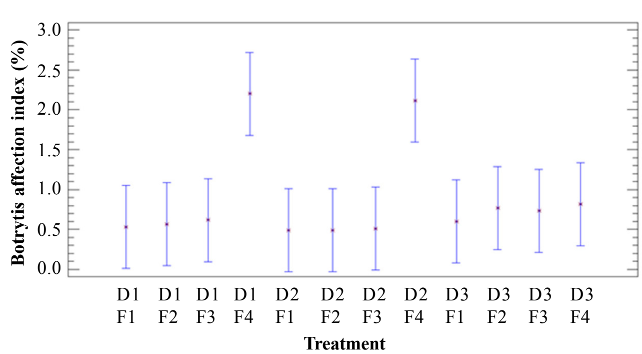

Figure 5. LSD intervals at a confidence level of 95% for analysis of the treatments in the evaluation performed at 90 days after first treatment.

No significant difference exists between treatments (except D1F4 and D2F4). Fig. 6 shows the average affection index achieved in the treatments compared to the average affection index in control plots. It can be seen that after 75 days of treatment the affection index of Botrytis can be considered negligible, therefore it has had complete control of the disease.

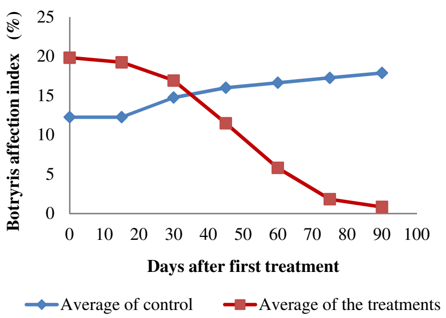

Figure 6. Evolution of the average affection index of Botrytis in the treatments (except D1F4 and D2F4) and control.

Economic analysis of treatments: Given that the dose factor does not affect at levels studied, the best treatments from economic viewpoint may be D1F3 or D3F4. Within the group of treatments that have proved more efficient (all except D1F4 and D2F4) D1F3 has the lowest dose and frequency with intervals of application most distanced. Although the D3F4 treatment has a higher dose, it has fewer applications. For the selection between the treatments D1F4 and D1F3, an economic analysis was performed.

Table 1 presents the composition of each dose. The blocks with frequency F3 received 5 applications, and blocks F4 received 4 applications. If the price per kg of $CuSO_4$ is defined as the price P and the price of the other components is defined as K, the total treatment costs D1F3 is given by equation 2 and D3F4 treatment cost is given by equation 3.

$$\text{CostD1F3} = 5 \times (0.5 \times P + K) \qquad (2)$$

$$\text{CostD3F4} = 4 \times (1.5 \times P + K) \qquad (3)$$

The breakeven point is determined by solving the equation (2) = (3), obtaining Equation 4:

$$P = \frac{2}{7} K \qquad (4)$$

When the price of $CuSO_4$ is less than 3.5 times the price of the other components D3F4 will be cheaper than D1F3. If the price of $CuSO_4$ is greater than 3.5 times the price of the other components will be cheaper in the D1F3 treatment.

Conclusions

In this work we tested different ways of implementing Bordeaux mixture in passion fruit (*Passiflora ligularis* Juss) growing for control of *Botrytis cinerea*. It has been demonstrated that treatment with 0.5 kg of $CuSO_4$ in 100 litres of water applied every 21 days gives good results for the control of gray mold. Complete removal of the Botrytis is achieved at 75 days after starting treatment. Treatments with higher dose level and the same frequency of application did not improve the results. On the other hand, if the applications were conducted every 28 days, the treatment efficacy decreased. If the applications were carried out every 28 days, it is necessary to increase the concentration of $CuSO_4$ with 1.5 kg per 100 litres of water. This treatment will only be more economical if the price of $CuSO_4$ is 3.5 smaller than that of the other components.

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