

Efficacy of chemical fungicides against the anthracnose disease caused by *Colletotrichum gloeosporioides* in *Carica papaya* fruits

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ABSTRACT

Objective: To evaluate the efficacy of chemical fungicides against *Colletotrichum gloeosporioides* in papaya fruits. **Design/methodology/approach:** The effect of four chemical fungicides (A+F=azoxystrobin+fludioxinil, C+F=cyproconil+fludioxinil, B+P=boscalid+pyraclostrobin and T=tiabendazole) at 250, 500, 750, and 1000 mg kg⁻¹ were assessed on severity and the area under the disease progress curve (AUDPC) of *Colletotrichum gloeosporioides* inoculated on papaya fruits.

Results: At 12 days after inoculation (dai) A+F achieved a range of effectiveness between 63.0 (250 mg kg⁻¹) to 77.52 (1000 mg kg⁻¹); while the range of effectiveness for T was 12.8% (250 mg kg⁻¹) to 74% (1000 mg kg⁻¹). Both fungicides achieved the highest effectiveness at 1000 mg kg⁻¹ that C+F (38.5%) and B+P (55.6%). The AUDPC achieved the same value at the four studied concentration in A+F, C+F, and B+P. Only 750 and 1000 mg kg⁻¹ of T achieved the lowest AUDPC than 250 and 500 mg kg⁻¹.

Findings/conclusions: The fungicides A+F and T achieved adequate control of anthracnose in papaya fruits and the use of the diagrammatic logarithmic scale is easy to use to give a quick estimate of the disease, as well as being easy to reproduce.

Keywords: fungal disease, chemical control, postharvest, severity.

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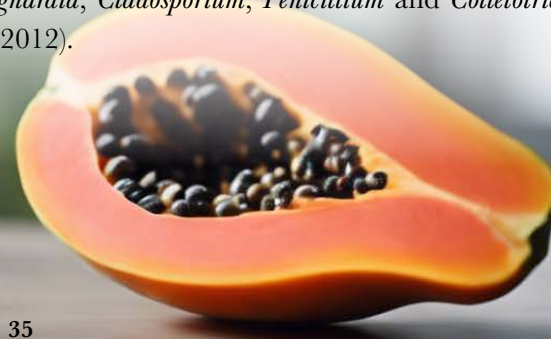
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INTRODUCTION

Papaya fruit (*Carica papaya* L.) is the most commercially important species within the Caricaceae family; its production is destined to the national and international markets because its consumption promotes health benefits and due to its exotic nature in some countries (Mailafia *et al.*, 2017). It is a climacteric fruit, whose ripening occurs quickly after its harvest, therefore it is considered as a highly perishable fruit. *C. papaya* is a fruit with a short shelf life and very susceptible to postharvest handling and decay, which causes significant losses in quantity and quality of fruit when handled improperly (Suárez-Quiroz *et al.*, 2013). Main phytopathogenic fungi, which cause postharvest diseases in papaya fruits worldwide, are the genera: *Alternaria*, *Fusarium*, *Ascochyta*, *Rhizopus*, *Mycosphaerella*, *Phomopsis*, *Lasiodiplodia*, *Guignardia*, *Cladosporium*, *Penicillium* and *Colletotrichum* (Chávez-Quintal *et al.*, 2011; Rathod 2012).



The anthracnose, which is caused by the micromycete *Colletotrichum gloeosporioides* Penz, is the most important fungal disease in *C. papaya*. It can manifest itself on leaves and petioles, but it is mainly a fruit disease (Molina-Chavez *et al.*, 2017). Symptoms on leaves show as gray to brown spots with darker margins and a yellow surrounding halo. The spots later enlarge and coalesce to form sizable necrotic areas. Small, light-colored spots appear first on the skin of fruits. As they mature, the spots grow considerably in size (up to 5 cm) and become round, dark brown lesions, often with a water-soaked or raised appearance. Pinkish to orange specks grow within the lesions in a concentric manner. Smaller, reddish-brown, sunken spots (up to 2 cm), referred to as “chocolate spots”, are also visible. Fruits tend to fall off prematurely. These symptoms might develop after harvest, particularly if the fruits are refrigerated (Maeda and Nelson 2014).

Colletotrichum gloeosporioides has a greater impact on the shelf life or postharvest of papaya fruits (Zavala-León *et al.*, 2005). Losses ranging from 20 to 50% caused by anthracnose and other diseases have been reported; during the period of flowering and berthing of fruit, therefore fungicides of different mode of action must be applied to avoid latent infections and reduce the risk of resistance of the fungus (Ferreira-Demartelaere *et al.*, 2017).

Among the alternatives to control the anthracnose are the use of hot air, hydrothermal treatments (Ayón-Reyna *et al.*, 2017a; Ayón-Reyna *et al.*, 2017b), modified atmospheres, ultraviolet light, plant extracts (*Allium sativum*, *Azadirachta indica*, *Cinnamomun zeylanicum*, *Thymus vulgaris*, and *Ricinus communis*), biological control agents (*Trichoderma* sp., *Gliocladium* sp., *Streptomyces* sp., *Bacillus* spp., and *Coniothyrium* sp.) (Hewajulie and Wilson 2010) and some active ingredients (*a.i.*) of chemical fungicides such as trifloxystrobin, imazalil, benomyl, azoxistrobin and prochloraz (Subramanian *et al.*, 2006; Santamaría-Basulto, 2011).

However, constant use of chemical fungicides causes resistance in the plant phytopathogenic fungi including *C. gloeosporioides* (Rathod, 2012); hence, one strategy to avoid the fungicide resistance is the rotation of the active ingredients, as well as the exploration of the new ingredients available in the market, to find the most effective ones to the phytopathogenic fungi (Salvatore and Ritenour, 2007). In addition, to determine the mean effective concentration to *C. gloeosporioides* under postharvest conditions is needed to the integrated management of fruit diseases. The present study raised the use of some chemical active ingredients of fungicides available in the agricultural market, which poses registration for papaya exportation by the environmental protection agency (EPA) from U.S.A. This study aimed to evaluate the efficacy of chemical fungicides to postharvest control of *C. gloeosporioides* in papaya fruits and to calculate the median effective (EC₅₀) concentration of each molecule.

MATERIALS AND METHODS

Fungal source

A single strain of *C. gloeosporioides* was used for bioassays, this strain was isolated from fruits with anthracnose symptoms. The strain was TSWT, which belongs to the collection of phytopathogenic fungi of the Faculty of Biological and Agricultural Science of the Colima University. Strain was reactivated in potato dextrose agar supplemented

with chloramphenicol (100 mg/L, Sigma-Aldrich, U.S.A.) under laboratory conditions (25 ± 3.0 °C, $75 \pm 5.0\%$ of relative humidity and 12:12 h light: darkness).

Diagrammatic logarithmic scale

Papaya cv. “Maradol” fruits (1.5 to 2.5 kg with a maturity stage 2) were used according to Santamaría-Basulto *et al.* (2009). Fruits were washed twice with a non-ionic detergent (Extran[®] 1.0%) and sodium hypochlorite (100 mg kg⁻¹), and then washed with distilled sterile water (Zavala-León and Cristobal-Alejo, 2012). Disinfected fruits were punctured with a sterile needle (0.8 mm) and inoculated with 15 µL of a conidia suspension of *C. gloeosporioides* (1.8×10^6 conidia/mL) using a micropipette. Inoculated fruits were incubated at laboratory conditions (25 ± 3 °C, $80 \pm 50\%$ of relative humidity and 12 h light: darkness) for 10 days to permit the fruit infection. After this period, fruits were photographed with a Kodak[®] Camera at 35 cm between the lens and the papaya fruit. Digital images were processed with the ImagenJ software (Rasband 2014) to measure the infected area (%) by anthracnose. The diagrammatic logarithmic scales with six classes were established to quantify the anthracnose severity caused by *C. gloeosporioides* in papaya fruits. Class 0 corresponded to healthy fruits, by other hand; the class 6 corresponded to an anthracnose percentage of 43.81% (Figure 1).

Chemical fungicides and doses-response bioassays

The fungicides Bankit[®] Gold (Syngenta), Switch[®] 62.5 WG (Syngenta), Cabrio[®] C (BASF) and Tecto[®] 60 (Syngenta) were used in this study. Each fungicide was evaluated

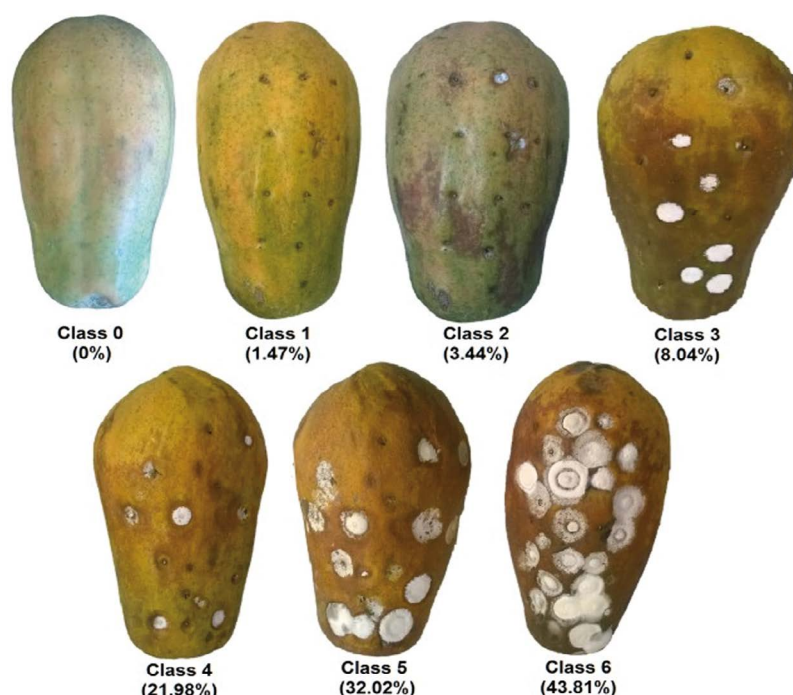


Figure 1. Diagrammatic logarithmic scale to evaluate the severity of anthracnose caused by *Colletotrichum gloeosporioides* in *Carica papaya* fruits.

using four concentrations (250, 500, 750, and 1000 mg kg⁻¹) through a dose-response bioassay. In addition, a control was established using distilled sterilized water. For bioassays, papaya fruits (maturity stage 2) were disinfected as was previously described, and then the fungicide treatments using four concentrations (Table 1) were diluted in distilled water plus 1 mL L⁻¹ of a pH buffer (Buffer XS[®], Mexico City). Fungicide concentration was applied by soaking the papaya fruits using a contained of 40 L of capacity with 20 L of the fungicides at the evaluated concentration. Fruits were soaked for three minutes and incubated at laboratory conditions. Fruits from the control were soaked only with distilled water with 1 mL/L of a pH buffer. Both the treated fruits with fungicides and the control were inoculated immediately with *C. gloeosporioides* as was mentioned previously (Zavala-León *et al.*, 2005; Santamaría-Basulto *et al.*, 2011). Treated fruits with fungicides and inoculated with *C. gloeosporioides* were incubated at laboratory conditions for 12 days. Response variables were evaluated as follow.

Response variables

Anthracoze severity by *C. gloeosporioides* on fruits was evaluated every four days using the diagrammatic scale, which was designed with six scales (Figure 1). The effectiveness of the fungicides concentrations was determined using the formula:

$$Effectiveness = (control - treatment) / control \times 100$$

The Area Under the Disease Progress Curve (AUDPC) was determined with the formula

Table 1. Chemical fungicides and concentrations.

Active ingredients	Chemical groups	Concentration (mg kg ⁻¹)	Key
azoxystrobin + fludioxonil	strobilurins fenilpirroles	250	A + F 250
		500	A + F 500
		750	A + F 750
		1000	A + F 1000
cyprodinil fludioxinil	anilinopirimidine fenilpirroles	250	C + F 250
		500	C + F 500
		750	C + F 750
		1000	C + F 1000
boscalid pyraclostrobin	anilidas strobilurins	250	B + P 250
		500	B + P 500
		750	B + P 750
		1000	B + P 1000
tiabendazole	benzimidazol	250	T 250
		500	T 500
		750	T 750
		1000	T 1000

$$AUDPC = \sum_{i=1}^n [(X_{i+1} + X_i) / 2] [t_{i+1} - t_i]$$

Where, X_i is the damage ratio of the host tissue i^{th} per day, t_i is the time (days) after the onset disease i^{th} (days) and n is the number of observations (Marin-Cortez *et al.*, 2019). The mean effective concentration (EC_{50}) was calculated through a Probit analysis using the fungicide concentration and the effectiveness percentage in the SAS software for windows.

Experimental design and data analysis

Independent experiments which involve the fungicides and concentrations were established through doses response bioassays. The studies concentrations and controls were established with the replicates, each of them with three treated fruits. The response variables: anthracnose severity and AUDPC was analyzed using an analysis of variance and a Tukey ($P \leq 0.05$) multiple comparison procedure. These analyses were performed using StatGraphics Plus for windows. The severity data of *C. gloeosporioides* on inoculated fruits of *C. papaya* did not show homogeneity of variance, therefore, they were transformed to $\sqrt{X+1}$ to develop the ANOVA.

RESULTS AND DISCUSSION

Diagrammatic logarithmic scale

The diagrammatic logarithmic scale generated to quantify the severity of *C. gloeosporioides* in *C. papaya* fruits has seven severity classes, where class 0 corresponded to fruits without damage, on the contrary, class 6 to fruits with the maximum percentage of damage or severity estimated with 43.81% (Figure 1).

Disease severity

Each fungicide was evaluated at four concentrations at the same time. At 4 dai of the concentrations of azoxystrobin+fludioxinil (A+F) achieved a significant difference ($F=25.96$, $P=0.00001$) between the concentrations of the fungicides and the control. However, no differences were found between the concentrations, the lowest severity values were found at 250 (0%), 750 (0%), and 1000 (0%) mg kg^{-1} . On the contrary, the highest severity was found in the control with 0.43% (Table 2). At the second evaluation (8 dai) the A+F concentrations, a significant difference ($F=13.78$, $P=0.0004$) was found for the fungicide concentrations and the control. However, no differences were found between the concentrations, the lowest value was obtained at 500 mg kg^{-1} with 1.01%. On the contrary, the highest severity was found in the control with 9.44% (Table 2). At 12 dai of A+F concentrations significant difference ($F=4.06$, $P=0.0330$) was achieved, the control together with concentrations 250, 750 and 1000 mg kg^{-1} showed the highest severity with 40.0, 14.8, 11.1 and 9.0%, respectively. By the other side, 500 of A+F registered the lowest severity (6.21%) (Table 2).

In the treatment boscalid+pyraclostrobin (B+P), at 4 dai, a significant difference ($F=5.60$, $P=0.125$) in severity was found, the control together with concentrations 750 and 1000 mg kg^{-1} showed the highest severity with 0.43, 0.13 and 0.13%, respectively.

While the concentrations 250 (0.0%) and 500 (0.07%) mg kg⁻¹ showed the least severity (Table 2). At 8 dai, B+P treatment achieved a significant difference (F=4.82, P=0.199) in the severity, the control with the concentrations 250 and 1000 mg kg⁻¹ showed the highest severity with 9.44, 3.50 and 2.38%, respectively. While the concentrations 500 and 750 mg kg⁻¹ showed the lowest severity with 1.67 and 1.44%, respectively (Table 3). At 12 dai, the B+P concentrations did not achieve significant differences (F=25.96, P=0.00001). The lowest severity was found at 500 (10.2%) mg kg⁻¹. On the contrary, the highest severity was found in the control with 40.0% (Table 2).

In the cyprodinil+fludioxinil (C+F) evaluations at 4 (F=2.69, P=0.0931), 8 (F=2.79, P=0.0855) and 12 (F=0.86, P=0.5177) dai, did not achieved significant differences between the concentrations of the fungicides and the control (Table 2).

For the tiabendazole (T) treatment, at 4 dai significant difference was found (F=5.13, P=0.0165) in severity, the control together with concentrations 250 and 500 mg kg⁻¹ showed the highest severity with 0.43, 0.25 and 0.31%, respectively. While the concentration of 1000 mg kg⁻¹ showed the lowest severity with 0.03% (Table 2). At the second evaluation

Table 2. Anthracnose severity in papaya fruits under the application of fungicides at four concentrations.

Active ingredients	Concentrations (mg kg ⁻¹)	Days after inoculation		
		4	8	12
A+F	250	0.0 a	1.57 a	14.82 ab
A+F	500	0.05 a	1.01 a	6.21 a
A+F	750	0.0 a	2.07 a	11.10 ab
A+F	1000	0.0 a	1.16 a	9.0 ab
Control	0	0.43 b	9.44 b	40.05 b
B+P	250	0.00 a	3.50 ab	18.20
B+P	500	0.07 a	1.67 a	10.2
B+P	750	0.13 ab	1.44 a	15.5
B+P	1000	0.13 ab	2.38 ab	17.7
Control	0	0.43 b	9.44 b	40.0
C+F	250	0.10	3.91	20.82
C+F	500	0.10	2.81	20.88
C+F	750	0.13	3.28	19.78
C+F	1000	0.09	3.42	24.61
Control	0	0.43	9.44	40.05
T	250	0.25 ab	7.34 bc	34.91 b
T	500	0.31 ab	3.52 ab	26.67 ab
T	750	0.05 a	1.68 a	11.71 a
T	1000	0.03 a	1.30 a	10.36 a
Control	0	0.43 b	9.44 c	40.05 b

Means followed by different letter are significant different from each other according to Tukey test (P≤0.05). A+F=Azoxystrobin+Fludioxinil, C+F=Cypronil+Fludioxinil, B+P=Boscalid+Pyraclostrobin, and T=Tiabendazole.

(at 8 dai) of T concentrations, a significant difference ($F=16.89$, $P=0.0002$) in severity was found, the control together with the 250 mg kg^{-1} concentration showed the highest severity with 9.44 and 7.34%, respectively. While the concentration of 1000 mg kg^{-1} showed the lowest with 0.03% (Table 2). At 12 dai of T, a significant difference ($F=7.63$, $P=0.0044$) was found the control and the concentrations of 250 and 500 mg kg^{-1} showed the highest severity with 40.05, 34.91, and 26.67%, respectively. By the other side, the concentration of 1000 mg kg^{-1} showed the lowest severity with 10.36% (Table 2).

Effectiveness

The A+F treatments at 4 dai, achieved a significant difference ($F=236.67$, $P=0.00001$) was found between the concentrations of the fungicides and the control. However, no differences were found between the concentrations, the highest effectiveness values were found at 250 (100%), 750 (100%) and 1000 mg kg^{-1} (100%). On the contrary, the lowest effectiveness was found in the control with 0.0% (Table 3).

At 8 dai, the concentrations of A+F showed a significant difference ($F=104.16$, $P=0.00001$). However, no differences were found between the concentrations; the highest effectiveness value was found at 500 mg kg^{-1} with 89.28%. On the contrary, the lowest effectiveness was found in the control with 0% (Table 3). At 12 dai of evaluation, A+F concentrations, a highly significant difference ($F=32.58$, $P=0.00001$) was found between the concentrations and the control. However, the highest effectiveness value was found at 500 mg kg^{-1} with 84.49%. On the contrary, the lowest effectiveness was found in the control with 0% (Table 3).

In the B+P treatment, significant difference ($F=14.65$, $P=0.0003$) was found at the 4 dai, the concentrations no differences were found, the highest effectiveness value was found at 250 mg kg^{-1} with 100%. On the contrary, the lowest effectiveness was found in the control with 0% (Table 3). At 8 dai of evaluation the B+P achieved significant difference ($F=11.57$, $P=0.0009$). However, no differences were found between the concentrations, the highest effectiveness value was found at 750 mg kg^{-1} with 84.7%. On the contrary, the lowest effectiveness was found in the control with 0% (Table 3). At 12 dai, the same behavior was found ($F=12.75$, $P=0.0006$), no differences were found between the concentrations, the highest effectiveness value was found at 500 mg kg^{-1} with 74.4%. On the contrary, the lowest effectiveness was found in the control with 0% (Table 3).

For C+F treatment, at 4 dai significant difference ($F=6.21$, $P=0.0089$) was achieved. The same effectivity was found between the concentrations, the highest effectiveness value was found at 1000 mg kg^{-1} with 78.1%. On the contrary, the lowest effectiveness was found in the control with 0% (Table 8). At 8 ($F=7.44$, $P=0.0048$) and 12 ($F=4.80$, $P=0.0203$) dai significant difference were found between the concentrations and the control. However, between the concentrations no differences were found, the highest effectiveness value was found at 500 mg kg^{-1} with 70.2% and in 1000 mg kg^{-1} with 38.5% (12 dai). On the contrary, the lowest effectiveness was found in the control with 0% (Table 3).

In the T treatment, significant difference was found at 4 dai ($F=7.29$, $P=0.0051$), the control together with concentrations 250 and 500 mg kg^{-1} showed the lowest effectiveness

with 0, 42.0, and 26.4%, respectively. While the concentration of 1000 mg kg⁻¹ showed the highest effectiveness with 92.9%. At 8 dai, the control and the 250 mg kg⁻¹ showed the lowest effectiveness with 0 and 22.2%, respectively (F=7.10, P=0.0056). While the concentration of 1000 mg kg⁻¹ showed the highest effectiveness with 86.2% (Table 3). At 12 dai of T concentrations, the control and the 250 mg kg⁻¹ application achieved the lowest effectiveness with 0.12.8% each one (F=27.43, P=0.00001). By the other side, the concentration of 1000 mg kg⁻¹ showed the highest effectiveness with 74.1% (Table 3).

Area under the disease progress curve (AUDPC)

Figure 2 shows the AUDPC of fungicides on *C. gloeosporioides* in *C. papaya* fruits. For A+F a highly significant difference was found (F=8.71, P=0.0027) between the concentrations and the control. However, the four studied concentrations did not show significant differences. Concentration of 500 mg kg⁻¹ achieved lowest value of ABCPE with 14.90. On the contrary, the highest area was found in control 30.15 (Figure 2). In B+P the four concentration reduced significantly (F=4.38, P=0.0265) the ABCPE. The lowest ABCPE was found at the concentration of 500 mg kg⁻¹ with 17.11. On the contrary, the

Table 3. Effectiveness of chemical fungicides at four concentrations against anthracnose caused by *Colletotrichum gloeosporioides* in papaya fruits.

Active ingredients	Concentrations (mg kg ⁻¹)	Days after inoculation		
		4	8	12
A+F	250	100.0 b	83.3 b	63.0 b
A+F	500	83.3 b	89.3 b	84.5 b
A+F	750	100.0 b	79.1 b	72.3 b
A+F	1000	100.0 b	87.7 b	77.5 b
Control	0	0 a	0 a	0 a
B+P	250	100.0 b	62.9 b	54.3 b
B+P	500	82.8 b	82.2 b	74.4 b
B+P	750	69.3 b	84.7 b	61.0 b
B+P	1000	70.3 b	74.7 b	55.6 b
Control	0	0 a	0 a	0 a
C+F	250	77.1 b	58.5 b	48.2 b
C+F	500	76.1 b	70.2 b	47.8 b
C+F	750	68.8 b	65.2 b	50.6 b
C+F	1000	78.1 b	63.8 b	38.5 ab
Control	0	0 a	0	0
T	250	42.0 ab	22.2 b	12.8 b
T	500	26.4 ab	62.7 b	33.4 b
T	750	87.2 b	82.2 b	70.7 b
T	1000	92.9 b	86.2 b	74.1 b
Control	0	0 a	0 a	0 a

Means followed by different letter are significant different from each other according to Tukey test (P≤0.05). A+F=Azoxystrobin+Fludioxinil, C+F=Cypronil+Fludioxinil, B+P=Boscalid+Pyraclostrobin and T=Tiabendazole.

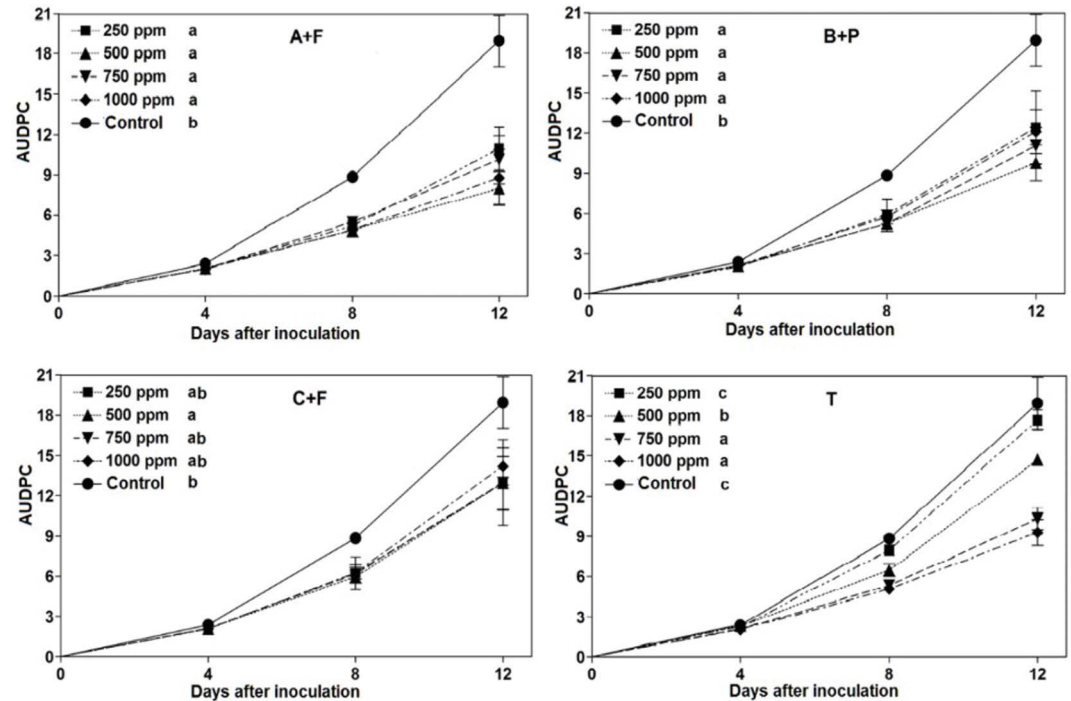


Figure 2. Area under the disease progress curve (AUDPC) of *Colletotrichum gloeosporioides* in *Carica papaya* fruits under different concentrations of chemical fungicides. A+F=Azoxystrobin+Fludioxinil, C+F=Cyproconil+Fludioxinil, B+P=Boscalid+Pyraclostrobin and T=Tiabendazole. Means (\pm standard error, $n=3$) with different literals are significantly different from each other (Tukey test, $P \leq 0.05$).

highest area was found in control 30.15 (Figure 2). For C+F, only the 500 mg kg^{-1} (30.15) archived a low ABCPE regarding to the control (21.0) (Figure 2). Finally, in T highly the concentration of 750 and 1000 mg kg^{-1} (<27.889) achieved lower values of AUDPC in comparison to the control (30.15) (Figure 2).

DISCUSSION

Carica papaya fruits immersed in A+F at 500 mg kg^{-1} at 12 dai showed the least severity (6.21%) and the highest effectiveness (84.49%), these results were similar to the reported by Gutiérrez-Alonso and Gutiérrez-Alonso (2003), Gutiérrez-Alonso *et al.* (2004) and Zavala-León *et al.* (2005), in studies with *Psidium guajava*, *Mangifera indica* and *C. papaya* fruits, respectively, inoculated with *C. gloeosporioides*. A concentration of 500 mg kg^{-1} of A+F was ideal to obtain the highest effectiveness and lowest severity at that concentration.

In another study, López-Navarrete *et al.* (2011), reported that pyraclostrobin solution at 500 mg kg^{-1} in *C. papaya* fruits inoculated with *C. gloeosporioides*, a greater effectiveness is obtained regarding the control (without pyraclostrobin), a similar result was achieved at the present study using B+P at 500 mg kg^{-1} at 12 dai. On the contrary, the results obtained by Pérez-León *et al.* (2015), did no differ from the results obtained at present study, because when application of boscalid+pyraclostrobin immediately after anthracnose inoculation, these authors obtained 100% effectiveness on the disease caused in *Sansevieria trifasciata*.

The low efficacy of tiabendazole to inhibit the growth of *C. gloeosporioides* at 250 and 500 mg kg⁻¹ were reported by Suárez-Quiroz *et al.* (2013), mentioning that tiabendazole was not able to inhibit fungal growth of papaya fruit disease such as *Fusarium crookwellense*, *F. oxysporum*, *C. gloeosporioides*, *Mucor hiemalis* and *M. circinell*. The tiabendazole has been reported with low effectivity against *C. gloeosporioides* in *Psidium guajava*. Despite these results, more investigation is needed to find the CL₅₀ of the chemical molecules on postharvest diseases in papaya fruits.

CONCLUSION

In conclusion, the application of A+F at 500 mg kg⁻¹ has the highest control of anthracnose caused by *C. gloeosporioides* in *C. papaya* fruits under laboratory conditions. In contrast, T at 250 mg kg⁻¹ showed reduced sensitivity to anthracnose (*C. gloeosporioides*) with a difference in severity to the control (5.14%), therefore its use is not recommended at that concentration. Finally, the use of the diagrammatic logarithmic scale is easy to use to give a quick estimate of the disease, as well as being easy to reproduce.

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