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Comparative *in vitro* and *in vivo* study of anthracnose in papaya (*Carica papaya*) caused by *Colletotrichum gloeosporioides* and *Colletotrichum truncatum*

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ABSTRACT

Background/Objetive. Globally, Mexico is the leading exporter of fresh papaya, with Oaxaca being the largest national producer. However, anthracnose, a disease caused by species of the *Colletotrichum* spp. complex, can affect up to 40% of production. The objective of the research was to identify the causal agent of anthracnose in producing localities of Oaxaca and to evaluate the effectiveness of different products (chemical and biological) in reducing the severity of this disease under *in vitro* and *in vivo* conditions.

Experimental Development. A sampling of fruits with anthracnose lesions was carried out at 12 sites in the municipalities of Villa de Tututepec de Melchor Ocampo and Santa María Huazolotitlán, Costa de Oaxaca. The isolates were identified by morphological analysis and microcultures. Three chemical fungicides (prochloraz, benomyl and azoxystrobin) and three biological fungicides (*Bacillus subtilis*, *Trichoderma* sp. and *Bacillus cereus*) were evaluated *in vitro* and *in vivo*, with a completely randomized design, with Duncan's mean comparison ($p \leq 0.005$).

Results. *Colletotrichum gloeosporioides* and *C. truncatum* were identified as being associated with anthracnose. In *in vitro* tests, prochloraz was the most effective treatment, with inhibition values of 86.42% against *C. truncatum* and 48.66% against *C. gloeosporioides*, achieving an overall effectiveness of 75%. Likewise, *B. subtilis* and *Trichoderma* sp. showed high *in vitro* efficacy (≥ 70%), demonstrating a potential for biological control under laboratory conditions.

Conclusions. *Colletotrichum gloeosporioides* (in 66.7% of the sampled sites) and *C. truncatum* (in 33.3% of the sampled sites) were confirmed as the cause of anthracnose in papaya in the Costa region of Oaxaca. In *in vivo* tests, prochloraz was the most effective treatment with 75% effectiveness against *C. truncatum* and 50% against *C. gloeosporioides*. Benomyl showed intermediate effectiveness, with 65% against *C. truncatum* and 45% against *C. gloeosporioides*, with severities of 60-73%. Biological treatments showed low effectiveness: *Trichoderma* sp. (35 and 15%) and *B. subtilis* (25 and 30%) for *C. gloeosporioides* and *C. truncatum*, respectively, with high severities between 73 and 93%. *B. cereus* and azoxystrobin showed intermediate behavior, with 30-40% effectiveness and severities of 75-86%. Overall, the results indicate that systemic fungicides outperformed biological agents by more than 30-40% in effectiveness, while biological agents showed less than 35% control and greater than 80% severity, confirming their limited performance in post-harvest.

Keywords: Biocontrol, *Carica papaya*, *Colletotrichum* spp., Synthetic pesticide

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INTRODUCTION

Mexico is the leading exporter of fresh papaya to the United States and Canada (OEC, 2022). The state of Oaxaca ranks first in national production, with an average yield of 100 t ha^{-1}. Within the state, the Costa region stands out for its significant contribution, accounting for about 80% of total production. The main producing municipalities—Villa de Tututepec, Santa María Huazolotitlán, Santiago Pinotepa Nacional, and Santiago Jamiltepec—together comprise a harvested area of 2 787 ha (SIAP, 2024). Papaya cultivation in this region represents an important source of economic activity, largely due to its high demand for labor. Each hectare is estimated to require around 500 workdays per production cycle, underscoring its regional economic impact (Alarcón-Pulido *et al.*, 2022).

Papaya production faces major phytosanitary challenges, particularly anthracnose, caused by *Colletotrichum* species. This disease is characterized by dark lesions on fruits accompanied by acervuli arranged in concentric rings (Tapia-Tussell *et al.*, 2008). It can affect up to 40% of total yield (Maharaj and Rampersad, 2012), leading to the intensive use of highly toxic fungicides, mainly to control *C. gloeosporioides* (Landero-Valenzuela *et al.*, 2016). However, recent evidence suggests that the disease may involve a complex of *Colletotrichum* species, highlighting the need for further research to confirm this (Zakaria, 2021).

Anthracnose management in tropical crops such as papaya, mango (*Mangifera indica*), avocado (*Persea americana*), and banana (*Musa* × *paradisiaca*) has relied primarily on synthetic fungicides. This dependence raises concerns about toxic residues in fruits and underscores the lack of comprehensive management strategies. Although biocontrol agents offer a more sustainable alternative, they still face challenges related to consistency and effectiveness (Crouch *et al.*, 2014). In this context, the present study aimed to identify the causal agent of anthracnose in papaya and assess the efficacy of biological and chemical products in reducing disease severity.

EXPERIMENTAL DEVELOPMENT

Collection of infected fruits. Fruits of papaya showing typical anthracnose symptoms were collected from 12 sites located in the municipalities of Villa de Tututepec de Melchor Ocampo and Santa María Huazolotitlán, in the Costa region of Oaxaca.

Fungal isolates. Fruits showing visible symptoms were washed several times with sterile distilled water and liquid soap to remove dust and impurities, then disinfected with 96% ethyl alcohol for three minutes. Brown to black lesions with orange-black acervuli were selected. From each lesion, a 1-mm fragment of symptomatic tissue was excised and placed on Petri dishes containing Potato Dextrose Agar (PDA, BIDICO®) medium. The plates were labeled and incubated for seven days to promote fungal growth. All plates were incubated at 25 °C in complete darkness. After four days of growth, actively growing hyphal tips from disinfected tissues were subcultured onto fresh PDA plates to obtain axenic cultures.

Taxonomic identification. Identification of the isolates was carried out at the Soil Laboratory of CIIDIR-IPN, Oaxaca. For cultural and morphological characterization, the microculture technique described by Aquiahuatl-Ramos *et al.* (2012) was used. This method involves culturing small portions of mycelium on PDA agar blocks placed on microscope slides inside a humid chamber, allowing microscopic observation of hyphae, conidiophores, and reproductive structures with minimal disturbance and under conditions resembling natural growth. Taxonomic identification was performed using the keys of Ainsworth *et al.* (1973), Sutton (1992), and Barnett and Hunter (1998).

***In vitro* sensitivity and effectiveness analysis.** To evaluate *in vitro* sensitivity, the agar disk diffusion method was used following the procedure described by Davis and Stout (1971), as adapted by Adaskaveg and Hartin (1997). Three commonly used field fungicides and three biological control agents were tested, each applied at three concentrations (high, medium, and low). The specific doses for each treatment are detailed in Table 1.

Table 1. Evaluated concentrations of chemical fungicides and biological agents used against *Colletotrichum gloeosporioides* and *C. truncatum* in papaya fruits.

Product / Microorganism	High concentration g L⁻¹	Medium concentration L⁻¹	Low concentration g L⁻¹
Prochloraz	3.0	2.0	1.0
Benomyl	0.6	0.4	0.3
Azoxystrobin	0.3	0.2	0.1
Trichoderma sp.	1.5 9.0×10^6 conidia mL^{-1}	1.2 7.2×10^6 conidia mL^{-1}	1.0 6.0×10^6 conidia mL^{-1}
Bacillus subtilis	4.0×10^9 UFC mL^{-1}	3.0×10^9 UFC mL^{-1}	2.0×10^9 UFC mL^{-1}
Bacillus cereus	1.5×10^8 UFC	1.2×10^8 UFC	1.0×10^8 UFC

Two previously identified fungal strains (*C. gloeosporioides* and *C. truncatum*) were used. PDA plates were inoculated with 200 µL of a spore suspension adjusted to 1×10^6 spores mL^{-1}, prepared by counting in a Neubauer chamber and adjusted to the required concentration using the dilution equation:

$$V2 = C1 * \frac{V1}{C2}$$

Where C_1 is the initial concentration, V_1 the initial volume, C_2 the desired final concentration, and V_2 the required final volume.

On each plate, four filter paper disks (6 mm) were placed and impregnated with 10 µL of each treatment, leaving one disk with distilled water as a control. The plates were incubated at 25 ± 1 °C for seven days, after which the inhibition halos were measured. Effectiveness (%) was calculated using Abbott's formula (1925), comparing the growth of each treatment with that of the control.

$$\text{Effectiveness}(\%) = (\frac{X_c - X_t}{X_c}) * 100$$

Where X_c = value observed in the control treatment, and X_t = value observed in the tested treatment.

***In vivo* assays in papaya for the control of *Colletotrichum* spp. under laboratory conditions**. Five fruits per treatment were selected at ripeness stage 5 according to Salazar (2002). After washing and disinfection, the fruits were allowed to dry for 10 minutes, and the treatments described in Table 1 were applied by spraying; the control consisted of fruits treated only with sterile distilled water. The fruits were incubated for 10 days at 25 ± 1 °C and 75% relative humidity. The experimental design was factorial, considering six antifungal treatments and four evaluation times. Product effectiveness was determined after 10 days based on the number of positive infections per fruit, using Abbott's formula (1925). Disease severity was assessed by measuring the diameter of lesions caused by *C. gloeosporioides* and *C. truncatum* and classifying them according to the five-grade scale of Castellanos *et al.* (2014), based on the affected area (mm²) (Table 2).

Table 2. Visual and quantitative scale for classifying anthracnose damage in papaya fruits according to lesion area.

Grade	Injury area (mm)	Damage description
1	0	No visible damage
2	1 – 15	Very small injuries
3	16 – 30	Moderate injuries
4	31 – 45	Extensive injuriess
5	> 45	Very severe injuries

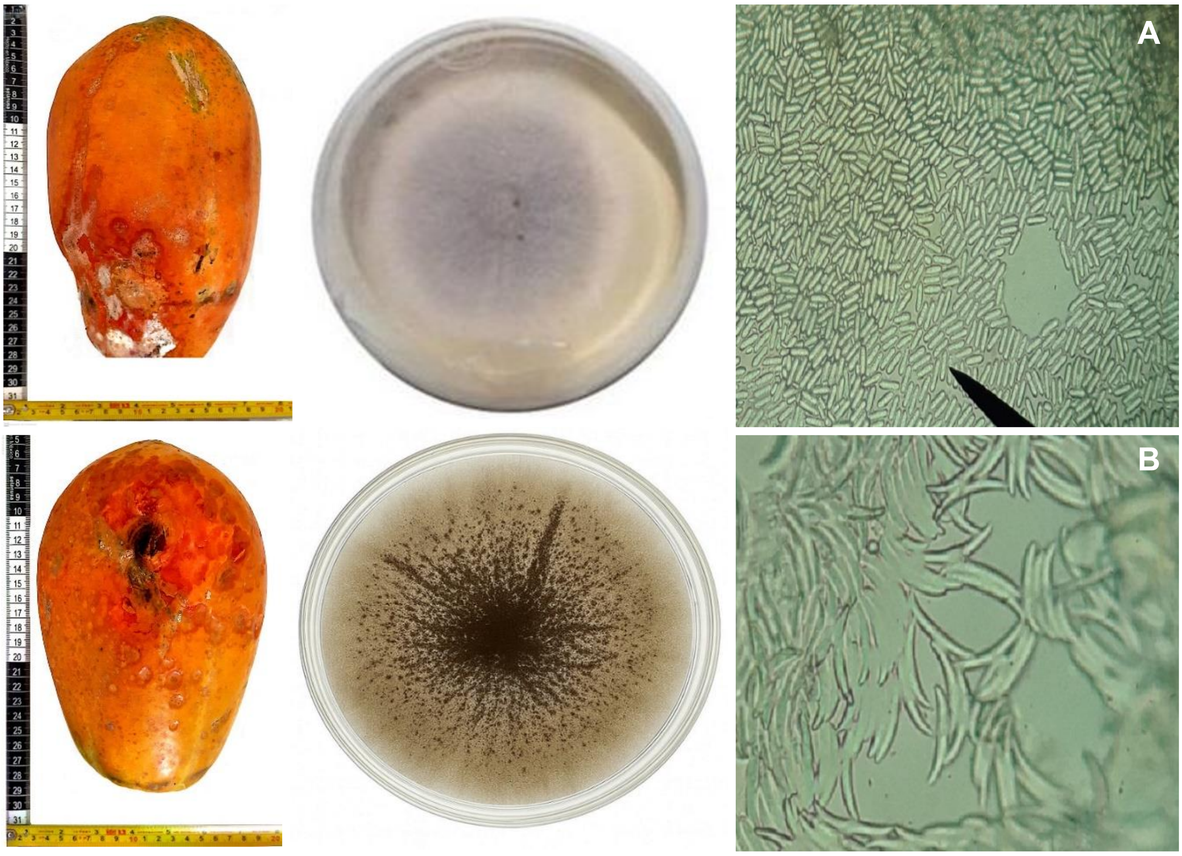
Treatment effectiveness was evaluated 10 days after inoculation using Abbott's formula (1925). Disease severity was quantified by measuring lesion diameter and classifying fruits according to the five-grade scale of Castellanos *et al.* (2014). In addition, the severity index was calculated using the formula of Anculle and Álvarez (1999), considering both the frequency and degree of damage in the evaluated fruits:

$$IS(\%)=\frac{(\sum nF\ x\ g)}{N*G}*100$$

Where IS = severity index; Nf = number of fruits in each grade; G = damage grade; N = total number of fruits evaluated; and G_{max} = highest grade on the scale.

Data analysis. Data on *in vitro* and fruit inhibition were transformed (√(x+1) and cosine, respectively) and analyzed under a completely randomized design. For the fruit assay, a factorial arrangement (6 × 4) was applied, followed by ANOVA and mean comparison (Tukey or Duncan, $p \leq 0.05$) using SAS® 9.4. The severity index was used solely as a descriptive indicator.

Identification of isolates. *C. gloeosporioides* was identified in 66.7% of the sites and *C. truncatum* in the remaining 33.3%, based on morphological characteristics, as associated with anthracnose in papaya fruits. *Colletotrichum gloeosporioides* produced cylindrical-fusiform conidia (14.5–17.02 µm) and white-to-black colonies (29.55–56.38 mm after seven days). In contrast, *C. truncatum* exhibited curved, hyaline conidia (22.8–23.8 µm) and white-to-black colonies with radial growth (22.3–41.1 mm after seven days) (Figure 1). Molecular identification of the isolates is necessary for confirmation.



Harm on the fruits | Colonies | Conidium

Figure 1. Morphological characteristics of *Colletotrichum gloeosporioides* (1A) and *C. truncatum* (1B) isolated from papaya fruits.

***In vitro* sensitivity and effectiveness of fungicides and biological agents**. Table 3 shows the results of the *in vitro* assays, which revealed clear differences in the sensitivity of *C. gloeosporioides* and *C. truncatum* to the evaluated treatments. Prochloraz was the most effective, producing inhibition halos of 14 mm (48.66%) in *C. gloeosporioides* and 25–27 mm (86.42%) in *C. truncatum*.

Table 3. Analysis of variance of the effect of different products on lesions in *Carica papaya* var. Maradol fruits caused by *C. truncatum* 10 days after inoculation.

Source of variation	DF	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Product	5	1.141**	3.225**	4.588**	7.880**	9.209**	13.157**	17.345**	17.985**
Injury	3	1.393**	5.048**	8.410**	9.234**	8.945**	13.873**	17.345**	24.967**
Product* Injury	15	0.318ns	0.321ns	0.365ns	0.720ns	0.703ns	1.229ns	17.345ns	1.773ns
Mistake	96	0.306	0.430	0.991	1.204	1.504	1.716	17.345	2.213
Total	119								
CV (%)		46.723	47.071	55.330	58.024	59.319	57.459	50.705	48.269

Where: ($P < 0.05$) according to Tukey's multiple range test; * = significant at 0.05, ** = highly significant at 0.001, ns = not significant.

In the experiment with *C. gloeosporioides*, high-dose azoxystrobin produced similar inhibition halos (14 mm; 29.81%), while *B. subtilis* and *B. cereus* achieved 11–13 mm (73.47% and 50.87%, respectively). *Trichoderma* sp. showed intermediate inhibition against *C. gloeosporioides* (9–11 mm; 27.65%), whereas benomyl and low-dose azoxystrobin were the least effective (<7 mm; <20%). For *C. truncatum*, *B. subtilis* (25–27 mm; 28.97%) and *Trichoderma* sp. (20–23 mm; 71.34%) were the most notable, with prochloraz being the most effective treatment against both species. Overall, *B. subtilis* and *Trichoderma* sp. exhibited greater control potential for *C. truncatum* (Figure 2A and B).

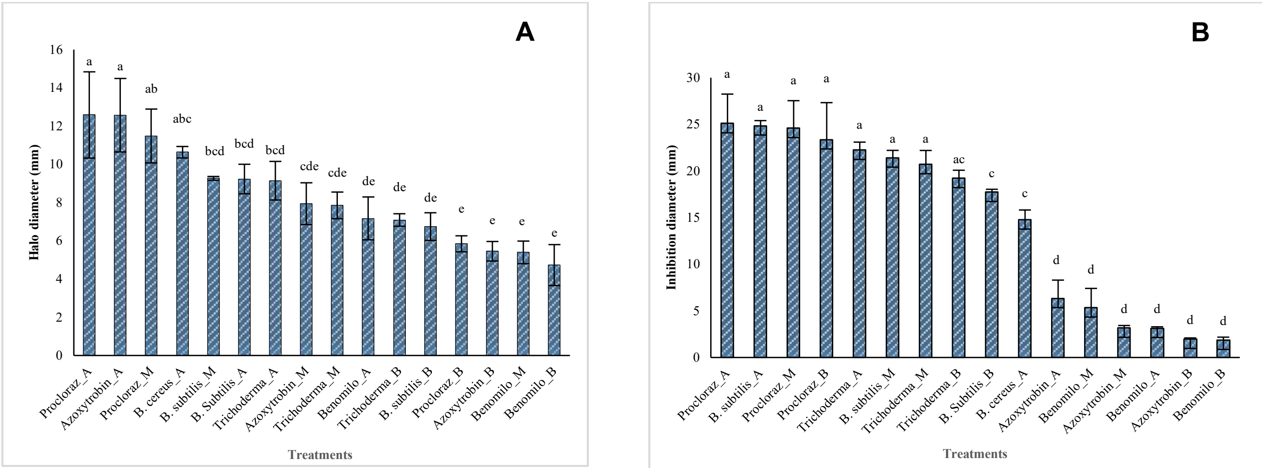


Figure 2. Inhibition diameter after seven days of growth for *C. gloeosporioides* (**A**) and *C. truncatum* (**B**) with three doses and six fungicides using the disk diffusion technique. Evaluated doses: prochloraz, H = high 3 g L^{-1}; M = medium 2 g L^{-1}; L = low 1 g L^{-1}; benomyl, H = high 0.6 g L^{-1}; M = medium 0.4 g L^{-1}; L = low 0.3 g L^{-1}; azoxystrobin, H = high 0.3 g L^{-1}; M = medium 0.2 g L^{-1}; L = low 0.1 g L^{-1}; *B. subtilis*, H = high 4 g L^{-1}; M = medium 3 g L^{-1}; L = low 2 g L^{-1}; *Trichoderma* spp., H = high 1.5 g L^{-1}; M = medium 1.2 g L^{-1}; L = low 1 g L^{-1}; *B. cereus* 1.5 × 10^8 CFU mL^{-1}. Means followed by the same letter within each species are not significantly different (Duncan, 0.05).

Effectiveness and severity of fungicides in *Carica papaya* var. Maradol fruits. Analysis of variance revealed highly significant differences (P < 0.001) among treatments throughout the evaluation period (3–10 days after inoculation), indicating a marked effect on the inhibition and progression of lesions caused by *C. truncatum*. Disease severity also showed a continuous and significant increase (Table 3).

For *C. gloeosporioides*, analysis of variance showed significant differences among treatments from day 5 post-inoculation (P < 0.05; P < 0.001), indicating their effect on lesion progression. The lesion factor was highly significant, with no treatment × lesion interaction (P > 0.05), reflecting uniform behavior across sites. The coefficients of variation (44.9–54.3%) correspond to the natural variability of fresh tissue (Table 4).

Table 4. Analysis of variance of the effect of different chemical and biological products on lesions in *Carica papaya* var. Maradol fruits caused by *C. gloeosporioides*, 10 days after inoculation.

Source of variation	DF	Day							
		3	4	5	6	7	8	9	10
Product	5	0.563^{ns}	1.505^{*}	3.969^{**}	4.229^{*}	7.361^{**}	6.744^{*}	5.882^{*}	8.296^{*}
Injury	3	3.378^{**}	8.904^{**}	17.604^{**}	22.457^{**}	30.895^{**}	32.674^{**}	40.082^{**}	43.353^{**}
Product*Injury	15	0.206^{ns}	0.4655^{ns}	0.420^{ns}	0.880^{ns}	0.990^{ns}	1.206^{ns}	1.555^{ns}	1.170^{ns}
Mistake	96	0.442	0.669	0.958	1.351	1.705	2.207	2.153	2.606
Total	119								
CV (%)		49.726	52.811	52.284	54.337	54.134	53.807	46.713	44.897

Where: (P < 0.05) according to Tukey's multiple range test; * = significant at 0.05, ** = highly significant at 0.001, ns = not significant.

In fruits inoculated with *C. truncatum*, significant differences were observed among treatments. *B. cereus* showed the largest lesions (2.74–20.02 mm), whereas prochloraz exhibited the least progression (<1.0 mm initially; 1.54–5.58 mm on day 10), followed by benomyl. *Trichoderma* sp. and *B. subtilis* presented intermediate values, with site 1 being the most affected (17.73 mm) compared to site 4 (6.28 mm) (Table 5).

Table 5. Effect of different chemical and biological products on lesions in *Carica papaya* var. Maradol fruits caused by *C. truncatum*, 10 days after inoculation.

Product	Day			
	3	4	5	6
B. cereus	2.739±1.096^{a}	5.006±1.263^{a}	7.441±1.475^{a}	9.26±1.885^{a}
B. subtilis	0.920±0.646^{ab}	0.702±0.533^{b}	3.329±1.256^{ab}	4.414±1.530^{ab}
Benomyl	0.686±0.523^{ab}	1.0775±0.614^{b}	1.795±0.735^{b}	1.548±0.854^{b}
Azoxytrobin	0.284±0.284^{b}	1.3975±0.652^{b}	4.241±1.205^{ab}	5.301±1.497^{ab}
Trichoderma	0±0^{b}	0.979±0.56^{b}	3.07±0.985^{b}	4.094±1.172^{ab}
Prochloraz	0±0^{b}	0.341±0.341^{b}	0.977±0.551^{b}	0.521±0.52^{b}
Injury:				
1	1.9243±0.633^{ab}	3.987±0.962^{a}	6.464±0.881^{a}	7.844±1.141^{a}
2	0.842±0.639^{ab}	1.724±0.565^{b}	3.69±0.932^{ab}	3.392±0.877^{b}
3	0.842±0.319^{ab}	0.329±0.2349^{b}	2.073±0.915^{b}	3.264±1.377^{b}
4	0±0^{b}	0.2943±0.294^{b}	1.674±0.825^{b}	2.257±1.0359^{b}
Product	**Day**			
	7	**8**	**9**	**10**
B. cereus	10.488±1.831^{a}	13.469±2.395^{a}	17.736±2.355^{a}	20.024±2.826^{a}
B. subtilis	6.812±1.897^{ab}	7.145±2.103^{a}	11.137±2.342^{ab}	13.954±2.477^{ab}
Benomyl	1.724±0.986^{b}	2.123±1.161^{ab}	4.043±1.501^{bc}	5.579±1.921^{b}
Azoxytrobin	5.62±1.532^{ab}	6.793±1.783^{b}	8.102±1.948^{bc}	11.048±2.398^{ab}
Trichoderma	4.851±1.440^{ab}	8.346±1.761^{ab}	8.892±2.267^{bc}	15.273±2.444^{a}
Prochloraz	1.541±1.064^{b}	1.844±1.271^{b}	3.014±1.773^{c}	5.311±2.286^{b}
Injury:				
1	8.105±1.397^{a}	10.564±1.486^{a}	14.717±1.897^{a}	17.726±1.743^{a}
2	6.386±1.23^{ab}	7.878±1.466^{ab}	10.922±1.920^{a}	14.584±2.091^{ab}
3	3.506±1.237^{b}	4.605±1.543^{b}	5.167±1.439^{b}	8.868±2.065^{bc}
4	2.694±1.235^{b}	3.433±1.675^{b}	4.475±1.442^{b}	6.281±2.124^{c}

Means within columns followed by the same letter are not significantly different ($P < 0.05$) according to Tukey's multiple range test. where: * = significant at 0.05; ** = highly significant at 0.001; ns = not significant.

In fruits inoculated with *C. gloeosporioides*, significant differences were observed among treatments (Table 6). Prochloraz produced the largest lesion diameters throughout the evaluation period (2.38–24.58 mm), whereas benomyl and *Trichoderma* sp. recorded the smallest values (1.10–8.23 mm). Site 1 exhibited the highest severity (23.99 mm) and site 4 the lowest (6.36 mm). In terms of effectiveness, prochloraz was the most efficient (50–75%), followed by benomyl (45–65%), while *Bacillus* spp. and azoxystrobin showed limited control (<40%) and higher severity (>70%).

Table 6. Effect of different products on lesions in *Carica papaya* var. Maradol fruits caused by *C. gloeosporioides*, 10 days after inoculation.

Product	**Day**			
	3	**4**	**5**	**6**
Prochloraz	2.38±0.69^{a}	4.19±1.04^{a}	7.61±1.19^{a}	9.07±1.76^{a}
B. subtilis	1.06±0.50^{a}	2.89±0.95^{a}	3.37±1.27^{ab}	5.82±1.73^{ab}
B. cereus	0.94±0.55^{a}	2.26±0.94^{a}	4.25±1.46^{ab}	6.77±1.67^{ab}
Trichoderma	1.57±0.84^{a}	1.72±0.95^{a}	2.70±1.35	2.63±1.51^{b}
Azoxytrobin	0.61±0.42^{a}	1.36±0.74^{a}	3.63±1.21^{ab}	5.26±1.58^{ab}
Benomyl	1.1±0.61^{a}	1.25±0.69^{a}	2.04±0.82^{b}	3.47±1.00^{b}
Injury**:**				
1	3.10±0.654^{a}	5.42±0.90^{a}	8.50±1.22^{a}	10.87±1.40^{a}
2	1.12±0.497^{b}	2.51±0.72^{b}	5.07±0.96^{b}	7.09±1.27^{ab}
3	0.55±0.388^{b}	0.82±0.47^{b}	1.62±0.71^{c}	3.10±1.07^{bc}
4	0.34±0.241^{b}	0.36±0.36^{b}	0.54±0.40^{c}	0.95±0.56^{c}
Product	**Day**			
	7	**8**	**9**	**10**
Prochloraz	13.22±2.24^{a}	15.56±2.56^{a}	18.72±2.80^{a}	24.58±3.90^{a}
B. subtilis	6.550±1.95^{ab}	9.55±2.32^{ab}	12.96±2.48^{ab}	16.88±2.85^{ab}
B. cereus	8.07±1.99^{ab}	6.97±2.26^{b}	11.93±2.53^{ab}	15.71±2.77^{ab}
Trichoderma	4.63±2.01^{b}	6.61±2.45^{b}	9.05±2.63^{b}	13.67±2.78^{b}
Azoxytrobin	8.54±2.16^{ab}	10.46±2.49^{ab}	11.61±2.52^{ab}	14.47±2.58^{bc}
Benomyl	3.34±1.06^{b}	5.66±1.26^{b}	7.93±1.61^{b}	8.23±1.78^{b}
Injury:				
1	13.6±1.62^{a}	15.59±1.76^{a}	19.66±1.51^{a}	23.99±1.53^{a}
2	9.85±1.58^{a}	12.13±2.06^{ab}	15.33±2.16^{a}	19.07±2.50^{ab}
3	4.52±1.45^{b}	6.65±1.72^{bc}	8.73±1.88^{b}	12.94±2.46^{bc}
4	1.56±1.02^{b}	2.17±1.05^{c}	4.40±1.49^{b}	6.36±1.95^{c}

Means within columns followed by the same letter are not significantly different ($P < 0.05$) according to Tukey's multiple range test. where: * = significant at 0.05; ** = highly significant at 0.001; ns = not significant.

The results demonstrated variability in the efficacy of the evaluated treatments for managing *C. gloeosporioides* and *C. truncatum* in papaya fruits. Overall, chemical fungicides showed better performance than biological agents. Prochloraz stood out as the most effective treatment, achieving 75% effectiveness against *C. truncatum* and 50% against *C. gloeosporioides*, with relatively low severity levels (60–75%), confirming its ability to reduce disease progression and limit visible fruit damage. Benomyl showed intermediate performance, with 65% effectiveness against *C. truncatum* and 45% against *C. gloeosporioides*, while also maintaining low severity values (60–73%), suggesting that it remains a viable alternative under postharvest conditions, although less effective than prochloraz.

In contrast, the biological treatments showed limited effectiveness. *Trichoderma* sp. and *Bacillus* spp. exhibited control percentages below 35% for both pathogens, reflecting a reduced ability to halt infection progression at this phenological stage of the fruit. Moreover, the associated severity values were high, particularly for *B. subtilis* and *Trichoderma* sp., which reached 93 and 73% in *C. gloeosporioides*, and 86 and 93% in *C. truncatum*, respectively. These results indicate that, under postharvest evaluation conditions, their efficacy was insufficient to contain lesion development—possibly due to the rapid colonization of fruit tissue by *Colletotrichum* spp., whose aggressiveness poses a challenge for biological agents when applied in a curative manner.

The values recorded for azoxystrobin and *B. cereus* place these treatments at an intermediate response level, with 30–40% effectiveness and severity ranging from 75–86%, indicating some suppressive activity, although insufficient for effective postharvest control (Table 7).

Table 7. Effectiveness and severity in *Carica papaya* var. Maradol fruits of six fungicides seven days after inoculation with *C. truncatum* and *C. gloeosporioides* species.

Treatments	Effectiveness in fruit (%)		Severity (%)	
	C. gloeosporioides	***C. truncatum***	***C. gloeosporioides***	***C. truncatum***
Benomyl	45	65	73	60
Trichoderma sp.	35	15	73	93
Procloraz	50	75	75	60
Azoxytrobin	30	40	86	80
B. cereus	30	10	86	75
B. subtilis	25	30	93	86

Overall, the results suggest that the preventive application of chemical fungicides such as prochloraz and benomyl remains the most effective strategy for managing anthracnose in papaya, while biocontrol agents could serve as complementary tools within an integrated management program, particularly at preharvest stages or under repeated application schemes to enhance their establishment and antagonistic activity.

Anthracnose severity in papaya fruits was significantly influenced by treatment and time after inoculation ($P < 0.001$), with progressive lesion development observed up to day 10. Prochloraz was the most effective treatment, achieving 75% control against *C. truncatum* and 50% against *C. gloeosporioides*, followed by benomyl with intermediate efficacy. In contrast, azoxystrobin and the biological agents (*Bacillus* spp. and *Trichoderma* sp.) exhibited effectiveness below 50% and severity above 70%, indicating limited postharvest control. These results confirm that systemic fungicides remain the most effective strategy for curative postharvest management, whereas biocontrol agents may be better suited for preventive applications within integrated disease management programs.

CONCLUSIONS

Anthracnose in papaya fruits from the coastal region of Oaxaca was caused by *C. gloeosporioides* in 66.7% of the sampled sites and by *C. truncatum* in 33.3% of the sites, with both species differing in their response to fungicides and biological antagonists. In the *in vitro* assays, prochloraz was the most effective treatment, showing 14 mm inhibition in *C. gloeosporioides* (48.66%) and 25–27 mm in *C. truncatum* (86.42%). High-dose azoxystrobin produced 14 mm inhibition in *C. gloeosporioides* (29.81%). The biological agents exhibited intermediate to low control: in *C. gloeosporioides*, *B. subtilis* (11–13 mm; 73.47%) and *B. cereus* (11–13 mm; 50.87%), while *Trichoderma* sp. reached 9–11 mm (27.65%). In *C. truncatum*, *Trichoderma* sp. performed best with 20–23 mm inhibition (71.34%), surpassing *B. subtilis* (25–27 mm; 28.97%).

In fruit assays, prochloraz was the most effective treatment, achieving 75% control against *C. truncatum* and 50% against *C. gloeosporioides*, followed by benomyl, which showed moderate control. The biological agents exhibited low postharvest effectiveness (<35%) and high disease severity (>70%), particularly *B. subtilis* and *B. cereus*. Overall, the results confirm that prochloraz is the most reliable alternative for curative postharvest management, whereas biocontrol agents require preventive applications within an integrated management program to improve their effectiveness.

Limitations

The *in vivo* assays were conducted under controlled conditions and therefore do not fully represent the environmental variability and dynamics present in the field. Moreover, the persistence of the treatments and their long-term effects were not evaluated, highlighting the need for additional studies under real cultivation conditions with extended monitoring.

Conflict of Interest

The authors declare no conflict of interest in the conduct and publication of this study.

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