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Inhibition of mycelial growth and conidium germination of *Colletotrichum* sp. for organic and inorganic products

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ABSTRACT

Objective: To evaluate the effect of hydrogen peroxide, potassium sorbate, sodium bicarbonate, and chitosan on mycelial growth and *in vitro* germination of *Colletotrichum* sp., to be used for future management of anthracnose disease in postharvest cv. Ataulfo mango fruit.

Design/Methodology/Approach: The effectiveness of the treatments was evaluated using the poisoned culture method. The evaluated concentrations of hydrogen peroxide and potassium sorbate were 1.0, 0.8, 0.6, 0.4, 0.2, 0.16, 0.12, 0.08, and 0.04%; sodium bicarbonate, 1.0, 0.8, 0.6, 0.4 and 0.2%; and chitosan, 2.5, 2.0, 1.5, 1.0 and 0.5%. A 6-day disk of *Colletotrichum* sp. mycelial growth was placed in each poisoned culture medium. The inhibition of mycelial growth and the germination of *Colletotrichum* sp. conidia were evaluated. The experimental design was completely randomized with five repetitions for mycelial growth and four for conidium germination. The results were analyzed using the Kruskal-Wallis test and the comparison of average ranges. The CE_{50} and CE_{95} of each product was estimated using Probit analysis with the results of mycelial growth inhibition.

Results: The mycelial growth inhibition (100%) of the *Colletotrichum* sp. strain was reached starting at concentrations of 0.16, 0.2, 1.0, and 2.5% for hydrogen peroxide, potassium sorbate, sodium bicarbonate, and chitosan, respectively. The inhibition of conidium germination was only observed in treatments with hydrogen peroxide and potassium sorbate. The CE_{50} and CE_{95} for hydrogen peroxide was 0.1 and 0.12%; for potassium sorbate, 0.10 and 0.19%; for sodium bicarbonate, 0.16 and 0.88%; and for chitosan, 1.20 and 2.18%.

Findings/Conclusions: The evaluated treatments represent an effective and viable ecological alternative for the control of *Colletotrichum* sp., causal agent of anthracnosis in mango fruit.

Key words: Anthracnosis, mango, *in vitro* control, ecological alternatives.

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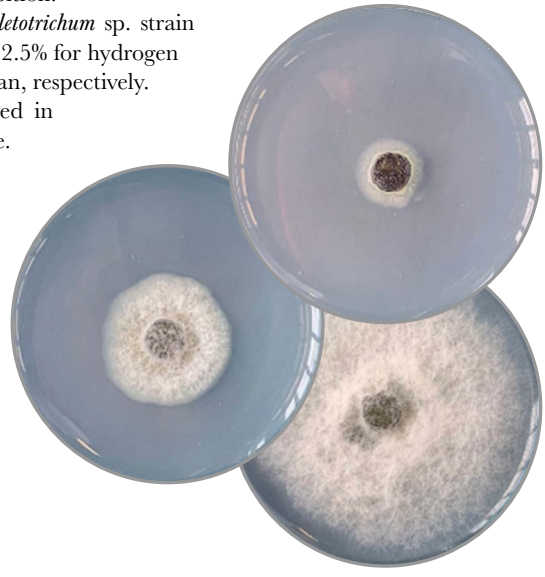

INTRODUCTION

One of the diseases with greatest economic importance in mango (*Mangifera indica* L.) farming is anthracnosis or cankers, caused by the fungus *Colletotrichum gloeosporioides* (Sharma and Kulshrestha, 2015). During postharvest, this disease appears as small, rounded lesions, brown to black in color, with undefined outlines that are slightly sunken into the fruit's flesh. The lesions increase in size as the fruit ripens until joining together, and in severe cases, they cover the entire surface (Siddiqui and Ali, 2014). The control of anthracnosis in postharvest mango fruit is generally done with synthetic fungicides. However, due to the demands of the international market, these have ceased to be used due to the possible risks for human health and environmental contamination (Landero-Valenzuela *et al.*, 2016). Because of the restriction of pesticide use, currently the market has proposed control alternatives such as hydrothermal treatment, use of inorganic salts, storage in controlled and modified environments, biological control strategies, products of organic origin, and vegetable extracts, among others (Dessalegn *et al.*, 2013). Among the products of organic origin, the use of chitosan has shown an inhibitory effect on the development of the disease in postharvest mango fruit cv. Tommy Atkins (Gutiérrez-Martínez *et al.*, 2017). However, there are reports that the effectiveness of chitosan depends on the pathogenic strain evaluated, the molecular weight of the product, the concentration used, and its degree of deacetylation, among other variables (Bautista-Baños *et al.*, 2006; Li *et al.*, 2008). Other organic alternatives for the management of anthracnosis are the use of sodium bicarbonate and potassium sorbate, which have shown total control of the disease in the postharvest of papaya (Ferreira *et al.*, 2018) and olives (El-Sayed *et al.*, 2014). The use of hydrogen peroxide has been reported as inorganic alternative, whose use in the laboratory has shown promising results for control of the pathogen (Muangdech, 2014). Based on the above, the study evaluated the *in vitro* biological effectiveness of hydrogen peroxide, potassium sorbate, sodium bicarbonate, and chitosan on the mycelial growth and germination of *Colletotrichum* sp., with the aim of applying the findings in future studies on the management of anthracnosis in postharvest mango fruit cv. Ataulfo.

MATERIALS AND METHODOLOGY

The study was carried out in the Phytopathology Laboratory of the Rosario Izapa Experimental Field, belonging to the INIFAP based in Tuxtla Chico, Chiapas.

The pathogenic strain (6523) of *Colletotrichum* sp. used in this study was obtained from mango inflorescences (*Mangifera indica* L.) with symptoms of anthracnosis, collected in Huehuetán, Chiapas, Mexico. This strain was selected given its previous evaluation for pathogenicity and aggressiveness (Martínez-Bolaños *et al.*, data not published). The evaluation of treatment effectiveness was done using the poisoned culture method. For this, individual flasks (one per treatment) were prepared with potato-dextrose-agar (PDA) medium and sterilized at 120 °C for 15 min, and then each treatment was added once the medium reached an average temperature of approximately 40 °C, followed by transferring the growth medium into Petri dishes. The evaluated products were hydrogen peroxide and potassium sorbate in concentrations of 1.0, 0.8, 0.6, 0.4, 0.2, 0.16, 0.12, 0.08, and 0.04%;

and sodium bicarbonate in concentrations of 1.0, 0.8, 0.6, 0.4, and 0.2%. Each combination of product/dose was considered one treatment. Additional treatments consisted of chitosan of low molecular weight (Sigma-Aldrich) in five concentrations (0.5, 1.0, 1.5, 2.0, and 2.5%) (Ghaouth *et al.*, 1991), for which a PDA medium was prepared; after its solidification, 1000 μL of each concentration of chitosan were added to form a film approximately 1 mm in thickness on the growth medium.

After the growth medium solidified, a disk (5 mm diameter) of the strain's mycelial growth (6 days old) was deposited on the medium's surface, in the central area; and, finally, the dishes were incubated at room temperature (25±2 °C) for a period of 6 d. As a control treatment, mycelial growth disks were used on PDA without adding any treatments.

A completely randomized experimental design was used with five repetitions for each one. The evaluated response variable was the percentage of effectiveness for each treatment, expressed as a percentage of inhibition of mycelial growth (PIMG) of the *Colletotrichum* sp. strain, with the following formula:

$$PIMG = \frac{Control\ growth - Treatment\ growth}{Control\ growth} * 100$$

To evaluate the effect of each of the treatments on the germination of fungal conidia, two additional Petri dishes were used for each treatment, and 100 μL of the 6523 strain conidia suspension (concentration of 1×10^5 conidia/mL) were deposited and dispersed on the surface of the poisoned growth medium. The Petri dishes were incubated at room temperature (25±2 °C) for 24 h and then 100 conidia were counted and the total germination percentage was determined under a compound microscope (40x). A conidium was considered germinated when the length of its germination tube was greater than that of the conidium itself.

The results on inhibition of *Colletotrichum* sp. conidia growth and germination were analyzed using the Kruskal-Wallis test and a comparison of average ranges (P=0.05), given that the errors were not normally distributed. The effective concentration of the products to inhibit 50 and 95% of mycelial growth (CE_{50} and CE_{95}, respectively) was estimated using a Probit analysis.

RESULTS AND DISCUSSION

The concentrations of hydrogen peroxide, potassium sorbate, sodium bicarbonate, and chitosan demonstrated a significant inhibitory effect on the mycelial growth of *Colletotrichum* sp. (P≤0.05). The hydrogen peroxide showed inhibition of more than 95% on the mycelial growth of the fungus at a dose of 1.12 to 1.0% (Table 1 and Figure 1). These results were statistically different from the other concentrations (difference with the average ranges test). In the lowest dose of this inorganic product (0.04%), there was little effectiveness (15.7%). Similar results were obtained with the evaluation of potassium sorbate, as it totally inhibited the development of the pathogen at concentrations from 0.2 to 1.0% (Figure 2).

Table 1. Inhibition of mycelial growth and conidia germination of *Colletotrichum* sp. *in vitro*, under different concentrations of hydrogen peroxide (HP), potassium sorbate (PS), sodium bicarbonate (SB), and chitosan (Q).

Treatment	Concentration (%)	Mycelial growth inhibition (%)	Average range	Conidia germination (%)	Average range
PH	1.00	100.0[1]	33.5 a*	0[2]	18.5 b
	0.80	100.0	33.5 a	0	18.5 b
	0.60	100.0	33.5 a	0	18.5 b
	0.40	100.0	33.5 a	0	18.5 b
	0.20	100.0	33.5 a	0	18.5 b
	0.16	100.0	33.5 a	0	18.5 b
	0.12	95.7	30.0 b	0	18.5 b
	0.08	20.0	12.4 c	0	18.5 b
	0.04	15.7	8.6 d	0	18.5 b
	0.00	0.0	3.0 e	100	38.5 a
	To		47.0 **		39.0 **
	DMSr		3.5		0.2
SP	1.00	100.0	37.0 a	0	18.5 b
	0.80	100.0	37.0 a	0	18.5 b
	0.60	100.0	37.0 a	0	18.5 b
	0.40	100.0	37.0 a	0	18.5 b
	0.20	100.0	37.0 a	0	18.5 b
	0.16	83.0	27.4 b	0	18.5 b
	0.12	67.0	18.6 c	0	18.5 b
	0.08	25.0	11.8 d	0	18.5 b
	0.04	14.8	9.2 d	0	18.5 b
	0.00	0.0	3.0 e	100	38.5 a
	To		46.7 **		39.0 **
	DMSr		4.1		0.2
BS	1.00	100.0	28.0a	56.5	2.5 b
	0.80	92.9	22.8 b	100	14.5 a
	0.60	79.6	18.2 c	100	14.5 a
	0.40	63.7	12.4 d	100	14.5 a
	0.20	54.2	8.6 e	100	14.5 a
	0.00	0.0	3.0 f	100	14.5 a
	To		28.0 **		22.8 **
	DMSr		2.3		0.7
Q	2.50	100.0	24.5 a	100	12.5 a
	2.00	90.6	21.3 a	100	12.5 a
	1.50	85.9	21.3 a	100	12.5 a
	1.00	20.8	11.8 b	100	12.5 a
	0.50	8.2	8.4 bc	100	12.5 a
	0.00	0.0	4.0 c	100	12.5 a
	To		14.9 *		-
	DMSr		6.5		-

To=Statistic for Kruskal-Wallis test, DMSr=Minimum significant difference of ranges, [1]Average of five repetitions, [2]Average of four repetitions, *Values with the same letter are not different in the comparison of average ranges (P=0.05).

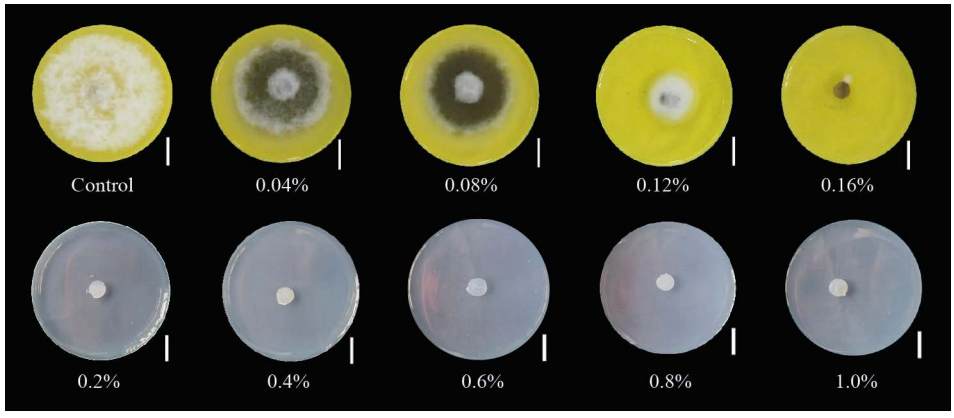

Figure 1. Mycelial growth of *Colletotrichum* sp. at different concentrations of hydrogen peroxide. Scale bar: 1 cm.

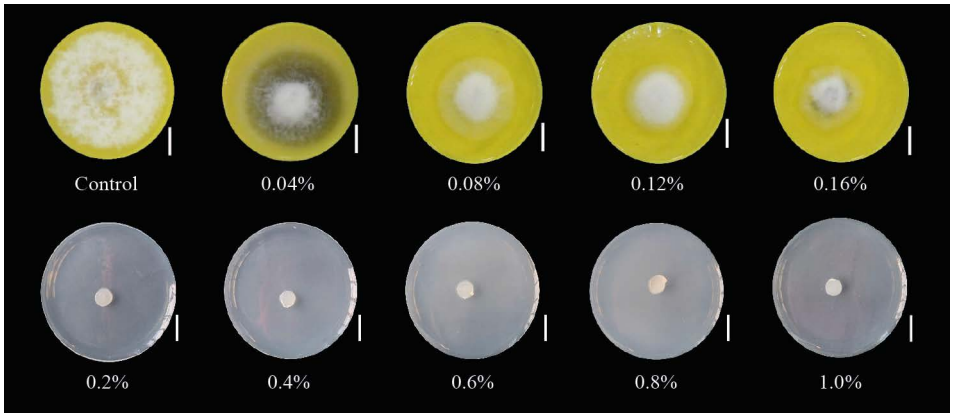

Figure 2. Mycelial growth of *Colletotrichum* sp. at different concentrations of potassium sorbate. Scale bar: 1 cm.

Concentrations at less than 0.2% demonstrated a 14.8 to 83.0% inhibition. With respect to sodium bicarbonate, the total inhibition of mycelial growth of the fungus was reached only at the highest dose (1.0%), followed by the concentration of 0.8% with 92.9% effectiveness (different from the average ranges test at a concentration of 1.0%), while at the lowest dose (0.2%), effectiveness was 54.2% (Figure 3). Finally, chitosan in the highest concentration (2.5%) did not allow the growth of the mycelial pathogen, but there was no statistical difference with concentrations at 2.0 and 1.5%, with an inhibition of 90.6 and 85.9%. In the lowest dose of chitosan (0.5%), the effect was minimal (8.2%) (Figure 4).

In the germination tests of *Colletotrichum* sp. conidia, a significant effect was observed from concentrations of hydrogen peroxide, potassium sorbate, and sodium bicarbonate ($P \leq 0.05$) compared to the control, with total inhibition of germination when using the different concentrations of hydrogen peroxide and potassium sorbate, and with 43.5% inhibition of germination when using sodium bicarbonate at 1.0%. Finally, there was no observed inhibitory effect on conidia germination when the different concentrations of chitosan were used, or in the control.

Figure 3. Mycelial growth of *Colletotrichum* sp. at different concentrations of sodium bicarbonate. Scale bar: 1 cm.

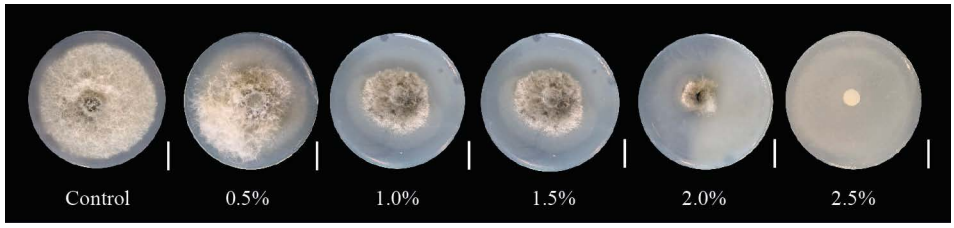

Figure 4. Mycelial growth of *Colletotrichum* sp. at different concentrations of chitosan with low molecular weight. Scale bar: 1 cm.

Similar results of the effectiveness of hydrogen peroxide on the inhibition of mycelial growth of *C. gloeosporioides* were reported by Muangdech (2014) when using concentrations of 0.5% and 0.25%. The inhibitory effect of hydrogen peroxide can be attributed to its capacity for producing highly reactive oxygen free radicals, which adhere to and damage some of the cellular components, including membrane rupture, enzymatic inhibition, nucleoside oxidation, disruption of protein synthesis, and finally, cellular death (Finnegan *et al.*, 2010). Its inhibitory effect on fungal cells has been shown in different fungi species and is attributed to the peroxidase enzyme. Together with an adequate concentration of peroxide as an oxygen donor, this enzyme directly affects the proteins of the spores and mycelium by forming a lignin barrier in the cell walls and in so doing, limiting the development of the fungus (Joseph *et al.*, 1998).

The effectiveness of potassium sorbate for inhibiting the growth of *Colletotrichum* was previously reported by Jabnoun-Khiareddine *et al.* (2016), who obtained total inhibition of the mycelial growth of *C. coccodes* with the use of potassium sorbate at concentrations of 0.5, 1.0, and 1.5%. The principal mode of action in most of the compounds based on potassium salts was a reduction in the turgor pressure of the fungi, which causes a collapse and contraction of the hyphae (Fallik *et al.*, 1997a; Palmer *et al.*, 1997).

The sensitivity of *Colletotrichum* sp. to sodium bicarbonate in the present study is consistent with that obtained by Hasan *et al.* (2012), who observed greater sensitivity of *C. gloeosporioides* with the increase in concentration of this compound. The authors reported more than 60% inhibition of mycelial growth at concentrations of 1.0%, and total inhibition of 2.0, 2.5, and 3.0%. However, the effect of sodium bicarbonate on the germination of spores observed in this study differed partially from that reported by Hasan *et al.* (2012), who mentioned an inhibitory effect only at doses above 2.0%, while in the case of *Colletotrichum* sp., inhibition was observed starting at 1.0% in this study.

The inhibitory and antifungal effect of sodium bicarbonate is attributed to its different modes of action. Sodium bicarbonate has the capacity to elevate the pH of its surroundings, it can deactivate the extracellular enzymes of fungi, and it can interact directly with cellular membranes and interrupt cellular physiology (Palou *et al.*, 2001). Additionally, the salts in sodium bicarbonate increase osmotic stress, reducing the turgor pressure of fungal cells, which results in the collapse of hyphae and spores (Fallik *et al.*, 1997b; De Costa and Gunawarhana, 2012).

The results obtained with the use of chitosan in the inhibition of mycelial growth of *Colletotrichum* sp. are similar to those reported by Berumen *et al.* (2015). However, they differ from that reported by these authors in relation to its effect on conidia germination: they reported an effect at concentrations of 1.0, 1.5, and 2.0%, while no effect was observed for this variable under the doses evaluated in this study.

The inhibition of the growth of this fungus is due to the groups of free aminos in chitosan that produce changes in cellular permeability and cellular disequilibrium of ionic homeostasis of K^+ and Ca^{2+}, among others, which cause the hyphae to atrophy, deform, and collapse (Jun *et al.*, 2011; Peña *et al.*, 2013). In addition to the aforementioned changes, it has been shown that chitosan produces a physical barrier for diverse pathogens in different fruits, while also increasing firmness and delaying ripening in strawberry, tomato, peach, and papaya (Luna *et al.*, 2001; Bautista-Baños *et al.*, 2003).

Hydrogen peroxide reached the lowest CE_{50} and CE_{95} for the mycelial growth of *Colletotrichum* sp. with 0.1 and 0.12%, followed by potassium sorbate with 0.1 and 0.19%, while chitosan of low molecular weight reached the highest CE_{50} and CE_{95} with 1.2 and 2.18%, respectively (Table 2).

Table 2. CE_{50} and CE_{95} values of potassium sorbate, hydrogen peroxide, sodium bicarbonate, and chitosan for the mycelial growth of *Colletotrichum* sp. isolated from mango inflorescences.

Product	CE_{50} (%)	CE_{95} (%)
Hydrogen peroxide	0.10	0.12
Potassium sorbate	0.10	0.19
Sodium bicarbonate	0.16	0.88
Chitosan	1.20	2.18

CONCLUSIONS

The inhibitory effect observed on mycelial growth and conidia germination of *Colletotrichum* sp., with the use of hydrogen peroxide, potassium sorbate, sodium bicarbonate, and chitosan, suggest their possible use as ecological alternatives for the postharvest management of anthracnosis in mango fruit var. Ataulfo.

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