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SCIENTIFIC ARTICLE

Assessing hydrothermal treatment and antagonistic yeasts combination for mango anthracnose control

Evaluación de la combinación del tratamiento hidrotérmico y levaduras antagonistas para el control de la antracnosis en mango

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ABSTRACT

Anthracnose caused by *Colletotrichum* species is the main post-harvest disease in mango in almost all production areas in the world. Hydrothermal treatment (HT) and antagonism of yeasts *Pichia guilliermondii* Wick., *Candida oleophila* Montrocher, and *C. quercitrusa* S. A. Mey. & Phaff were evaluated *in vitro* and *in vivo* in order to determine its effectiveness on anthracnose in 'Ataulfo' mango. The pathogen and the yeasts were prepared at concentrations of 10^5 conidia•mL^{-1} and 10^8 cells•mL^{-1}, respectively. The most effective strains in *in vitro* assays and HT were evaluated in 'Ataulfo' mango inoculated with *Colletotrichum* spp. In *in vitro* assays, *P. guilliermondii* strain CDBB-932 was most effective in controlling the mycelial growth of the pathogen, whereas in *in vivo* assays, the combination HT with *C. quercitrusa* (strain 42) showed the greatest effectiveness. Hydrothermal treatment in combination with yeasts could be implemented as preventive control of anthracnose in mango postharvest.

KEYWORDS

Colletotrichum spp.; microbial antagonists; biological control agents; 'Ataulfo' mango; heat treatment.

RESUMEN

La antracnosis, causada por especies de *Colletotrichum*, es la enfermedad postcosecha más importante en el mango a nivel mundial. Se evaluaron el tratamiento hidrotérmico (HT) y el antagonismo de levaduras de *Pichia guilliermondi*i Wick., *Candida oleophila* Montrocher y *C. quercitrusa* S. A. Mey. & Phaff, *in vitro* e *in vivo*, para determinar su efectividad sobre antracnosis en mango 'Ataulfo'. El patógeno y las levaduras se prepararon en concentraciones de 10^5 conidios•mL^{-1} y 10^8 células•mL^{-1}, respectivamente. Las cepas con mayor efectividad en los ensayos *in vitro* y el HT se evaluaron en mango 'Ataulfo' inoculado con *Colletotrichum* spp. En ensayos *in vitro*, la cepa CDBB-932 de *P. guilliermondii* tuvo mayor inhibición del crecimiento micelial del patógeno, y en ensayos *in vivo*, la combinación HT con *C. quercitrusa* (cepa 42) mostró mayor eficacia. El tratamiento hidrotérmico en combinación con levaduras podrían implementarse como control preventivo de antracnosis en mango.

PALABRAS CLAVE

Colletotrichum spp.; antagonistas microbianos; agentes de control biológico; mango 'Ataulfo'; tratamiento con calor.

Introduction

The mango (*Mangifera indica* L.) is an important crop for the international market due to its flavor, aroma, color, and nutritional properties (Evans et al. 2017). It has been reported that 'Ataulfo' mango has a higher level of β-carotene, ascorbic acid, total phenols, and antioxidant activity than other commercial varieties such as Haden, Kent, Keitt, and Tommy Atkins (Kabir et al. 2017; Mercado-Mercado et al. 2018). Mango fruit has high moisture content and a nutrient-rich profile, making it highly susceptible to different pathogens, including fungi and bacteria. Anthracnose, caused mainly by *Colletotrichum* species (De Souza-Pollo and De Goes 2017; Wu et al. 2020), is the primary postharvest disease in mango; it is considered a critical point that destabilizes the fruit value chain in almost all production areas (Konsue et al. 2020). It has been reported that 87 *Colletotrichum* species are associated with anthracnose symptoms in mangoes (Wu et al. 2020). Fungicides, either as pre or postharvest control methods, have been mainly used to reduce the loss caused by anthracnose. However, the use of chemicals is increasingly restricted due to the induction of resistance in the pathogens and public concern over the toxic residues that are harmful to the environment and public health, which has generated the search for other control options (Gómez-Maldonado et al. 2020; Vilaplana et al. 2020).

Hydrothermal treatment (HT) is a safe and non-chemical method that has been used to control postharvest disease in some fruit species (Usall et al. 2016). It has been proposed that HT, alone or with chemical products, can also control anthracnose in mango in postharvest. In Mexico, HT is an established practice as a condition for exporting mango to the United States, Japan, Chile, New Zealand, and Australia (Luna et al. 2006; Brecht and Yahia 2017). The quality of 'Ataulfo' mango subjected to HT (46.1 °C for 75 min) has been evaluated (Luna et al. 2006). According to the study, HT accelerated the ripening process, fruits developed better color and less titratable acidity, and the sensory characteristics did not vary significantly under preservation at temperatures above 13 °C during two weeks.

In recent years, the search for alternatives for anthracnose control has increased, and biological control has become essential for successful postharvest application (Dukare et al. 2019). Previous research has reported the use of bacteria and yeast such as *Stenotrophomonas rhizophila* Wolf et al. (Hernández-Montiel et al. 2017; Reyes-Pérez et al. 2019), *Burkholderia cepacia* (Palleroni and Holmes) Yabuuchi et al. (De los Santos-Villalobos et al. 2012), *Debaryomyces hansenii* (Zopf) Lodder & Kreger-van Rij, *Meyerozyma caribbica* (Vaughan-Mart. et al.) Kurtzman & Suzuki (Aguirre-Güitrón et al. 2019), and *Cryptococcus laurentii* (Kuff.) C. E. Skinner (Bautista-Rosales et al. 2014) for the control of this disease. The postharvest phase is considered a suitable environment for the successful application of biological control agents; however, their success depends on their compatibility with other practices to achieve integrated management, among other factors (Di Francesco et al. 2016). Several studies previously conducted (Leverentz et al. 2000; Zhao et al. 2010; Liu et al. 2010; Wei et al. 2016) have integrated thermal treatment and biological control to increase the efficacy of controlling the disease.

Taking the previous reports on anthracnose into consideration, the objective of this study was to evaluate the efficacy of hydrothermal treatment and the antagonistic action of *Pichia guilliermondii* Wick., *Candida oleophila* Montrocher, and *C. quercitrusa* S. A. Mey. & Phaff strains in assays *in vitro* and *in vivo* for anthracnose control in 'Ataulfo' mango.

Materials And Methods

Antagonists and pathogen

We used strains 71 (Genbank JX455762), 89 (Genbank JX993808), MN (Genbank JX993815), MNH (Genbank JX455757), and MZC (Genbank JX993807) of *P. gui-*

lliermondii, strain 9 of *C. oleophila* (Genbank KX981191), and strain 42 of *C. quercitrusa* (Genbank KX98119), all isolated from huitlacoche galls in a previous study where it was observed that they were able to inhibit the growth of *Ustilago maydis* (DC.) Corda (Guevara-Vázquez et al. 2009). We also used two commercial *P. guilliermondii* reference strains: strain CDBB-932 (Wickerham 1966), which was obtained from the National Collection of Microbial Strains and Cell Cultures of CINVESTAV-IPN, and strain C-04568 (Laitila et al. 2007) obtained from the VTT Culture Collection of the Technical Research Centre of Finland. Strains preserved in potato dextrose agar (PDA) and mineral oil were reactivated in Petri dishes with YM medium (1 L of distilled water containing 10 g of glucose, 3 g of yeast extract, 3 g of malt extract, 5 g of peptone, and 20 g of agar). The pathogen *Colletotrichum* spp. was isolated from 'Ataulfo' mango fruits with typical anthracnose symptoms and cultured in Petri dishes with PDA medium (Wu et al. 2020; Weir et al. 2012). The fungus was incubated for six days in darkness at 25 ± 1 °C. The pathogen was identified by its distinctive morphological structures and purified with the hypha-tip technique.

Hydrothermal treatment and antagonistic yeasts for the control of *Colletotrichum* spp.

The effect of HT (46.1 °C for 70 min) and yeasts *P. guilliermondii, C. oleophila,* and *C. quercitrusa* on *Colletotrichum* spp. were evaluated in *in vitro* and *in vivo* assays. The *in vitro* assays were performed in three study conditions to define the *in vivo* assays experimental design.

***In vitro* assay**

The yeast strains were inoculated in Erlenmeyer flasks with 250 mL of NYDB liquid culture. These were kept in a rotary incubator (Lab Companion SI-300, Illinois, USA) at 150 rpm for 72 h at 25 °C. The medium was then centrifuged (Mikro 220 Hettich Zentrifugen, Tuttlingen, Germany) at 1,735.1 g for 10 min, and the pellet formed was washed with sterile distilled water. A cell suspension in distilled water was done with the help of a stirrer (Mixer VM-300, Gemmy Industrial Corporation, Taipei, Taiwan), and a solution with 10^8 cells•mL^{-1} (Liu et al. 2010) was prepared with the aid of a Neubauer chamber (Marienfield, Laboratory Glassware, Kônigshofen, Germany). The *Colletotrichum* spp. conidia with six days of growth were removed from the culture surface with sterile distilled water and a magnetic stirrer. The suspension was homogenized and adjusted to 10^5 conidia•mL^{-1} (Kefialew and Ayalew 2008; Liu et al. 2010). The HT was applied in half the volume obtained from each prepared solution (*Colletotrichum* spp., *P. guilliermondii, C. oleophila,* and *C. quercitrusa*) in bain-marie equipment with temperature control.

The effect of HT and the antagonistic yeasts of *P. guilliermondii*, *C. oleophila,* and *C. quercitrusa* on *Colletotrichum* spp. were evaluated under three conditions:

$C_{noHT}Y_{noHT}$: Effect of yeast on the growth area of *Colletotrichum* spp. For this purpose, a filter paper disc containing *Colletotrichum* spp. (C) and another disc with yeast (Y) were placed in Petri dishes, both without HT. In this test, the most effective strains in the control of the fungus were determined.

$C_{HT}Y_{noHT}$: Effect of yeast on the growth area of *Colletotrichum* spp. previously subjected to HT. A disc with *Colletotrichum* spp. subjected to HT, and another disc with yeast without HT were placed in Petri dishes.

$C_{HT}Y_{HT}$: Effect of yeast on the growth area of *Colletotrichum* spp. both previously subjected to HT. A disc with *Colletotrichum* spp. and another with yeast, both subjected to HT were placed in dishes.

In this latter condition, we observed the resistance of each of the strains to temperature, an important factor in antagonists biocontrol efficacy. High temperatures affect the viability of yeasts in biological control, in both pre or postharvest applications; therefore, it is essential to know the tolerance and response of yeasts to thermal stress for a successful application and biocontrol efficacy (Sui and Liu 2014).

The discs used were made of Whatman no. 1 sterile filter paper (5 mm in diameter). They were immersed in the antagonist solutions (with or without HT) and the pathogen (with or without HT) and were placed at two reference points, keeping the same distance in all dishes. The nine antagonistic strains (treatments) and one control treatment (*Colletotrichum* spp. disc without HT) were analyzed in each phase with three replicates at 25 °C. The results of the experiment concluded when the fungus invaded the Petri dishes in the control.

The pathogen and the antagonist growth areas were measured through photographs with ImageJ Image Analysis Software (Image Processing and Analysis in Java, version 1.52, National Institute of Health, USA). Pictures were taken with a dark background to contrast with the light color of the growth areas.

***In vivo* assay**

A hydrothermal treatment of 46.1 °C for 70 min and the antagonism of *P. guilliermondii*, *C. oleophila*, and *C. quercitrusa* were evaluated, alone and combined, for the control of anthracnose in 'Ataulfo' mango fruits artificially inoculated with the pathogen. Strains CDBB-932 and MZC of *P. guilliermondii*, strain 9 of *C. oleophila*, and strain 42 of *C. quercitrusa* were selected due to the greater efficacy in the control of *Colletotrichum* spp. *in vitro*. The yeast and pathogen suspensions were prepared with a concentration of 10^8 cells•mL^{-1} (Zhao et al. 2010) and 10^5 conidia•mL^{-1} (Liu et al. 2010), respectively, as described in the *in vitro* assays.

The mango was collected in orchards established in Nueva Italia, Michoacán, México. The fruits were selected by size, uniform color, and absence of visual defects. Mangoes were disinfested by immersion in 2.5 % sodium hypochlorite solution for 3 min, followed by a rinse with potable water (Vilaplana et al. 2020). Fruits were inoculated by immersion in the pathogen solution for 3 min and incubated for 24 h in plastic boxes. Subsequently, the fruits infected with the fungus were treated with yeast immersion for 3 min (T1-T5), HT (T6), or a combination of both (T7-T11). Therefore, the following treatments were formed: T1) *C. oleophila* 9, T2) *C. quercitrusa* 42, T3) *Pichia* CDBB-932, T4) *Pichia* MZC, T5) Yeast mixture (9 + 42 + CDBB-932 + MZC), T6) HT, T7) HT + *C. oleophila* 9, T8) HT + *C. quercitrusa* 42, T9) HT + *Pichia* CDBB-932, T10) HT + *Pichia* MZC, T11) HT + yeast mixture (9 + 42 + CDBB-932 + MZC). One treatment (T12) was used as a negative control (without HT and untreated).

Each treatment was applied to five fruits with three replicates. Fruits were stored for up to 20 days at room temperature (20 ± 2 °C) in plastic boxes kept at 95 % relative humidity. Anthracnose severity (AS) was evaluated as percentage reduction of the damaged area by the fungus, determined as the difference between the damaged area in the negative control (N) and the damaged area in treatments (T) (Oliveira et al. 2018) using the formula: AS (%) = [(N - T) / N] * 100.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) with SAS version 9.1 statistical software (SAS 2004), using a completely randomized design (CRD) with the statistical model Yij = μ + Ti + •ij. Yeast growth area data were transformed with the logarithmic function, and the percentage (%) severity was subjected to the Box-Cox transformation with $\lambda = 0.01$. Transformations were made to satisfy the conditions of normality and homogeneity of variance. Comparison of means was performed with the Tukey test ($P \leq 0.05$), and an adjustment of α was made with the Bonferroni test for protection against type I error.

Results And Discussion

***In vitro* assay: Efficacy of hydrothermal treatment and antagonistic yeasts**

In the condition $C_{noHT}Y_{noHT}$, the results showed that strains CDBB-932, 9, 42, MZC, C-04568 and MN had greatest efficacy ($P = 0.05$) in the control of the pathogen (10 - 18.6 %) (Table 1). These results are satisfactory and differ from what was reported in another study, where *P. guilliermondii* (10^8 cells•mL^{-1}) could not control *Colletotrichum acutatum* J. H. Simmonds 1965 (Liu et al. 2010). Of the strains mentioned above, C-04568 has been reported to suppress the growth of several species of *Fusarium* (Laitila et al. 2007). On the other hand, the yeast growth area of strain 42 of *C. quercitrusa* was the highest growth when it interacted with *Colletotrichum* spp. By contrast, strain CDBB-932 of *P. guilliermondii*, which showed the greatest antagonistic effect on the fungus, had the lowest growth (Table 1).

Antagonistic microorganisms have several modes of action for pathogen control. Some of them are competition for space and nutrients, production of lytic enzymes, parasitism, oxidative stress, and induced resistance (Di Francesco et al. 2016). Figure 1 shows that, apparently, the most effective *P. guilliermondii* yeasts used different mechanisms for the control of *Colletotrichum* spp. strain CDBB-932 of *P. guilliermondii*

Table 1. Effect of strains of *Pichia guilliermondii*, *Candida oleophila*, and *Candida quercitrusa* yeasts on mycelial growth of *Colletotrichum* spp. *in vitro* after 13-day storage at 25 °C.

Strain	mycelial growth (mm^2)	yeast growth (mm^2)
Control	5104.7 ± 171.9 a	-
Pichia guilliermondii		
MNH	4918.4 ± 99.0 ab	138.71 ± 8.4 ab
89	4716.7 ± 141.3 a-c	140.36 ± 34.8 ab
71	4669.0 ± 143.1 a-c	162.19 ± 15.4 ab
MN	4593.8 ± 94.6 b-d	155.37 ± 12.9 ab
C-04568	4508.4 ± 83.5 b-d	142.23 ± 16.2 ab
MZC	4411.2 ± 86.8 cd	145.28 ± 6.6 ab
CDBB-932	4154.3 ± 49.3 d	106.10 ± 0.4 b
Candida oleophila		
9	4180.0 ± 100.2 d	145.91 ± 23.9 ab
Candida quercitrusa		
42	4276.5 ± 20.7 d	203.07 ± 21.7 a

Data correspond to the mean value ± standard deviation. a,b,c,d Different superscripts in the same column indicate that the means differ significantly ($P \leq 0.05$).

presented a small inhibition halo of approximately 0.5 cm, being the only one that showed such behavior. This inhibition halo was light in color, but due to dark background photography, dark tones are observed. This mode of action can be attributed to the secretion of some yeast inhibitory substances, if it is considered that yeasts can produce exogenous chemicals that cause adverse effects on some pathogens (Guo et al. 2015). Similar behavior was reported in a study that evaluated *Brevundimonas diminuta* (Leifson and Hugh 1954) Segers et al. 1994, *Stenotrophomonas maltophilia* (Hugh 1981) Palleroni and Bradbury 1993, a member of Enterobacteriaceae, *Candida membranifacien*s (Lodder & Kreger) Wick. & K. A. Burton, 1954, and the yeast isolated to control *C. gloeosporioides* (Penz.) Penz. & Sacc., 1884 (Kefialew and Ayalew 2008). The results of this study showed that filtrated antagonist cultures had a more significant antagonistic effect than cell suspensions; the authors attributed this result to the possible presence of inhibitory substances.

Regarding strains 9 (*C. oleophila*), MZC, and C-04568 (*P. guilliermondii*), the pathogen maintained its growth when it joined the colonies margin of these yeasts. A similar response was found when *P. guilliermondii* Moh 10 was evaluated for the control of *Botryodiplodia theobromae* (Pat.) Griffon & Maubl., 1909 (Mohamed and Saad 2009). Finally, strains 42 (*C. quercitrusa*) and MN (*P. guilliermondii*) were slightly invaded by the fungus. Still, they also had the most significant growth, so the mode of action could be competition for space.

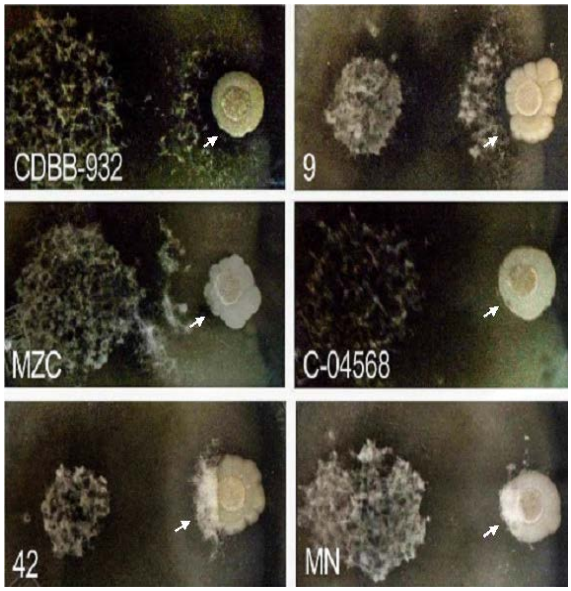

Figure 1. *In vitro* interaction of *Colletotrichum* spp. and the most effective antagonistic yeasts (pointed by the arrow) in pathogen control after 13-day storage. Inhibition halo: CDBB-932 (*Pichia guilliermondii*). Inhibition at the margin: 9 (*Candida oleophila*), MZC, and C-04568 (*Pichia guilliermondii*). No inhibition: 42 (*Candida quercitrusa*) and MN (*Pichia guilliermondii*).

When evaluating the combination of HT and antagonistic yeasts ($C_{HT}Y_{no\text{-}HT}$ and $C_{HT}Y_{HT}$) on the fungal mycelial growth, it was observed that the postharvest pathogen was inhibited. This behavior is consistent with that reported in a study on the use of heat treatment to control *Botrytis cinerea* Persoon, 1794 (Di Francesco et al. 2018) and *Fusarium* spp. (Petreš et al. 2020). In the present study, HT inhibited fungal growth; however, a limitation of heat treatment is that it has little effect when pathogen contamination occurs in the post-treatment storage period (Leverentz et al. 2000). Therefore, and based on the results of the first condition (both organisms without HT), it can be argued that the complementary use of yeasts may be a way to prevent the growth of *Colletotrichum* spp. in the event of post-HT contamination.

Results indicate that the yeasts resist high temperatures; however, there is a significant reduction ($P \leq 0.05$) in the growth area when they are subjected to HT (Table 2). Yeasts showed greater growth in the $C_{HT}Y_{no\text{-}HT}$ combination. This can be attributed to the fact that HT inhibited Colletotrichum spp. Consequently, the yeasts could develop without problems due to a lack of competition for space and nutrients. On the other hand, HT ($C_{HT}Y_{HT}$) significantly damaged *C. oleophila* (strain 9) and *C. quercitrusa* (strain 42) since they did not develop growth, whereas the evaluated *P. guilliermondii* strains were not affected to such an extent; apparently, the latter were thermotolerant. This contrasts with another study where it was reported that heat treatment at 38 °C for 24 h had a harmful effect on *P. guilliermondii* (Zhao et al. 2010).

The evaluation of the growth of the nine strains together with *Colletotrichum* spp. ($C_{noHT}Y_{noHT}$, $C_{HT}Y_{noHT}$ and $C_{HT}Y_{HT}$) was useful to define the most appropriate form of applying the treatments in the *in vivo* assays. Initially, the option of applying the yeasts together with HT in the fruits was considered. Still, because *C. oleophila* (strain 9) and *C. quercitrusa* (strain 42) did not resist heat, it was decided first to apply the HT and then the yeasts.

Although statistically strains CDBB-932, 9, 42, MZC, C-04568, and MN had the same control ability against the pathogen (Table 1), only the first four were selected for *in vivo* evaluation.

In vivo assay: Efficacy of hydrothermal treatment and antagonistic yeasts

Fruits treated only with yeasts had the highest degree of severity (37.4 to 71%) and were not statistically different from each other (Table 3). This is consistent with the results *in vitro* assay, where there was also no statistically significant difference among them; however, the order of the most effective strains did not coincide. De Capdeville et al. (2007) evaluated antagonistic microorganisms for the control of anthracnose on papaya fruits. They found that the most effective strains in *in vitro* and *in vivo* assays were not the same, similar to the present research. This behavior may be due to the fact that the culture medium conditions are not the same as those found in the fruits and, therefore, each strain shows different capacities. The yeast mixture (9 + 42 + CDBB-932 + MZC) had no effect on the control of anthracnose; the effect of *P. guilliermondii* strain MZC was more significant than that produced by the mixture with 37.4%.

An inhibitory effect of anthracnose on mangoes treated with the MZC strain was observed (Figure 2B) with respect to control (Figure 2A), although statistically, the effect was the same. In the mangoes treated only with HT, and in those where HT was combined with antagonistic yeasts, we observed a better control

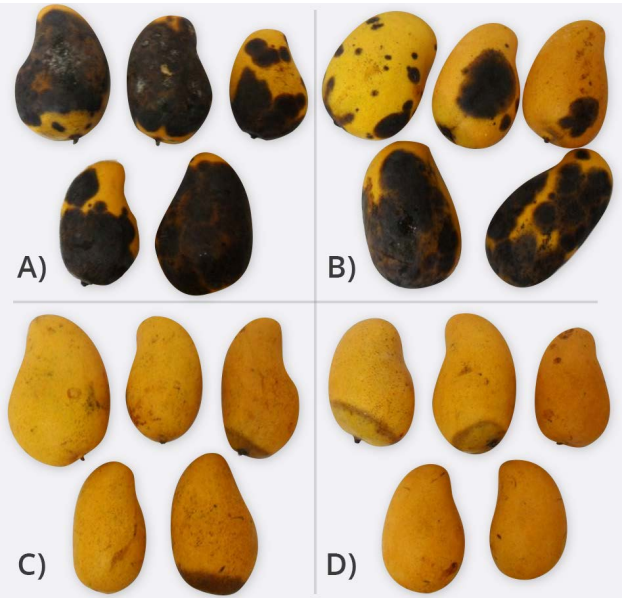

Figure 2. Anthracnose severity after 20-day storage at room temperature (20 ± 2 °C) in 'Ataulfo' mango inoculated with *Colletotrichum* spp.: A) Control (56.2 %), B) *Pichia guilliermondii* MZC (37.3%), C) HT (3.2%), D) HT + *Candida quercitrusa* 42 (1.8 %). HT: Hydrothermal treatment (46.1 °C for 70 min).

Table 2. Growth area of nine *Pichia guilliermondii* strains in interaction with *Colletotrichum* spp. for 13 days storage at 25 °C evaluated under three *in vitro* conditions.

Condition	Yeast growth (mm^2)
$C_{HT}Y_{non\text{-}HT}$	181.50 ± 37.5 a
$C_{no\text{-}HT}Y_{non\text{-}HT}$	147.95 ± 28.1 b
$C_{HT}Y_{HT}$	112.66 ± 61.5 c

HT: 46.1 °C for 70 min. $C_{HT}Y_{non\text{-}HT}$: *Colletotrichum* spp. with HT and yeast without HT. $C_{non\text{-}HT}Y_{non\text{-}HT}$: *Colletotrichum* spp. without HT and yeast without HT. $C_{HT}Y_{HT}$: *Colletotrichum* spp. with HT and yeast with HT. Data correspond to the mean value ± standard deviation. ^{a,b,c} Different superscripts in the same column indicate that the means differ significantly ($P \leq 0.05$).

Table 3. Effect of hydrothermal treatment (HT), antagonist yeasts of *Pichia guilliermondii* (MZC and CDBB-932), Candida oleophila (strain 9), and *Candida quercitrusa* (strain 42) or a combination of both on anthracnose severity in mango fruits after 20-day storage at room temperature (20 ± 2 °C).

Treatment	Severity (%)	Efficacy (%)
T_1: 9	70.94 ± 13.5 a	-
T_3: CDBB-932	63.95 ± 12.2 a	-
T_{12}: Control	56.24 ± 10.9 a	0.0
T_5: Yeast mixture	55.89 ± 21.9 a	0.6
T_2: 42	55.76 ± 21.3 a	0.9
T_4: MZC	37.35 ± 30.0 a	33.6
T_{11}: HT + Yeast mixture	3.60 ± 1.6 b	93.6
T_7: HT+ 9	3.55 ± 1.5 b	93.7
T_6: HT	3.27 ± 1.5 b	94.2
T_{10}: HT + MZC	3.10 ± 0.8 b	94.5
T_9: HT + CDBB-932	2.59 ± 2.0 b	95.4
T_8: HT + 42	1.79 ± 0.6 b	96.8

HT: 46.1 °C for 70 min. Yeast mixture: 9 + 42 + CDBB-932 + MZC. Data correspond to the mean value ± standard deviation. a,b Different superscripts in the same column indicate that the means differ significantly ($P \leq 0.05$).

of anthracnose (Figure 2C, 2D). We did not observe significant differences between them ($P \leq 0.05$), but we did with the control and individual application of yeasts. According to the results, the most effective treatment was HT + *C. oleophila* 42 with 96.8 % efficacy in the anthracnose control, although a similar effect was observed with hydrothermal treatment only.

Yeasts were not as effective in controlling *Colletotrichum* spp. compared to the effect of *P. guilliermondii* on other pathogens such as *B. theobromae* in guava (Mohamed and Saad 2009) and *Colletotrichum capsici* (Schwein.) Andrus & W. D. Moore, 1935 (Chanchaichaovivat et al. 2007); *C. oleophilla* on *Colletotrichum musae* (Berk. & M. A. Curtis) Arx, 1957 in banana (Bastiaanse et al. 2010); and *C. quercitrusa* on *C. capsici* in chilli (Chanchaichaovivat et al. 2007). On the other hand, the effect of HT was greater than that of the yeasts for controlling the fungus, thereby agreeing with another study conducted in mangoes from different ecologies of Ethiopia (Kefialew and Ayalew 2008). The result of this work was significant since the anthracnose could be controlled with HT, considering that there are reports where this same method also controls the fruit fly in 'Ataulfo' mango (Luna et al. 2006). It has been pointed out that, in postharvest systems, an acceptable level of harm is less than 5 % (Janisiewicz and Korsten 2002). Therefore, the combination of HT with the antagonistic yeasts

proved to be very effective because it resulted in damages of only 1.9 % after 20-day storage at 20 °C. Heat treatment can help eradicate fungal spores at the time of application, but they only have a slight effect in protecting the fruit from future infections. By contrast, antagonists do have this property, so the use of yeasts could complement preventative control in postharvest mango storage (Sui et al. 2016).

Conclusions

In the *in vitro* assay, HT (46.1 °C for 70 min) completely inhibited *Colletotrichum* spp. Regarding the yeasts, strains 9 (*C. oleophila*), 42 (*C. quercitrusa*), MZC, MN, CDBB-932, and C-04568 (*P. guilliermondii*) were the most effective in controlling (between 10 and 18.6 %) mycelial growth of the pathogen. In the *in vivo* assay, the combination of HT and *C. quercitrusa* (strain 42) controlled anthracnose in 'Ataulfo' mango with an efficacy of 96.8 % for 20 days. The hydrothermal treatment combined with yeasts could be implemented for the preventative control of the anthracnose in 'Ataulfo' mango.

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Conflict Of Interest

There is no conflict of interest in the present investigation.

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