

Microbiological evaluation of coatings starch-based extracted from tropical fruits during postharvest storage

Evaluación microbiológica de recubrimientos a base de almidón extraído de frutos tropicales durante el almacenamiento postcosecha

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ABSTRACT

Starch-based edible coatings, in addition to preserving organoleptic properties, also allow for gas exchange. This study aimed to evaluate the microbiological activity of three starch-based coatings. Starch was extracted from banana, mango, and soursop fruit and applied to stenopermocarpic 'Ataulfo' mango fruit during postharvest storage. From a batch of mango fruit, 1 cm² segments of affected tissue were sectioned, placed on potato dextrose agar, and incubated for seven days and selecting strains. A pathogenicity test was carried out by inoculating fruit with 10 µL of a spore suspension at 1x10⁶ mL⁻¹ of each of the eight previously isolated strains. These were labeled CP (Pathogenic Strain). A highly aggressive strain was morphologically identified and selected for qualitative and quantitative damage evaluation. An *in vivo* microbiological evaluation was made using stenopermocarpic 'Ataulfo' mango fruit coated with 2 % (w/v) starch and inoculated with CP₃. Moderate damage was observed in the control fruit and slight visible damage was observed in the coated and inoculated fruit. Mango coating inhibited the CP₃ strain damage by 66.91 % at eight days of storage. Starch-based coatings for horticultural products help to reduce damage caused by microorganisms during postharvest handling.

KEY WORDS: Anthracnose, *Colletotrichum* sp., *Mangifera indica*, pathogenicity, stenopermocarpy.

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RESUMEN

Los recubrimientos a base de almidón, además de poseer propiedades organolépticas, permiten el intercambio de gases. El objetivo fue evaluar la actividad microbiológica de recubrimientos a base de almidón extraído de frutos de plátano, mango y guanábana, aplicados a frutos de mango 'Ataulfo' estenospermocárpico durante su almacenamiento postcosecha. A partir de un lote de frutos de mango, se seccionaron segmentos de 1 cm² de tejido afectado, colocandolos en agar papa dextrosa e incubandolos por un periodo de siete días y seleccionando cepas. Se realizó una prueba de patogenicidad, inoculando los frutos con 10 µL de una suspensión de esporas de 1x10⁶ mL⁻¹ de cada una de las cepas previamente aisladas (ocho) asignándoles la nomenclatura CP (Cepa Patógena); seleccionando e identificando morfológicamente la CP de alta agresividad, evaluando los daños cualitativa y cuantitativamente. Se hizo una evaluación microbiológica *in vivo* con frutos de mango 'Ataulfo' estenospermocárpico recubiertos con almidón al 2 % (p/v) e inoculados (CP₃). Se observaron daños moderados (en frutos testigo) y ligeramente visibles en frutos recubiertos e inoculados. El recubrimiento de mango redujo el daño en un 66.91 % por CP₃ hasta los ocho días de almacenamiento. Los recubrimientos a base de almidón de productos hortícolas coadyuvan a disminuir los daños ocasionados por microorganismos durante el manejo postcosecha.

PALABRAS CLAVE: Antracnosis, *Colletotrichum sp.*, estenospermocarpia, *Mangifera indica*, patogenicidad.

Introduction

Mango plants (*Mangifera indica* L.) belong to the Anacardiaceae family, a species of great economic importance, whose fruits are globally accepted for fresh consumption due to their characteristic smell, flavor, and nutritional aspects (Le *et al.*, 2022). The main producing countries of this fruit are Mexico, Thailand, Brazil, Peru, and the Netherlands (FAOSTAT, 2020). In 2021, Mexico was the country with the highest mango exports with a production of 1'096,690.89 t (SIAP, 2021) which is mainly based on the cultivars of 'Tommy Atkins' (11, 314.99 t), manila (17, 702.31 t) and 'Ataulfo' (43,175.36 t); the 'Ataulfo' is distinguished by its excellent organoleptic characteristics. The main producing entities of the 'Ataulfo' mango are Chiapas (208,484.64 t) and Nayarit (110, 307.40 t) according to the report by SIAP (2021). Although various factors cause the loss of fruits in postharvest, it has been documented that the main cause is phytopathogenic fungi (Zhang *et al.*, 2021). The fruits of tropical origin present problems during postharvest storage; estimating losses of 30 – 50 % due to fungi and bacteria (Danh *et al.*, 2021; Choudhury *et al.*, 2018). Most of the typical postharvest pathogenic fungi cause damage to the host tissue through enzymes

or toxins that degrade the cell wall; subsequently, they absorb nutrients from the host's dead cells, causing tissue decomposition (Dukare et al., 2019). The main pathogenic fungi that occur in postharvest include *Botrytis cinerea*, *Penicillium* spp., *Monilinia* spp., *Alternaria alternata*, *Rhizopus stolonifer*, *Trichothecium roseum*, *Fusarium* spp., *Colletotrichum* spp. among others (Zhang et al., 2021). *Colletotrichum* spp. is the causal agent of anthracnose, a disease that attacks the 'Ataulfo' mango, affecting 60 % of the fruit production (Tovar-Pedraza et al., 2020).

The route of infection of *Colletotrichum* spp. is by direct penetration, remaining inactive and causing latent infections, interrupting the latency period when the fruit reaches the climacteric peak, which affects the quality parameters, decreasing its commercial value (Sivakumar et al., 2021). Anthracnose is controlled with chemical fungicides that are used indiscriminately during pre- and post-harvest, generating restrictions on its use due to its toxic effects, also inducing the development of resistance in the pathogen, which harms the environment and public health (Danh et al., 2021; Hu et al., 2014). Due to greater consumer awareness regarding chemical residues in food, the search for natural and environmentally friendly alternatives has been motivated; the use of edible coatings is one of the technologies that has emerged for the control of postharvest diseases as well as extending the shelf life of the products (Romanazzi & Moumni, 2022; Sapper & Chiralt, 2018). An edible coating is a thin continuous matrix that is applied to the food surface, creating a modified atmosphere in the fruit due to a barrier that occurs in gas exchange and water loss control, in addition to decreasing the respiration rate, enzymatic darkening and the release of volatile compounds into the environment, improving the appearance and maintaining the integrity of the fruit (Armghan et al., 2022; Oyom et al., 2022). Edible coatings are made of proteins, lipids, polysaccharides, and mixtures of these. Polysaccharides can be obtained from plants and marine species (crustaceans, red and brown algae), of which the industry most demands are cellulose, starches, alginates, polyalanine, chitosans, gums, and carrageenan (Kocira et al., 2021). Starch is the biopolymer that has drawn attention to the industry for its acceptable aesthetics, biodegradability, and thermoplastic properties; it contains considerable amounts of amylose, which positively affects its coating and edible film-forming properties, is inexpensive, and is abundant in nature. Starch-based coatings have organoleptic (odorless and tasteless), optical (transparent and colorless) properties, as well as a good gas exchange barrier (CO₂ and O₂ permeability) due to the arrangement of the hydrogen bond network (Singh et al., 2022; Oyom et al., 2022; Hassan et al., 2018). Oliveira et al. (2016) carried out an investigation (Short note) using four concentrations of cassava (*Manihot esculenta*) starch (1, 2, 3, and 4 %) as an edible coating to control anthracnose in papaya (*Carica papaya*); reporting that the coating made with 2 % cassava starch was the most effective in the control of anthracnose due to the lower cost, even considering that the four concentrations presented a 100 % control of anthracnose. Another study conducted by De Freitas et al. (2022) evaluated the effect of edible coatings based on cassava and corn (*Zea mays*) starch; and the combination of these at concentrations of 1, 2, 3, and 4 % for the control of anthracnose in avocado fruits (*Persea americana* Mill.). The fruits were covered by immersion and artificially inoculated with a conidial suspension of the pathogen that causes anthracnose. The researchers reported that the coating of cassava starch at 2 and 3 % combined with corn starch was

efficient in restricting the increase in damaged areas in avocado fruits by anthracnose. Due to the above, the objective of the present investigation was to evaluate the microbiological activity of coatings based on starch extracted from stenospermocarpic banana 'Pera', soursop, and mango 'Ataulfo' fruit, applied to stenospermocarpic 'Ataulfo' mango fruits during postharvest storage.

Material and Methods

Plant material

The research was carried out in the microbiology analysis laboratory of the Food Technology Unit (UTA), of the Autonomous University of Nayarit (UAN) during the spring-summer cycle of 2021. Stenospermocarpic 'Ataulfo' mango fruits used in the present study were harvested at physiological maturity from the Atonalisco, municipality of Tepic, Nayarit, located at 234 masl with coordinates 21° 42' N, 104 ° 51' W and transported to the UTA of the UAN the same day of harvest. The fruits were washed with water and 2 % sodium hypochlorite (NaClO) for 10 min, rinsed with distilled water, and subsequently allowed to dry for 30 min at room temperature.

Isolation of the fungus causing anthracnose

The pathogens were isolated from 10 stenospermocarpic 'Ataulfo' mango fruits that were purchased at the Tepic, Nayarit food market at physiological maturity without visible mechanical damage or signs of disease. The fruits were washed with tap water and 2 % sodium hypochlorite (NaClO) for 10 min, rinsed with distilled water and subsequently allowed to dry for 30 min at room temperature and incubated at 28 °C and at a relative humidity (RH) greater than 90 %. Once the fruits developed signs of disease, 1 cm² segments of affected and unaffected tissue (50 %) were taken. The segments were disinfected with 1 % NaClO by immersion (3 min) and washed three times with sterile water to remove NaClO residues; allowed to dry on sterile absorbent paper to remove the excess water. Subsequently, the tissue was placed in the center of Petri dishes with potato-dextrose agar (PDA Dibico^{MR}) and then incubated for seven days at 28 °C, performing frequent reseeding to obtain and preserve the pure strains (Mulkay *et al.*, 2010).

Inoculum preparation

A total of eight monosporic cultures were obtained. The initials CP with a subscript were assigned to each of the isolated strains, which were used as inoculum in the pathogenicity test. To each monosporic culture obtained from eight days of growth in PDA, 10 mL of sterile distilled water was added, to later be scraped and shaken until the disintegration of the colony in a shaker (Vortex-Genie II Mixer SI-0236, USA) at 600 rpm for 30 s. Once the suspension was obtained, it was decanted and filtered through sterile gauze into a sterile glass tube. A 10 µL aliquot of the spore suspension was placed per space in a Neubauer chamber. The following equation was used to calculate the spore suspension at the desired concentration.

$$\text{Total number of cells} = \frac{\text{Number of spores} \times \text{dilution} \times 10^4}{\text{Number of area counted mm}^2} \quad (\text{Eq. 1})$$

The concentration of the spore suspension was adjusted to 1×10^6 mL⁻¹ spores when necessary (Oliveira *et al.*, 2016). Likewise, in the *in vivo* microbiological evaluation, the same procedure was carried out to prepare the inoculum of the strain that presented the highest pathogenicity.

Pathogenicity test

The importance of the pathogenicity test or Koch's Postulates lies in the fact that the symptoms present in an artificially infected host should coincide with the symptoms (relevant morphological and biological traits) initially observed in a naturally infected host (Danh *et al.*, 2021). In this bioassay, 36 stenospemocarpic 'Ataulfo' mango fruits with four replicates per pathogen were selected. The visually healthy fruits in a state of physiological maturity were washed and disinfected with a 2 % NaClO solution for two minutes and then rinsed and allowed to dry at room temperature. Two wounds were made to the fruit with a sterile punch of 0.4 mm in diameter. Subsequently, the fruits were inoculated with a conidial suspension of 1×10^6 spores mL⁻¹ of each of the microorganisms previously isolated and 8 d after sowing in a PDA culture medium. Four fruits were inoculated for each organism and four fruits were used as control fruits. The fruits were placed at 28°C and an RH ≥ 90 %, adequate conditions for the fruiting of the pathogen. The pathogen that presented the greatest aggressiveness was selected, evaluating the damage qualitatively (observation) and quantitatively by daily measuring the diameter of the lesion using a Vernier (caliper) and documenting the development of the microorganism, as well as the severity of the symptom-based on the diameter of the lesion (Gañan *et al.*, 2015). The area of damage caused by the pathogen was calculated using the following formula and was expressed in cm²:

$$A = \pi r^2 \quad (\text{Eq. 2})$$

Where A is the area of damage caused by the pathogen, π is the constant 3.1416 and r^2 is the radius of the affected site in mm.

Morphological identification

For the morphological identification of the species with the highest pathogenicity, macroscopic and microscopic observations of the isolated culture were made. The pathogen was macroscopically identified by documenting the characteristics of the culture such as color, texture, and growth rate of the pathogen. For microscopic identification, structures such as conidia shape, formation of the appressorium and mycelium were observed, as well as any other structure that could identify the pathogen; for this, three microcultures were made by placing 20 μ L of PDA medium on a slide that was inoculated with the pathogen once the culture medium had solidified. A gauze was placed at the bottom of the Petri dish adding 2 mL of sterile distilled water to create adequate conditions for the proliferation of the pathogen. The microcultures were incubated at 28 °C for 6 d; subsequently, the structures of the pathogen were observed in a Motic model BA310 microscope (Motic, British Columbia, Canada) with the 40X objective of the unrecognized fungus that was used for inoculation

in the pathogenicity test and *in vivo* microbiological evaluation (Barrientos-Martínez *et al.*, 2022).

***In vivo* microbiological evaluation**

In the *in vivo* microbiological evaluation, 160 stenopermocarpic 'Ataulfo' mango fruits at physiological maturity were used, selecting the fruits by uniform size and homogeneous color, discarding those that presented physical or mechanical damage or some phytopathological affection. Stenopermocarpic 'Ataulfo' mango fruits were washed and disinfested with 2 % NaClO, then rinsed and allowed to dry at room temperature. The visually healthy fruits underwent a 0.4 mm diameter wound in the epicarp with a sterile awl. The fruits were coated with 2 % (w/v) starch, extracted from banana 'Pera', soursop, and stenopermocarpic 'Ataulfo' mango. Once the fruits were covered, they were allowed to dry at room temperature for one hour. Next, the fruit were artificially inoculated. The wound site was inoculated with 10 µL of conidial suspension of the pathogen that presented the greatest severity of damage to the fruit at a concentration of $1 \times 10^6 \text{ mL}^{-1}$ conidia. Negative controls were treated with 10 µL of distilled water (Table 1). Once the fruits were inoculated, they were placed in a humid chamber at 28 °C and a relative humidity > 80 % (Mulkay *et al.*, 2010). The fruits were evaluated by observing and measuring the damage of the fruit in the cuticle and dark brown spots that appear due to physiological disorders produced by microorganisms. The evaluations were made according to the following scale: 0 = no visible damage; 1 = slightly visible (≤ 25 % of the cuticle); 2 = moderate (26-50 % of the cuticle); and 3 = severe (> 50 % of the cuticle) (Palou *et al.*, 2007). The wound was measured in mm using a Vernier or caliper. The development of the pathogen and the appearance of signs of pathogenicity were observed and documented. The inhibition percentage was calculated using the following equation:

$$\% I = \frac{R1-R2}{R1} \times 100 \quad (\text{Eq. 3})$$

Where R1 is the radial growth of the pathogen in the absence of inhibition (positive control), R2 is the decreased radial growth of the pathogen compared to R1 which reflects the inhibition of the opposite colony; % I indicate radial colony growth that is inhibited in the presence of the opposite colony (Jacobs *et al.*, 2003).

Statistical analysis

A multivariate analysis by the Bonferroni method with an $\alpha \leq 0.005$ was performed, using the Bartlett test for equal variances. The area damaged by the pathogen was considered a dependent variable in the analysis of variance (ANOVA). The STATA 11 software was used for the statistical analysis. The study consisted of a complete experimental design with a factorial arrangement (3x2), generating 6 treatments with two controls (positive and negative) with a total of 8 treatments. 20 fruits per treatment were used for this experiment; the treatments were distributed as shown in Table 1.

Treatments

Table 1. Treatments generated in this study.

Treatment	Starch-based coating at 2 %	Inoculation of the pathogen CP ₃
T1	Banana 'Pera'	No
T2	Banana 'Pera'	Yes
T3	Soursop	No
T4	Soursop	Yes
T5	Stenospermocarpic Mango 'Ataulfo'	No
T6	Stenospermocarpic Mango 'Ataulfo'	Yes
T7	Without coating (negative control)	No
T8	Without coating (positive control)	Yes

Results and discussion

Isolation of the fungus causing anthracnose

The fungal strains that caused the greatest damage to stenospermocarpic 'Ataulfo' mango fruits were compared and selected according to the description of anthracnose disease lesions reported by Sivakumar *et al.* (2021). A total of eight monoconidial isolates were obtained, to which the nomenclature was assigned: CP₂, CP₃, CP₄, CP₅, CP₆, CP₇, CP₈, and CP₉.

Pathogenicity test

Anthrachnose symptoms on a fruit appear as dark brown to black sunken lesions; likewise, when the fruit is exposed to high relative humidity, prominent pinkish-orange spores appear in the lesions (Sivakumar *et al.*, 2021; Tovar-Pedraza *et al.*, 2020). Figure 1 shows the signs of disease that appeared from the fourth day on in fruits that were artificially inoculated, except CP₃, being the pathogen that presented the greatest damage. The visual damage presented by the CP₃ pathogen was moderate at 8 d after inoculation (26 - 50 % of the cuticle) according to Palou *et al.* (2007),

presenting the signs of disease from the second day. The pathogenicity of the isolated fungus was confirmed, since the inoculated fruits presented typical symptoms of anthracnose disease (dark brown to black sunken lesions), similar to the pathogen that naturally infected the fruit, caused by the strain isolate assigned as CP₃, fulfilling Koch's postulates. These results coincide with those presented by Camargo-Piñeres *et al.* (2021).

The results from the pathogenicity test are shown in Figures 2 and 3, strains CP₂, CP₄, CP₅, CP₇, and CP₉ were located within group **a**, which behaved similarly to control fruits (CP₁). In group **b**, the three strains with the highest pathogenicity were located: CP₃, CP₆, and CP₈; finding a significant difference between CP₆ and CP₈ concerning CP₃, CP₃ showed the highest pathogenicity (CP₈ < CP₆ < CP₃), which was re-isolated and preserved for later use.

The damages caused in the CP₃, CP₆, and CP₈ strains are probably due to the climatic conditions to which the fruits were exposed once they were inoculated; same that favor the development and growth of the pathogen (25 - 28 °C and an RH > 90 %). In group **c**, CP₆ and CP₂ were located, which behaved similarly between them, presenting significant differences from the rest of the groups.

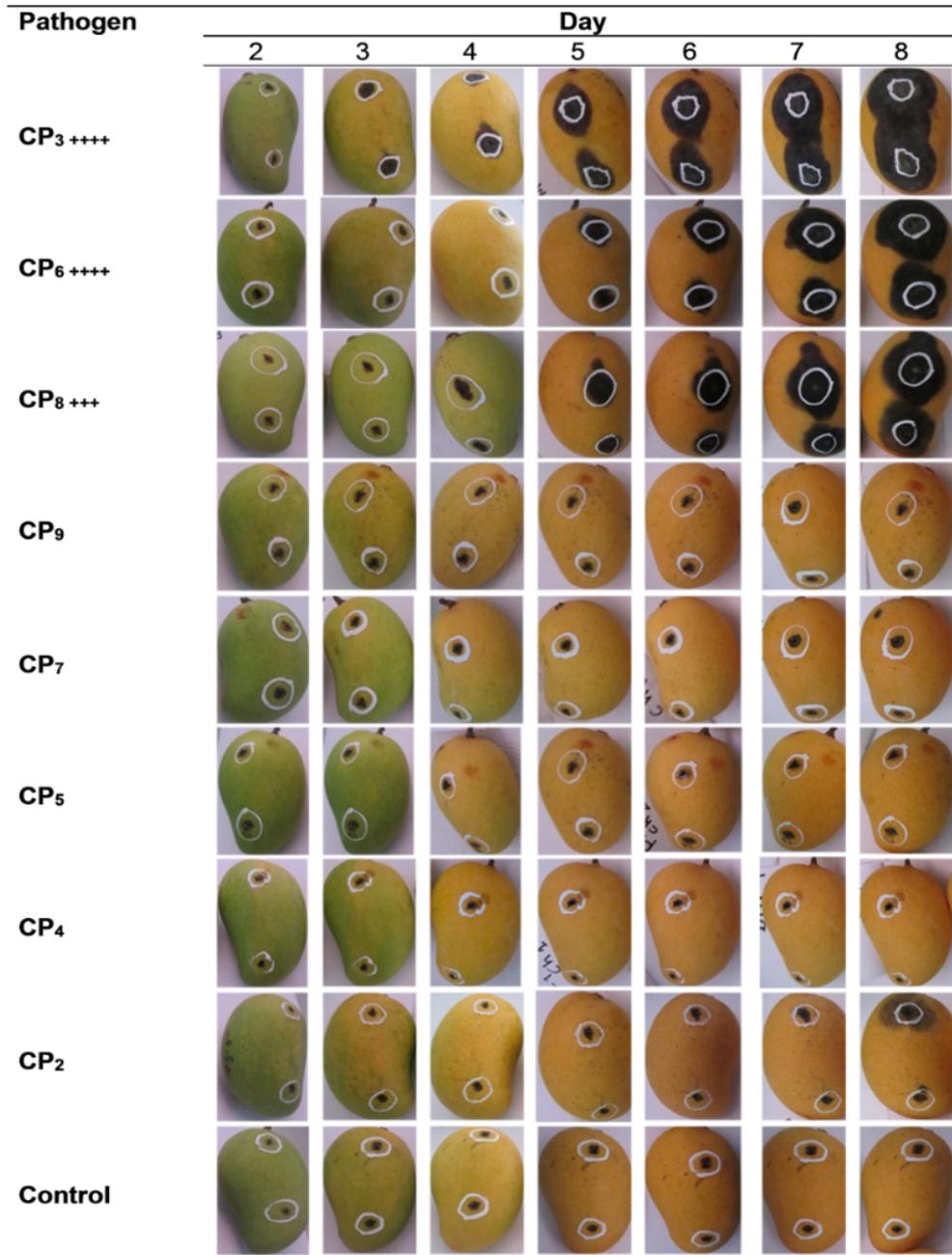


Figure 1. Pathogenicity test. CP nomenclature assigned to pathogenicity strains. Most pathogenic strains (CP+++), intermediate pathogenicity strains (CP++), and less pathogenic strains (CP+).

In Table 2, the Correlation between each of the phytopathogen microorganisms that damage the stenospermocarpic 'Ataulfo' mango.

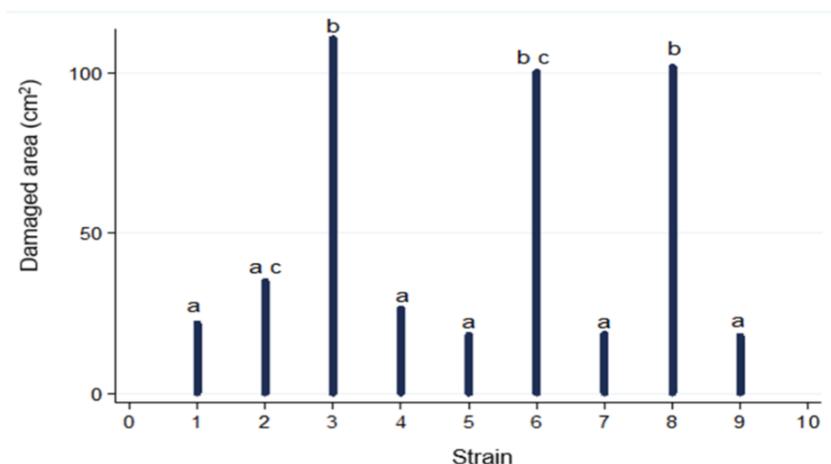


Figure 2. Fruit damage by pathogenic microorganisms inoculated in stenospermocarpic 'Ataulfo' mango fruit for pathogenicity test. Different letters indicate statistically significant differences ($P < 0.001$).

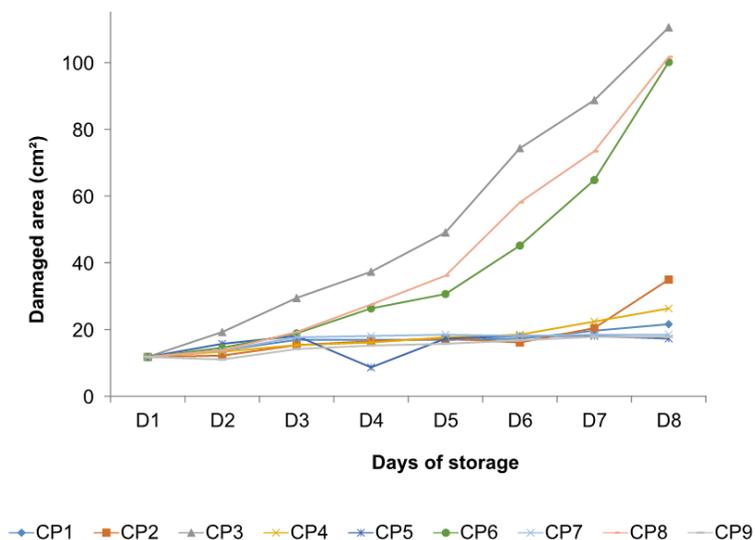


Figure 3. The behavior of pathogenic microorganisms inoculated in stenospermocarpic 'Ataulfo' mango fruit for the pathogenicity test per day of storage.

The CP initials are the assignment given to each isolated pathogen.

Table 2. Correlation of area of damage by the strain of phytopathogenic microorganism using the Bonferroni method.

Cepa	CP ₁	CP ₂	CP ₃	CP ₄	CP ₅	CP ₆	CP ₇	CP ₈
CP ₂	1.22719 (1.0) a							
CP ₃	35.728 (0.014)	34.5006 (0.021)						
CP ₄	0.93662 (1.0) a	-0.29452 (1.0) a	-34.7951 (0.019)					
CP ₅	-0.736313 (1.0) a	-1.9635 (1.0) a	-36.4641 (0.011)	-1.66898 (1.0) a				
CP ₆	22.4821 (0.775)	21.2549 (1.0) c	-13.2457 (1.0) b	21.5494 (0.983)	23.2184 (0.639)			
CP ₇	0.245437 (1.0) a	-0.98175 (1.0) a	-35.7823 (0.015)	-0.687225 (1.0) a	0.98175 (1.0) a	-22.2366 (0.825)		
CP ₈	26.1145 (0.289)	24.8874 (0.407)	-9.6132 (1.0) b	25.1819 (0.375)	26.8509 (0.234)	3.63247 (1.0) b	25.8691 (0.309)	
CP ₉	-2.06168 (1.0) a	-3.28886 (1.0) a	-37.7894 (0.007)	-2.99434 (1.0) a	-1.32536 (1.0) a	-24.5437 (0.447)	-2.30711 (1.0) a	-28.176 (0.158)

*Values with different letters indicate statistically significant differences ($P < 0.05$) using Bartlett's test for equal variances.

The damage caused by the most aggressive pathogen (CP₃) in a stenospermocarpic 'Ataulfo' mango is shown in Figures 4A and 4B.

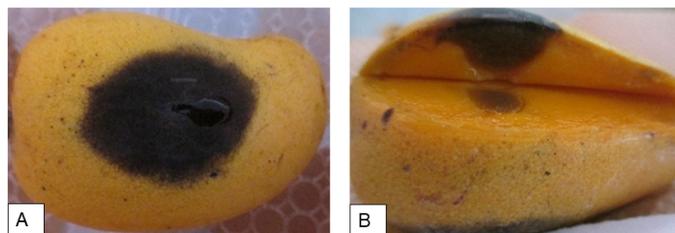


Figure 4. Stenospermocarpic 'Ataulfo' mango fruit inoculated with the pathogen CP₃ presented greater aggressiveness on day 7. A) Lesion on whole affected fruit B) Longitudinal section of the affected fruit.

Morphological identification

In the PDA culture medium, macroscopically, the isolate of the pathogen CP₃, presented on the obverse (Figure 5Aa), a brown-pink center with a beige to cream central ring and dense cottony white aerial mycelium, while on the reverse (Figure 5Ab), the pathogen presented an appearance of a grayish-white center with black dots and concentric rings. Unicellular and cylindrical spores with rounded ends and some with slightly narrow ends were observed at the microscopic level. The measurements of the conidia ranged from 10 ~11.53 μm x 3 ~ 4.5 μm (n=25) (Figure 5B). The pathogen presented the sexual or perfect teleomorphic phase of *Colletotrichum* sp., dark brown, ovate, slightly irregular or lobed melanized appressoria (Figure 5Ca and 5Cb, respectively) and abundant and septate mycelium (Figure 5D). Additionally, CP₃ has a high sporulation capacity, which increases its capacity for survival and rapid development of subsequent infections (Gutiérrez-Alonso *et al.*, 2003).

Kim *et al.* (2009) isolated a total of 82 *Colletotrichum* species, identifying their cultural and morphological characteristics, 26 of the *C. gloeosporioides* isolates produced their teleomorph *Glomerella cingulata* in the PDA culture medium, coinciding with some of the main morphological characteristics of that study with those of the present investigation, inferring that the CP₃ pathogen is a species of the genus *Colletotrichum*, the causative agent of anthracnose.

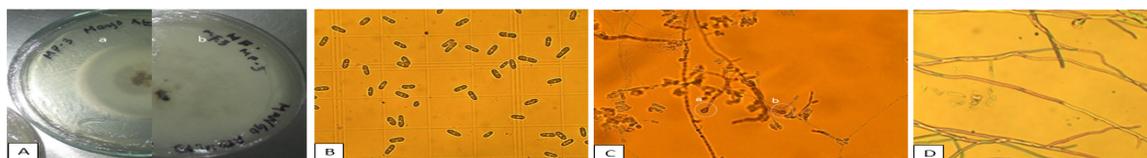


Figure 5. Macroscopic description of *Colletotrichum* sp. A) Isolated strain causing the greatest damage to stenospermocarpic 'Ataulfo' mango fruit. a) Obverse and b) reverse. Microscopic description of *Colletotrichum* sp. B) Conidia C) Sexual or perfect teleomorphic stage of *Colletotrichum* sp. (*Glomerella cingulata*) b) appressorium D) Mycelium.

In vivo microbiology evaluation

In the analysis of variance, the damaged area caused by the pathogen that origin the anthracnose was considered as the dependent variable, finding significant differences between the groups ($F = 13.1$, $P < 0.001$) where the Bartlett test for equal variances was applied to determine of specific groups.

Treatments 1, 3, and 5 were found in group **(a)** (banana 'Pera' coating, soursop and stenospemocarpic mango 'Ataulfo' without pathogen) which behaved similarly to treatment 7 (negative control: fruits without coatings and without pathogen, respectively) observing minimal damage to them (Figure 5). In group **(b)**, treatments 2, 4, and 6 were located (banana 'Pera', soursop, and stenospemocarpic mango 'Ataulfo' with the pathogen). Within this group, the damage caused by *Colletotrichum* sp. was remarkable. The treatments of group **(c)** T5 and T6 (stenospemocarpic 'Ataulfo' mango coating without and with the pathogen) behaved similarly between them. The damage intervals that occurred within these two groups were T5 = 12.88, 13.86, 14.23, and 23.17 cm² of the damaged area on days 5, 6, 7, and 8, respectively, and T6 = 21.68, 29.61, 46.89 and 57.21 cm² of the damaged area on days 5, 6, 7 and 8, respectively. That is, the T6 presented the lowest susceptibility to the pathogen at 66.91 % compared to the control T8 (positive control: fruits without coatings with the pathogen) which presented damages of 172.90 cm² on the eighth day of storage while the treatment T2 and T4 inhibited 42.68 and 46.44 %, respectively. Finally, the fruits of T8 were located within group **(d)** (positive control: fruits without pathogen coating). These fruits suffered severe damage, presenting a significant difference from the rest of the groups.

On the other hand, Figure 6 shows the damage in the fruits from the fourth day with a notable increase in damage from the sixth day in the fruits that were covered and inoculated; distinguishing less damage in the fruits of the treatments that were covered and that were not inoculated with the CP₃ pathogen.

Figure 7 shows the damage caused by anthracnose in stenospemocarpic 'Ataulfo' mango fruits that were treated with 2 % starch from stenospemocarpic banana 'Pera', soursop, and 'Ataulfo' mango fruits per day of storage in which they were stored, presenting moderate damage to the fruits in the presence of the pathogen and slightly visible damage to the fruits to which the coating was applied, but which were not inoculated, even though a wound was made in the epicarp of the fruits, which favors the favorable conditions for the pathogen to enter the fruit tissue (Figure 8).

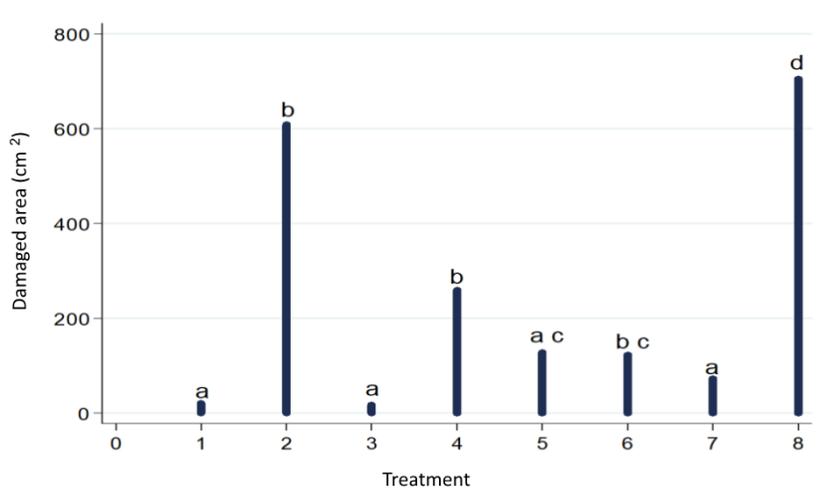


Figure 6. Damage caused by *Colletotrichum* sp. in stenopermocarpic mango 'Aaulfo' fruit with a 2 % starch-based coating. Different letters indicate statistically significant differences ($P < 0.001$).

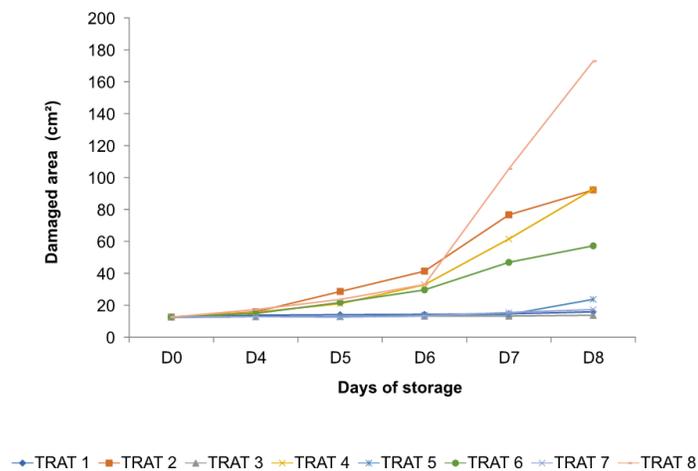


Figure 7. Damage caused by *Colletotrichum* sp. in stenopermocarpic mango 'Aaulfo' fruit with 2 % starch-based coating per day of storage.

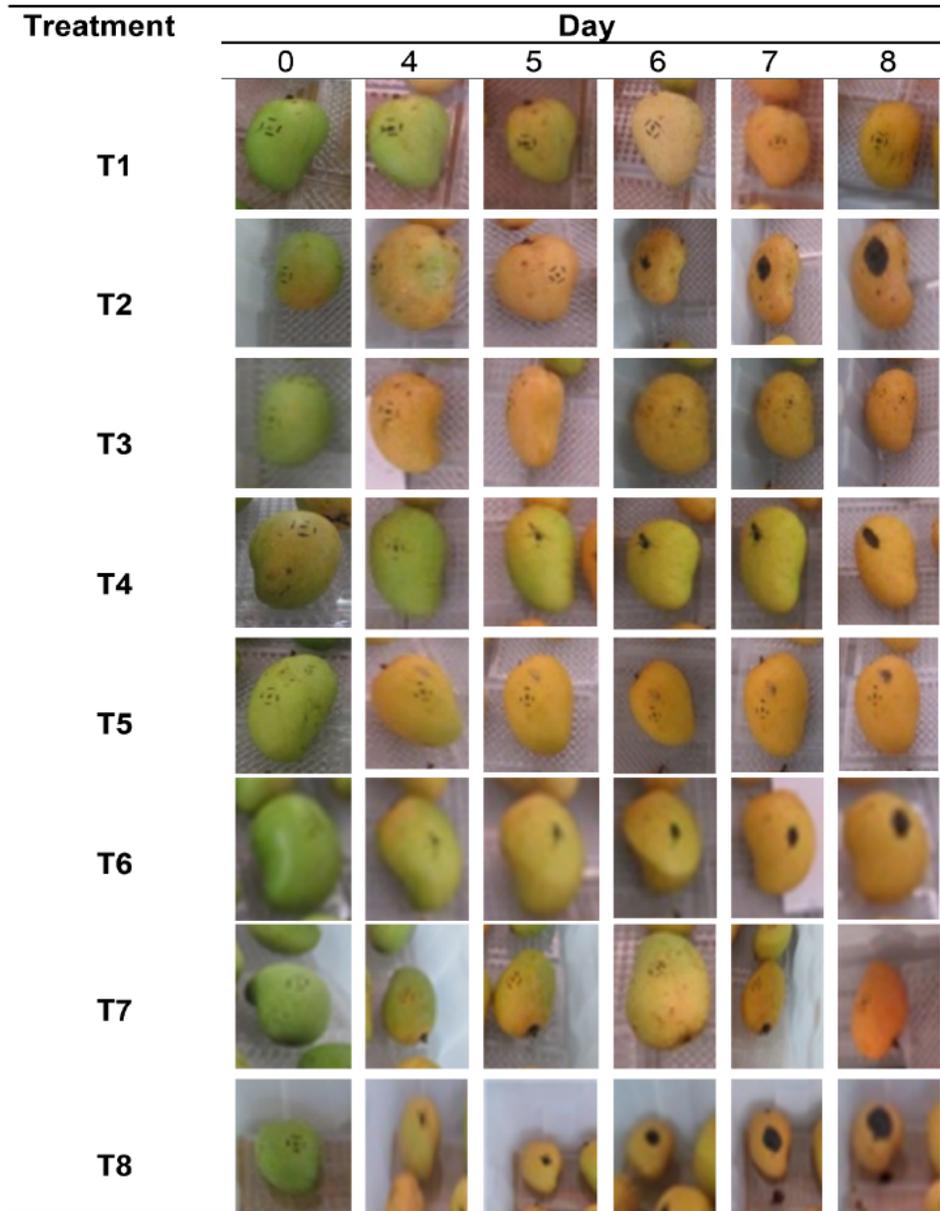


Figure 8. Pathological damage by treatment: T1, T3 y T5 (based coating of banana 'Pera', soursop and 'Ataulfo' stenospemocarpic mango fruit without pathogen, respectively), T7 (negative control: fruit without coatings and without pathogen) and T8 (positive control: fruit without coating and inoculated with the pathogen).

In Table 3, the Correlation between each of the phytopathogen microorganisms that damage the stenospermocarpic 'Ataulfo' mango.

Table 3. Correlation of treatments of the area of damage by treatment using the Bonferroni method.

Treatment	T1	T2	T3	T4	T5	T6	T7
T2	29.2335 (0.001)						
T3	-1.15929 (1.0) ^a	-30.3928 (0.0)					
T4	24.6845 (0.0)	-4.54891 (1.0) ^b	25.8438 (0.006)				
T5	1.22224 (1.0) ^a	-28.0112 (0.004)	2.38154 (1.0) ^a	-23.4623 (0.039)			
T6	16.0518 (0.545)	-13.1817 (1.0) ^b	17.2111 0.387	-8.63277 (1.0) ^b	14.8295 (1.0) ^c		
T7	-0.268608 (1.0) ^a	-29.5012 (0.001)	0.890687 (1.0) ^a	-24.9531 (0.009)	-1.49085 (1.0) ^c	-16.3204 (0.518)	
T8	46.5928 (0.00)	17.3594 (0.333)	47.7521 (0.000)	21.9083 (0.041)	45.3706 (0.00)	30.5411 (0.0)	46.8614 (0.0) ^d

*Values with different letters indicate significant differences statistically ($P < 0.05$) using Bartlett's test for equal variances.

Ali *et al.* (2020) searched for starch-based materials with antimicrobial effects, reporting that starch has been recognized as the most important of several polymers in the field of the food industry due to its biodegradability, biocompatibility, edible properties, and sustainability. However, they mention that presents limitations to be used in food packaging due to its reduced mechanical, barrier, and processing characteristics. The authors mention that to reduce these limitations, different plasticizers are mixed with the starch, in addition to the inclusion of materials such as clay, zinc oxide, titanium dioxide, and magnesium oxide, which can improve the microbial barrier and physical stability, chemical, mechanical and thermal properties of starch, alluding that oils extracted from plants provide good and safe antimicrobial properties. The authors conclude that different starch-based nanocomposite materials, which are available in nature, may be

promising candidates in the field of the food packaging industry. Rodríguez *et al.* (2020) & Arauz (2000) reported that different analyzes have been proposed to help control anthracnose during postharvest; however, climacteric fruits such as mangoes, present a quiescent infection and exhibit an increase in their respiratory rate during their ripening period, which accelerates the production of ethylene, a mechanism that the pathogen uses as a signal to reactivate the process of infection with the fungus *Colletotrichum* spp. According to Torres-Leon *et al.* (2016), the mango fruit is rich in water, sugars, fiber, minerals, vitamins, and antioxidants; its seed contains bioactive compounds such as phenolic compounds (mangiferin, isomangiferin, homomangiferin, quercetin, kaempferol and anthocyanins), phenolic acids (gallic, protocatechuic, ferulic, caffeic, coumaric, ellagic, 4-caffeoylquinic) and antioxidant minerals (K, Cu, Zn, Mn, Fe, Se). In this regard, these researchers report an investigation on mango seeds grown in Egypt where they used high-performance liquid chromatography (HPLC) to determine phenolic compounds, identifying a high content of tannins (20.7 %) and vanillin (20.2 %), acid gallic (6 %), coumarins (12.6 %), caffeic acid (7.7 %), ferulic acid (10.4 %) and cinnamic acid (11.2 %), concluding that the mango seed has antimicrobial activity attributed to its high capacity antioxidant, the content of various compounds and antioxidants. On the other hand, Yadav *et al.* (2022) in a review article report that mango peel is a good source of polyphenols, carotenoids, dietary fiber, and vitamins E and C. Researchers mention that the content of phenols, flavonoids, gallic acid, mangiferin, and antioxidant capacity is much higher (53.3 %) compared to the content of these compounds in the mango pulp and its content will depend on the cultivar, extraction method and stage of maturity in which the fruit is found. In the present investigation, three coatings based on starch at 2 % w/v were used, which were extracted from the tropical fruit of banana 'Pera', soursop, and stenospermocarpic mango 'Ataulfo', which reduced the incidence and severity of anthracnose in fruit of stenospermocarpic 'Ataulfo' mango during postharvest. It should be noted that in this study whole 'Ataulfo' stenospermocarpic mango fruit at physiological maturity were used to obtain starch in the preparation of the edible coating of treatments T5 and T6. The starch-based coating extracted from stenospermocarpic 'Ataulfo' mango fruits (T6) applied to stenospermocarpic 'Ataulfo' mango fruits presented the best result in terms of inhibition of anthracnose, controlling the growth of anthracnose on the eighth day of storage by 66.91 % and that, according to the scale proposed by Palou *et al.* (2007), the damage caused by the pathogen was from 0 to 1 (no visible damage to slightly visible damage).

Conclusion

The 2 % starch-based coatings extracted from banana 'Pear', soursop, and mango 'Ataulfo' diminished the damage caused by *Colletotrichum* sp. in stenospermocarpic mango 'Ataulfo' during postharvest storage. The starch-based coat extracted from stenospermocarpic mango 'Ataulfo' presented the lowest damage (66.91 %) in the fruits during postharvest storage, preserving the organoleptic and microbiology quality of the fruit, leading to fresh consumption.

Author contributions

Conceptualization (HGSE, BMR). Methodology (HGSE). Software management (HGSE, BMR, JZJO). Experimental validation (HGSE, BMR). Formal analysis (HGSE, BMR, LGGG, BRPU, JZJO, LRCE). Data curation (HGSE, BMR, JZJO). Writing and original draft (HGSE, BMR). Writing, reviewing, and editing (HGSE, BMR, LGGG, BRPU, JZJO, LRCE). Project administration (BMR). Funding acquisition (BMR).

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Conflict of interest

The authors declare no conflict of interest.

References

- Ali, S. W., Bairagi, S., & Banerjee, S. (2021). Starch-based antimicrobial materials. In *Advanced Antimicrobial Materials and Applications* (pp. 43-60). Ed. Springer, Singapore. https://doi.org/10.1007/978-981-15-7098-8_2#DOI.
- Arauz, L.F. (2000). Mango Anthracnose: Economic impact and current options for integrated management. *Plant Disease*, 84(6), 601-611. <https://doi.org/10.1094/PDIS.2000.84.6.600>.
- Armghan Khalid, M., Niaz, B., Saeed, F., Afzaal, M., Islam, F., Hussain, M., Mahwish, H., Salman Khalid M., Siddeeg, A., & Al-Farga, A. (2022). Edible coatings for enhancing safety and quality attributes of fresh produce: A comprehensive review. *International Journal of Food Properties*, 25(1), 1817-1847. <https://doi.org/10.1080/10942912.2022.2107005>.
- Barrientos-Martínez, A., Mir, S. G. L., Hernández, M. V., Tapia, M. C., Ochoa, S. A., Acuayte-Valdés, E., Bermudez-Barrientos, J.R., & Barrientos-Priego, A. F. (2022). Pruebas de patogenicidad de hongos en plantas de aguacate 'Hass' relacionados a complejos ambrosiales. *Tropical and Subtropical Agroecosystems*, 25(2022), 041. <http://dx.doi.org/10.56369/tsaes.3727>.

- Camargo-Piñeres, Y., Zambrano Montenegro, G., Ortega-Cuadros, M., Gutierrez Montero, D. J., & Yepes, J. A. (2021). Actividad antifúngica *in vitro* del aceite esencial de *Swinglea glutinosa* Merr sobre *Colletotrichum* sp. patógeno de mango (*Mangifera indica* L.). *Revista Colombiana de Biotecnología*, 23(1), 62-71. <https://doi.org/10.15446/rev.colomb.biote.v23n1.88025>.
- Choudhury, D., Dobhal, P., Srivastava, Seweta, S., Saha, S., & Kundu, S. (2018). Role of botanical plant extracts to control plant pathogens-A review. *Indian Journal of Agricultural Research*, 52 (4), 341-346. <https://arccjournals.com/journal/indian-journal-of-agricultural-research/A-5005>.
- Danh, L. T., Giao, B. T., Duong, C. T., Nga, N. T. T., Tien, D. T. K., Tuan, N. T., Tuan, T. N., Huong, B. T. C., Nhan, C. T., & Trang, D. T. X. (2021). Use of Essential Oils for the Control of Anthracnose Disease Caused by *Colletotrichum acutatum* on Post-Harvest Mangoes of Cat Hoa Loc Variety. *Membranes*, 11(9), 719. <https://doi.org/10.3390/membranes11090719>.
- De Freitas, A. S., de Oliveira Soares, M. G., Barros, A. V., da Silveira, A. L., Silva, S. S. C., & Alves, E. (2022). Postharvest control of anthracnose in avocado with cassava starch and corn starch films. *Comunicata Scientiae*, 13, e3729-e3729. <https://doi.org/10.14295/CS.v13.3729>.
- Dukare, A. S., Paul, S., Nambi, V. E., Gupta, R. K., Singh, R., Sharma, K., & Vishwakarma, R. K. (2019). Exploitation of microbial antagonists for the control of postharvest diseases of fruits: a review. *Critical Reviews in Food Science and Nutrition*, 59(9), 1498-1513. <https://doi.org/10.1080/10408398.2017.1417235>.
- Food and Agriculture Organization of the United Nations Statistics (FAOSTAT) (2020). <https://www.fao.org/faostat/en/#home>.
- Gañán, L., Álvarez, E., & Castaño-Zapata, J. (2015). Identificación genética de aislamientos de *Colletotrichum* spp. causantes de antracnosis en frutos de aguacate, banano, mango y tomate de árbol. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales*, 39(152), 339-347. <http://dx.doi.org/10.18257/raccefyn.192>.
- Gutiérrez-Alonso, J.G., Gutierrez-Alonso, O., Nieto-Angel, D., Téliz-Ortiz, D., Zavaleta-Mejía, E., Delgadillo-Sánchez, F., & Vaquera-Huerta, H. (2003). Resistencia a benomil y tiabendazol en aislamientos de *Colletotrichum gloeosporioides* (Penz.) Penz. y Sacc. obtenidos de mango (*Mangifera indica* L.) en cinco regiones de México. *Revista Mexicana de Fitopatología*, 21(3), 260-266. <https://www.redalyc.org/pdf/612/61221303.pdf>.
- Hassan, B., Chatha, S. A. S., Hussain, A. I., Zia, K. M., & Akhtar, N. (2018). Recent advances on polysaccharides, lipids and protein based edible films and coatings: A review. *International Journal of Biological Macromolecules*, 109, 1095-1107. <https://doi.org/10.1016/j.ijbiomac.2017.11.097>.
- Hu, M., Yang, D., Huber, D. J., Jiang, Y., Li, M., Gao, Z., & Zhang, Z. (2014). Reduction of postharvest anthracnose and enhancement of disease resistance in ripening mango fruit by nitric oxide treatment. *Postharvest Biology and Technology*, 97, 115-122. <https://doi.org/10.1016/j.postharvbio.2014.06.013>.
- Kim, W. G., Hong, S. K., Choi, H. W., & Lee, Y. K. (2009). Occurrence of anthracnose on highbush blueberry caused by *Colletotrichum* species in Korea. *Mycobiology*, 37(4), 310-312. <https://doi.org/10.4489/MYCO.2009.37.4.310>.
- Jacobs, H., Simon N. G., & Crump, D. H. (2003). Interactions between nematophagous fungi and consequences for their potential as biological agents for the control of

- potato cyst nematodes. *Mycological Research*, 107(1), 47-56. <https://doi.org/10.1017/s0953756202007098>.
- Le, T. D., Viet Nguyen, T., Muoi, N. V., Toan, H. T., Lan, N. M., & Pham, T. N. (2022). Supply chain management of mango (*Mangifera indica* L.) fruit: A review with a focus on product quality during postharvest. *Frontiers in Sustainable Food Systems*, 5, 571. <https://doi.org/10.3389/fsufs.2021.79943>.
- Kocira, A., Kozłowicz, K., Panasiewicz, K., Staniak, M., Szpunar-Krok, E., & Horthyńska, P. (2021). Polysaccharides as edible films and coatings: Characteristics and influence on fruit and vegetable quality-A review. *Agronomy*, 11(5), 813. <https://doi.org/10.3390/agronomy11050813>.
- Mulkay, P. A., Aranguren, M., & Herrera, O. (2010). Diagnóstico de las enfermedades fungosas de mayor incidencia durante la poscosecha de la papaya (*Carica papaya* L.), el mango (*Mangifera indica* L.) y el aguacate (*Persea americana* Miller) en tres localidades frutícolas de Cuba. *Revista CitriFrut*, 27(2), 23-30. <https://agris.fao.org/agris-search/search.do?recordID=CU2011900017>.
- Oliveira, B. F., Cruz, A. F., & Alves, E. (2016). Cassava starch coatings for postharvest control of papaya anthracnose. *Phytopathologia Mediterranea*, 55(2), 276-284. http://doi.org/10.14601/Phytopathol_Mediterr-15214.
- Oyom, W., Zhang, Z., Bi, Y., & Tahergorabi, R. (2022). Application of starch-based coatings incorporated with antimicrobial agents for preservation of fruits and vegetables: A review. *Progress in Organic Coatings*, 166, Article 106800. <https://doi.org/10.1016/j.porgcoat.2022.106800>.
- Palou, L., Crisosto, H. C., & Garner, D. (2007). Combination of postharvest antifungal chemical treatments and controlled atmosphere storage to control gray mold and improve storability of 'Wonderfull' pomegranates. *Postharvest Biology and Technology*, 43 (1), 133-142. <https://doi.org/10.1016/j.postharvbio.2006.08.013>.
- Romanazzi, G., & Moumni, M. (2022). Chitosan and other edible coatings to extend shelf life, manage postharvest decay, and reduce loss and waste of fresh fruits and vegetables. *Current Opinion in Biotechnology*, 78, Article 102834. <https://doi.org/10.1016/j.copbio.2022.102834>.
- Sapper, M., & Chiralt, A. (2018). Starch-based coatings for preservation of fruits and vegetables. *Coatings*, 8(5), 152. <https://doi.org/10.3390/coatings8050152>.
- Servicio de Información Agroalimentaria y Pesquera (SIAP) (2021). Acceso el 13 de febrero del 2021. <https://nube.siap.gob.mx/cierreagricola/>.
- Singh, G. P., Bangar, S. P., Yang, T., Trif, M., Kumar, V., & Kumar, D. (2022). Effect on the properties of edible starch-based films by the Incorporation of additives: A review. *Polymers*, 14(10), 1987. <https://doi.org/10.3390/polym14101987>.
- Sivakumar, D., Tuna Gunes, N., & Romanazzi, G. (2021). A comprehensive review on the impact of edible coatings, essential oils, and their nano formulations on postharvest decay anthracnose of avocados, mangoes, and papayas. *Frontiers in Microbiology*, 12, Article 2150. <https://doi.org/10.3389/fmicb.2021.711092>.
- Stata Corp. (2011). *Stata Statistical Software: Release 12*. College Station, TX: StataCorp LP.
- Tapia, R. A., Ramírez, D. J. F., Salgado, S. M.L., Castañeda V. Á., Maldonado, Z. F. I., & Díaz, A. V. L. (2020). Distribución espacial de antracnosis (*Colletotrichum gloeosporioides* Penz) en aguacate en el Estado de México, México. *Revista Argentina de Microbiología*, 52(1), 72-81.

<https://doi.org/10.1016/j.ram.2019.07.004>.

- Torres-León, C., Rojas, R., Contreras-Esquivel, J. C., Serna-Cock, L., Belmares-Cerda, R. E., & Aguilar, C. N. (2016). Mango seed: Functional and nutritional properties. *Trends in Food Science & Technology*, 55, 109-117. <https://doi.org/10.1016/j.tifs.2016.06.009>.
- Tovar-Pedraza, J. M., Mora-Aguilera, J. A., Nava-Díaz, C., Lima, N. B., Michereff, S. J., Sandoval-Islas, J. S., Cámara, M.P.S., Téliz-Ortíz, D., & Leyva-Mir, S. G. (2020). Distribution and pathogenicity of *Colletotrichum* species associated with mango anthracnose in Mexico. *Plant disease*, 104(1), 137-146. <https://doi.org/10.1094/PDIS-01-19-0178-RE>.
- Yadav, D., Pal, A. K., Singh, S. P., & Sati, K. (2022). Phytochemicals in mango (*Mangifera indica* L) parts and their bioactivities: A Review. *Crop Research*, 57(1y2), 79-95. <http://dx.doi.org/10.31830/2454-1761.2022.012>.
- Zhang, Z. Q., Chen, T., Li, B. Q., Qin, G. Z., & Tian, S. P. (2021). Molecular basis of pathogenesis of postharvest pathogenic fungi and control strategy in fruits: progress and prospect. *Molecular Horticulture*, 1(1), 1-10. <https://doi.org/10.1186/s43897-021-00004-x>.