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Morphological, pathogenic and molecular characterization of fungal species associated with mango fruits in Mexico

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The characterisation of the causal agents of postharvest diseases, as well as their pathogenicity in different mango cultivars of economic importance in Mexico was evaluated. In total, 37 fungi were isolated from diseased mango (*Mangifera indica* L.) of the cvs Ataulfo, Hayden, Manila, Peach, Haden and creole. For the morphological characterization, the isolates were seeded in potato dextrose agar media and described by 4 variables. For the molecular characterization, DNA extraction was performed using the commercial Kit. For amplification, primers ITS5 and ITS4 were used and phylogenetic tree was built using RaxML. Six species were identified, namely *Aspergillus niger*, *Colletotrichum asianum*, *Lasiodiplodia theobromae*, *Neofusicoccum oculatum*, *Pestalotiopsis mangiferea* and *Talaromyces variabilis*. The most abundant group was the genus *Aspergillus*, with an appearance frequency of 0.35. Phylogenetic analysis showed that *C. asianum* and *P. mangiferae* belong to the families Glomerellaceae and Pestalotiopsidaceae, respectively, whereas *A. niger* and *T. variabilis* belong to the family Trichocomaceae. The fungi *L. theobromae* and *N. occulatum* belong to the family Botryosphaeriaceae. The pathogenicity of all isolates was demonstrated, except for *T. variabilis*. In contrast, *L. theobromae* and *N. oculatum*, were the most pathogenic isolates in all evaluated cultivars. Susceptibility to each pathogen differed among the cultivars, and Creole was most susceptible to the fungi evaluated.

Key words: Cultivars, disease postharvest, *Mangifera indica* L., phylogenetics analysis.

INTRODUCTION

Mango is a popular and economically important tropical fruit throughout the world, mostly because of its excellent eating quality and nutritional composition (Kim et al., 2009). Global production reached 50.65 million tons in 2017 (Shahbandeh, 2018), and India is the principal mango producer with 35% of the world's production (13.6 million tons), followed by China, Thailand, Indonesia, Mexico and others (FAOSTAT, 2009). The Ataulfo,

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Haden, Tommy Atkins, Kent and Keitt varieties are the most in demand (Luna-Esquivel et al., 2006). Of these, the Ataulfo mango is one of the perennial cultivars with the largest cultivation area in Mexico (114,403 ha planted, 77,993 ha harvested), with an annual production of 510,700 t, and has been listed by Servicio de Información Agroalimentaria y Pesquera (SIAP, 2019) as one of the most important cultivars due to its growing demand on the foreign market. A significant amount, however, is wasted, estimated at 2 to 33% because of fruit drop cracking, immaturity and postharvest decay, mainly due to anthracnose and stem end rot (Nuevo and Apaga, 2010). Anthracnose is caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (Dodd et al., 1997; Arauz, 2000; Kamle and Kumar, 2016) whereas stem-end rot is caused by *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl. (Prusky et al., 1997; Kobiler et al., 2001). Infection of fruit occurs during production and postharvest operations (Johnson and Hofman, 2009); it compromises storage life of the fruit leading to a decline in market value. In this study, due to a lack of information on this fungus in the host, this investigation was carried out, in order to detect the presence of the pathogens with certainty. Because of this, the aim of our investigation was to characterize pathogenically and molecularly different isolations obtained from diseased mango (*Mangifera indica* L.) and determine the causal agent.

MATERIALS AND METHODS

Sampling, isolation and morphological identification

The investigated mango fruits were of the commercial cultivars Ataulfo and Manila from the state of Nayarit, cv. Hayden, Peach and Haden from the state of Guerrero and Creole from the state of Morelos, México. The fruits were of top quality and uniform in size. Six fruits per variety, without physical damage or diseases caused by pathogens, were selected and taken to the Postharvest Technology Laboratory for Agricultural Products of CEPROBI-IPN for processing.

Isolation of phytopathogenic fungi from mango fruits

The mango fruits were washed with soap and water; this will reduced the number of contaminants. Six fruits placed into single humid chamber (95% humidity) and incubated at room temperature (28 ± 2°C), every 2 days were reviewed for a period of 10 days. Once fungal development was observed, mycelium samples were taken with the help of a dissection needle under aseptic conditions, seeded in Petri dishes with potato-dextrose agar (PDA) and incubated at room temperature. Once the colonies had developed, they were purified by reseeding in new PDA dishes and incubated at a temperature of 28 ± 2°C.

Establishment of morphological groups

Each morphology found was grouped according to its cultural characteristics, taking into account the colour of the colony and its appearance, zoning, colour of the underside and edge. After the grouping of each morphology, the frequency of appearance was calculated using the following equation:

$$fa1=N1/Nt \quad (1)$$

where N1 is the number of times a morphology appears, and Nt is the total number of morphologies found (Pérez-Bocourt et al., 2010).

Cultural and morphological characterisation

The cultural characterisation of six fungi was determined from the growth rate of the isolates in the culture media Sabouraud-Dextrose (SDA), nutrient agar (AN), cellulose agar (AC), Czapek agar (Cz), cornmeal agar (AHM), flour agar oatmeal (AHA), water agar (AA) and PDA as control. Mycelium discs with a diameter of 5 mm and a growth period of 15 days in PDA were seeded in the centre of the 50-mm Petri dishes containing each culture medium in triplicate. The dishes were then incubated at room temperature (28 ± 2°C) until the colony reached its maximum mycelial growth, and the colony diameter was measured daily. The data were graphed, and the growth rate was determined by an equation of the straight line of each growth curve for each fungus in the different culture media. A Scott-Knot multivariate analysis was carried out using Infostat 2020.

Micromorphological characterisation was carried out by preparing microcultures in duplicate with two revisions at eight and fifteen days. These consisted of 8-mm discs of PDA, AA and SDA, placed on the ends of slides and inside sterile Petri dishes. They were inoculated with 10 uL of spore solution at a concentration of 1×10^7 mL^{-1} of each fungus, and each disk was covered with a cover slip. Two slides were prepared for each culture medium.

For the analysis of the microcultures, the coverslip was placed on a new slide with a drop of lactophenol blue at 1% v/v and sealed with enamel. The microcultures were observed under an optical microscope (Nikon Olympus CI, Japan) with a 10 and 40x objective. Taxonomic structures such as conidiogenic cells, conidiophore type, conidium type and mycelium type were determined and compared with specialised bibliography.

Molecular identification

Molecular characterisation was performed from an isolate of each of the species obtained from mango fruits. From each selected isolate, 10-day-old mycelium was recovered with the help of a sterile spatula and placed in a 2-mL microtube. Subsequently, DNA extraction was performed using the commercial DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA), following the manufacturer's instructions. The DNA integrity was verified by 1.5% w/v agarose gel electrophoresis, and the DNA was quantified by spectrophotometry using a Nanodrop® ND-1 V 3.2.1 (Thermo Fisher Scientific, Waltham, MA, USA); the concentration was adjusted to 50 ng/µL. For amplification, the primers ITS5 and ITS4 (White et al., 1990) were used, which delimit the Internal Transcribed Spacer 1 and 2, as well as the 5.8 gene of the nuclear ribosomal RNA.

Amplification was performed using a Mastercycler Pro thermocycler (Eppendorf, Hamburg, Germany) with a total reaction volume of 25 µL, with two replicates. The amplification conditions consisted of an initial denaturation step at 94°C for 4 min, followed by 36 denaturation cycles at 94°C for 60 s, annealing at 55°C for 60 s and extension at 72°C for 60 s; final extension was performed at 72°C for 10 min. The amplified products were separated by ethidium bromide-stained 1% agarose gel electrophoresis and visualised using a Chemi Genius 2 Bio Imaging System photodocumenter (Syngene International Ltd, Karnataka, India). The PCR products were purified using the QIAquick PCR

Table 1. Morphological group and frequency of appearance of fungi in different cultivars mango.

Morphological group	Genus	Frequency of appearance	cvs Mango
GM1	*Aspergillus*	0.35	Ataulfo, Hayden, Melocotón and Haden
GM2	*Colletotrichum*	0.12	Haden and creole
GM3	*Lasiodiplodia*	0.20	Ataulfo, haden, Hayden and Melocotón
GM4	*Neofusicoccum*	0.10	Ataulfo, Hayden and Haden
GM5	*Pestalotiopsis*	0.02	Hayden
GM6	*Talaromyces*	0.02	Hayden, Peach and Ataulfo

purification Kit, following the manufacturer's instructions. Purified products were sent for sequencing in both directions to Macrogen (Macrogen Inc., Seoul, Korea), using the same primers as those used for amplification.

Sequence quality was verified by reviewing the electropherograms. The "forward" and "reverse" sequences were aligned using the BioEdit v. 7.2.5 software (Hall, 1999) to obtain the consensus sequences of approximately 560 to 600 bp. The sequences of the isolates were compared with those deposited in the National Center for Biotechnology Information (NCBI), using the BLAST tool. Sequences were deposited in the NCBI Nucleotide Database

Phylogenetic tree

For phylogenetic studies, the molecular marker Internal Transcribed Spacer (ITS) was used, which represents a highly conserved region within mitochondrial DNA, highly repetitive with a slow evolution, where mutations are scarce. Despite this, this marker has a low resolution in terms of discrimination among species, most likely because it also has an area with greater variation (Suárez-Contreras et al., 2022). The orthologue search was performed using a Biopython script with NCBIWWW, with a p-value parameter of 1e-20. The BLAST results were processed with Python and Perl scripts to facilitate multiple alignments. The Mafft v.7.475 program (Yamada et al., 2016), was employed to obtain multiple alignments with the --reorder--auto parameters. Each alignment was edited with Trimal v. 1.4.rev 22 (Capella-Gutiérrez, 2009) with the parameters -gt 0.3 -st 0.001. We used the script Fasta_to_phylip.py to convert to Phylip format https://github.com/josephhughes/Sequence-manipulation), and the phylogenetic tree was built using RaxML v. 8.2.12 (Stamatakis 2014), with the following parameters: -f a -T 15 -m GTRGAMMA -N 100 -x 12345 -p 54321. Finally, the tree was plotted with iTOL (https:/ /itol.embl.de/login.cgi).

Pathogenicity tests

Six isolates were chosen as representatives for the species identified. Inoculation was conducted on healthy mango fruits of the cvs Ataulfo, Creole, Manila and Haden. The fruits were washed with soap and water, disinfected with 1% v/v sodium hypochlorite for 3 min and rinsed three times with water. For inoculation, we used 5-mm-diameter mycelium discs from 8 to 15 days old, grown in PDA medium. Five fruits per variety were inoculated with two discs from the fungi, one per side in the equatorial zone of each fruit. For control, we used un-inoculated fruits, which were kept in a humid chamber with a relative humidity of 100% at room temperature (28 ± 2°C) for 10 days.

The percentage of disease incidence (% I) was determined for each fungus, using the following equation:

$$\%Incidence=Nd/Nt \times 100 \quad (2)$$

where Nd is the number of disks that caused symptoms, Nt is the total number of disks inoculated in mango fruits (Pérez Bocourt et al., 2010).

The area of damage was determined by measuring the width and height of each symptom with the help of a Vernier; for calculation, the following equation was used:

$$Area = Equatorial\ diameter \times Longitudinal\ diameter\ mm^2 \quad (3)$$

RESULTS

Establishment of morphological groups

The frequencies of appearance of the six fungi are shown in Table 1. Morphological Group 1 (GM1) showed black to pale yellow colonies and barely visible white mycelium with a granular to floppy texture, especially in the central zone, similar to *Aspergillus* colonies. In Group 2 (GM2), the colonies had a cottony appearance, dark grey mycelium in the centre with orange spores forming rings, smooth edges and concentric growth; they were included within the genus *Colletrotrichum*. Morphological Group 3 (GM3) presented typical characteristics of the genus *Lasiodiplodia*, with colonies from white to black, with a fluffy-cotton appearance, irregular concentric growth and an irregular border. Morphological Group 4 (GM4) showed colonies similar to those of the genus *Neofusicoccum*, with olive-brown woolly mycelium, irregular growth, brown underside and irregular growth. Morphological Group 5 (GM5) showed white mycelium growing in rings and with a woolly appearance. In the centre, we observed black spores and irregular growth, coinciding with the characteristics of the genus *Pestalotiopsis*. Morphological Group 6 (GM6) presented regular concentric growth, smooth edges and mycelium with a powdery appearance due to the quantity of olive-green to lemon-green spores. Some colonies were reddish on the underside, characteristic of the genus *Talaromyces.* The frequency of appearance differed between the genera isolated. *Aspergillus* presented the highest frequency of appearance (0.35) and *Pestalotiopsis* and *Talaromyces* the lowest one (0.02).

Table 2. Growth rate of the six fungi species in the different culture media evaluated.

Species	Growth rate (mm/day)							
	PDA†	SDA	NA	CzA	AA	CELA	CMA	OMA
Aspergillus niger	12.17	11.80	9.11	11.14	8.28	7.58	9.84	6.87
Colletotrichum asianum	6.28	9.06	6.75	9.75	6.10	6.47	8.81	7.91
Lasiodiplodia theobromae	18.50	21.43	19.42	20.55	15.88	20.84	19.44	24.03
Neofusicoccum oculatum	2.53	19.70	8.56	15.74	10.39	14.78	14.94	16.98
Pestalotiopsis mangifereae	8.45	9.08	9.47	10.60	10.05	8.38	11.22	9.44
Talaromyces variabilis	2.93	3.25	2.53	2.73	2.73	2.46	2.81	3.23

†PDA: potato dextrose agar, SDA: Sabouraud dextrose agar, NA: nutrient agar, CzA: Czapek agar, AA: water agar, CELA: cellulose agar, CMA: cornmeal agar, OMA: oatmeal agar.

The Ataulfo and Hayden varieties were infected by the six fungi isolated.

Cultural and morphological characterisation

Growth rates (mm/day) differed among the six species in the eight selected culture media. The species *L. theobromae* (Pat.) Griffon & Maubl (15.88 to 24.03), *Neofusicoccum oculatum* Syd & P. Sid (2.53 to 19.70), *Aspergillus niger* v. Tieghem (6.87 to 12.17) and *Pestalotiopsis mangifereae* P. Henn (8.45 to 11.22) presented the highest growth rates, whereas *Colletotrichum asianum* Prihastuti (6.10 to 9.75) and *Talaromyces variabilis* C.R. Benj (2.46 to 3.23) showed the lowest values (Table 2). A high cultural variability was observed among the pathogens that cause various diseases in mango. These results not only demonstrate genetic diversity among populations, they are also preferentially distributed in varieties mango and have great potential to colonize and cause disease symptoms in some varieties more than others. For example *L. theobromae* and *N. oculatum* showed 100% incidence in all varieties evaluated. However, *C. asianum* presents 100% incidence in two varieties.

A total of 37 isolates of fungi were recovered from lesions of mango fruits, and six species were morphologically identified as *A. niger* (14 isolates), *C. asianum* (5 isolates), *L.theobromae* (8 isolates), *N. oculatum* (5 isolates), *P. mangiferea* (1 isolates) and *T. variabilis* (4 isolates). Microscopic taxonomic structures were described; *A. niger* showed conidiophores hyaline and smooth, forming spherical conidial heads, morphology biseriate with globose hyaline phialides, and bottle-shaped metulae attached to the spore chain radially. The conidia are globose, with a surface wrinkled black (4 µm). *C. asianum* presents hyaline phialides and conidia without septa, hyaline in the form of a stick (fusiform) of 8 to 12 µm. The brown appressoria, semicircular 10-15 µm; *L. theobromae* with subovoid conidia with round apices, brown in color and a septum. They have longitudinal striae (myelin deposits) 15 to 20 µm. *N. oculatum* does not present reproduction structures only mycelial development, reason why it is not included in Figure 1. *P. mangiferea* presents conidia formed by five cells, two hyaline at the ends and three versicolor central. Fusoid, ellipsoid or straight, 12 to 15 µm, basal cell conical, hyaline; with basal and apical tubular hyaline appendages. *T. variabilis* showed right conidiophores, hyaline biverticillates, with ampulla-shaped phialides and straight metulas. Globose conidia of green color and smooth walls, 3.5-4 µm, in chains (Figure 1).

Molecular identification

Coverage and identity values for all strains exceeded 99%, based on analyses performed on all sequences using the Targeted Loci Nucleotide BLAST tool (Table 3). For phylogenetic studies, the molecular marker ITS (Internal Transcribed Spacer) was used, which represents a highly conserved region within mitochondrial DNA, highly repetitive with a slow evolution, where mutations are scarce. Despite this, this marker has a low resolution in terms of discrimination among species, most likely because it also has an area with greater variation (Suárez-Contreras et al., 2022).

The results obtained from the phylogenetic analysis for each of the six isolates is as shown in Figure 2. In each of them, the name and accession number assigned by NCBI are indicated in red. The clades or branches closest to it indicate sequences similar to the fungi identified in this work, obtaining a more precise approach to the name of the species. The isolates are grouped in correspondence to the genetic distance between them. Three clades (branches) were formed, and in each of them, two bifurcations were formed, indicating two different species. In the case of *C. asianum* and *P. mangiferae*, they are genetically closer compared to *A. niger* and *T. variabilis,* and the genetic distance between *L. theobromae* and *N. occulatum* is even smaller with respect to the other isolates. This is because the fungi identified belong to the same classes, that is, *C. asianum* and *P. mangiferae* belong to the class Sordariomycetes

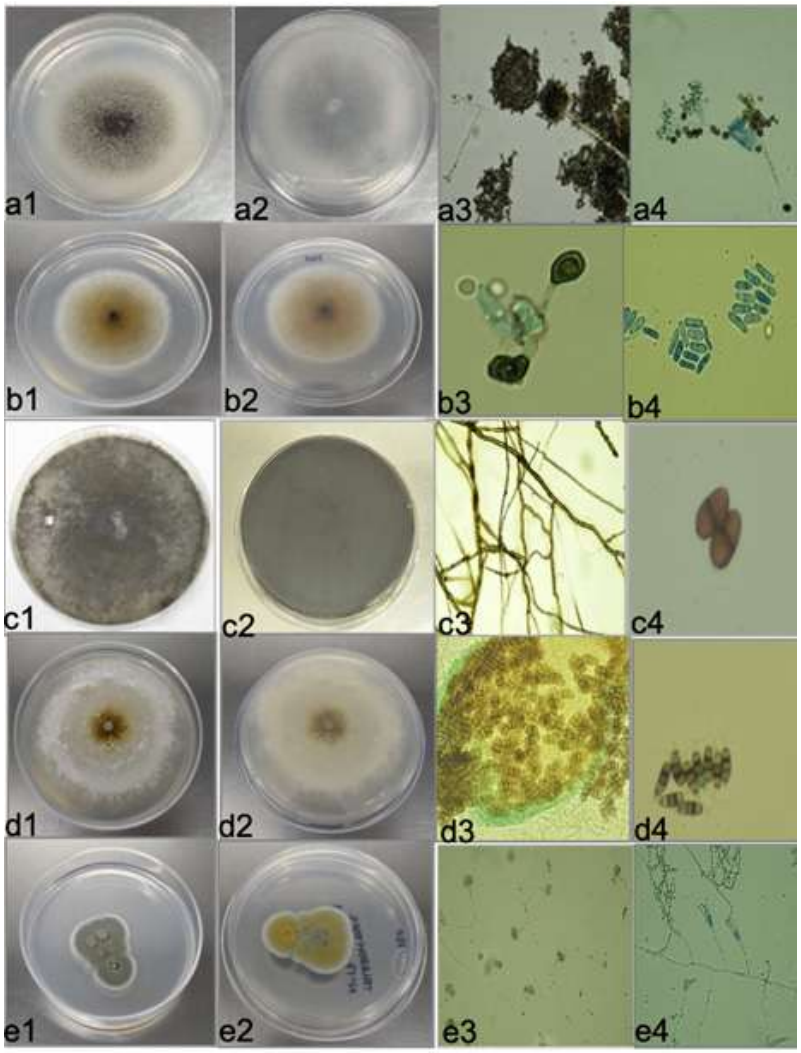

Figure 1. Macroscopic and microscopic characteristics of fungi species from mango fruit. *Aspergillus niger*: Colony upper surface (a1); Colony lower surface (a2); Conidiophore (a3); Phialide (a4). *Colletotrichum asianum*: Colony upper surface (b1); Colony lower surface (b2); Conidio (b3); Apresorio (b4). *Lasiodiplodia theobromae:* Colony upper surface (c1); Colony lower surface (c2); Mycelio (c3); Conidio (c4); *Pestalotiopsis mangifereae:* Colony upper surface (d1); Colony lower surface (d2); Picnidio (d3); Conidio (d4); *Talaromyces variabilis:* Colony upper surface (e1); Colony lower surface (e2); Conidiophore (e3); Phialide and Conidiophore (e4).

Table 3. Molecular identification using ITS markers of fungi isolated from mango fruits.

Género	No. Accession[±]	Morphological identification	Molecular identification	Coberage (%)	Identity (%)
Neofusicoccum	ON003476.1	*Neofusicoccum* spp.	*Neofusicoccum oculatum*	100	100
Aspergillus	ON003479.1	*Aspergillus niger*	*Aspergillus niger*	100	99.83
Colletotrichum	OM892874.1	*Colletotrichum* spp.	*Colletotrichum asianum*	100	99.82
Lasiodiplodia	ON003477.1	*Lasiodiplodia* spp.	*Lasiodiplodia theobromae*	100	100
Penicillium	ON003478.1	*Penicillium* spp.	*Talaromyces variabilis*	99.83	100
Pestalotiopsis	ON003480.1	*Pestalotiopsis* spp.	*Pestalotiposis mangiferae*	100	99.82

[±]Accession number in the NCBI database.

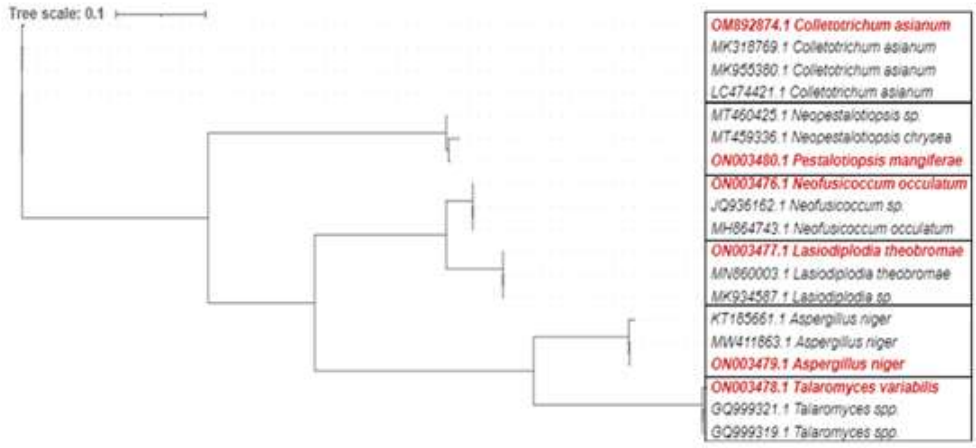

Figure 2. Phylogenetic tree of the six fungi isolated from fruits of different mango cultivars. The isolates identified in this research are marked in red with the NCBI accession code and the species name. Black are the accessions with which these sequences were aligned. Analysis was performed with Maximum likelihood.

Table 4. Incidence of fungi inoculated in mango fruits of the cv Ataulfo, Manila, Haden and criolle after 10 days.

Species	Incidence (%)†			
	Ataulfo	Criolle	Manila	Haden
Aspergillus niger	60	0	60	0
Colletotrichum asianum	0	100	0	100
Lasiodiplodia theobromae	80	100	100	100
Neofusicoccum oculatum	100	100	100	100
Pestalotiopsis mangifereae	0	0	0	0
Talaromyces variabilis	0	0	0	0

†O (%) Calculated from five fruits per variety, two wounds per fruit, total: 10 wounds.

but to the families Glomerellaceae and Pestalotiopsidaceae, respectively. The same occurs with *A. niger* and *T. variabilis*, which belong to the class Eurotiomycetes and to the same family, Trichocomaceae. The fungi *L. theobromae* and *N. occulatum* belong to the family Botryosphaeriaceae. All three classes belong to the division Ascomycetes and are therefore located in the central branch of the tree.

Pathogenicity tests

Based on the results of the pathogenicity test, isolates of *L. theobromae* and *N. oculatum* were responsible for the development of symptoms associated with peduncle scar rot disease and dieback in cvs Ataulfo, Criollo, Manila and Haden. In our study, *A. niger* caused infection on fruits of the cvs Ataulfo and Manila, and *C. asianum* was pathogenic for the cvs Haden and Criolle, with high percentages of incidence. The fungi *P. mangifereae* and *T. variabilis* did not cause symptoms in the three cvs and in Criolle (Table 4).

Figure 3 shows that *N. oculatum* induced damage on the fruit surface, with areas of 53.60 mm^2 in Ataulfo, 40.58 mm^2 in Manila, 37.51 mm^2 in Haden, and 7.74 mm^2 in Criolle. The tissue of these fruits turned dark brown at the point of inoculation, becoming lighter as the disease progressed, and abundant white mycelium and amber gutules developed on the skin. To the touch, the fruit felt firm, the colour of the pulp turned light brown, and a strong odour of ripe or rotten fruit was noticed. The fungus *L. theobromae* produced damage areas of 28.72 mm^2 for Ataulfo, 38.0 mm^2 for Manila, 95.11 mm^2 for Haden and 11.23 mm^2 for the Criolle on the surface of the fruits, with necrotic lesions on the entire surface with small yellow patches and concentric growth. Abundant white mycelium was observed at 10 days, along with the presence of small transparent gutules. The fungus *C. asianum* only caused infection on the cv Haden, with a damage area of 2.83 mm^2, and on Criolle, with an area of

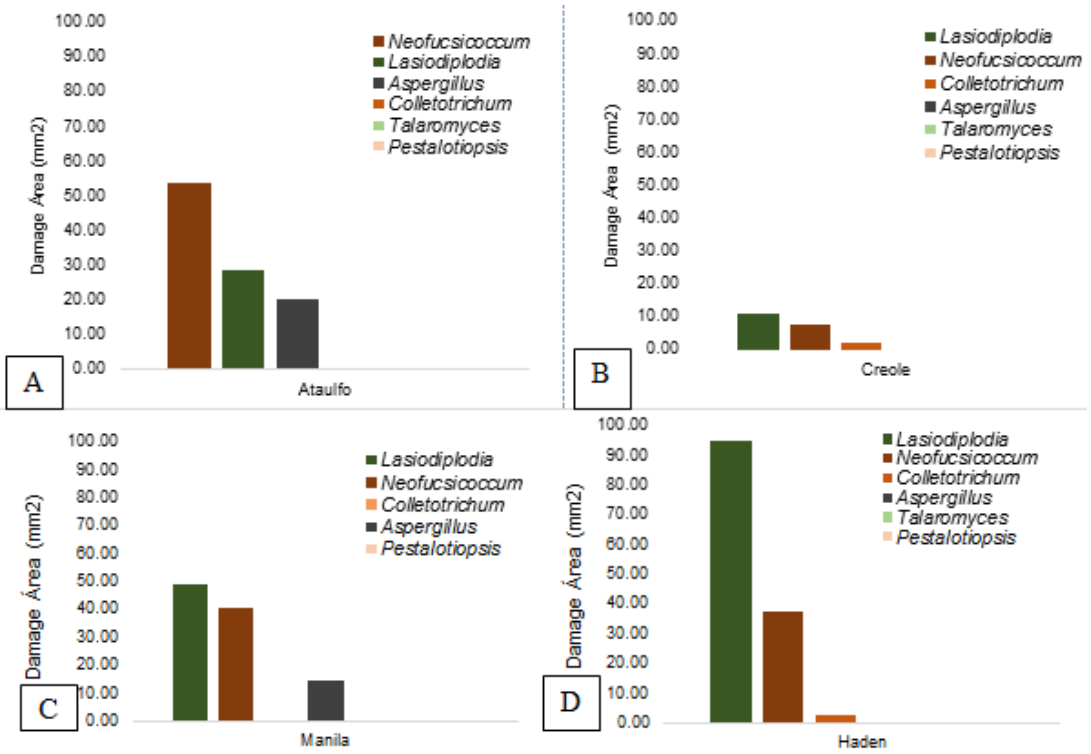

Figure 3. Damage area caused by fungi phytopathogenic in mango fruits. A) cv Ataulfo; B) Creolle; C) cv Manila; D) cv Haden.

2.09 mm^2. Necrotic and sunken lesions were observed at the inoculation site, with concentric growth. Signs such as grey or white mycelium growing in the wounds were observed. The species *A. niger* caused damage in an area of 19.75 mm^2 of the fruit surface of Ataulfo and of 14.75 mm^2 in Manila. The development of a light brown concentric sunken lesion and a darker halo was observed as the disease progressed. At the point of inoculation, white mycelium and abundant black spores were observed. Overall, we observed a high cultural, morphological and pathogenic variability among the isolates.

DISCUSSION

Several species of fungi associated with dieback in mango plantations in Gran Canaria have been reported, such as *Fusarium* spp., *Pestalotiopsis* spp., *Alternaria alternata*, *C. gloesporioides*, *Cladosporoium* spp., *Dothiorella* spp., *Penicillium* spp. and *Phomopsis* spp., with different incidence values depending on the sample type (leaf, stem or fruit), mango variety and collection season (Rodríguez et al., 2008). Santos-Mezones (2019) identified postharvest fungi from 486 fruits collected in a fruit packaging facility in Peru; the authors found two most frequent types of symptoms and identified the genera associated with these: *Alternaria* (50%), *Curvularia* (7%), *Colletotrichum* (6%), *Stemphylium* (6%), *Bipolaris* (4%), and *Pestalotia* (4%) from fruit spots and *Aspergillus* (54%), *Lasiodiplodia* (38%) and *Penicillium* (8%) from peduncular rot. These results are closely related to the findings of the present study, where most of the isolates from the brown spots on the peduncle were identified as *Lasiodiplodia, Alternaria, Aspergillus* and *Pestalotiopsis* and isolated directly from the progression of the disease *Colletotrichum* and *Neofusicoccum.* Another of the diseases of great importance in mango cultivation is "anthracnose", which has been reported in Asia and Latin America (Valenzuela et al., 2022). Anthracnose causes between 30 and 60% of losses in the field, and in some cases, up to 100% of the production is lost in humid conditions (Felipe et al., 2022). *C. asianum* may be the most important species in mango, described in Australia, Brasil, Florida (USA), Ghana, Mexico, the Philippines, South Africa and Thailand (Weir et al., 2012). In Mexico, anthracnose for varieties such as Ataulfo does not represent a serious postharvest problem compared to varieties from Florida, such as petacones (Tommy Atkins, Kent, Keiit, Haden). The latter explains the high percentage of occurrence for *Colletotrichum* in the cv Haden (19 isolates) and the absence for the cv Ataulfo. Krishnapillai and Wijeratnam (2014) were the first to report the presence of *C. asianum* in mango varieties in Sri Lanka; the pathogenicity tests carried out in varieties of petacones showed that the cvs Tommy Atkins and

Keiit were resistant to this fungus, whereas the Haden and Kent varieties were susceptible. Felipe et al. (2022), using molecular methods, reported a gene related to mango resistance to anthracnose. This was detected through the β-1,3-GLU2 gene, which synthesises the β-1,3-glucanase enzyme related to abiotic and biotic response processes in plants. The authors conclude that this gene is one of the first mango responses against *C. gloeosporioides* attack. Species of the genus *Pestalotiopsis* have been reported on mango trees in Italy (Ismail et al., 2013). *P. mangiferae* is a pathogen, that requires wounds to achieve infection and is often found as a saprophyte or associated with other diseases of the stem during postharvest storage. In addition, *P. glandicola* was reported as the causative agent of a postharvest disease of mango in Bangalore. In the present study, both *C. asianum* and *P. mangiferae* were genetically close in the phylogenetic analysis since both fungi belong to the same class, albeit to different families. However, there is not sufficient evidence for a resistance of mango cultivars to *P. mangiferae*.

Based on the results of the pathogenicity test, isolates of *L. theobromae* and *N. occulatum* produced symptoms on three cultivars and criolle. The species *L. theobromae* caused more damage to Ataulfo, Haden and Criolle on the surface of the fruits, whereas *N. occulatum* induced damage on the cvs Ataulfo, Manila and Haden. These fungi belong to the family Botryosphaeraceae, whose species are cosmopolitan, causing different diseases in different crops, such as dieback, trunk cancer and fruit rot being responsible for economic losses in fruit production; they have been reported in most mango producing areas in Asia, Africa and America. The *N. occulatum* isolate found in this research aligns with sequences reported in the NCBI database for *Neofusicoccum* isolates from mango in different areas in Mexico. Sandoval-Sánchez et al. (2013) isolated *N. parvum* from the mango cv. Ataulfo. In Egypt, *Lasiodiplodia* is considered to be the main causal agent of fruit deterioration, stem deterioration, panicle brown rot and stem dieback (Abdalla et al., 2003). Coutinho et al. (2017) identified the genus *Lasiodiplodia* through symptoms and pathogenicity in its different hosts. Taxonomic characteristics such as conidia, colony morphology, changes in coloration due to the effect of temperature, as well as changes in growth rate and molecular markers such as ITS, genes such as β-tubulin and elongation factor-α allowed the identification of more species that affect mango cultivation, emphasising that for the family Botryosphaeriacea, the use of several markers is necessary to separate the species of this group.

A. niger has been reported in fruits imported from Puerto Rico and Venezuela to England (Snowdon, 1991) placing “black rot” as an important disease in mango cultivation. Together with fungi of the genus *Penicillium* (*Talaromyces teleomorph*), they are saprophytic species and of particular importance when the fruits have a previous infection and a high degree of deterioration. The cultivars susceptible to artificial inoculation of *A. niger* were Ataulfo and Manila. As discussed by Yilmaz et al. (2014), the genus *Penicillium* is a monophyletic group with an asexual subgenus called *Biverticillium* and a sexual one called *Talaromyces*. However, Samson et al. (2011) recombined the subgenus *Biverticillium* within the genus *Talaromyces*, which allowed this group to have species that reproduce sexually and asexually, which is why the genus *Talaromyces* is reported in this research, isolated and identified in the first instance as the genus *Penicillium*. Together with *A. niger*, they are within the family Trichocomceae, with a lower genetic distance in the phylogenetic tree with respect to the remaining fungi identified.

In this work it is shown that diversity of fungal species is not only subject to the fungi that cause the symptoms, but also associated with a greater number of fungal species that are in a cryptic form and are only expressed when the physiology of the fruits changes. Also the chemical composition of each fruit is different, the cvs ataulfo presents a high variety of antifungal compounds in peel and pulp (Istúriz et al., 2022) and was more resistant to the fungi evaluated. Disease management in mango is challenging since several species or subspecies of the same genus converge with differences in terms of morphology and pathogenicity, so it is recommended to carry out the taxonomic identification of the isolates in addition to implementing the use of resistant varieties. Accordingly, such information is relevant because it can assist in the implementation of disease control measures more effectively.

Conclusions

In this study, we determined the pathogenicity and incidence of six postharvest phytopathogens on the most important commercial mango cultivars in Mexico. The greatest affectation corresponds to Haden, Manila, Creole and cv. "Ataulfo" being the most resistant, most likely because it is an endemic cultivar. However, all cultivars were susceptible to fungi of the family Botryosphareaceae and differed in their susceptibility to *A. niger* (Ataulfo and Manila) and to *C. asianum* (Criollo and Haden). We assume that the resistance or susceptibility of cultivars depends on their origin and genetics.

CONFLICT OF INTERESTS

The authors have not declared any of conflict of interests.

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