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Natural resistance of two mango *Mangifera indica* L. commercial cultivars to anthracnose caused by *Colletotrichum gloeosporioides* Penz. Penz. & Sacc

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Abstract

Mexico ranks first worldwide as the largest mango exporter is one of the more produced tropical fruits worldwide. The need for strategies for the protection of crops, environment and people leads us to investigate disease control methods. The use of resistant cultivars is the most important. In addition, it is advantageous for the producer because it will reduce the cost for disease control. The objective of this research was to evaluate the natural resistance of two mango cultivars against anthracnose, using different spore doses and times of evaluation. The sampling was performed on April 15th, 2018 at an open market placed south of Saltillo, Coahuila, Mexico. Pathogen was isolated from fruits of Tommy Atkins and Ataulfo mango cultivars showing advanced degrees of black spots collected out from at open markets in Saltillo, Coahuila, Mexico. The pathogen purification was done by monoconidial cultures and identification was done with morphocultural criteria. Spore suspension was prepared and inoculated on ripe mango fruits using different doses and disease severity was evaluated 6 and 10 days after inoculation using millimetric sheets. A complete random factorial design of three factors was used, where: factor A stands for mango cultivars (Tommy Atkins and Ataulfo), factor B is treatments and factor C stands for times of evaluation. The results are shown as percentage and data was analyzed with factorial analysis using the SAS® 9.1 software. *C. gloeosporioides* was identified in mango cultivars; with hyaline conidia of 16.90 to 25.12 µm length and 4.33 to 5.18 µm width. The results show cultivars resistant to anthracnose in the study area. Natural resistance against anthracnose was as 80.00 to 93.67 %, thus, mango cultivars showed a certain resistant degree.

Keywords: Doses**,** incidence, severity, % of damage, crop.

Introduction

Mango *Mangifera indica* L. is one of the more produced tropical fruits worldwide. It is also known as the 'King of fruits' (Purnachandra and Saritha, 2013) belonging to the Anacardiaceae family (Nawab et al., 2017). *Colletotrichum* Corda corresponds to genus of phytopathogenic fungi that causes diseases and mainly anthracnose in different hosts. The genus *Colletotrichum* consists of approximately 600 species including most studied species like *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., *Colletotrichum capsici* (Schweinitz) Andrus & W. D. Moore, *Colletotrichum acutatum* J.H. Simmonds, *Colletotrichum dematium* (Persoon) Grove, *Colletotrichum nigrum* Ellis & Halsted and *Colletotrichum coccodes* (Wallroth) S. J. Hughes (Glomerellaceae). The species of this genus infect more than 3200 species of monocot and dicot plants (O'Connell et al., 2012).

Anthracnose caused by these fungal pathogens can negatively impact both yield and fruit quality in post-harvest (Sutton, 1980; Arauz, 2000). This disease is more serious in all mango growing regions of the world (Smooth and Segall, 1963; Tandon and Singh, 1968; Muirhead and Grattidge, 1984; Johnson et al., 1989). The anthracnose caused by *C. gloeosporioides* reduces the shelf life of mature fruit (Wu et al., 2020; Ahmad et al., 2021). This pathogen belongs to the Family: Glomerellaceae; Order: Glomerellales, Class: Sordariomycetes, Phylum: Ascomycota and Kingdom: Fungi (Chen et al., 2015). Also, it manifests itself as dark brown or black spots, which grow and form larger and more cleft ones (Sangeetha and Rawal, 2008). One species of this fungi is *Glomerella cingulate* (Stoneman) Spauld. & H. Schrenk (Glomerellaceae), (López-Vásquez and Castaño-Zapata, 2010), which causes the inflorescence to fall, affecting the fruits, leaves and young branches (Álvarez et al., 2006), causing direct and indirect losses (Jeffries et al., 1990; Ploetz, 1994; Prusky et al., 2000).

Production of mango *M. indica*, mangosteens *Garcinia mangostana* L. (Clusiaceae), and guava *Psidium guajava* L. (Myrtaceae) during the 2018 cycle was 55,383,785 t worldwide and 2,178,927 t in Mexico. Paez (2003) and Bustamante (2006) showed that losses by anthracnose in

papaya *Carica papaya* L. (Caricaceae) and mango ranges between 15 to 50% up to 60% (Vega-Piña, 2006; León, 2007). If that 50% loss during that cycle is taken, 1,089,463.5 t was lost in Mexico and 27,691,892.5 t worldwide. The main growers are India, China and Thailand (FAOSTAT, 2020). The control of *Colletotrichum* spp. represents a challenge for the farmers, where the preventive protection strategy and the time factor are essential elements given the short incubation period of the fungi and its high sporulation capacity in young tissues (Abd-Alla and Wafaa, 2010). *Colletotrichum* is a genus of fungi which is a cosmopolitan distribution and predominantly in tropical and subtropical regions (Xiao et al., 2004; Cannon, et al., 2012; Lei, et al., 2016). It was recently voted as the eighth more important group of plant pathogenic fungi in the world, based on perceived scientific and economic importance (Dean et al., 2012).

Identification of *Colletotrichum* spp. is essential for effective execution strategy as well as genetic improvement programs aiming to manage and control the disease (Than et al., 2008). There is a variation regarding plant susceptibility to either *Colletotrichum* or *Gloeosporium* Desmazières & Montagne (Dermateaceae) and strains of these microorganisms possess different pathogenicity to a given plant (Agrios, 2005). The objective of this research was to evaluate the natural resistance of two cultivars of mango against anthracnose, using different spore doses and times of evaluation.

Results and discussion

Conidia hyaline and cylindric shape with an average length of 16.90 to 25.12 µm and width of 4.33 to 5.18 µm were found in a suspension of 2.55×10^{7} spores/mL^{-1}; colonies were white and the mycelium hadving at least 4 mm height (Table 1). The pathogen possessed acervuli with mushrooms and septate hyphae, distinctive characteristics of *C. gloeosporioides,* similar to those reported by Wharton and Diéguez-Uribeondo, (2004), Tozze et al. (2006), Andrade et al. (2007), Pérez et al. (2003), Muñoz et al. (2003), Montero et al. (2010), Barquero et al. (2013), Trinidad-Ángel et al. (2017). Statistical analysis showed significant difference among factors; cultivars mango (Factor A, p= 0.0012), treatments (Factor B, p= 0.000) and times (Factor C, p= 0.000) with a variance coefficient of 21.35, as well as factor interaction AXBXC (p= 0.001).

Tommy Atkins showed a disease severity of 7.33 to 20.00%, whereas in Ataulfo it was 6.33 to 14.33% (Table 2, Figure 2). The natural resistance of Tommy Atkins was about 80 to 93.67% and Ataulfo resistance was 82.67 to 85.67%, where the most severe damage was observed using 3000 µL of spore suspension evaluated days 10 after treatment. Ploetz (2008) mentioned that Tommy Atkins variety isresistant to anthracnose, into a certain degree. Using fungicide has a direct control effect, but it is likely to induce resistance in the fungi (Hu et al., 2015). Nowadays, the increase in development of resistance to synthetic fungicides, as well as the growing consumer demand for food without pesticides stresses the need for alternative strategies to control phytopathogens (Zhou et al., 2003).

Almada-Ruiz et al. *(*2003) mentioned that the benomyl in combination with multisite non-mobile protectant fungicides, e.g; mancozeb or copper hydroxide, is currently one of the primary strategies used to control fruit anthracnose preharvest. However, in some cases, their continued use has led to the development of resistance (Gutiérrez-Alonso et al., 2003). For instance, chemical control of anthracnose requires biweekly or monthly application of fungicides, which could be damaging to the environment, and frequent use of chemical fungicides could lead to the development of fungicide-resistant strains (Onyeka et al., 2006). Ningthoujam et al. (2009) mentioned that the chemical practices to overcome plant disease problem have adverse environmental effects on non-target organisms, causing health hazards to humans, besides demanding high costs. The compulsive use of synthetic fungicides has caused different types of environmental and toxicological problems, such as environmental pollution and health-illness. Therefore, there is a crucial need to find out a sustainable and eco-friendly alternative to address concerned issues (Chung et al., 2006). The integration of a number of practices will reduce or eliminate negative side effects of chemicals being used to control major mango diseases. This is is the most realistic option for solving the problem (Chowdhury and Rahim, 2010). However, the genetic resistance is the only economically viable method of controlling the disease and current research efforts emphasized the search for resistant cultivars (Mignouna et al., 2001). Moreover, sources of inoculum can be avoided by not planting other host plants of postharvest pathogens near to mango crops. This practice eliminates other sources of inoculum such as diseased twigs and diseased fruits (Fittzell and Peak, 1986; Sangchote, 1991).

Low damage severity in mangoes is probably due to the phenological stage of cultivars and ripe mangoes which were used in this study. Villanueva et al. (2006) mentioned that immature fruit of custard apple *Annona cherimola* Miller (Annonaceae) were more susceptible to *Colletotrichum sp*. Padron (1991) showed that during the immature stage, mango fruits highly increase its respiratory rate, rendering them more susceptible to disease since most of its strength is channeled to maturation. On the other hand, Bailey and Jeger (1992) reported a high genetic variation among isolates of *C. gloeosporioides* from avocado *Persea Americana* Miller (Lauraceae), *C. papaya*, plantain *Musa x paradisiaca* L. (Musaceae) and mango. Zainuri et al. (2001) demonstrated that low severity of anthracnose caused by *C. gloeosporioides* in post-harvest mango was due to the presence of salicylic acid, having an average of 0.1 – 0.25 mg of salicylates every 100 g of mango (Ngw, 2020).

Valdés et al. (2017) demonstrated that mango cultivars Haden, Bizcochuelo, Delicioso, San Diego, Señora and Macho are susceptible to isolates of *C. gloeosporioides* a 1×10^{5} spores/mL^{-1} suspension, whereas in this research a 2.55×10^{7} spores/mL^{-1} suspension was used, which is a difference of 2.54×10^{7} spores/mL^{-1}. On the other hand, Orlando et al. (2013) showed different isolates of *C. gloeosporioides* at 1×10^{6} spores/mL^{-1} in passion fruit *Passiflora edulis* Sims (Passifloraceae) crops at 40 days old in the greenhouse reached the maximum disease severity (70%) in only 30 days, thus, presented a natural resistance of 30%.

Materials and methods

Sampling

The sampling was performed on April 15th, 2018 at an open market placed south on Saltillo(Figure 1). Mangoes of Tommy Atkins and Ataulfo cultivars with advanced maturity showing black spots collected out from at open markets in Saltillo, Coahuila, Mexico. Showing the

Table 1. Conidia means corresponding to *C. gloeosporioides.*

Length (µm)	Mean	SD
16.90 to 25.12	21.8171	3.14106985
Width (µm)	Mean	SD
4.33 to 5.18	3.14106985	0.33002191

SD= Standard deviation.

Fig 1. Location of the study area, S=Saltillo, Coahuila, Mexico.

Table 2. Evaluation times of severity of *C. gloeosporioides* in cultivars mango.

	TE 6			TE 10		
Cultivar	Trat.	Sev. (%)	SD	Trat.	Sev. (%)	SD
Tomy Atkins	1	5.66	0.577350269	1	10.66	1.154700538
	2	7.33**	1.154700538	2	11.33	1.154700538
	3	4.66	4.163331999	3	20.00**	2.00000000
	4	0.00	0.000000000	4	0.00	0.00000000
Ataulfo	1	4.34	0.577350269	1	8.66	1.154700538
	2	5.33	1.527525232	2	11.66	1.527525232
	3	6.33**	1.154700538	3	14.33**	1.154700538
	4	0.00	0.000000000	4	0.00	0.000000000

TE 6= Evaluation time at six days; TE 10= Evaluation time at 10 days; Trat.= Treatments, 1= 2.55×10^{7} spores/mL^{-1} (1000 µL),2= 2.55×10^{7} spores/mL^{-1} (2000 µL), 3= 2.55×10^{7} spores/mL^{-1} (3000 µL) and 4= Control (sterile water); Sev. (%)= % de severity; SD= Standard deviation. **= Treatments with higher severity

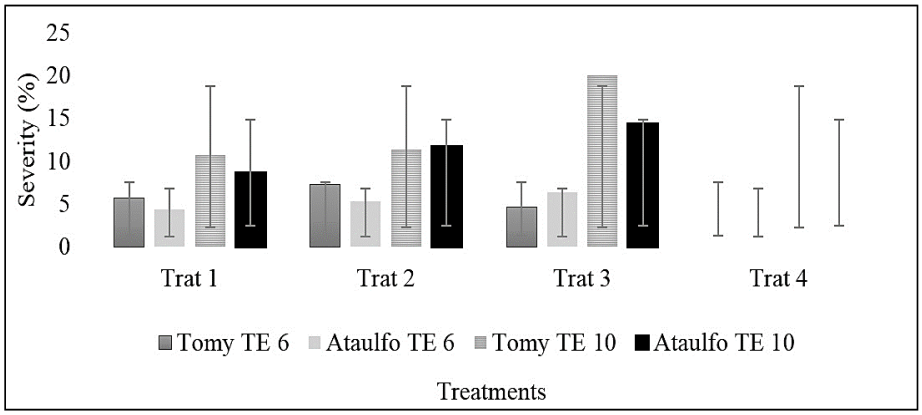

Fig 2. Severity of *C. gloeosporioides* in cultivars mango. Trat 1= 2.55×10^{7} spores/mL^{-1} (1000 µL), Trat 2= 2.55×10^{7} spores/mL^{-1} (2000 µL), Trat 3= 2.55×10^{7} spores/mL^{-1} (3000 µL) and Trat 4= Control (sterile water), TE 6= Evaluation time at six days; TE 10= Evaluation time at 10 days.

characteristic "sunken black spots" of anthracnose were selected.

Isolation and purification of the pathogen

Three cuts of approximately 0.5 cm in length and 0.3 cm width were performed on healthy and sick tissue of mangoes. Subsequently, the samples were washed with water and liquid soap to remove any possible pesticide residue, and disinfected with 3% hypochlorite solution for 1 min, 90% alcohol for 1 min and washed with distilled water. Tissue was left to dry on sterile absorbent paper at room temperature (25 ± 2 °C) for 3 min. Next, the sample was transferred to a sterile hood and 3 cuts were equidistantly placed in a Petri dish with PDA + antibiotic (Gentamicin, 1 mL L^{-1}), with 10 replicates. Petri dishes were sealed, labeled, kept at 25°- 30 °C for 168 h. Purification of the isolates was performed by monoconidial cultures in PDA (Bioxon + antibiotic), which then were preserved at 4 to 6 °C.

Morphocultural identification of the pathogen

Identification was performed with a microscope using the DinoCapture 2.0 software (Dino-lite, 2020). Based on colony characteristics (color and shape), conidia (color, length, and width; 100 conidia were analyzed) with the taxonomic keys

from Barnett and Hunter (2006) to identify genus and the references of Sutton (1992) for species.

Inoculum acquisition and conservation using suspension in sterile distilled water

An explant with 0.5 cm diameter was taken and placed in a previously sterilized mortar containing 20 mL of sterile distilled water. Subsequently, the sample was macerated, and spores were counted from the suspension using a Neubauer chamber.

Pathogenicity tests

Healthy fruits were inoculated by puncturing and sprinkle a given spore suspension (i.e., treatments) using a dropper (1000, 2000 and 3000 µL); with a control (sterile water), using 10 replicates per treatment and mango cultivar with 5 maturity degrees.

Evaluation of severity (%)

Percent damage severity caused by *C. gloeosporioides* in mangoes was examined at day 6 and 10 after treatment using a millimetric sheet.

Experimental design

A complete random factorial design of three factors was used, where: factor A stands for mango cultivars (Tommy Atkins and Ataulfo), factor B is treatments and factor C stands for times of evaluation (at day 6 and 10). Data was analyzed by Turkey test to 0.01 of significance (α=0.01), using the statistical software SAS® 9.1 (SAS 2002; versión 9.1, SAS Institute, Cary, North Carolina, USA).

Conclusion

Mango cultivars Ataulfo and Tommy Atkins with 5 maturity degrees showed a natural resistance from 80.00 to 93.67% to anthracnose (*C. gloeosporioides*). They are considered to a certain extent as resistant. The need for strategies for the protection of crops, environment and people leads us to investigate disease control methods. The use of resistant cultivars is the main one. In addition, it is advantageous for the producer because the cost for disease control it would be less.

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