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Conservation

Characterization morphological, cultural and pathogenic of isolated *colletotrichum sp.* anthracnose producing in mango (*mangifera indica* L.).

Caracterización morfológica, cultural y patogénica de aislados de *colletotrichum sp.* produciendo antracnosis en mango (*mangifera indica* L.).

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Abstract

Anthracnose is considered the main fungal disease affects mango. Cause considerable damage to leave, flowers and fruits. The filamentous fungi of the genus *Colletotrichum* and its teleomorph *Glomerella* pathogens are considered the most widely distributed plants worldwide, causing this disease. In this investigation were obtained 22 isolates of *Colletotrichum* from different localities of the country western region, in six varieties of mango. Cultural characterization was varied in the growth of colonies on PDA, with presence and absence of exudation at different temperatures, 24 degrees being optimal for growth. The observed in isolated sexual stage shows few perithecia. Conidia concentration ranged between 107 and 105 measured Neubauer chamber while appresoria and conidia are within the ranges for the species *Colletotrichum gloeosporioides*. Variability in pathogenicity showed that all species investigated wizards are susceptible to microorganism. The statistical analysis shows the grouping of isolates by locality with similar pathogenic characteristics.

Keywords: *Glomerella cingulata*, anthracnose, appressoria, conidia pathogenicity.

Resumen

La antracnosis es considerada la principal enfermedad fúngica que afecta al mango. Causa considerables daños en las hojas, las flores y los frutos. Los hongos filamentosos del género *Colletotrichum* y su teleomorfo *Glomerella* son considerados los patógenos de plantas más ampliamente distribuidos a nivel mundial, causantes de esta enfermedad. En esta investigación se obtuvieron 22 aislados del género *Colletotrichum* de diferentes localidades de la región occidental país, en seis variedades de mango. La caracterización cultural fue diversa en el crecimiento de colonias en PDA, con presencia y ausencia de exudaciones a diferentes temperaturas, siendo los 24 grados la óptima para el crecimiento. La fase sexual observada en algunos aislados muestra pocos peritecios. La concentración de conidios varió entre 107 y 105 medidos Cámara de Neubauer mientras los apresorios y conidios están dentro de los rangos para la especie *Colletotrichum gloeosporiodes*. La variabilidad en la patogenicidad mostró que todas las especies de mangos investigadas son susceptibles al microorganismo. El análisis estadístico arroja el agrupamiento de los aislados por localidad con características patogénicas similares.

Palabras claves: *Glomerella cingulata*, antracnosis, apresorios, conidios, patogenicidad.

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1 Introduction

Mango (Mangifera indica L.) is a crop well adapted to the conditions of Cuba (Capote et al., 1989; Valdés et al., 2015). Anthracnose is the main disease that attacks this crop in the country. It is caused by the fungus Colletotrichum gloeosporioides (Penz) Penz & Sacc. Glomerella cingulata, which causes the fall of the inflorescence, affects the fruits, leaves and young branches (Álvarez et al., 2006). It also appears as a post-harvest disease of ripe fruits during storage. As a consequence, poor harvests are obtained with low quality fruits (Rebouca, 2002; Bruwer et al., 2006), which leads to a decrease in the price of the fruit, both in the domestic market and in the international market (Arauz, 2000; Rodríguez et al., 2002).

The control of diseases caused by the fungus represents a challenge for the farmer, where the preventive protection strategy and the time factor are indispensable elements given the short incubation period of the fungus and its high sporulation capacity in young tissues (Infoagro, 2006). , Abd-Alla and Wafaa, 2010). Therefore, it is necessary to integrate management measures such as the use of varieties with a certain degree of tolerance or resistance (Carrillo et al., 2005).

At times, implementing control measures is difficult due to the convergence in the same host of several species or subspecies of fungi of the genus Colletotrichum and the great variety of morphological forms of these in relation to the environmental variations (Santiago et al., 2005; Copping, 2000). In order to solve this situation the studies have been focused, first towards the taxonomic identification of species and secondly to the characterization of sub-populations of each species. Which is essential for the implementation of effective control strategies (Santiago et al., 2005, Jayasinghe and Fernando, 2009).

The present work aimed to characterize morphological, cultural and pathogenic strains of Colletotrichum sp. that coexist in mango plantations in several of the western provinces of Cuba.

2 Materials and method

Collection of isolates: Vegetable samples with anthracnose symptoms from the municipality of Alquízar, province of Havana, and from private plots of the municipalities Escambray and Jaruco, located in the provinces of Cienfuegos and La Habana, were collected. The affected portions were washed with tap water, disinfected with 1% sodium hypochlorite for 1 min and seeded in Petri dishes with Potato-Dextrose-Agar (PDA) culture medium, incubated at 30 ° C until mycelial growth . From these, monosporic cultures were grown at 30 ° C in the dark. Once grown, they were stored in the test tubes at 4 ° C.

Cultural characterization of the isolates: The isolates of the strain were planted in Petri dishes with PDA and incubated at 27 ± 2oC, for 7 days. Once the colonies were grown, discs were taken with punches of 5 mm diameter and placed in the center of Petri dishes with PDA, 11 cm in diameter. Three replicates were evaluated per isolate at temperatures of 20, 24, 27 and 30 oC for 7 days. After this time the observation of the cultural characteristics and measurement of the colonies was made following the methodology of Freeman et al. (1998). Data on the diameter of the colony were analyzed by bifactorial variance with two treatments and three replicates per treatment. The averages were compared by Duncan's Multiple Rank Test (Cigarroa, 1985).

Observation of the sexual phase: The isolates of the strain were planted in boxes of 11 cm in diameter with PDA and incubated at 30 ° C under a cycle of 12 light hours and 12 hours of darkness. From these boxes weekly observations were made, for 2 months, to determine the formation of perithecia and ascospores of the sexual phase (Glomerella cingulata).

Determination of conidial concentration: To evaluate conidia concentration per isolate, suspensions were prepared by adding 5 mycelial discs, obtained from colonies of 15 days incubation at 30 ° C, in 10 ml of sterile distilled water as described above. From these the concentration in Neubauer's Chamber was determined. The obtained data was analyzed by simple classification variance (ANOVA) and the means were compared by Duncan's Multiple Range Test (Cigarroa, 1985).

2.1 Morphometric characterization of the isolates

Conidia morphometry: From colonies with 7 days of incubation at 30 °C, 5 disks of 5 mm diameter were taken per isolate and were added in 10 ml of sterile distilled water, placed in a vortex for 1 min to promote the release of conidia. The concentration of the

suspensions was adjusted to 1 x 105 conidia.ml^{-1}. From these preparations were made and 50 conidia were measured per isolate, their shape being described according to (Gutiérrez et al., 2001). This was done with the help of the Olympus optical microscope with a magnification of 100x.

Aperture morphometry: Slides coated with a thin film of Agar-Water were prepared and 50 μl of suspensions adjusted to 1 x 105 conidia.ml^{-1} were added. Three replicates were performed per isolate. The slides were incubated at room temperature for 24 hours, after which they were observed under the Olympus optical microscope, with magnifiers of 40 and 100x, measuring and describing 50 appressions for each isolate, according to Gutiérrez et al., 2001.

Pathogenicity test: The infectious capacity of the isolates was evaluated in leaves of mango plants of the Haden, Filipino, Señora and Keitt varieties, from the mango collection of the Experimental Station of Fruits in the municipality of Alquízar, Artemisa province. Ninety young leaves of each variety were collected. These were washed with water, detergent and 70% alcohol to remove dirt and disinfected respectively. They were then placed in petri dishes containing slides and filter paper moistened with sterile water.

Three replicates and one control were mounted for each variety and each isolate, making 6 incisions with a needle in each leaf. As inoculum 10 μl of a dilution of 1 x 105 conidia.ml^{-1} was used for each isolate. In the case of the controls, 10 μl of sterile distilled water was applied instead of the suspension. The boxes were incubated at room temperature and observed daily for 5 days, following the appearance of symptoms and measuring the lesion diameter (strain virulence). The diameter data of the lesion by isolate and variety were processed using the transformation (x + 1) and analyzed following a trifactorial variance analysis. The means were compared by Duncan's Multiple Range Test (Cigarroa, 1985).

Statistical analysis for clustering of isolates: A statistical analysis of main components (PCA) was performed with the obtained results to determine the variable that contributed the most to diversity. A clustering analysis was performed with the three variables that most contribute using the Euclidean distance, based on the unweighted arithmetic mean and the SAHN algorithm of the NTSys version 2.1 program package.

3 Results and discussion

3.1 Isolates acquiring

According to their origin and the mango variety with which it was worked, 22 fungal isolates were found that are listed in Table 1.

3.2 Cultural characterization of the isolates

In general for the temperatures of 20, 24, 27 and 30 °C the growth was always circular and with regular edges except for the isolates 9 and 20 that presented irregular borders. Colony colorations varied between white (Figure 1: D and E), greenish gray (Figure 1: A and B) and pale gray (Figure 1: C and F).

Mostly growth sectors were found (Figure 1: A, C and D) and in very few concentric growth halos apeared (Figure 1: B and E). Both types of zoning were also commonly observed on the backs of the colonies and in the case of isolates 4, 5, 11, 13 and 17 the sectors formed a star. Different types of textures were observed in the colonies: (Figure 1: F), woolly (Figure 1: A and B), cottony (Figure 1: C and D) and plush (Figure 1: E) presenting texture differences at the margin of the colony (Figure 1: A). Only 10, 13, 14 and 15 isolates had salmon pink pigments. The presence of exudations on the mycelium manifested itself variably between the isolates at different temperatures and therefore cannot be classified as a distinctive factor of a strain or temperature. These results coincide with those reported by Sangeetha and Rawal in 2008 who obtained isolates with similar characteristics. The temperature of 27 °C favored sporulation compared to the others. The conidial mass presented mainly as orange, viscous exudations that developed from mature acervuli without setae; In agreement with Arauz (2000) and Gutiérrez et al. (2001).

3.3 Observation of the sexual phase

In general, in the isolates that formed sexual phase were observed few perithecia and most of them were immature. Only isolates 2, 3 and 5 presented black and erumpent color in the middle of the culture.

The morphology and dimension of the ascospores allows establishing that the peritecial strains be-

Table 1. Relationship of Colletotrichum sp. Obtained according to their location and variety of mango plants

Isolate	Origin	Variety	Observed symptomatology
1 2 3 4 5	Jaruco	Haden	Young leaves with spots of irregular shape, ash-brown color at the tip and margin, with the appearance of burns. Formation in the back of the patches of yellowish excrescences in conditions of high humidity.
6 7 8	Alquízar	Bizcochuelo	Irregular brown spots from the apex to the base of the leaf.
9 10 11 12 13		Delicioso	Angular spots from dark brown to light brown, from the edge towards the center of the leaf without reaching the nerves.
14 15		San Diego	Circular stains of more or less regular form, Carmelite-ash color in the center and a dark Carmelite fringe.
16	Jaruco	Haden	Young leaves with spots of irregular shape, ash-brown color at the tip and margin, with the appearance of burns. Formation in the back of the patches of yellowish excrescences in amarillentas en condiciones de alta humedad.
17 18	Alquízar	Delicioso	Angular spots from dark brown to light brown, from the edge towards the center of the leaf without reaching the nerves.
19	Alquízar	Señora	Large, regular gray ash stains on the tip and margin of new leaves of the vegetative sprout.
20	Escambray	Mango macho	Brown spots on the fruit with crevices in the center.
21	Alquízar	Señora	Small, circular patches of diameter 3 to 4 mm with a dark brown stripe on the edge and yellow center on new buds.
22			Circular spots 5 to 6 mm in diameter in new leaves, brown with a darker center.

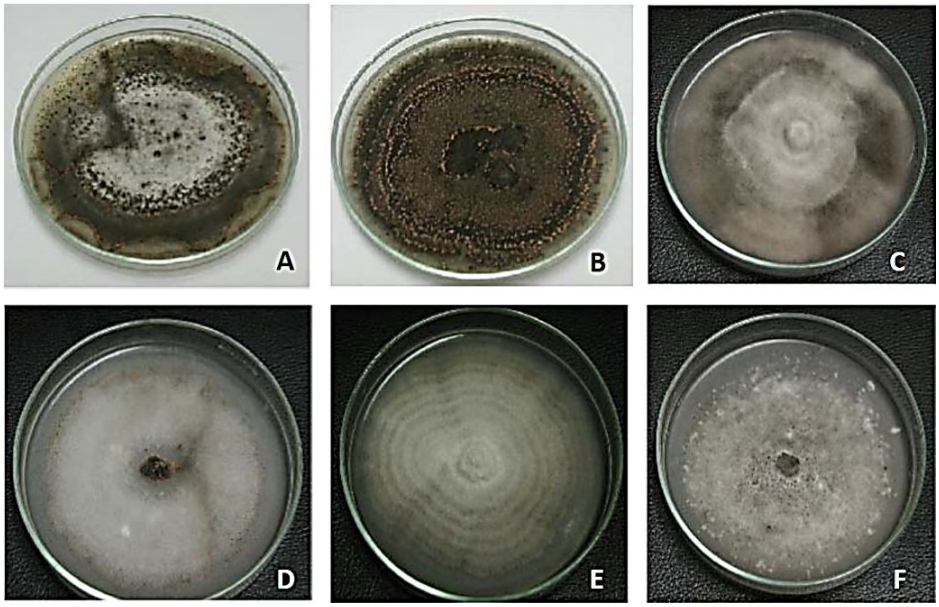

Figure 1. Different types of growth of colonies of isolates in Petri dish with PDA at 27 ± 2 °C, after 7 days incubation during study

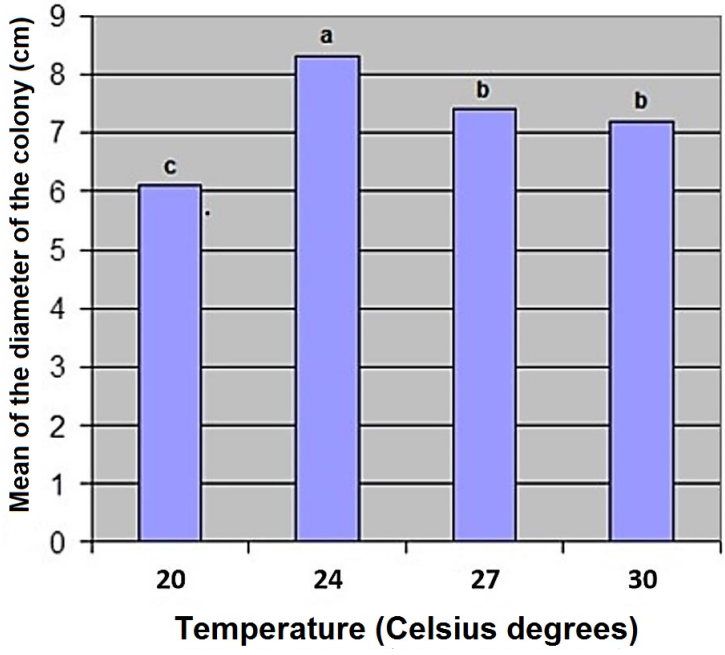

Figure 2. Mean of microbial growth measured in centimeters according to the different temperatures in PDA culture medium (CV: 0.61, S: 0.0443)

long to the species Glomrella cingulata (Stonem) according to published by Freeman et al. (1998); Gutiérrez et al. (2001) and Carrillo et al. (2005). In the isolates 2 and 3, mature broilers were observed with ascospores of 12-20 x 5-6 µm, hyaline, rounded, multivacuolated and curved (Cerón et al., 2007).

3.4 Determination of conidial concentration

The results of the conidia count in the Neubauer chamber are shown in Table 2. Significant differences ($P < 0.05$) were found between the isolates in the number of produced conidia. In all cases the concentration of conidia was found to be above 1 x 105 conidia.ml^{-1} coinciding with that reported by Sanders et al., 2000 and Sangeetha and Rawal (2008). The isolates 4 and 5 stand out with concentrations of the order of 107 conidia.ml^{-1}.

3.5 Morphometric characterization of the isolates

3.5.1 Morphometry of conidia

The isolates formed hyaline conidia, cylindrical and straight. Their length varied between 15.7 and 11.1 µm and their width between 3.0 and 4.5 µm (Table 3). Most had both rounded ends although a considerable percentage had at least one tapered end which coincides with that found by Sanders et al. (2000) and Sangeetha and Rawal (2008). All conidia presented abundant spherical bodies as published by Kuo (1999). In isolates 6, 8, 13 and 19 between 1 and 3% of the conidia were spindle-shaped.

3.6 Morphometry of appressors

There were lobular or irregular appendages, nails and ovoid, dark brown. The irregular shape predominated over the rest as shown in Table 3. In all cases, terminal appressors were observed in germinative tubes with sizes in a range of 9.7-14.5 x 5.5-10.1 µm. This coincides with Páez (1996); Arauz (2000) and Sangeetha and Rawal (2008) who observed lobed, dark brown appressors that could be terminal in germinative tubes or directly out of conidia.

These characteristics coincide with the information recorded in the description of the species Colletotrichum gloeosporiodes (Penz) Penz & Sacc by Sutton (1980), who defines that it presents straight, cylindrical conidia of 9.0-24.1 x 3.0-6.7 µm and appressors of 6 -21 x 4-12.7 µm, nailed or irregular. On the other hand, Carrillo et al. (2005) suggest that most Colletotrichum gloeosporioides isolates form conidia with rounded iota and less than 10% with tapered iota.

3.7 Pathogenicity test

The in vitro study of the virulence of the 22 isolates showed that the experimental infections were positive, examples illustrated in Figure 3. The leaves showed brown spots, with regular shape, they gradually increased and in some cases completelly covered the organ of the plant during the study period. At the end of the 5 days, there were emerging mycelial growths in the lesions and acervuli appeared with abundant conidia production immersed in chromogenic masses, mostly orange and salmon only in the case of the isolate 21. In no case were perithecia typical of the Species Glomrella cingulata (Stonem) Spauld & Schrenk. The materials used as controls remained always free of infection.

The lesions with the highest growth rates for all varieties were those caused by strains 21, 19, 3, 2, 17 and 5 in that order, respectively. Within these are both peritalecial strains and only conidia producers, contrasting with the results obtained by Gutiérrez et al. (2001) who argue that lesions caused by the peritoneal strain, grow faster than those caused by the conidial.

Significant differences ($P < 0.05$) were observed among the varieties (Figure 4). The Filipino variety was the most affected, contradicting Cañizares (1966) and Capote et al. (1989) who include it among the most resistant to anthracnose. These differences with respect to the other varieties may be due to the fact that the Filipino mango variety is considered by the farmers as an early variety, that their maturation period begins in the month of April, perhaps this low susceptibility is referred to not a genetic resistance, but that the fruits are produced and mature early, outside the rainy season in which the infection by the pathogen is favored, therefore, they do not contradict the obtained results. The Señora variety showed a high susceptibility to the pathogen coinciding with Capote et al. (1989).

Among the Haden and Keitt varieties there was no significant difference. Both presented ïn vitro"low level of susceptibility. These results coincide with what was proposed by Capote et al. (1989), for

Figure 3. In vitro tests of the pathogenicity of the 22 isolates and their corresponding controls on leaves of different varieties of mangoes. (A-isolated 21 Philippine variety, B-isolated 7 variety Lady, C-isolated 13 Philippine variety, D-isolated 10 variety Keitt, E-isolated 12 Haden variety; H-isolated 6 Keitt variety).

Table 2. Measurement of conidia concentration per ml obtained in Neubauer chamber from colonies of 15 days of incubation at 30°C ± 2°C in PDA culture medium with statistical analysis of P <0.05

Isolates	Amount of Conidia per ml
5	1.47 x 107 a
4	1.14 x 107 a
17	7.75 x 106 b
19	7.35 x 106 b
11	5.00 x 106 b
22	3.70 x 106 b
21	3.60 x 106 b
2	2.45 x 106 b
1	1.75 x 106 b
3	1.70 x 106 b
7	1.35 x 106 b
20	1.30 x 106 b
9	9.00 x 105 c
16	9.00 x 105 c
8	7.50 x 105 c
13	7.00 x 105 c
6	6.00 x 105 c
15	5.00 x 105 c
18	5.00 x 105 c
10	4.00 x 105 c
14	3.00 x 105 c
12	2.00 x 105 c

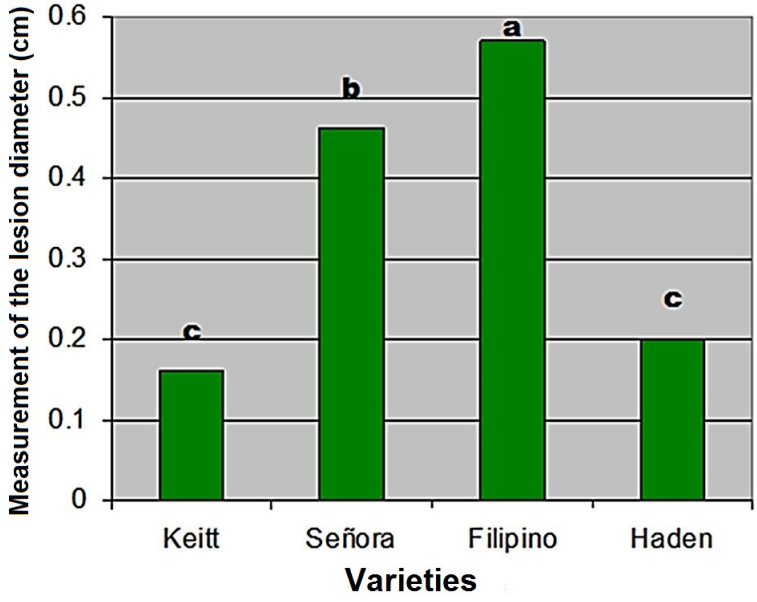

Figure 4. Measurement of sensitivity of mango varieties resulting from the pathogenicity tests of the 22 isolates of Colletotrichum gloeosporiodes (Penz) Penz & Sacc.

Table 3. Relation of the measurements of the conidia and appressor structures of the isolates with their corresponding shape criteria expressed in percent

Isolate	Conidia		Appressor	
	Size (μm)	Ends (spindle or rounded)	Form	Size (μm)
1	11.2-14.4 x 3.2-6.4 x = 12.1 x 3.9	89 % rounded 11 % both	60 % irregulares 38 % tapered 2 % rounded	7.5-17.5 x 5.0-10 x = 11.5x 6.7
2	9.6- 14.4 x 3.2-6.4 x = 12.0 x 3.9	88 % rounded 12 % both	80 % irregulares 16 % tapered 4 % rounded	7.5-17.5 x 5.0-10 x = 12.6 x 6.7
3	11.2-14.4 x 3.2-4.8 x = 11.9 x 3.8	92 % rounded 8 % both	89 % irregulares 10 % tapered 1 % rounded	7.5-17.5x5.0-12.5 x = 11.6 x 6.8
4	11.2-14.4 x 3.2-4.8 x = 12.4 x 4.1	85 % rounded 15 % both	94 % irregulares 6 % tapered	7.5-17.5 x 5.0-7.5 x = 11.7 x 5.9
5	11.2-14.4 x 3.2-4.8 x = 12.2 x 4.5	82 % rounded 18 % both	95 % irregulares 3 % tapered 2 % rounded	7.5-12.5 x 5.0-10 x = 10.5 x 5.4
6	11.2-16 x 1.6-4.8 x = 13.6 x 3.3	87 % both 3 % spindled 10 % rounded	70 % irregulares 29 % tapered 1 % rounded	8.6-21.5 x 4.3-17.2 x = 14.0 x 9.9
7	9.6-14.4 x 3.2-4.8 x = 11.1 x 3.4	90 % both 10 % rounded	80 % irregulares 20 % tapered	8.6-21.5x4.3-17.2 x = 14.3 x 8.8
8	11.2-16 x 1.6-6.4 x = 13.0 x 3.5	89 % both 10 % rounded 1 % spindled	80 % irregulares 18 % tapered 2 % rounded	12.5-21.5x4.3-17.2 x = 14.5 x 10.1
9	11.2-16.0 x 3.2-4.8 x = 13.6 x 3.8	70 % rounded 30 % both	77 % irregulares 15 % tapered 8 % rounded	7.5-17.5 x 5.0-10 x = 11.5 x 7.3
10	8.0-16.0 x 3.2-4.8 x = 13.2 x 4.2	75 % rounded 25 % both	86 % irregulares 14 % tapered	8.6-21.5x 4.3-17.2 x = 14.1 x 9.5
11	11.2-16.0 x 3.2-4.8 x = 13.9 x 3.9	79 % rounded 21 % both	76 % irregulares 24 % tapered	7.5-21.5x4.3-21.5 x = 12.2 x 7.9
12	11.2-16.0 x 3.2-4.8 x = 13.4 x 4.1	77 % rounded 23 % both	78 % irregulares 20 % tapered 2 % rounded	8.6-17.2x4.3-12.9 x = 13.8 x 8.4
13	11.2-16.0 x 3.2-4.8 x = 13.1 x 3.9	75 % rounded 24 % both 1 % spindled	70 % irregulares 28 % tapered 2 % rounded	8.6-21.5x4.3-17.2 x = 13.9 x 8.9
14	9.6-14.4 x 3.0-3.2 x = 11.9 x 3.2	80 % both 20 % rounded	81 % irregulares 19 % tapered	5.0-12.5x2.5-12.5 x = 9.8 x 6.6
15	8.0-16.0 x 1.6-3.2 x = 11.3 x 3.1	88 % both 12 % rounded	83 % irregulares 12 % tapered 5 % rounded	7.5-20.0x5.0-10.0 x = 10.4 x 7.2
16	8.0-16.0 x 3.2-4.8 x = 11.2 x 3.5	72 % both 28 % rounded	89 % irregulares 11 % tapered	8.6-21.5 x 8.6-12.9 x = 13.8 x 7.9
17	9.6-12.8 x 1.6-4.8 x = 11.5 x 3.3	94 % rounded 6 % both	75 % irregulares 25 % tapered	10.0-15.0 x 5.0-10.0 x = 12.6 x 7.4
18	8.0-16.0 x 3.2-4.8 x = 12.7 x 3.5	95 % rounded 5 % both 88 % both	70 % irregulares 30 % tapered 80 % irregular	7.5-12.5 x 5.0-10.0 x = 10.6 x 6.6
19	12.8-17.6 x 3.2-6.4 x = 15.6 x 3.5	10 % rounded 2 % spindled	19 % tapered 1 % rounded	9.6-15.0 x 5.0-11.2 x = 12.4 x 7.8
20	12.8-19.2 x 1.6-3.2 x = 15.2 x 3.0	68 % both 32 % rounded	84 % irregular 16 % tapered	7.5-12.8 x 4.8-7.5 x = 9.7 x 5.5
21	14.4-17.6 x 3.2-4.8 x = 15.7 x 3.3	74 % both 26 % rounded	75 % irregular 25 % tapered	6.4-12.8 x 3.2-8.0 x = 10.8 x 6.0
22	14.4-16.0 x 3.2-4.8 x = 15.5 x 3.6	76 % both 24 % rounded	77 % irregulares 23 % tapered	9.6-16.0 x 4.8-8.0 x = 12.9 x 6.3

Table 4. Relación de las medidas de las estructuras conidios y apresorios de los aislados con sus correspondientes criterios de forma expresados en porciento

Isolate	Original data	Transformed data
21	0.69	1.69 a
17	0.59	1.59 b
3	0.55	1.55 b
22	0.54	1.54 b
1	0.53	1.53 b
19	0.51	1.51 c
2	0.50	1.50 bc
5	0.49	1.49 bc
18	0.41	1.41 cd
10	0.37	1.37 d
20	0.34	1.34 d
4	0.33	1.33 d
16	0.22	1.22 e
15	0.20	1.20 e
14	0.19	1.19 e
11	0.19	1.19 e
13	0.17	1.17 e
7	0.17	1.17 e
9	0.15	1.15 e
12	0.14	1.14 e
8	0.14	1.14 e
6	0.12	1.12 e

Table 5. Statistical results in the analysis of main components up to the fourth component, with contributions of qualitative and quantitative variables.

Variables	CP1	CP2	CP3	CP4
Color of the colony	0.375	1.16	**-0.732**	-0.096
Number of conidia	0.418	**-0.719**	-0.233	0.216
Exudations	0.0091	**0.759**	-0.241	0.311
Shape of appressor	0.146	-0.266	**-0.704**	-0.261
Color of the conidial massl	**-0.774**	0.160	0.042	-0.401

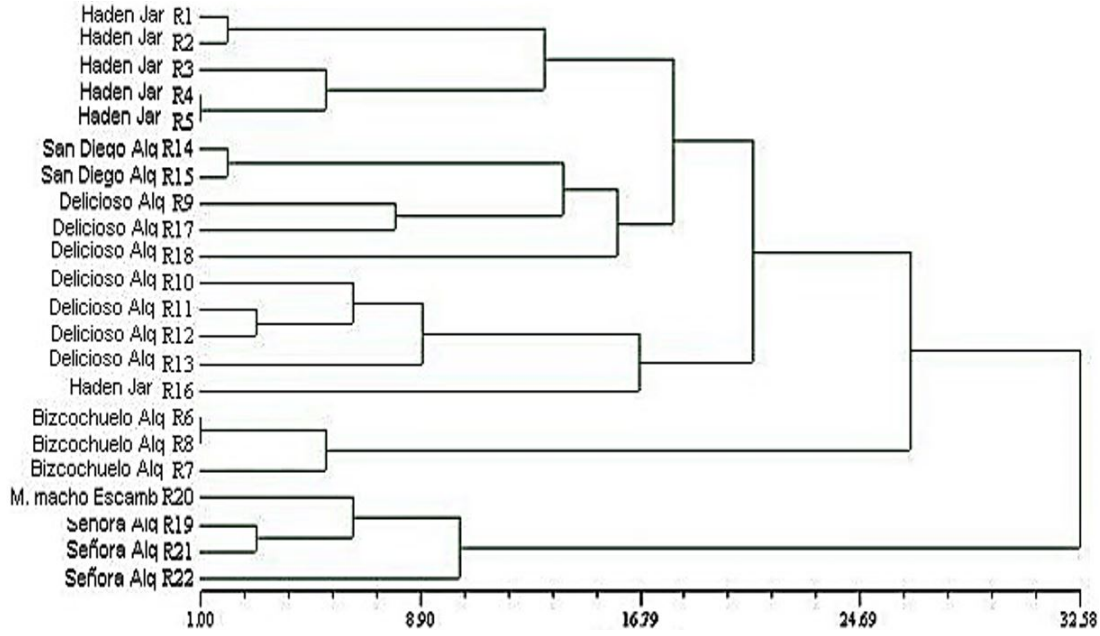

Figure 5. Grouping of the 22 isolates of Colletotrichum gloeosporioides (Penz) Penz & Sacc. Obtained from the six varieties of mango in the different localities of western Cuba.

whom these are grouped within varieties with low susceptibility to anthracnose in the field. However, Campbell (1992) and Rodríguez et al. (2002) published sensitivity to the disease for the Haden variety. These divergences may be based on the influence of climatic differences on the disease cycle and fruit ripening, because the studies were carried out in different regions.

The virulence of the isolates is presented in Table 4, where the least pathogenic are those that produce less conidia taking into account the results of the count. The isolates obtained from Haden and Señora were more pathogenic in these than in the other varieties, in agreement with Sangeetha and Rawal (2008) and Denoyes et al. (2003) who argue that the strains obtained from a particular host were more pathogenic in this one than in other hosts.

The same symptoms observed in the field samples were reproduced and the results showed the same characteristics as the originals. According to Koch's postulates, the isolated strains were responsible for the anthracnose in the collected samples

3.8 Statistical analysis for group differentiation

The factors that most influence the clustering of the isolates correspond to the color of the colony, the amount of conidia, the exudations to the medium, the form of the appressor and the color of the conidial mass as shown in Table 5.

Figure 5 shows the dendogram of C. goleosporioides isolates; these results indicate that the isolates are grouped mainly according to the locality from where they were obtained in the investigation. In the first group (I) formed by the isolates of Jaruco var Haden that differ perfectly from group (II), which are mostly constituted by the isolates obtained from different varieties of mango collected in the locality of Alquilar. There is an isolation from Escambray that is grouped at the end and does not correspond to any of the groups mentioned above. In this sense, we agree with Torres (1995), who argued that the different climatic regions of Cuba determined the behavior of the crop, Gutiérrez et al. (2001) found that there are isolates of C.gloeosporioides that are grouped more than by their variability in the area where the isolation was carried out, indicating that the environment can modify the behavior of the strains.

4 Conclusions

The 22 strains isolated in the western region of Cuba belong to the species Colletotrichum gloeosporioides (Penz.) Penz. And Sacc with an optimum temperature of growth above $24 \pm 2\,^{\circ}C$. The dimensions of the structures measured: conidia and aphorisms, are among the limits established worldwide for this species. The in vitro pathogenicity of the isolates is shown in agreement with the plant variety, with the isolate 21 having the highest degree of virulence.

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