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Research note

Morphological identification of fungi isolated from *Garcinia mangostana* plants

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Abstract

Mangosteen fruits possess several nutritional and nutraceutical properties important for human health, their cultivation is considered a production alternative for the tropical regions of Mexico. The objective of this research was to characterize morphologically pathogenic or antagonistic fungi isolated from plant tissues of mangosteen plants located in the municipality of Cacahoatán, Chiapas. In 2022, leaves and roots were collected from healthy plants, without characteristics of disease symptoms, fungi were isolated using the filter paper screening technique to obtain monosporic cultures, and morphological characterization was performed by scanning electron microscopy. Three fungal cultures were obtained, one from the rhizosphere *Trichoderma asperellum* and two in the leaves *Rhizopus oryzae* and *Botrytis cinerea*. A total of 25 measurements were taken to calculate the length and width of the following structures: sclerotia, columellae, conidia, conidiophores, phialides, and chlamydospores. The correct identification of the specific structures of the causative agents can help to propose strategies for disease control and prevention in mangosteen crops in the Soconusco region of the state of Chiapas, Mexico.

Keywords:

Botrytis cinerea, *Rhizopus oryzae, Trichoderma asperellum*, mangosteen, rhizosphere.

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Mangosteen (*Garcinia mangostana* L.) is a highly branched tree 6 to 25 m tall (Corner, 1988), with dark green evergreen leaves approximately 10 to 30 cm long and 3 to 15 cm wide (Campbell, 1966; Yaacob and Tindall, 1995; Osman and Rahman, 2006). Mangosteen plants have excellent adaptation, production and profitability in tropical regions and their fruits can reach quite high prices (Suárez *et al*., 2018). The mangosteen fruit contains 18 types of flavonoids, which, when ingested, convert into peroxide or copper radicals, making it an antioxidant fruit (Williams *et al*., 1995; Pedraza-Chaverri *et al*., 2008).

In 2022, mangosteen production in the state of Chiapas, Mexico, was 38 t from a 65 ha sowing distributed in the municipalities of Tapachula and Tuxtla Chico, with a production value of $2 835 000.00 pesos (SIAP, 2022). In the state of Veracruz, the mangosteen crop was introduced in the late 1960s and in the El Palmar Experimental Agricultural Field, research work began with this fruit tree as a production alternative for the humid tropical regions of Mexico (Díaz-Fuentes and Picón, 2007). Most of the commercial plantations cultivated in the period from 2013 to 2017 began productive life between the years 2019 and 2023 (Díaz *et al*., 2019). For this reason, there is not enough information on the productive behavior of mangosteen crops.

In this sense, the isolation and precise identification of the fungi associated with this crop will be decisive to know their behavior and identify which ones act as potential pathogens or antagonists for *Garcinia mangostana* L. The objective of this research was to characterize morphologically pathogenic and antagonistic fungal strains isolated from plant tissues of mangosteen plants from the state of Chiapas, Mexico.

We used six fungal strains isolated from mangosteen plants from an experimental plantation located in the municipality of Cacahoatán, Chiapas, Mexico, during 2022. This municipality is part of the Soconusco region, where 98% of the area planted with mangosteen in Mexico is concentrated (Díaz-Fuentes and Díaz-Hernández, 2011).

Plant tissue (leaves and rhizosphere) samples were sterilized for 1 min with a 3% sodium hypochlorite solution, washed two times with sterile distilled water, then leaf and rhizosphere samples were dried with (sterile) filter paper and placed in Petri dishes with potato dextrose agar culture medium (PDA, Bioxon®). The dishes were incubated at 28 °C for seven days under dark conditions or until the colonies covered the entire area of the culture medium (Cuervo-Parra *et al*., 2022). Once monosporic cultures were obtained, fungal strains were kept at -4 °C in PDA and as spore suspension at -87 °C until use.

The morphological descriptions of the fungal isolates were based on comparison with other isolates of *Trichoderma asperellum* (García-Núñez *et al*., 2017; Sánchez *et al*., 2021; Cuervo-Parra *et al*., 2022), *Rhizopus oryzae* (Hernández-López *et al*., 2019), and *Botrytis cinerea* (Terrones-Salgado *et al*., 2019; Montiel-Frausto and Vásquez-López, 2021), complemented by new observations. Fungal strains were grown in PDA culture medium, incubated for seven days, and kept at 28 °C during morphological characterization.

A total of 25 measurements were taken for each trait evaluated to calculate the length and width of the following structures: sclerotia, columellae, conidia, conidiophores, phialides, and chlamydospores. Measurements of the morphological characteristics of the isolated strains were performed using micrographs obtained with a scanning electron microscope (SEM; JEOL, model IT300, Boston, United States of America) located in the facilities of the Apan Higher School (ESAp-UAEH).

Colonies of the strains: JEAB01, JEAB02 and JEAB03 of *Trichoderma* covered the surface of the PDA culture medium for 3-4 days and showed a diameter of 80-83 mm. They showed hyaline growth at the beginning and over time acquired an olive green coloration due to the production of abundant spores. After seven days of growth, the colonies sporulated and produced white conidial pustules, which become dark green as they accumulate, distributed in concentric circles or completely covering the culture medium (Figure 1A-B).

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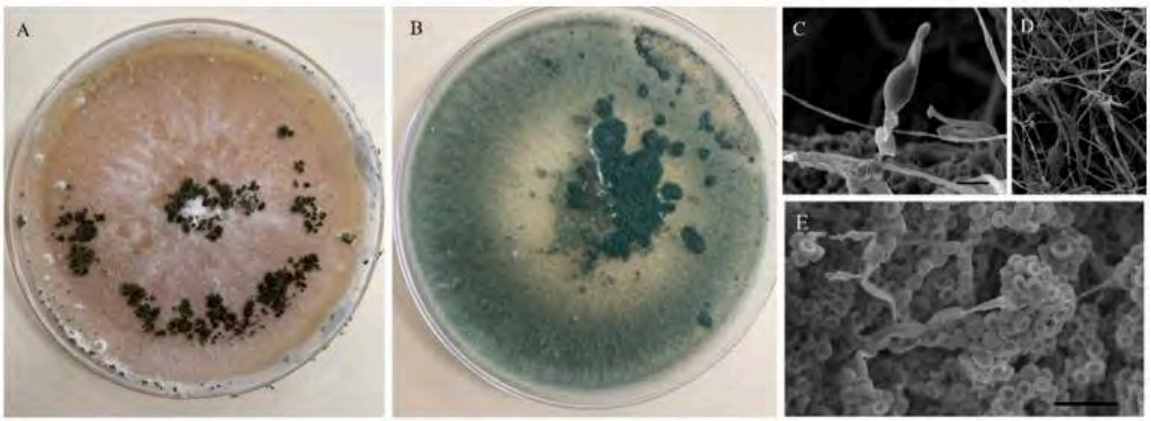
Figure 1. Strain of *Trichoderma asperellum* isolated from the rhizosphere of mangosteen, A-B) presence of pustules after five days of growth in PDA medium; C-D) SEM micrographs of phialides, conidiophores and conidia of *T. asperellum*; and E) SEM micrographs of conidia in groups. Scale bar: C= 5 µm; D= 50 µm; and E= 10 µm.



Micrographs showed hyaline hyphae with highly branched conidiophores 15.89-79.95 µm long × 2.08-4.6 µm wide (average values of 30.44 ±16.08 µm long, 3.16 ±0.79 µm wide). The phialides apically shaped, in whorls, alone or in groups (Figure 1C-D), 4.01-11.41 µm long × 1.08-3.01 µm wide (average values 7.98 ±1.6 µm long, 1.8 ±0.59 µm wide). The conidia showed ovoid shape, 2.22-3.58 µm long × 1.18-3.51 µm wide (average values 3.01 ±0.36 µm long, 2.08 ±0.48 µm wide), arranged in groups of 6 or more conidia (Figure 1E).

In the observed strains, the presence of chlamydospores was not identified, but when they are present, they are interspersed between hyphae, between 4.49-15.3 µm long by 3.67-2.3 µm wide (Cuervo-Parra *et al*., 2022). Derived from the morphological characteristics observed, the strains: JAEB01, JAEB02 and JAEB03 correspond to isolates of *T. asperellum* (Rai *et al*., 2016; García-Núñez *et al*., 2017; Sánchez *et al*., 2021).

The colonies of the JEAB04 and JEAB05 strains grown in PDA showed a diameter of 80-85 mm with hyaline mycelium at the beginning, which over the days became dense or dispersed with a cottony texture, from pale gray to grayish brown, with long white sporangia that reached the edge of the Petri dish and when mature acquired a black color at the ends of the hyphae due to the accumulation of conidia (Baraona and Sancho, 1992).

The reverse side of the colony is colorless and the hyphae were colorless, non-septate, and broad. The stolons were hyaline, with rhizoids at their ends of between 4-6 arms, brown in color and between 1-5 conidiophores with stipes more than 1500 µm long, usually not bifurcated, spherical in shape with measurements of 30.68-75.33 µm long by 1.04-2.59 µm wide (average values of 41.95 ±8.21 µm long, 1.61 ±0.35 µm wide).

The columellae were pale brown, spherical in shape, 59.66-168.1 µm long × 56.89-117.1 µm wide (average values 97.99 ±19.69 µm long, 86.43 ±13.03 µm wide), with brown conidia, variable in size, from ellipsoidal to slightly fusiform or with an irregular angle, commonly 2.65-3.92 µm long × 2.14-3.74 µm wide (average values of 3.28 ±0.37 µm long, 2.92 ±0.41 µm wide) with fluted walls. These observations are consistent with the descriptions reported by other authors (Pitt and Hocking, 2009; Hernández-López *et al*., 2019). Placing JEAB04 and JEAB05 strains as novel isolates of *R. oryzae* in crops of *G. mangostana* L. in Chiapas, Mexico.

Fungi of the genus *Rhizopus* are characterized by growing rapidly on the tissues of the plants they affect, causing rot of the affected tissues (Zhang *et al*., 2023). It is a phytopathogenic fungus that spreads by wind or insects and can affect the fruits of the hosts where it develops in a maximum

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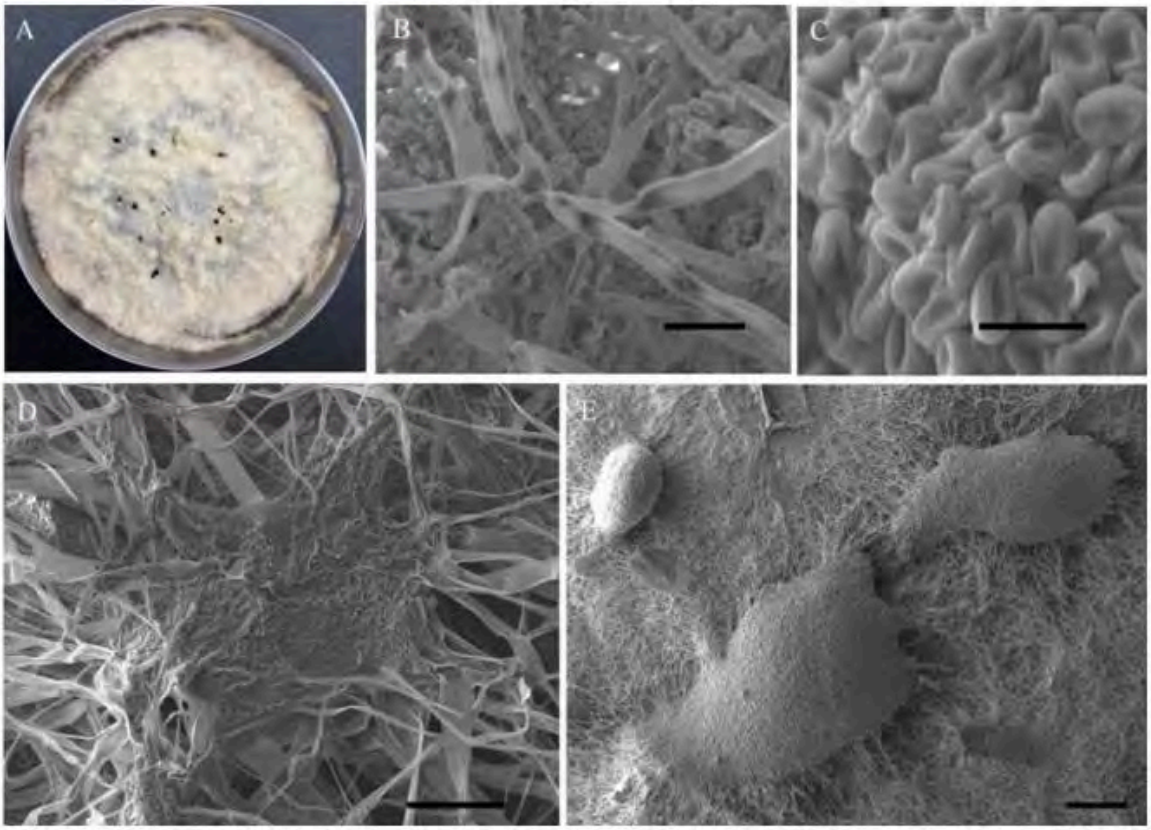
time of four days (Northover and Zhou, 2002). Its spores can remain viable for long periods of time under unfavorable conditions (low moisture and high temperatures), germinating on plant tissues when conditions are favorable, causing plant tissue rot (Zhang *et al*., 2023).

On the other hand, colonies of *Botrytis cinerea* strain JEAB06 grown in PDA medium covered the surface of the culture medium in 5 days and had a diameter of 83-85 mm. During the early stages of growth, the texture of the colony was cottony, white to grayish white, which over time develops dark brown to black sclerotia and has an irregular distribution over the culture medium (Figure 2A).

Figure 2. *Botrytis cinerea* isolated from mangosteen. A) morphological characteristics after seven days of growth in PDA; B) SEM micrographs of *B. cinerea* conidiophores; C) SEM micrographs of *B. cinerea* conidia; D) SEM micrographs of *B. cinerea* pseudoparenchyma; and E) SEM micrographs of *B. cinerea* sclerotia. Scale bars: B= 10 µm; C= 5 µm; D= 50 µm; and E= 200 µm.



The conidiophores observed are long, thin, branched, originating mainly from the hyphae or from the sclerotia (Figure 2B). With dimensions of 853.1-1895.6 µm long by 7.03-18.02 µm wide (average values 1 594.43 ±341.32 µm long, 16.33 ±2.71 µm wide). Conidia are abundant, ovoid, globose, or elliptic, formed from the budding of precursor cells of conidia 9.822-14.51 µm long by 4.687-5.962 µm wide (average values 11.88 ±1.38 µm long, 5.49 ±0.37 µm wide) formed in sterigmata (Figure 2C).

The mycelium is made up of cylindrical hyphae, which, when multiplying vegetatively, give rise to the formation of the pseudoparenchyma (Figure 2D) and from which sclerotia are formed (Figure 2E). They are irregularly shaped, 693.4-3 467.7 µm long × 529.5-2 312.7 µm wide (average values 1 713.32 ±828.1 µm long, 1 046.61 ±395.71 µm wide).

The morphological observations described in Figure 2 are similar to the reports made by other authors (Terrones-Salgado *et al*., 2019; Montiel-Frausto and Vásquez-López, 2021), and confirm the identification of the JEAB06 strain as a novel isolate of *B. cinerea* from the mangosteen plant in Chiapas, Mexico. Gray mold is caused by *B. cinerea* and is a common disease during harvest and

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postharvest and is responsible for causing damage to the soft tissues of more than 200 different species of plants, affecting their roots, stems, and buds (Williamson *et al*., 2007; Díaz *et al*., 2014).

Conclusions

The microscopic characterization of the differential structures of the identified species will allow proposing integrated management and control strategies to avoid losses caused by gray mold and tissue rots, caused by *B. cinerea* and *R. oryzae* in mangosteen crops in the Soconusco region of the state of Chiapas.

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