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Scientific Article

In vitro control of *Colletotrichum gloeosporioides*, causal agent of anthracnose on grapefruit (*Citrus paradisi*), using plant extracts

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ABSTRACT

Background/Objective. Citrus (*Citrus* spp.) production is affected annually by anthracnose, associated with *Colletotrichum* spp. This disease can occur preharvest and postharvest and is generally controlled with synthetic fungicides, which pose health problems for farmers and consumers, negatively impact the environment, and lead to the emergence of resistant phytopathogen populations. An alternative to these problems is the application of plant extracts with antifungal properties, which could contribute to the production of safe food. Therefore, the objective of this study was to evaluate the effect of plant extracts under *in vitro* conditions against *Colletotrichum gloeosporioides*, causal agent of anthracnose on grapefruit (*Citrus paradisi*).

Materials and Methods. Extracts were obtained from 17 plant species. The effectiveness of the extracts was evaluated using the poisoned PDA culture technique (PDA + 10% plant extract), in which 5 mm diameter discs of the fungus were inoculated onto Petri dishes. The fungicide Benomyl (10%) was used as a positive control, and pure PDA as a negative control. A completely randomized experimental design with 19 treatments and three replicates was used. The percentage of inhibition of *C. gloeosporioides* mycelial growth was recorded when the negative control completely covered the surface of the Petri dish. Data were analyzed using the statistical software SAS version 9.0.

Results. The best extracts were zopilote seed (*Swietenia macrophylla*), bull blood tree bark (*Bocconia arborea*), and bull blood tree leaf and stem (*B. arborea*), and moringa seed (*Moringa oleifera*), inhibiting the growth of *C. gloeosporioides* by 69.2, 67.5, 44.1, and 35.6%, respectively. The positive control, Benomyl, inhibited *C. gloeosporioides* growth by 100%.

Conclusion: The best in vitro inhibitory effect on *Colletotrichum gloeosporioides* was obtained with *Swietenia macrophylla* seed extracts (69.2%), followed by *Bocconia arborea* bark (67.5%), *B. arborea* leaf and stem (44.1%), and *Moringa oleifera* (35.6%).

Keywords: Biorational management, Fungal diseases, Biofungicide

INTRODUCTION

Citrus (*Citrus* spp.) is produced in temperate and tropical regions worldwide (Spiegel-Roy and Goldschmidt, 2008). In Mexico, citrus production is of great importance, as it is carried out in 23 states of the country, where it generates numerous jobs and significant economic income. However, citrus anthracnose caused by *Colletotrichum* spp. limits citriculture, as it reduces yields during preharvest and affects fruit quality during postharvest, thereby hindering commercialization (Phoulivong *et al.*, 2012; Ghanei and Mollazade, 2023). In Mexico, *C. gloeosporioides* is the main agent associated with citrus anthracnose (Rojo-Báez *et al.*, 2017; Cruz-Lagunas *et al.*, 2020).

The most common method for its preharvest and postharvest management is chemical control, mainly through the application of synthetic fungicides such as copper-based products, strobilurins, dithiocarbamates, chlorothalonil, among others (Bordoh *et al.*, 2020; Ciofini *et al.*, 2022). However, the use of these fungicides in agricultural products intended for human consumption poses a risk to human health; moreover, they contaminate soil and water and adversely affect non-target organisms (Ncama *et al.*, 2019). In addition, misuse, as well as the frequent use of a single fungicide with the same mode of action, has promoted the development of resistant phytopathogenic strains (Gan and Wickings, 2017; Damalas, 2018; Zhang *et al.*, 2020).

Therefore, it is necessary to develop biorational alternatives for disease management in order to reduce the negative impact of agrochemicals. In this context, the use of plant extracts is considered a sustainable alternative to mitigate phytosanitary problems, decrease economic losses, and reduce the impact of human activity on the environment (Cerqueira *et al.*, 2016; Bernal *et al.*, 2005). Plant extracts have been evaluated for their biofungicidal effect, which is attributed to the presence of various secondary metabolites, such as phenolic compounds, flavonoids, quinones, tannins, coumarins, among others (Vig *et al.*, 2009; Ncama *et al.*, 2019); moreover, their use has greater societal acceptance by avoiding the secondary risks mentioned above (Tomazoni *et al.*, 2016). In accordance with this background, the objective of the present study was to evaluate the effect of 17 ethanolic extracts against *C. gloeosporioides*, the causal agent of anthracnose in grapefruit fruits, under *in vitro* conditions.

MATERIALS AND METHODS

Location of the experiment. The research work was conducted in the Plant Physiology and Biotechnology Laboratory of the Faculty of Agricultural and Environmental Sciences at the Autonomous University of Guerrero, located in Iguala de la Independencia, Guerrero, Mexico. The city belongs to the Northern Region and is situated at an altitude of 731 m above sea level, at the geographic coordinates 18°21′21.8″ N and 99°32′57.6″ W.

Evaluated phytopathogen. The phytopathogenic fungus *Colletotrichum gloeosporioides* was used, identified morphologically, through Koch's postulates, and molecularly as the causal agent of anthracnose in grapefruit (*Citrus paradisi*), isolate COLTOR1 (Cruz-Lagunas *et al.*, 2020).

Collection and preparation of ethanolic extracts. Samples of bark, leaves, stems, and seeds were collected from the Central and Northern regions of Guerrero (Table 1). The samples were prewashed with soap and water and then air-dried at room temperature in the shade for seven days; in the case of seeds, they were stored in glass containers until processing. Fifty grams of dried plant material and 100 g of seeds were weighed using a digital analytical balance (Denver

Instrument®). Subsequently, a prewash was performed with a 0.5% NaClO solution for 2 minutes, followed by four rinses with sterile distilled water. The material was then spread on paper towels and kept at room temperature to remove excess moisture. The plant material was ground using a conventional blender, and the resulting powder was soaked in 96% ethanol (Ochoa *et al.*, 2012) for approximately 24 hours. Subsequently, the ethanolic extracts from each sample were filtered using sterile gauze and placed in a digital drying oven (Novatech®) to remove the ethanol. The plant extracts were transferred to plastic containers and stored under refrigeration at 6 °C until further use.

Preparation of poisoned culture medium. To evaluate the effect of the extracts, the poisoned culture medium technique was used (Wilson *et al.*, 1997). For this purpose, PDA culture medium was prepared following the manufacturer's instructions (MCD LAB®), and 2 mL of gentamicin plus 2 mL of chloramphenicol per liter were added. Subsequently, the mixture was poured into Petri dishes containing the plant extract at 10% (Shamim and Chowdhury, 2016), consisting of a mixture of 22.5 mL of PDA medium and 2.5 mL of the plant extract. For the positive control, the commercial fungicide Benomyl at 10% was used (22.5 mL of PDA medium + 2.5 mL of Benomyl), whereas PDA medium alone (25 mL) without plant extract was used as the negative control. The experimental design was completely randomized with 19 treatments (Table 1) and three replicates per treatment. In each Petri dish prepared with the corresponding treatment, a fungal plug (5 mm in diameter) of *C. gloeosporioides* was placed at the center. Subsequently, the dishes were sealed, labeled, and incubated under laboratory conditions.

Evaluated variable. To determine fungal growth inhibition, the diameter of mycelial growth of each colony was measured using a graduated ruler after the negative control had completely covered the surface of the Petri dish. Subsequently, the radial mycelial diameter was recorded, the diameter of the initially transferred phytopathogenic fungal disc (5 mm) was subtracted, and the mean and the percentage of mycelial growth inhibition were calculated (Rebolledo-Prudencio *et al.*, 2025).

$$\% \text{ inhibition} = \frac{\text{mycelial diameter of the control} - \text{mycelial diameter of the treatment}}{\text{mycelial diameter of the control}} \text{ X } 100$$

Statistical analysis. Using the data generated for the percentage of mycelial inhibition, an analysis of variance was performed, and when statistical differences were detected, means were separated using the LSD (Least Significant Difference) test, at a 95% probability level ($p \leq 0.05$). All analyses were conducted using SAS software, version 9.0.

Table 1. Collection of plant material in the Central and Northern regions of Guerrero and treatments.

Number	Common name	Cientific name	Part used	Treatment	Location	Altitude	Reference of antimicrobial activity
1	Árbol de zopilote	*Swietenia macrophylla*	Seed	T1 = SZop	Iguala, Gro., Northem Región.	731 m.a.s.l.	(Tan *et al*., 2021)
2	Moringa	*Moringa oleifera*	Seed	T2 = SMor			(Riad *et al*., 2014)
3	Chicalote	*Argemone ochroleuca*	Leaf and stem	T3 = ChHT			(Juárez-García *et al*., 2020)
4	Castor been	*Ricinus communis*	Leaf	T4 = HHig			(Montes-Belmont, 2009)
5	Eucalyptus	*Eucalyptus globulus*	Leaf and stem	T5 = EHT	Zoquitipa, municipality of Chilapa de Álvarez, Gro., Central Región	1511 m.a.s.l.	(Baños *et al*., 2004)
6	Lemon grass	*Cymbopogon citratus*	Leaf	T6 = ZLH			(Scalvenzi *et al*., 2016)
7	Common ash	*Fraxinus excelsior*	Leaf and stem	T7 = FHT			Without registration
8	Quina	*Hintonia latiflora*	Leaf and stem	T8 = QHT			Without registration
9	Jarilla	*Barkleyanthus salicifolius*	Leaf and stem	T9 = JHT			(Hernández-Albíter *et al*., 2007)
10	Cornejo sedoso	*Cornus sericea*	Leaf	T10 = HCS	Acayahualco, municipality of Tepecoacuilco, Gro., Northem Región	800 m.a.s.l.	Without registration
11	Cornejo sedoso	*Cornus sericea*	Seed	T11 = SCS			Without registration
12	Guazuma	*Guazuma ulmifolia*	Leaf	T12 = HGzm			(Ramírez-Salcedo *et al*., 2019)
13	Guazuma	*Guazuma ulmifolia*	Fruit	T13 = FGzm			(Ramírez-Salcedo *et al*., 2019)
14	Bull blood	*Bocconia arborea*	Leaf and stem	T14 = STHC	Coaxtlahuacán, municipality of Mochitlán, Gro., Central Región	1716 m.a.s.l.	(Yu *et al*., 2014)
15	Bull blood	*Bocconia arborea*	Bark of tree	T15 = STC			(Yu *et al*., 2014)
16	Field horse tail	*Equisetum arvense*	Stem and leaf	T16 = CCTH			(Garcia *et al.,* 2013)
17	Cubata	*Acacia cochliacantha*	Leaf, stem and thorn	T17 = CHTE	Tomatal, municipality of Iguala, Gro., Región Norte.	866 m.a.s.l.	Without registration
18	-------	-------	--------	T18 = CNP[y]	--------	------	------
19	-------	-------	--------	T19 = CPB[z]	--------	-------	------

[y]Negative Control = PDA pure
[z]Positive Control = Benomyl

RESULTS AND DISCUSSION

Statistical analysis detected significant differences in the percentage of mycelial growth inhibition (PMGI) of *Colletotrichum gloeosporioides* as an effect of the evaluated treatments. For PMGI, statistical results indicated that the positive control Benomyl (CPB) and the zopilote seed extract (T1 = SZop) (*Swietenia macrophylla*) were statistically the most effective treatments ($p \leq 0.05$), achieving 100 and 69.2% inhibition of *Colletotrichum gloeosporioides* mycelial growth, respectively. These were followed by bull blood tree bark (T15 = STC) (*Bocconia arborea*) with 67.5%, bull blood tree leaf–stem (T14 = STHC) with 44.1%, and moringa seed (T2 = SMor) (*Moringa oleifera*) with 35.6% (Figures 1 and 2).

On the other hand, the extracts that showed a moderate to minimal effect on the PMGI of *C. gloeosporioides* were the leaf–stem extract of chicalote (T3 = ChHT) (*Argemone ochroleuca*), leaf–stem common ash (T7 = FHT) (*Fraxinus excelsior*), leaf–stem of quina (T8 = HTQ) (*Hintonia latiflora*), castor bean leaf (T4 = HHig) (*Ricinus communis*), and leaf–stem of eucalyptus (T5 = EHT) (*Eucalyptus globulus*), which showed PMGI values ranging from 19.8 to 6.3%. The remaining treatments showed no effect on the mycelial growth of *C. gloeosporioides* (0% PMGI) (Figure 1).

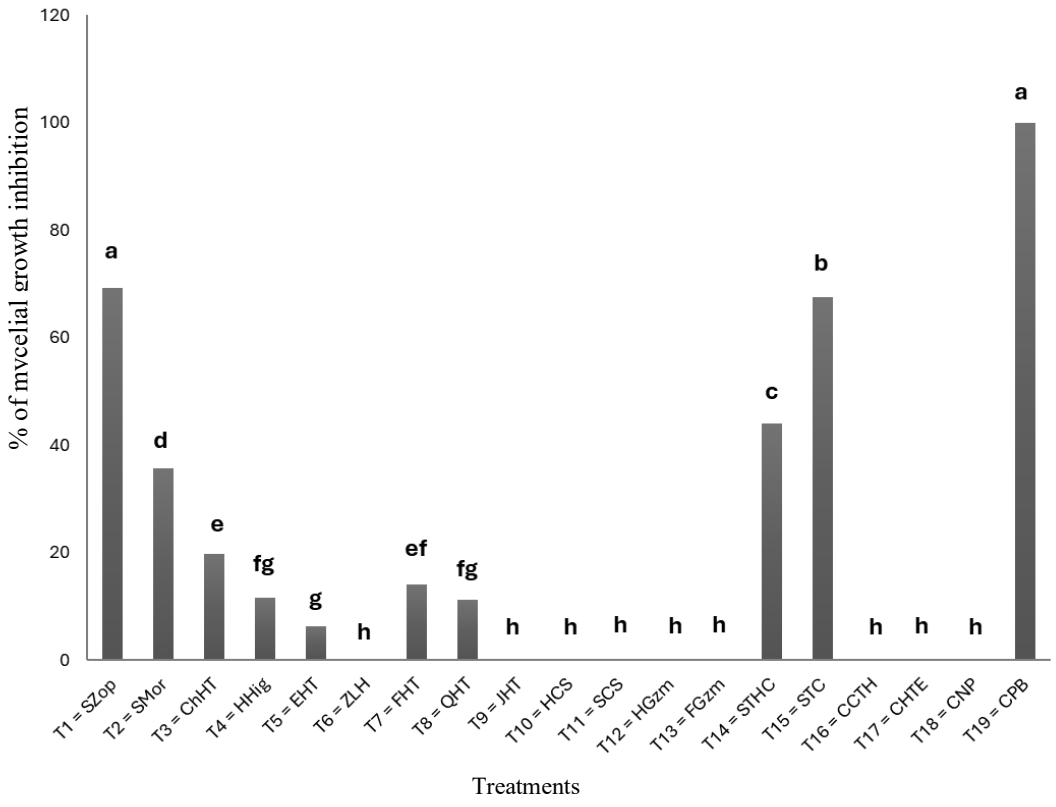

Figure 1. Effect of extracts from 17 plant species on the mycelial growth of *Colletotrichum gloeosporioides*, isolate COLTOR1. Means sharing the same letter are not statistically different according to the LSD test ($p \leq 0.5$).

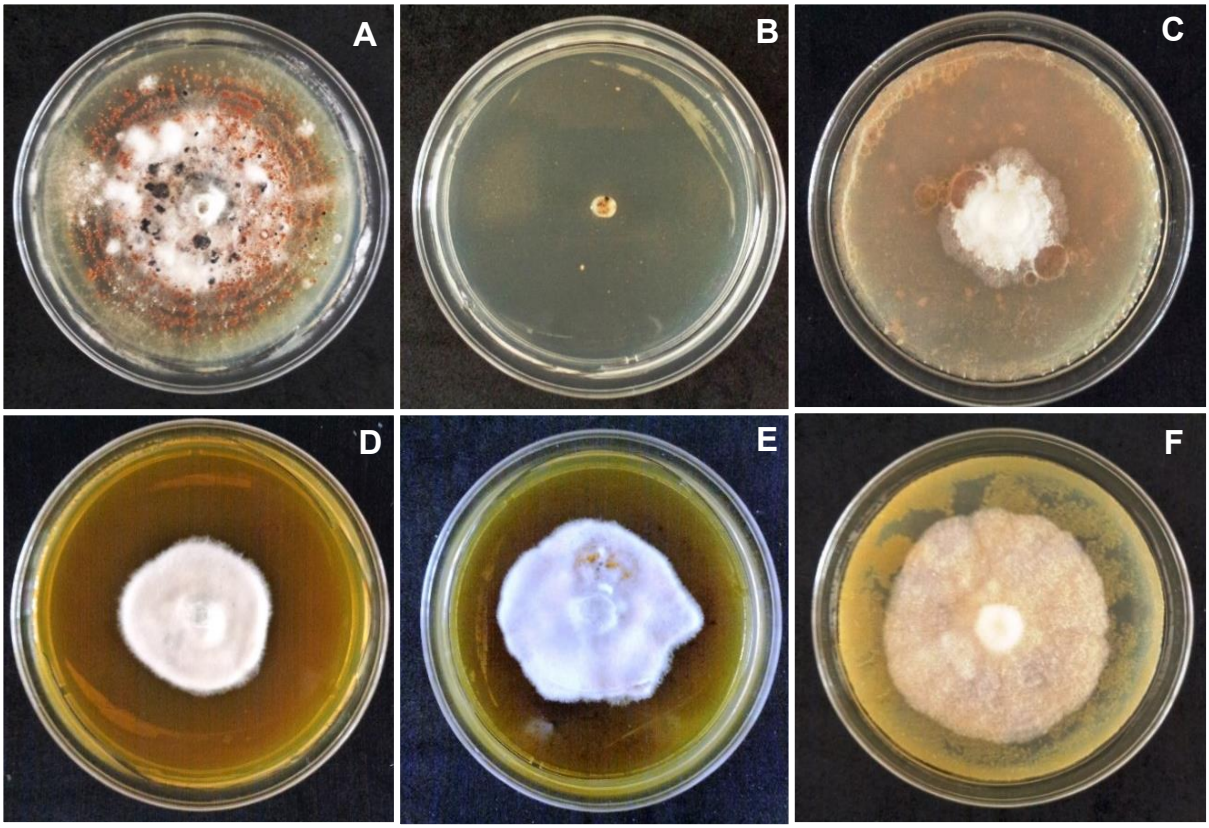

Figure 2. Effect of botanical extracts against *Colletotrichum gloeosporioides*. A = Negative control (PDA) (T18); B = Positive control (T19) (Benomyl); C = Vulture seed (*Swietenia macrophylla*) (T1 = SZop); D = Bloodroot bark (*Bocconia arborea*) (T15 = STC); E = Bloodroot stem–leaf (*B. arborea*) (T14 = STHC); and F = Moringa seed (*Moringa oleifera*) (T2 = SMor).

In 2021, Tan *et al.* (2021) evaluated the effect of methanolic extracts from *Swietenia macrophylla* seeds against the phytopathogens *Magnaporthe oryzae*, *Phytophthora capsici*, and *P. palmivora*, and determined high antifungal effectiveness, which was mainly attributed to the presence of limonoid compounds. Likewise, Mukherjee *et al.* (2011) demonstrated high effectiveness of *S. macrophylla* seeds in controlling *C. gloeosporioides* under *in vitro* conditions, with results similar to those obtained in this study. On the other hand, ethanolic extracts of bark, leaf, and stem of bull blood tree showed an inhibitory effect on the radial growth of *C. gloeosporioides*. Although no reports were found regarding their effectiveness against phytopathogens, Navarro and Delgado (1999) and Yu *et al.* (2014) reported that the antimicrobial effect of *Bocconia arborea* is due to the presence of various alkaloids with antibacterial and antifungal activity.

The ethanolic extract of moringa seeds (*Moringa oleifera*) showed a moderate inhibitory effect (35.5%) on the mycelial growth of *C. gloeosporioides*. In this regard, Riad *et al.* (2014) determined that moringa seed extracts at 10 and 20% inhibited the mycelial growth of *Fusarium oxysporum*, *F. solani*, *Alternaria solani*, and *A. alternata* by 66.7 to 95.5%. It is worth noting that the antifungal effect of moringa extract is associated with its phenolic nature and the presence of volatile coumarins, saponins, triterpenes, steroids, flavonoids, and tannins (Linares-Rivero *et al.*, 2018).

The antifungal activity of the ethanolic leaf–stem extract of chicalote (*Argemone ochroleuca*) inhibited *C. gloeosporioides* by 19.8%. In this regard, Juárez-García *et al.* (2020) evaluated the effect of ethanolic extracts of *A. ochroleuca* against *Fusarium*

oxysporum at three concentrations (13, 23, and 31%) and reported 66% inhibition using the 13% extract. When concentrations were increased to 23 and 31%, they reported 100% inhibition of mycelial growth of the fungus. Its biological activity against various phytopathogenic fungi is known to be attributed to the presence of chemical compounds such as alkaloids, terpenoids, flavonoids, and phenols (Brahmachari *et al.*, 2013). An ethanolic leaf–stem extract common ash (*Fraxinus excelsior*) showed low *in vitro* antifungal activity against *C. gloeosporioides* (PMGI = 14.0%). Although, to date, no studies have reported high *in vitro* activity of ethanolic extracts of *Fraxinus excelsior* against phytopathogenic fungi, studies have been published demonstrating high antibacterial activity of these extracts due to the presence of coumarins, secoiridoids, phenylethanoid glycosides, lignans, flavonoids, phenolic compounds, sterols, and triterpenes (Häuser *et al.*, 2025).

The antifungal activity of the castor bean leaf extract (*Ricinus communis*) inhibited *C. gloeosporioides* by 11.7%. Studies have reported antifungal activity of *R. communis* extracts against phytopathogenic fungi of the genera *Colletotrichum*, *Fusarium*, *Rhizoctonia*, *Pythium*, and *Sphaerotheca* (Schlösser, 1980; Montes-Belmont, 2009). Vargas *et al.* (2009) reported that castor bean has antifungal capacity to reduce black Sigatoka disease of banana caused by *Mycosphaerella fijiensis*. The antifungal activity of this extract is attributed to various substances such as flavonoids, cyanogenic glycosides, saponins, oxalate, alkaloids, and tannins (Yeboah *et al.*, 2020).

The antifungal activity of the leaf–stem extract of quina (*Hintonia latiflora*) inhibited the mycelial growth of *C. gloeosporioides* by 11.3%. To date, no scientific evidence has been found demonstrating an antifungal effect of ethanolic extracts derived from quina. The leaf–stem extract of eucalyptus (*Eucalyptus globulus*) showed minimal antifungal activity, with a PMGI of 6.3% against *C. gloeosporioides*. Baños *et al.* (2004) reported a reduction in mycelial growth of *C. gloeosporioides* (39%) using a botanical extract of white eucalyptus (*Eucalyptus globulus*). Leaves and roots of species of the genus *Eucalyptus* inhibit the growth of phytopathogenic fungi due to the presence of chlorogenic, ellagic, caffeic, ferulic, gallic, and gentisic acids; in addition, *Eucalyptus* essential oil is composed of tannins and flavonoids (Celis *et al.*, 2008). The positive control consisted of the application of the fungicide benomyl, which inhibited *C. gloeosporioides* by 100%. For the remaining treatments, the effect was null (0%); however, their antifungal potential at higher concentrations cannot be ruled out. Therefore, future studies could focus on evaluating other extraction methods and higher doses.

CONCLUSIONS

It was determined that the ethanolic extracts showing the highest percentage of mycelial growth inhibition (PMGI) of *Colletotrichum gloeosporioides* under *in vitro* conditions were zopilote seed (SZop) with 69.2%, followed by bull blood tree bark (STC) with 67.5%, bull blood tree leaf and stem (STH) with 44.1%, and moringa seed (SMor) with 35.6% PMGI. Extracts of zopilote seed, bull blood tree bark and stem–leaf, and moringa seed could be evaluated under preharvest and postharvest conditions in grapefruit fruits for the control of *C. gloeosporioides*, in order to assess their antifungal potential under these conditions.

Limitations. Not applicable.

Conflict of interest. The authors declare no conflict of interest.

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