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ORIGINAL ARTICLE

Antifungal activity of weed aqueous extracts on Persian lime anthracnose caused by *Colletotrichum gloeosporioides*

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Abstract

Citrus anthracnose caused by *Colletotrichum gloeosporioides* is an economically important disease around the world affecting the pre- and postharvest stages. While fungicides have been used to control this disease, integrated management systems associated with biological control techniques are a sustainable alternative. In the present study, we report the in vitro effect of leaf aqueous extracts (at 1, 2 and 4% w/v) of *Argemone mexicana*, *Datura discolor* and *Amaranthus palmeri* collected from northern Sinaloa (Mexico), against the growth of *C. gloesporioides*. The *D. discolor* extract inhibited fungal mycelial growth by 52–73% and did not differ from the chemical treatment (carbendazim-1 ppm). In addition, the *D. discolor* application at 4% reduced anthracnose in Persian lime fruit similar to fungicide treatment. These results indicate that the aqueous extract of *D. discolor* has the potential to control citrus anthracnose in Persian lime fruits. Our findings thus open the pathway for future research focusing on strategies to manage citrus anthracnose caused by *C. gloeosporioides*.

Keywords Biological control · Anthracnose · Persian lime fruit · Postharvest

Introduction

The genus *Colletotrichum* includes important plant pathogenic fungi in the world (Dean et al. 2012). Also, *Colletotrichum* spp. cause citrus fruit anthracnose, one of the major disease in many global citrus-growing regions (Pérez-Mora et al. 2021). *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is considered the most aggressive species to affect leaves, flowers, fruit, and twigs of citrus (Guarnaccia et al. 2017). Anthracnose disease losses in fruits can reach up to 100% under favorable conditions (Rojo-Báez et al. 2017); assessment of postharvest losses by *C. gloeosporioides* in India revealed that the pathogen contributed 21–26% of the total losses (Naqvi 2004). In Mexico, losses caused by *C. acutatum* in Mexican lime in Colima, under field conditions reached 40–60% in winter (Orozco-Santos et al. 2006); however, the losses caused by *C. gloeosporioides* on citrus orchards in Sinaloa (Pérez-Mora et al. 2021) have not determined.

Although fungicides are widely used to control anthracnose, there is increasing concern regarding the resistance of *Colletotrichum* spp. strains to these compounds (Forcelini et al. 2016; Luo et al. 2021).

Plants content a lot of chemical compounds with direct antimicrobial activity or that induce systemic resistance resulting in reduction of disease development (Kagale et al. 2004); e.g. PAL, phenolics, PR-proteins, terpenes and saponins as well as phytohormones. Furthermore are easily biodegradable (Qasem and Abu-Blan 1966).Thus they can

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provide us a source of natural agrochemicals for an eco-friendly disease management of plant-pathogens.

Plant-based fungicides have therefore gained interest, and several studies have focused on the antifungal activity of plant extracts against anthracnose in different fruits. For example, weeds include a broad group of plants with a wide distribution, and their extracts present potential antifungal activity (Mushatq et al. 2012). Ademe et al. (2013) studied the in vitro effect of nineteen plant ethyl acetate extracts against *C. gloeosporioides*, and found that *Echinops* sp. and *Lantana camara* L. extracts were the most effective in inhibiting the pathogen's mycelial growth and reducing the spore germination. In addition, aqueous extracts of *Echinops* sp. at concentrations of 10% and 25% exerted an effective control against postharvest anthracnose on papaya fruit. Alemu et al. (2014) evaluated the effect of twenty plant extracts, including several weed species, in which methanolic extracts from *Datura stramonium* L. inhibited in vitro conidial germination and reduced mycelial growth of the pathogen. Furthermore, the aqueous extract of *D. stramonium* at 50% was shown to reduce the incidence and severity of anthracnose in mango. Finally, Karim et al. (2017) reported that extracts of *Datura metel* L. caused significant reduction of mycelial radial growth in the same fungus in vitro.

The objectives of the present study were to: (a) determine the in vitro effect of aqueous extracts of the weeds *Argemone Mexicana* L., *Datura discolor* Bernh., and *Amaranthus palmeri* S. Wats. (collected in northern Sinaloa) against *C. gloeosporioides*; and (b) evaluate the efficacy of the extract in the control of anthracnose in Persian lime fruit (*Citrus* × *latifolia*).

Material and methods

Molecular identification of weeds used to obtain leaf extracts

Samples of Mexican prickly poppy (*A. mexicana*), desert thorn-apple (*D. discolor*) and palmer amaranth (*A. palmeri*) were collected at the flowering stage during July and August 2019 in the municipality of Ahome, Sinaloa, Mexico (25°54–55' N and 108–109°02–55' W).

Genomic DNA was extracted using the CTAB method (Doyle 1990). Subsequently, the ITS region was amplified using the ITS1 (5'-TCCGTAGGTGAACCTGCGG-3')/ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primer set (White et al. 1990). The PCR mix contained 1 µL (30–50 ng) of DNA, 1X of reaction buffer, 1 mM of $MgCl_2$, 0.5 mM of each primer, 500 µM of deoxynucleotide triphosphate (dNTPs) and 0.5 U of Taq DNA polymerase (Invitrogen, CAS: 10342–046) in a total volume of 25 µL. Amplification was performed in a C1000 thermal cycler (Bio-Rad®, Germany) under the following conditions: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 95 °C, 58 °C for 30 s, 72 °C for 40 s and a final extension at 72 °C for 5 min. The PCR products were separated by agarose gel electrophoresis (2%) at 80 V for 40 min in 0.5 × Tris–acetate-EDTA (TAE) buffer and visualized with UV light after ethidium bromide staining. The products were subsequently purified and then sequenced by Macrogen (Seoul, South Korea).

Sequences were edited in BioEdit v 7.0.5.3 (Hall 1999) and compared to sequences in GenBank using the BLASTn algorithm. MEGA X (Kumar et al. 2018) was used for alignment and the phylogenetic analysis of each genus. Multiple sequence alignment was performed using the MUSCLE alignment program (Edgar 2004) implemented in MEGA X. The Akaike information criterion (AIC) was used for substitution model selection, and the phylogenetic trees were constructed using the maximum likelihood (ML) method. The *Argemone* tree was constructed using the Tamura-Nei model with among-site rate modeling (four gamma categories; TN93 + G). The General Time Reversible model with among-site rate modeling (four gamma categories) and invariant sites (GTR + G + I) was used for phylogenetic reconstruction of *Datura,* and *Amaranthus* phylogeny was constructed using the Tamura 3-parameter model with among-site rate modeling (four gamma categories; T92 + G). Tree topology support was assessed by 1000 bootstrap replicates. All trees were edited in FigTree (Rambaut 2010).

Preparation of aqueous extracts

A. mexicana, *D. discolor* and *A. palmeri* leaves were disinfested with sodium hypochlorite (1%; v/v), rinsed three times with distilled water, and dried for 19 h at 60 °C. Next, 4 g of leaf samples were ground and mixed with 40 ml of distilled water to obtain a 1:10 dilution of each extract. Subsequently, each extract was boiled at 120 °C for 10 min and centrifuged at 4,500 rpm for 10 min. Finally, the supernatant was recovered and stored at 4 °C until further use (Baka and Mousa 2020).

Pathogenicity test

In order to determine the pathogenicity of *C. gloeosporioides* (isolate FAVF355), six Persian lime fruits, at maturity stage, were first washed with neutral soapy water, rinsed with tap water, and then immersed in 70% ethanol for 3 min. This was followed by immersion in 1% NaCl for 5 min, and then rinsing three times with sterile distilled water. Subsequently, three wounds were made on each fruit with a sterile toothpick. The fruits were then inoculated with 0.5 mL of conidial suspension (1×10^6 conidia/mL), while control fruits were treated with 0.5 mL of sterile distilled water.

The fruits were placed in a humidity chamber, incubated at 28 °C with 95% relative humidity (Baka and Mousa 2020). Seven days post inoculation, pathogenicity was determined by measuring the diameter and the depth of the lesions. In order to fulfill Koch's postulates, fungal colonies were re-isolated from the lesions of inoculated fruits the morphology of the colonies and conidia were similar to the original isolates. The treatments were arranged in a completely randomized design, and the experiment was performed twice.

In vitro *assay*

In order to determine the in vitro effect of aqueous leaf extracts of *A. mexicana* (*Am*), *D. discolor* (*Dd*), and *A. palmeri* (*Ap*), extracts were diluted to a concentration of 1%, 2%, or 4% (v/v) in autoclaved potato dextrose agar (PDA; Bioxon, Cuautitlán Izcalli, Estado de Mexico, Mexico) at 45 °C. The medium was poured into 90-mm diameter Petri dishes. Next, one mycelial plug (5 mm in diameter) from an 8-day-old colony of *C. gloeosporioides* was transferred to the center of plates containing the different extract concentrations. An additional treatment consisting of the fungicide carbendazim (1 ppm) was included, and Petri dishes containing PDA without any plant extracts or fungicide were included as a control. Five replicates (i.e. five Petri dishes) were used per treatment and incubated at 28 °C, and the growth of the colony was recorded every 24 h. Experiments were concluded once mycelial growth in the control plates reached 90 mm in diameter. The percentage of inhibition (PI%) was calculated as $PI\% = [(C\text{-}T)/(C)] \times 100$, where C is the radius of the fungus in the control plate and T is the radius of the fungus in the presence of the extract or fungicide (Paneerselvam et al. 2012). The treatments were arranged in a completely randomized design, and the experiment was repeated once.

In vivo *assay*

Healthy detached Persian lime fruits uniform in size and comparable in color were used in the study. Six fruits were superficially disinfected and inoculated as in the pathogenicity test assay. Fifteen hours post inoculation with *C. gloeosporioides*, fruit were sprayed with 1%, 2% or 4% (w/v) aqueous leaf extract of *D. discolor* or carbendazim (1 ppm). The control fruits were sprayed with sterile distilled water (Koomen and Jeffries 1993). A second application of the treatment was performed seven days post inoculation.

The efficacy of the extract and fungicides was determined at seven- and fourteen-days post inoculation, by measuring the diameter of the lesion caused by the pathogen on the fruit surface as well as the depth of the lesions with a TRUPER® digital caliper (CALDI-6MP). The treatments were arranged in a completely randomized design, and the experiment was carried out twice.

qPCR quantification

Samples of the infected areas (1 cm^3 of damaged tissue) were taken from each fruit. Subsequently, genomic DNA was extracted using the CTAB method (Doyle 1990). DNA was quantified on a Thermo Scientific NanoDrop™ One Spectrophotometer (Thermo Fisher Scientific, USA). Three damaged areas from each fruit were pooled and considered as a single sample, and three fruits per treatment were assessed.

PCR products were amplified using a CFX96™ thermal cycler (Bio-Rad, Germany). The thermocycling program consisted of one cycle at 95 °C for 3 min followed by 50 cycles at 95 °C (30 s) and 60 °C (30 s). The reaction mixture in each well contained 10 µL of Supermix (SsoAdvanced Universal Probe; Bio-Rad, cat. no. 1725281), 6 µL of nuclease-free water, 1 µL to 2 µM of each primer/probe and 1 µL (10 ng) of DNA; a negative control and a healthy control sample were also included in each qPCR run. Three biological replicates with three technical replicates were made for each treatment. A concentration curve, extending from 0.01 to 100 ng, was made with DNA from *C. gloeosporioides*. The ColF3/ColR1/ColP1 primers used for pathogen detection (Rahman et al. 2019) are shown in Table 1. In addition, the COXfpr primers, based on the citrus mitochondrial cytochrome oxidase gene, were used as an endogenous control (Hu et al. 2013).

Table 1 Primers used in this study

Primer	Sequence (5´-3´)	Amplicon/target	References
ColTqF3	GCTTGGTGTTGGGGCCCTAC	127 pb/ ITS *C. gloeosporioides*	Rahman et al. 2019
ColTqR1	GGTTTTACGGCAAGAGTCCCT		
ColTqP1	[FAM]CCCTCCCGGAGCCTCCTTTGCGTA[BHQ]		
COXf	GTATGCCACGTCGCATTCCAGA	68 bp, COX *Citrus* spp.	Hu et al. 2013
COXr	GCCAAAACTGCTAAGGGCATTC		
COXpc	[FAM]ATCCAGATGCTTACGCTGG [BHQ]		

Shelf life of *D. discolor* aqueous extract

A shelf life test of the *D. discolor* aqueous extract was conducted using the most effective concentration from the in vitro tests (4% aqueous extract). The extract was stored at 4 °C, and the evaluations were carried out three times on a monthly basis. These experiments were performed in the same way as in the in vitro assay described above.

Statistical analyses

All data were subjected to normality tests: for the in vitro assay, data were analyzed by the Kruskal–Wallis and Conover tests. Data obtained from fruit and the shelf life of *D. discolor* aqueous extract were analyzed by one-way ANOVA, and mean separation was performed using Tukey's test (Little and Hills 1973) with a value of $\alpha = 0.05$. To allow for zeros values in some treatments, lesion data from artificially inoculated fruits were transformed by $\sqrt{x+1}$ as previously described (Gomez and Gomez 1984). All data were analyzed using SPSS software (IBM SPSS Statistics for Windows, version 25.0. IBM, Armonk, NY, USA).

Results

Molecular identification of weeds

The ITS sequences from weeds were compared to the NCBI database. Amaranth sample showed 100% identity with the *Amaranthus* sequence of *A. palmeri* (GenBank accession numbers KY968864 and KY968865), whereas the *Argemone* sequence had a 99.40–100% identity with *A. mexicana* voucher TuTY1485 (MH768272) and MIB:SASS 0108 (MZ489728); furthermore, the sequence of *Datura* displayed a 99.10–100% identity with *D. discolor* (GenBank accession numbers MG693017 and JX467605). Phylogenetic inference confirmed the identity of each weed as *A. mexicana* (81.3% bootstrap, Fig. 1a), *D. discolor* (100% bootstrap, Fig. 1b) and *A. palmeri* (95.5% bootstrap, Fig. 1c), since all clustered with the respective reference sequence of each botanical species with high bootstrap support.

In vitro *assay of the aqueous extracts*

The results of the in vitro tests revealed that the *Dd*-1%, *Dd*-2% and *Dd*-4% aqueous leaf extracts of *D. discolor,* respectively, reduced the mycelial growth of *C. gloeosporioides* by 52%, 55% and 73% relative to the control (Fig. 2). Furthermore, the efficacy of these extracts was not significantly different ($P = 0.05$) from that of carbendazim (1 ppm), which exhibited 86% mycelial inhibition. The aqueous extracts of *A. mexicana* (*Am*-1%, *Am*-2% and *Am*-4%) and *A. palmeri* (*Ap*-1%, *Ap*-2% and *Ap*-4%) did not display any significant differences in the growth reduction of the pathogen relative to the control (Table 2). Therefore, these extracts were not included in subsequent studies.

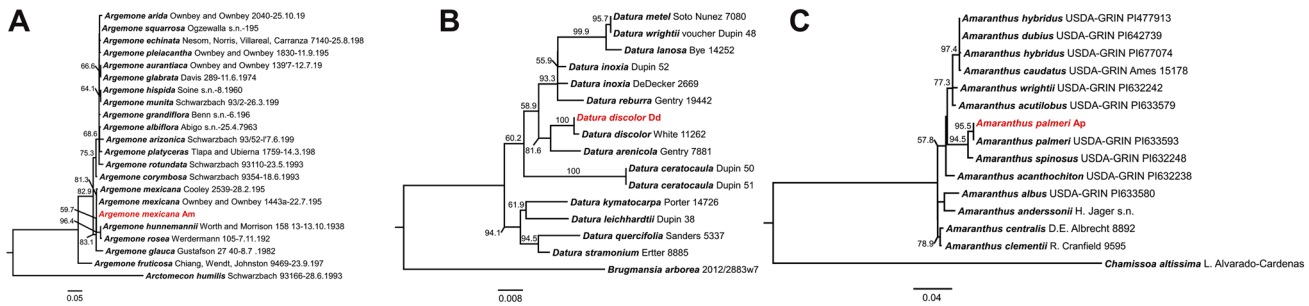

Fig. 1 Maximum likelihood trees based on the ITS region. Isolates characterized in this study are indicated in red bold font: **A** *A. mexicana*, **B** *D. discolor*, and **C** *A. palmeri*. Numbers at the branches indicate support values greater than 50%. Tree topology support was assessed by 1000 bootstrap replicates

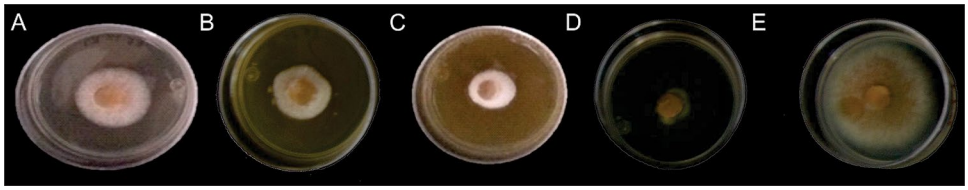

Fig. 2 Effect of aqueous extracts of *Datura discolor* on *C. gloeosporioides* growth. **A** *D. discolor* at 1%; **B** *D. discolor* at 2%; **C** *D. discolor* at 4%; **D** chemical control (carbendazim-1 ppm); **E** control

Table 2 In vitro inhibition of mycelial growth of *Colletotrichum gloeosporioides*. Treatments shown are for aqueous leaf extracts of three weeds and carbendazim

Treatment	Mycelial growth (cm)	Inhibition (%)*
Am-1%	3.1 ± 0.3	0[a]
Am-2%	3.2 ± 0.0	0[a]
Am-4%	3.4 ± 0.1	0[a]
Dd-1%	1.5 ± 0.1	52[bc]
Dd-2%	1.4 ± 0.5	55[bc]
Dd-4%	0.8 ± 0.0	73[bc]
Ap-1%	2.7 ± 0.4	11[ab]
Ap-2%	2.7 ± 0.1	13[ab]
Ap-4%	2.7 ± 0.2	13[ab]
Carbendazim-1 ppm	0.4 ± 0.3	86[c]
Control	3.1 ± 0.2	0[a]

*Means followed by different superscript letters are significantly different (P = 0.05; n = 6). Control = PDA without weed extract or fungicide; carbendazim-1 ppm = PDA + fungicide. For aqueous extracts, *Am* = *Argemone Mexicana*, *Ap* = *Amaranthus palmeri*, and *Dd* = *Datura discolor*; extracts were tested at different concentrations (1%, 2% and 4%)

Antifungal effect of the aqueous extract of *D. discolor* in Persian lime fruits

Fourteen days post inoculation and seven days after the second treatment of the fruits with *Dd*-1%, *Dd*-2%, *Dd*-4% and carbendazim-1 ppm (as well as the inoculated and non-inoculated controls), the lesion diameter ranged from 0.0 to 3.2 mm. The depth of the lesions in fruits treated with aqueous extracts and carbendazim-1 ppm varied from 1.9 to 5.2 mm. By contrast, the inoculated and non-inoculated controls presented lesions of 6.6 and 0.0 mm, respectively, with significant differences among the treatments ($P = 0.05$) (Fig. 3; Table 3).

qPCR quantification of *C. gloeosporioides*

The reduction of anthracnose in Persian lime fruit by *Dd*-4% was corroborated by the qPCR detection of fungus DNA copies. Concentration curve from 0.01 to 100 ng of pathogen DNA is shown in Fig. 4a. The amplification curve obtained in fruit samples infected with the pathogen (C +) was similar to the amplification of 10 ng of *C. gloeosporioides* (10 ng-*Cg*). Fruit samples treated with the chemical control carbendazim-1 ppm (Cc) and samples treated with the *Dd*-4% extract displayed a level of amplification similar to 0.01 ng of *C. gloeosporioides* (0.01 ng-*Cg*; Fig. 4b). The estimation of pathogen molecules revealed a smaller quantity in the Cc and *Dd*-4% treatments as compared to the control inoculated with the pathogen (Fig. 4c).

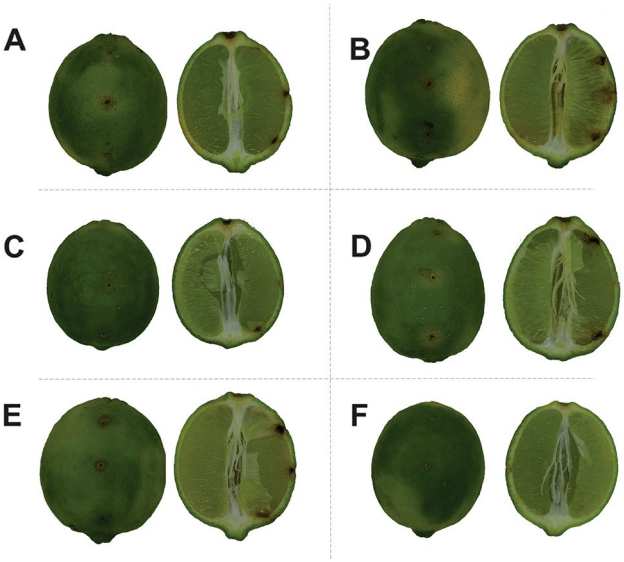

Fig. 3 Effect of *Datura discolor* extracts on surface and depth growth of *C. gloeosporioides* in Persian lime (*Citrus* × *latifolia*). **A** *D. discolor* at 1%; **B** *D. discolor* at 2%; **C** *D. discolor* at 4%; **D** chemical control (carbendazim-1 ppm); **E** fruit with pathogen; **F** fruit treated with water in the absence of the pathogen

Shelf life of *D. discolor* aqueous extract

In order to determine the shelf life of the *D. discolor* extract, in vitro experiments were performed on a monthly basis with the aqueous extract at the most effective concentration (*Dd*-4%). The extract was able to reduce the growth in vitro of *C. gloeosporioides* up to 3 months after its preparation (Table 4), but with less effectiveness. Fresh leaf extract inhibited mycelial growth by 73% as indicated above, whereas the inhibition effect of the *Dd*-4% treatment over

Table 3 Biocontrol activity of aqueous extracts of *Datura discolor* against anthracnose caused by *Colletotrichum gloeosporioides* on detached Persian lime fruits, 7 and 14 days after inoculation (dai)

Treatment	Lesion (mm)			
	7 dai		14 dai	
	Surface	Depth	Surface	Depth
Dd-1%	2.5 ± 0.3[b]	3.7 ± 1.3[b]	3.0 ± 0.3[c]	5.2 ± 2.4[cb]
Dd-2%	4.2 ± 1.5[b]	4.1 ± 0.7[b]	2.9 ± 0.4[c]	4.5 ± 1.1[cb]
Dd-4%	2.4 ± 0.3[b]	3.9 ± 0.3[b]	1.9 ± 0.3[b]	2.8 ± 0.2[ba]
Carbendazim-1 ppm	2.3 ± 0.5[ab]	2.1 ± 1.8[ab]	2.0 ± 0.1[b]	1.9 ± 1.3[ba]
Control inoculated	3.0 ± 1.3[b]	4.6 ± 0.9[b]	3.2 ± 0.6[c]	6.6 ± 2.8[c]
Control non-inoculated	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]

Data followed by different superscript letters in a column differ significantly according to Tukey's test ($p > 0.05$). *Dd* = *Datura discolor* aqueous extract at 1%, 2% and 4% concentrations

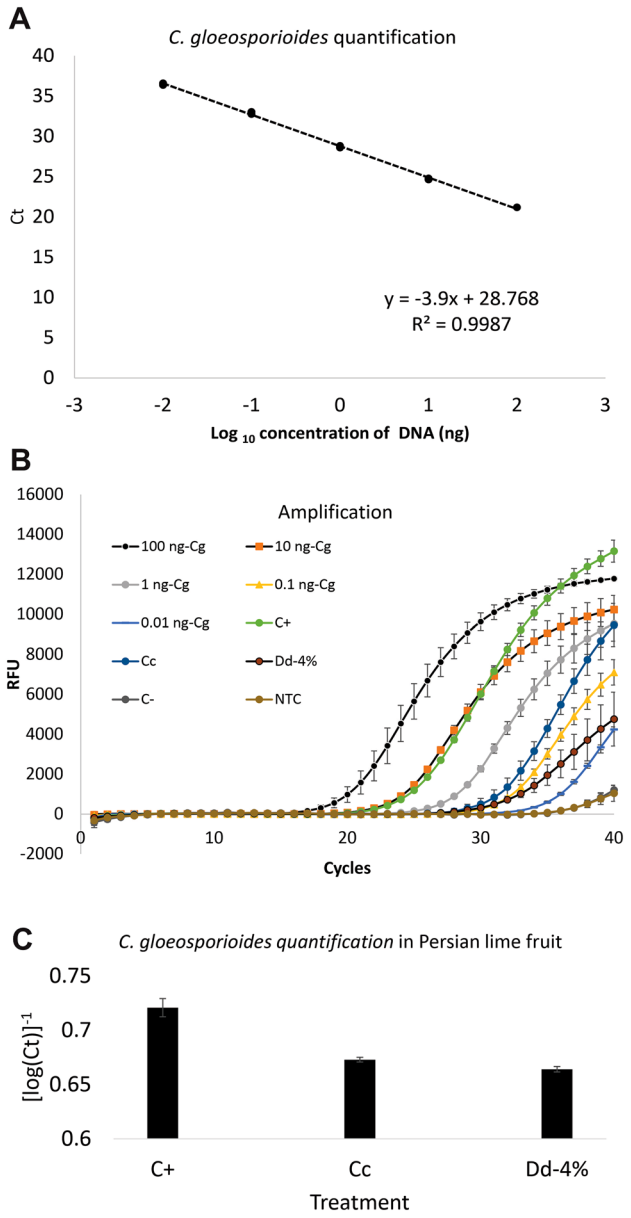

Fig. 4 Specific detection of *C. gloeosporioides* by qPCR. **A** Log starting quantity of *C. gloeosporioides* DNA (ng). **B** Amplification curves of qPCR reactions performed using the ColTqF1, ColTqR1 and ColTqP1 primers sets. Samples include pathogen DNA (with values ranging from 0.01 to 100 ng-*Cg*), fruit with pathogen (C+), fruit without pathogen (C−), fruit treated with fungicides (Cc), fruit treated with *D. discolor* at 4% (*Dd*-4%), and no template control (NTC). **C** Estimation of the amount of *C. gloeosporioides* molecules in the specified treatments of Persian lime fruits

time was 24.69% (1 month), 24.37% (2 months), and 28.08% (3 months).

Table 4 In vitro effect of aqueous extracts of *Datura discolor* on *Colletotrichum gloeosporioides* growth reduction (%) over time

Treatment	Period		
	Month 1	Month 2	Month 3
Dd-4%	24.69 ± 3.03[a]	24.37 ± 1.25[a]	28.08 ± 3.17[a]

Means are not significantly different according to Tukey's test ($p > 0.05$)

Discussion

Although the potential effect of weed leaf extracts against plant diseases has already been reported (Mushatq et al. 2012), research focused on this topic is still in its initial stages in Mexico. For this reason, the present study examined the antifungal potential of leaf extracts of *A. mexicana*, *D. discolor*, and *A. palmeri* against *C. gloeosporioides*, the causal agent of anthracnose in lime fruits. *Datura discolor* extracts significantly reduced mycelial growth of the fungus in vitro (52%, 55% and 73%; Table 2), whereas the extracts of *A. mexicana* and *A. palmeri* did not show any such effect.

Karim et al. (2017) evaluated the antifungal activity of methanolic extract from the leaves, seeds and roots of *D. metel* at 1.0, 1.5, 2.0, 2.5 and 3%. All concentrations significantly reduced the radial growth of *C. gloeosporioides*, although the seed extract at 1.5% showed the highest (80%) antifungal potential. In addition, leaf methanolic extracts of *D. stramonium* inhibited the mycelial growth of *C. gloeosporioides*, and the same extract also reduced spore germination by up to 15.7% (Alemu et al. 2014). Considering the results of the present study, further research should be focused on evaluating other *D. discolor* extracts, with special attention to different solvents (e.g. methanolic and ethanolic) and parts of the plant (e.g. roots and seeds). It would also be interesting to evaluate the effect of *D. discolor* extracts on the germination of pathogen conidia.

Previously, the aqueous extract of *D. stramonium* at 50% was shown to reduce anthracnose incidence and severity on postharvest mango fruit by 80% and 58.7%, respectively (Alemu et al. 2014). In this work, lesions on Persian lime fruit 14 days after inoculation reached a surface diameter of 3.2 mm and a depth of 6.6 mm in the control inoculated with the pathogen, while at the high treatment concentration (*Dd*-4%) the lesion had a surface diameter of 1.9 mm and a depth of 2.8 mm (Table 3). It is therefore possible that a greater reduction of anthracnose could be observed by increasing the concentration of the *D. discolor* extract. Furthermore, our molecular studies revealed that the amount of *C. gloeosporioides* DNA molecules was reduced when the Persian lime fruits were inoculated and sprayed with the 4% aqueous extract of *D. discolor* used to control anthracnose. This approach may also be followed to determine the effect

of different concentrations of aqueous extracts of *D. discolor* and other plants on disease control.

Species within the genus *Datura* are known to produce several phytochemical compounds with ethnopharmacological and antimicrobial characteristics (Cespedes-Mendez et al. 2021), and the antifungal activity of *D. discolor*, *D. metel* and *D. stramonium* has been evaluated. The methanolic and ethanolic extracts of *D. discolor* leaves and stems have been reported to inhibit the growth of *Aspergillus flavus* Link, *Aspergillus niger* P.E.L. van Tieghem, *Penicillium chrysogenum* Thom, *Penicillium expansum* Link, *Fusarium moniliforme* Sheldon, and *Fusarium poae* (Peck) Wollenw (Tequida-Meneses et al. 2002).

Datura discolor is a native plant and one of the most widely-distributed species in Mexico (Benítez et al. 2018), phytochemical studies of this species are scarce or null. But studies in *Datura* confirmed the presence of bioctive compounds as Daturilin a withanolide with antifungal activity (Choudhary et al. 1995; Kagale et al. 2004) found in *D. Ferox*, *D. metel*, *D. quercifolia*, and *D. stramonium* (Siddiqui et al. 1987; Kagale et al. 2004). Also, the accumulation of PR-proteins, the increase PAL activity and phenolic compounds have been observed in rice plants treated with leaf extract of *D. metel* and challenged with the pathogens *Rhizoctonia solani* and *Xanthomonas oryzae* (Kagale et al. 2004). Using GC–MS analysis, previous studies identified metabolites with antimicrobial activity such as n-hexadecanoic acid, phytol and octadecanoic acid, suggesting that the high antifungal activity of methanolic extract of *D. metel* might be due to the presence of these compounds (Karim et al. 2017; Hanif et al. 2022). These results suggest the possibility that aqueous leaf extracts of *D. discolor* might contain bioactive constituents with antifungal activity against *C. gloeosporioides*.

Knowledge of the shelf life of the aqueous leaf extracts is crucial for their use in plant disease management. The results in this study demonstrate that the in vitro inhibitory effect of *D. discolor* aqueous leaf extracts on *C. gloeosporioides* mycelial growth was maintained for up to 3 months. We also observed protective activity in Persian lime fruits when aqueous leaf extracts were applied two weeks post inoculation with the fungus in a 4% concentration at a seven-day interval.

Although aqueous leaf extract of *D. metel* at different concentrations (100, 200, 300, 400 and 500 µg/ml) has been reported causing deleterious effect on germination, growth and biochemical parameters in seedlings of *P. vulgaris* and *Z. mays* (Komolafe et al. 2021). In the present study, the fruits of Persian lime did not exhibit phytotoxicity after two weeks post application. This is the first report on the in vitro antifungal activity of *D. discolor* aqueous leaf extract against *C. gloeosporioides*. We also reported on the efficacy of this extract for controlling postharvest anthracnose caused by the same pathogen on Persian lime. Future research lines should focus on characterizing the bioactive compounds implicated in the antifungal activity in the aqueous extract of *D. discolor*.

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Author contribution X.E.V.C., J.C.M.A. and G.A.M.R. were involved in the conceptualization and design of the project, and drafted the manuscript; R.F.G. and S.P.D.C. revised and critically edited the manuscript; K.Y.L.M. performed the phylogenetic analysis to identify the weeds from which extracts were obtained; and C.R.I.S. and C.R.U. performed the statistical analysis and data interpretation. All authors have read and approved the manuscript.

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Data availability The authors declare that the data supporting the findings of this study are available within the article.

Code availability Not applicable.

Declarations

Conflicts of interest The authors declare no conflict of interest.

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