Mexican Journal of Phytopathology
ISSN: 2007-8080

Crossref
Open access

Phytopathological Note

In vitro antifungal activity of *Datura discolor* aqueous extracts obtained by High Pressure Processing

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Section:
Special Number

Received:
05 May, 2024
Accepted:
22 September, 2024
Published:
24 October, 2024

Citation:
Urias-Lugo DA, Martínez-Ereva OE, Romero-Urías CA and Ibarra-Sarmiento CR *et al.*, 2024. *In vitro* antifungal activity of *Datura discolor* aqueous extracts obtained by High Pressure Processing. Mexican Journal of Phytopathology 42(4): 35. https://doi.org/10.18781/R.MEX.FIT.2024-01

ABSTRACT

Background/Objective. The present work reports the *in vitro* effect of aqueous extracts (2, 4 and 6% w/v) of root, seed and leaf of *Datura discolor* obtained in two times (3 and 6 minutes) at High Pressure Processing, against *Sclerotium rolfsii*, *Sclerotinia sclerotiorum* and *Colletotrichum gloeosporioides*.

Materials and Methods. Extracts of roots, seeds and leaves of *D. discolor* were prepared in a 1:10 w/v ratio with distilled water. Two continuous treatments of high pressure (600 MPa) with pressure maintained for 3 min and another with pressure (600 MPa) maintained for 6 min were considered. The extracts were evaluated against *S. rolfsii*, *S. sclerotiorum* and *C. gloeosporioides*. The experiments were performed in Petri dishes with PDA medium. The efficiency of the extracts was evaluated by obtaining the percentage of inhibition.

Results. The results show variable percentages of inhibition of the extracts in the different anatomical parts of the plant and concentrations; The leaf extracts at 6%, regardless of the extraction time, show effectiveness against the three pathogens, with inhibition of 99 and 100%**,** 55 and 56%, and 43 and 36% for *S. rolfsii*, *S. sclerotiorum* and *C. gloeosporioides* at 3 and 6 minutes respectively**.**

Conclusion. The effectiveness of leaf extract at 6%, six months after its preparation, is similar to the observed with fresh extracts. These results pave the way for future research focused on the sustainable management of phytopathogens. Studies on the biological effectiveness of the extracts in the greenhouse and field are suggested.

Keywords: biological control, fungi, phytopathogen, antifungal activity.

Introduction

Sclerotium rolfsii, which causes root and stem rot, is a polyphagous pathogen reported in tropical and subtropical areas (Gholami *et al*., 2019). *Sclerotinia sclerotiorum* is a widely distributed and non-specific pathogen (Ordóñez-Valencia *et al*., 2018). *Colletotrichum gloeosporioides* causes anthracnose in leaves, flowers and citrus buds (Guarnaccia *et al*., 2017). The three phytopathogens are associated with significant losses in economically important crops, and their management is based on the use of chemical treatments.

The use of plant extracts with antimicrobial activity is of interest as part of the sustainable strategies to manage diseases in agricultural crops (Verdugo-Contreras *et al*., 2022). Methanolic, ethanolic and aqueous *Datura discolor* extracts have been evaluated *in vitro* against *Aspergillus flavus*, *A. niger*, *Penicillium chrysogenum*, *P. expansum*, *Fusarium moniliforme, F. poae* (Tequida-Meneses *et al*., 2002) and *C. gloeosporioides* (Verdugo-Contreras *et al*., 2022).

The traditional methods for the extraction of phytochemical compounds are based mainly on the use of organic solvents and thermal treatments in which the plant material is exposed to high temperatures for extended periods, which may cause the loss and degradation of thermolabile compounds with biological activity (Zhang *et al*., 2018). Strategies for the aqueous extraction of plant compounds through non-thermal High Pressure Processing (HPP) have gained attention in the pharmaceutical and food industries due to their high impact on the modification of the cell structure and the recovery of bioactive compounds, associated to the destruction of cell walls and other structural barriers (Le-Tan *et al*., 2022). However, no evaluations have been carried out on the effect of plant extracts obtained by HPP against plant pathogens.

The objective of this work was to determine the *in vitro* effect of aqueous *Datura discolor* extracts obtained by HPP against *Sclerotium rolfsii*, *Sclerotinia sclerotiorum* and *Colletotrichum gloeosporioides,* causal agents of soft rot, white mold and anthracnose, respectively.

Obtaining the extracts. The experiment used *D. discolor* roots, seeds and leaves, with identity confirmed molecularly by Verdugo-Contreras *et al*. (2022). The plant material was disinfested with a 1% v/v sodium hypochlorite solution, rinsed three times with distilled water and dried at 60 °C during 19 hours for its subsequent pulverization. The extracts were prepared from each anatomical part, the mixture of the plant material was prepared at a proportion of 1:10 p/v in distilled water, poured into polyethylene terephthalate (PET) with a high-density polyethylene (HDPE),

double seal cap and exposed to a high-pressure treatment at room temperature. Two continuous, high-pressure treatments were considered; one at 600 MPa maintaining this pressure for 3 min, and another at 600 MPa with the pressure maintained for 6 min. For HPP, the Hiperbaric 55 equipment with a 55-liter basket (14.5 gal) with a diameter of 200 mm (7.9 in) was used, with an integrated intensifier and a power of 62 KW. Subsequently, the samples submitted to HPP were centrifuged for 10 min at 4000 rpm and the supernatant was extracted. The extracts were stored at 4 °C until use.

Antifungal *in vitro* evaluation. The effect of the aqueous extracts of *D. discolor* leaves was evaluated one day after the processing against *Sclerotium rolfsii*, *Sclerotinia sclerotiorum* and *Colletotrichum gloeosporioides*, which were previously molecularly identified, and its pathogenicity was confirmed (Martínez-Álvarez *et al*., 2021; Martínez-Ereva, 2022; Pérez-Mora *et al*., 2021). The experiments were carried out in Petri dishes with PDA (Bioxon, Cuautitlán Izcalli, State of Mexico, Mexico); the extracts were diluted to a concentration of 2, 4 and 6% v/v in the culture medium. In the center, a 5 mm PDA plug with mycelium from the fungus with 5 days old was placed. An additional treatment was included, which consisted of the fungicide at 1 ppm (tebuconazole for *S. rolfsii* and *S. sclerotiorum* and carbendazim for *C. gloeosporioides*), Petri dishes containing PDA without any plant extracts or fungicide were included as a control. The petri dishes were incubated at 25 °C, the growth of the pathogens was recorded, the experiments were concluded once mycelial growth in the control plates reached the edge. The percentage of inhibition (PI%) was calculated as $PI\,\% = \frac{C\text{-}T}{C}\ x\ 100$, where C is the radius of the fungus in the control dish and T is the radius of the fungus in the presence of the extract or the fungicide (Paneerselvam *et al*., 2012). The experiments were conducted in a completely randomized arrangement with four repetitions per treatment; the experiments were conducted twice.

The shelf life of the extract with the most efficient concentration observed in the *in vitro* tests was evaluated (6% leaf extract). The extract was kept at 4 °C and the evaluation of biological effectiveness was carried out six months after it was obtained. These experiments were carried out in the same way as the *in vitro* test described earlier.

Statistical analysis. The data were subjected to the Shapiro-Wilk normality test and the non-parametric Kruskal-Wallis and Mann-Whitney, with a value of $p<0.05$. To allow values of zero in some treatments, the data were transformed with $\sqrt{x} + 1$ (Gomez and Gomez, 1984).

The results of the *in vitro* tests displayed variable percentages of mycelial growth inhibition of in *Sclerotium rolfsii*, *Sclerotinia sclerotiorum* and *Colletotrichum gloeosporioides,* by aqueous extracts of *D. discolor* obtained by (HPP) from the

different anatomical parts of the plants and concentrations. *Datura* species have been documented to have antifungal activity. However, this effect varies according to the concentration and types of solvents, as well as to the part of the plant from which they are obtained (Öz, 2017).

In *S. rolfsii*, inhibition fluctuated between 25 and 53%, 25 and 45% and between 77 and 100% with the root, seed and leaf extracts, respectively, in comparison with the control with no extract, with differences between treatments ($p < 0.001$). The efficiency of the 4 and 6% leaf extracts, regardless of the time of extraction, was not significantly different to the treatment with tebuconazole at 1 ppm which inhibited the growth of *S. rolfsii* by 100% (Table 1; Figure 1 A-D).

Table 1. *In vitro* inhibition of the mycelial growth of *Sclerotium rolfsii*, *Sclerotinia sclerotiorum* and *Colletotrichum gloeosporioides*. The treatments shown are with aqueous extracts of 2, 4 and 6% *Datura discolor* root, seed and leaf, obtained by High Pressure Processing in two times 3 and 6.

Treatment		Inhibition (%)		
		S. rolfsii	*S. sclerotiorum*	*C. gloeosporioides*
Root	2%-3	25 ^{BC}	0 ^{B}	0 ^{BC}
	2%-6	25 ^{BC}	0 ^{B}	0 $^{A-C}$
	4%-3	46 ^{B}	0 ^{B}	0 ^{C}
	4%-6	38 ^{B}	0 ^{B}	0 ^{BC}
	6%-3	50 ^{B}	0 ^{B}	0 ^{BC}
	6%-6	53 ^{B}	0 ^{B}	0 ^{C}
	CQ	100 ^{A}	72 ^{A}	78 ^{A}
	Control	0 ^{C}	0 ^{B}	0 ^{B}
Seed	2%-3	25 ^{CD}	0 ^{C}	56 ^{BC}
	2%-6	25 ^{CD}	0 ^{BC}	50 ^{BC}
	4%-3	37 ^{BC}	0 ^{C}	58 ^{AB}
	4%-6	41 ^{BC}	0 ^{C}	59 ^{AB}
	6%-3	45 ^{AB}	0 ^{C}	55 ^{BC}
	6%-6	45 ^{AB}	0 ^{C}	64 ^{AB}
	CQ	100 ^{A}	72 ^{A}	78 ^{A}
	Control	0 ^{D}	0 ^{B}	0 ^{C}
Leaf	2%-3	77 ^{B}	0 ^{C}	56 ^{B}
	2%-6	79 ^{B}	2 ^{BC}	46 ^{B}
	4%-3	97 ^{A}	15 ^{B}	43 ^{B}
	4%-6	96 ^{A}	50 ^{A}	39 ^{B}
	6%-3	100 ^{A}	55 ^{A}	43 ^{B}
	6%-6	99 ^{A}	56 ^{A}	36 ^{B}
	CQ	100 ^{A}	72 ^{A}	78 ^{A}
	Control	0 ^{C}	0 ^{BC}	0 ^{C}

Percentages with normal letters in superscripts for every anatomical part of the plant are not significantly different (P = 0.05; n = 8). Control = the pathogen in PDA without extract or fungicide; *CQ* = the pathogen in PDA + tebuconazole or carbendazim at 1 ppm.

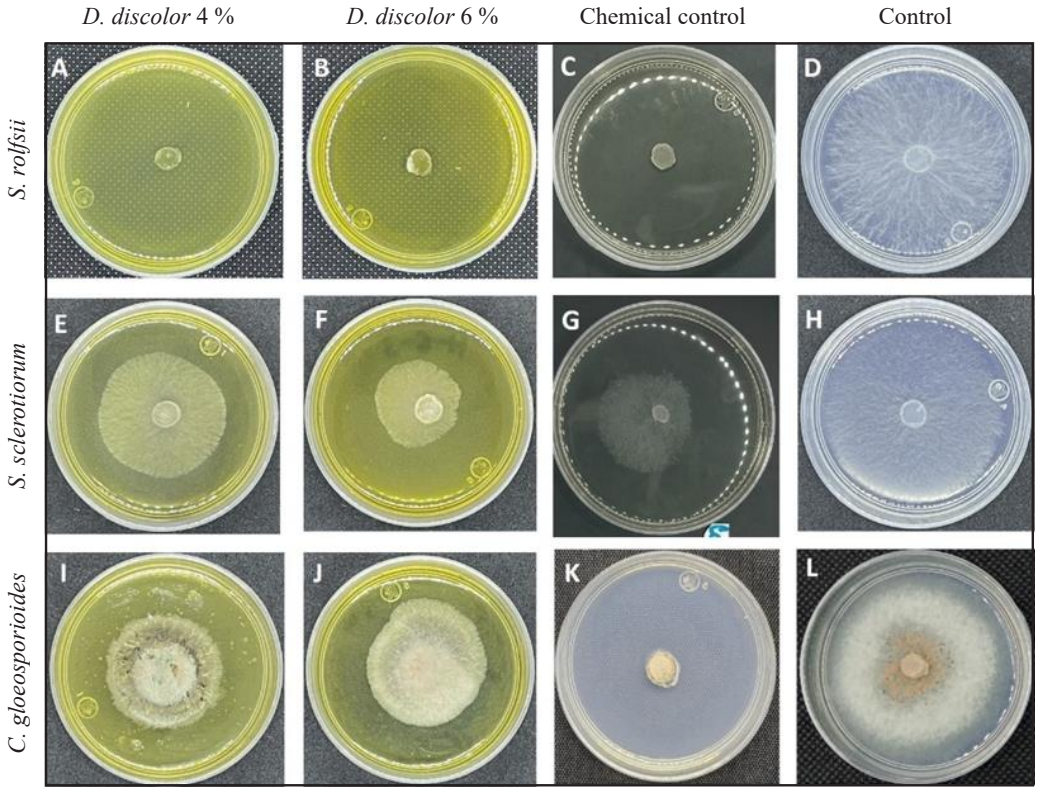

Figure 1. Effect of *Datura discolor aqueous* leaf extract obtained High Pressure Processing for 6 minutes. **A-D** *Sclerotium rolfsii*, **E-H** *Sclerotinia sclerotiorum*, **I-L** *Colletotrichum gloeosporioides.* Chemical control = tebuconazole or carbendazim 1 ppm).

An *in vitro* study carried out with a *D. metel* fruit methanolic extract at different concentrations (0.5 to 4.0%) displayed a significant reduction of the biomass of *S. rofsii* from 69 to 94% (Jabeen *et al*., 2014). In addition, Jabeen *et al*. (2022) evaluated the efficiency of the methanolic extract of *D. metel* leaves for the *in vitro* growth control of *S. rolfii*. The extract concentrations that fluctuated between 0.5 and 4.0% significantly controlled the growth of the pathogen from 29 to 88 % over the control. The results with aqueous leaf extracts obtained by HPP in this study surpass those reported with methanolic extracts from *D. metel* fruits and leaves.

No reduction was observed in the mycelial growth of *S. sclerotiorum* in any of the treatments with root and seed extracts, nor with 2% leaf extracts, whereas in the 4 and 6% leaf extracts, inhibition fluctuated between 15 and 56%. The efficiency of the leaf extracts at 4% (with 6 min) and 6% (in 3 and 6 min) was not significantly different to the treatment with tebuconazole at 1 ppm that inhibited 72% of the growth of *S. sclerotiorum* (Table 1; Figure 1 E-H).

Previous studies indicate that the aqueous *D. metel* extract inhibited *S. sclerotiorum* in comparison with the control (Sharma *et al*., 2015). The ethanolic *D. inoxia* leaf extract, which has a higher concentration of secondary phytochemicals and total flavonoids in comparison with the aqueous extracts displayed an inhibition of 100% of the mycelial growth of *S. sclerotiorum* in all the concentrations evaluated in comparison with the control, whereas the aqueous extract displayed inhibition from 29 to 94%, with the highest inhibition shown in the concentrations of 1600 and 2000 µg 100 mL^{-1} (Matías *et al*., 2020). Roy *et al*. (2021) evaluated three concentrations (5, 10 and 15%) of aqueous *D. stramonium* extracts *in vitro* against *S. sclerotiorum* and they reported percentages of inhibition of 28, 31 and 37%, respectively. Additionally, Kewate *et al*. (2020) reported a 28% inhibition of *S. sclerotiorum* with powdered extracts of *D. stramonium* at 10% in an *in vitro* test. The results reported by Matías *et al*. (2020) surpass the inhibition reported in our study for aqueous *D. discolor* leaf extracts obtained by HPP. However, the latter are higher than those reported for *D. stramonium* against *S. sclerotiorum*.

In the present study, the root extracts displayed no mycelial growth inhibition in *C. gloeosporioides*, whereas the seed extracts inhibited it by 50 to 64% and the leaf extracts, by 36 to 56%. The efficiency of the seed extracts was not significantly different to the treatment with carbendazim 1 ppm, which inhibited the growth of *C. gloeosporioides* by 78%. Although less efficient than the treatment with carbendazim 1 ppm, the leaf extracts significantly inhibited the pathogen regarding the control without an aqueous extract (Table 1, Figure 1 I-L).

Karim *et al*. (2017) evaluated the antifungal activity of methanolic *D. metel* leaf, seed and root extracts at concentrations of 1.0, 1.5, 2.0, 2.5 and 3.0%. All concentrations significantly reduced the radial growth of *C. gloeosporioides*. The 1.5% seed extract displayed the highest inhibition rate (80%). Similarly, *D. stramonium* leaf extracts obtained with methyl acetate and with methanol significantly inhibited the mycelial growth of *C. gloeosporioides* (Alemu *et al*., 2014). It was previously reported that aqueous *D. discolor* leaf extracts (at 1, 2 and 4% p/v) inhibited the mycelial growth of *C. gloeosporioides* by 52-73%, without significant differences with the treatment with carbendazim at 1 ppm (Verdugo-Contreras *et al*., 2022). The aqueous extracts of *D. discolor* leaves at 2, 4 and 6% obtained at high pressures inhibited the pathogen by 36 to 56%, whereas the seed extracts inhibited it by 50 to 64 % (Table 1). That is, the percentages of inhibition observed for *C. gloeosporioides* are greater with seed extracts, as reported by Karim *et al*. (2017). The percentages of inhibition with aqueous *D. discolor* leaf extracts against *C. gloeosporioides* report by Verdugo-Contreras *et al*. (2023) are greater than those reported in this study, which may be due to differences in the concentrations and types of bioactive compounds in the extraction processes, with the ability to inhibit *C. gloeosporioides*.

Methanolic and ethanolic extracts from the stem and leaves of *D. discolor* inhibited the mycelial growth of *Aspergillus flavus*, *A. niger*, *Penicillium chrysogenum*, *P. expansum*, *Fusarium moniliforme* and *F. poae* (Tequida-Meneses *et al*., 2002). The extracts with solvents such as ethanol, methanol and methyl acetate may, in some cases, display greater effectiveness against pathogens. Although it may be interesting to evaluate the effect of *D. discolor* extracts with solvents such as ethanol and methanol against *S. sclerotiorum* and *C. gloeosporioides*, alternatives are sought with the use of clean technologies.

Sharma *et al*. (2015) reported that the aqueous *D. metel* extract was effective against *S. sclerotiorum*, but not against *Rhizoctonia solani* or *Fusarium oxysporum*. The *D. inoxia* aqueous leaf extract inhibited *S. sclerotiorum* but did not affect the growth of *Fusarium solani* (Matias *et al*., 2020). *D. discolor* aqueous leaf extracts obtained by HPP inhibited *S. rolfsii*, *S. sclerotiorum* and *C. gloeosporioides*, displaying an antifungal ability of the extract with wide spectrum.

On the other hand, except for the 4% leaf extract treatment against *S. sclerotiorum*, no differences were observed in the inhibition of the different pathogens related to the extraction times, that is, extracts obtained by high pressures with a duration of 3 or 6 minutes of the process. This may be considered in subsequent evaluations against other pathogens, considering the 6% leaf extract, subjected to HPP for 3 minutes, which helps to streamline the process.

Despite the scarcity of *D. discolor* phytochemical studies, whithanolides datudiscolides A (8) and B (9) (González *et al*., 2023) have recently been isolated from this species. Additionally, the presence of whithanolides with antifungal activity has been confirmed in *D. ferox*, *D. metel*, *D. quercifolia* and *D. stramonium* (Siddiqui *et al.,* 1987; Kagale *et al*., 2004), therefore the inhibition in the growth of the evaluated phytopathogens could be attributed to whithanolides present in *D. discolor*, although additional studies are required to confirm this hypothesis. Phytochemical studies performed on other species report that the main secondary metabolites for the *Datura* genus are terpenoids, flavonoids, whithanolides, tannins, phenolic compounds, steroids and fatty acids (Céspedes-Méndez *et al*., 2021). The antifungal potential of *D. inoxia* leaf extracts against *S. sclerotiorum* have been related to the abundance of alkaloids and phenolic compounds (Matías *et al*., 2020). Conducting a phytochemical screening of *D. discolor* to complement research on its antifungal activity is relevant.

The 6% leaf extract evaluated six months after its processing reduced the *in vitro* growth of *S. rolfsii*, *S. sclerotiorum* and *C. gloeosporioides* by 99, 54 and 39%, respectively. The percentages observed are similar to those found in the evaluations with the fresh extract. Verdugo-Contreras *et al.* (2022) reported that the aqueous extract of the *D. discolor* leaf at 4% obtained by maceration with heat managed to reduce the *in vitro* growth of *C. gloeosporioides* up to 3 months

after its preparation, only less effectively. This suggests greater stability of the bioactive compounds obtained through HPP processes with effectiveness against the pathogens evaluated.

The 6% leaf extracts, regardless of the extraction time, show antifungal activity against the three fungi and their effectiveness remains active for at least 6 months. Future lines of research should focus on characterizing the bioactive compounds implied in the antifungal activity of the *D. discolor* aqueous extracts obtained through HPP, as well as in greenhouses and field studies to determine the potential of such extracts for the control of the phytopathogens evaluated *in vitro*.

Acknowledgments

The authors wish to thank IDEAS DE BERENJENA S.A. de C. V. for providing their facilities for the use of the hyperbaric chamber (Hiperbaric 55) and to the PIFIP-2022 Program of the Dirección de Investigación y Posgrado of the Universidad Autónoma de Occidente for the funding provided.

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