

Technical Note

IN VITRO INHIBITION OF *Fusarium* spp. ISOLATED FROM BEAN CROP USING *Argemone ochroleuca* EXTRACT

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ABSTRACT

Species of the genus *Fusarium* are the main cause of root rot in bean crops, resulting in yield losses of up to 50%. Synthetic fungicides are the primary tools for their control; however, they cause environmental damage. The objective of this study was to evaluate the effect of extracts from *A. ochroleuca* on *Fusarium* spp under *in vitro* conditions. During the spring-summer 2022 growing season, plants exhibiting symptoms of wilting and vascular browning were collected. The isolated fungi were purified through monosporic cultures and hyphal tip techniques, and their morphological features were characterized. To determine growth inhibition, a poisoned medium methodology was employed, evaluating two concentrations of extracts (13 and 23 %) in both ethanol and aqueous mediums. The obtained isolates corresponded to the morphological characteristics of *Fusarium*. The extract volumes that showed 100 % inhibition were the 23 % concentration in both ethanol and aqueous mediums. The 13 % ethanol extract exhibited inhibition ranging from 29 to 62 % between the first and tenth day of incubation. The 13 % aqueous extract displayed the lowest inhibition (25 to 43 %). The *Argemone ochroleuca* extract demonstrates antifungal properties against *Fusarium* spp. isolated from beans.

Additional keywords: Papaveraceae, plant extract, root rot

RESUMEN

Inhibición *in vitro* de *Fusarium* spp. aislado de cultivo de frijol con extracto de *Argemone ochroleuca*.

Especies del género *Fusarium* son la principal causa de la pudrición en la raíz del cultivo de frijol y causan pérdidas hasta del 50% en el rendimiento. Los fungicidas sintéticos son las principales herramientas para su control; sin embargo, estos causan daños al ambiente. El objetivo fue evaluar el efecto de extractos de *A. ochroleuca* en *Fusarium* spp. en condiciones *in vitro*. Durante el ciclo agrícola primavera-verano 2022 se recolectaron plantas con síntomas de secadera de marchitamiento vascular. Los hongos aislados se purificaron mediante cultivos monospóricos y por punta de hifa, y los aislados fueron caracterizados morfológicamente. Para determinar la inhibición del crecimiento, se utilizó la metodología de medio envenenado, donde se evaluaron dos concentraciones de extractos (13 y 23 %) en medio etanólico y acuoso. Los aislados obtenidos concordaron con características morfológicas de *Fusarium*. Los volúmenes de extracto que mostraron una inhibición del 100 % fueron las concentraciones del 23 % en medio etanólico y acuoso. El extracto etanólico al 13 % mostró una inhibición del 29 al 62 % entre el primer y décimo día de incubación, mientras que el extracto acuoso al 13 % mostró una inhibición menor (25-43 %). Se concluye que el extracto de *Argemone ochroleuca* presenta propiedades antifúngicas contra *Fusarium* spp. aislado del frijol.

Palabras clave adicionales: Extracto vegetal, Papaveraceae, pudrición de raíz

INTRODUCTION

Plant diseases caused by phytopathogenic fungi represent a significant threat to agriculture and food security worldwide. Among the most devastating pathogenic fungi are species of the genus *Fusarium*, known for their ability to infect a wide range of crops (Cota et al., 2023), including *Phaseolus vulgaris* (Benchimol et al., 2023).

Fusarium solani and *F. oxysporum* are recognized as the main root pathogenic species within the *Fusarium* genus. *F. solani* is the primary cause of root rot, while *F. oxysporum* causes bean yellowing (de Carvalho et al., 2022). In Mexico, the high frequency of *Fusarium* could be attributed to the practice of seed reuse by farmers. Additionally, there is a suggestion of the existence of multiple strains and subtypes of

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Fusarium, displaying morphological, pathogenic, and genetic variability within the populations (Martínez et al., 2014). Additionally, the state of Guanajuato records a high incidence of this genus of pathogenic fungi (Rangel et al., 2017; Juárez et al., 2021).

F. oxysporum is associated with wilting and yellowing of plants during flowering and grain filling stages, with this species invading the plant's root system from early stages and causing symptoms during the reproductive stages of the crop resulting in production losses (Guido et al., 2021).

To control these pathogens, chemical compounds such as triazoles (Li et al., 2022) are used, including tebuconazole, propiconazole, and epoxiconazole, which can be toxic to beneficial organisms, birds, aquatic organisms, especially when used in high concentrations or in locations near water bodies (Roman et al., 2021). In the long term, they can also cause resistance to fungal diseases (de Chaves et al., 2022).

Therefore, in recent years, there has been a growing interest in the use of natural products for plant disease control as an alternative to conventional chemical pesticides (Seepe et al., 2021). In this context, plants of the genus *Argemone* are used for pest and disease control in the agricultural sector (Hernández et al., 2020). Therefore, the objective of this study was to evaluate the in vitro inhibitory activity of *Argemone ochroleuca* extracts on the growth of *Fusarium* spp. isolated from bean crops.

MATERIALS AND METHODS

Morphological characterization of the fungus. During the spring-summer 2022 agricultural cycle in the town La Labor, municipality of Apaseo el Grande, Guanajuato Mexico (20.528° N, 100.694° W), five bean plants

of the Marcela variety (Flor de junio) were collected, showing symptoms of vascular wilting, sparse foliage, thin stems, and chlorosis (Figure 1a). Upon examining the roots, a reddish-brown discoloration was observed (Figure 1b).

For the classification of the *Fusarium* genus, the mycelial growth and pigmentation of the culture medium were observed. Temporary preparations were used to characterize microconidia, macroconidia, and chlamydoconidia. The cultural and morphological characteristics were compared with the descriptions by Nelson et al. (1983). One isolate was selected from each location and plated on Carnation Leaf Agar (CLA) medium (HYCEL), 20 g·L⁻¹ in distilled water.

Preparation of *Argemone ochroleuca* extract. Glaucous herbaceous plants with yellow latex; stems equipped with straight, whitish, widely spaced thorns of variable length, perpendicular to the surface, were collected in Irapuato, Guanajuato (latitude 20.723° N; longitude 101.338° W) and identified as *Argemone ochroleuca* based on the botanical description by Rzedowski and Rzedowski (2001).

The protocol described by Juárez et al. (2020) was followed, which involved weighing 500 g of fresh plant material, cutting the plant into 2 cm sections along the main axis, pre-washing the material with a 0.5% sodium hypochlorite solution for 2 minutes, and then rinsing it four times with distilled water. The plant material was divided into two parts to prepare an aqueous extract (250 ml of distilled water) and an ethanolic extract (250 ml of 96% ethanol), which were ground in a six-blade blender for 20 minutes at a temperature of 20 °C. Each obtained mixture was filtered using four layers of sterile gauze, followed by sterile filter paper for the final filtration. The total extract obtained was placed in amber glass bottles and refrigerated at 4°C for seven days until use.

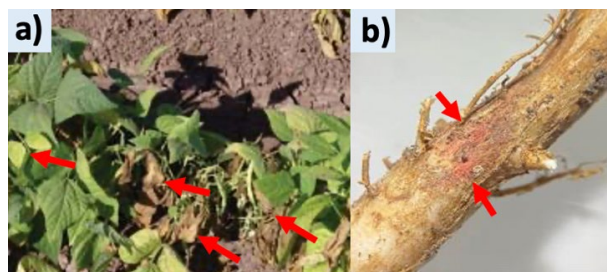


Figure 1. Marcela bean plants with symptoms of vascular wilting.

Assessment of radial growth inhibition. The evaluation of extract activity was performed by measuring the growth halo of the fungus compared to the negative control. Potato Dextrose Agar (PDA, BD Bioxon) was prepared according to the manufacturer's instructions, with 39 g·L⁻¹ distilled water, sterilized at 121 °C for 15 minutes; pH was adjusted to 5.0 with tartaric acid before pouring 20 mL of PDA into Petri dishes containing the concentrations of *A. ochroleuca* extract from the four treatments (13 and 23 % ethanol extract, and 13 and 23 % aqueous extract). The plates were allowed to solidify and then inoculated with 3 mm diameter circles of eight-day-old phytopathogenic fungal cultures. The circles were placed in the center of the dish and incubated at 26 ± 1°C. To evaluate the inhibition of fungal growth, the diameter of the mycelial growth was measured with a caliper at three, six, nine, and 12 days after inoculation. Three replicates were performed for all concentrations, including controls.

The percentage of growth inhibition was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{control mycelial } \emptyset - \text{treatment mycelial } \emptyset}{\text{control mycelial } \emptyset} \times 100$$

The growth data of halos were analyzed through a completely randomized experimental design using ANOVA and mean comparison by DMS test from SAS-JMP 16 software (Cary, NC, USA). Each replication consisted of four Petri dishes per assessment.

RESULTS

The identification of *F. oxysporum* in bean plant collections was carried out through the observation of cultural and morphological characteristics, which matched the descriptions by Nelson et al. (1983). The colonies initially exhibited a pink-violet color that evolved into violet tones. Additionally, ovoid and hyaline microconidia were observed, produced on short phialides, along with slightly curved macroconidia with 3 to 5 septa, ranging in length from 21 to 43 µm and a width of 3 to 5 µm. The chlamydospores were located terminally or intercalary (Figure 2)

The radial growth of the fungus was observed in the control group and in the treatments with a concentration of 13% aqueous and ethanolic extract of *A. ochroleuca*. The treatments with a concentration of 23% (aqueous and ethanolic extract) did not show any fungus development from day one to day ten of evaluation. It was not until day 11 that they exhibited growth (Figure 3).

The concentration of the extract had a statistically significant effect on fungal growth, resulting in different groups. From day one to day ten, three different groups were formed, with the greatest effects observed when using concentrations of 23 %, regardless of the aqueous or ethanolic medium. Similarly, the concentrations of 13 % were statistically equal within that time range. From day thirteen onwards, five statistically different groups were formed, with the treatment using 23 % ethanolic extract showing the highest effect and the treatment using 13 % aqueous extract showing the lowest effect (Table 1).

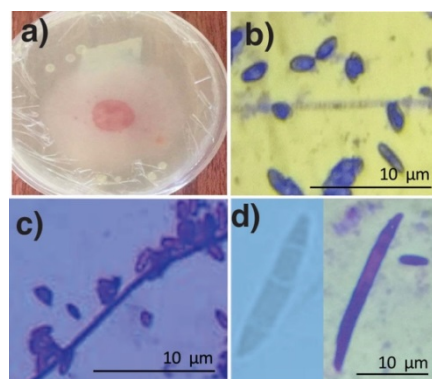


Figure 2. Characteristics of: a) colonies and morphological structures of *Fusarium* spp., b) chlamydospores, c) mycelium, d) microconidia.

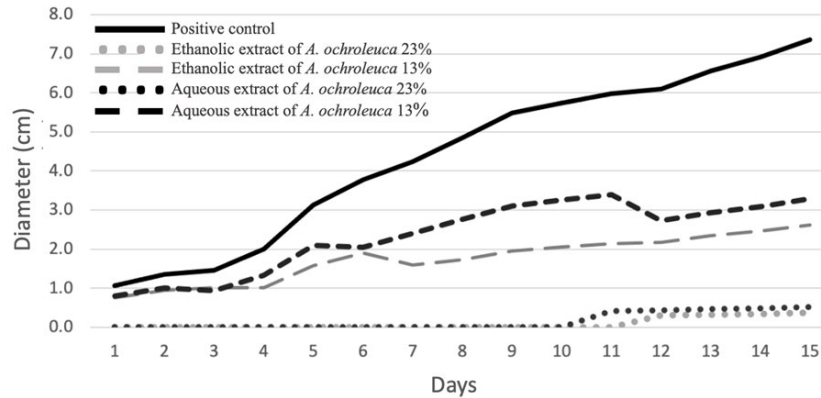


Figure 3. Radial growth curve in *F. oxysporum* treatments

Table 1. Multiple mean comparison for *Fusarium* growth halos (centimeters) according to different concentrations of *A. ochroleuca* extract.

Treatment	Percentage of inhibition in <i>F. oxysporum</i>							
	1	3	5	7	9	11	13	15
Testigo	1.06a	1.45a	3.13a	4.24a	5.47a	5.97a	6.55a	7.35a
EE 23%	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	0.32d	0.36d
EE 13%	0.75b	1.02b	1.57c	1.58b	1.95b	2.13b	2.33c	2.62c
EA 23%	0.00c	0.00c	0.00c	0.00c	0.00c	0.41c	0.45d	0.51d
EA 13%	0.79b	0.92b	2.08b	2.39b	3.11b	3.39b	2.92b	3.28b

EA= Aqueous extract of *A. ochroleuca*, EE = Ethanol extract of *A. ochroleuca*. Different letters in the same column indicate significant differences according to DMS test ($P \leq 0.05$). Each value corresponds to the average of three repetitions

DISCUSSION

The characteristics of the isolated strains presented canoe-shaped macroconidia, which are typical of *Fusarium*, where the species with the highest occurrence and responsible for root rot and yellowing of beans are *F. solani* and *F. oxysporum* (González et al., 2005; Mariscal et al., 2017). Additionally, the *Fusarium* genus presents pink colonies that turned violet with age, ovoid microconidia and hilarios produced in short phialides, and slightly curved macroconidia (Rangel et al., 2023).

In this study, a chemical characterization of the extract was not conducted. However, the antifungal effect of the *A. ochroleuca* extract is attributed to the compound dehydrocoridalmine (Singh et al., 2009), one of the 45 alkaloids reported in various organs of plants of the *Argemone* genus (Brahmachari et al., 2013).

In *A. ochroleuca*, the presence of 14

isoquinoline-type alkaloids is reported, among which dehydrocoridalmine is not found. On the other hand, dihydrochelerithrine ($C_{21}H_{19}NO_4$) and dihydrosanguirannine ($C_{20}H_{15}NO_4$) are the most abundant in seeds and vegetative tissue of the species, whose biological activity is related to antibacterial and molluscicidal activity (Hernández et al., 2020).

In the *in vitro* tests, complete inhibition of *F. oxysporum* was observed using ethanolic extracts from *A. ochroleuca*, beginning at concentrations of 23%. This finding aligns with the outcomes of the conducted research, where the 23% ethanolic extract demonstrated inhibition of fungus growth until day 11, paralleled by the aqueous extract, which exhibited inhibition until day 10 (Juarez et al., 2020). There are also reports where the latex of *A. ochroleuca* presents antifungal effects against isolates of phytopathogenic fungi such as *Alternaria alternata*, *Drechslera halodes*, *Fusarium oxysporum*, *Macrophomina phaseolina*,

Pythium ultimum, and *Rhizoctonia solani* (Moustafa et al., 2013).

A reduction in the infection capacity of fungi from the genera *Fusarium*, *Phyllosticta*, and *Sclerotium* is also reported when introducing *Argemone* sp. plants into the soil (Rizvi et al., 2012; Gahukar, 2016).

CONCLUSION

The extract of *Argemone ochroleuca* exhibits antifungal properties at concentrations of 23 %, since it inhibited the growth of the halo of *Fusarium* spp. isolated from beans.

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