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Actividad biológica *in vitro* del extracto acuoso de *Argemone mexicana* L. en un hongo fitopatógeno: *Sclerotinia sclerotiorum*

Biological activity *in vitro* of the aqueous extract of *Argemone mexicana* L. in a phytopathogenic fungus: *Sclerotinia sclerotiorum*

Iridiam Hernández Soto [a], Judith Prieto Méndez [a], Alfredo Madariaga Navarrete [a], Rafael Germán Campos Montiel [a], Rubén Jiménez Alvarado [a], Alma Delia Hernández Fuentes [a]*

Abstract:

Sclerotinia sclerotiorum is a phytopathogenic fungus that causes the disease known as cotton rot that infects crops of agronomic interest. The objective of this study was to evaluate the antifungal activity of the aqueous extract of *Argemone mexicana* L. commonly known as chicalote against *Sclerotinia sclerotiorum*, the extract was obtained from the leaves of chicalote and three concentrations were tested: 500, 1000, 2000 ppm, using the dilution method of the extract in agar, in addition the mean lethal concentration (LC_{50}) of the extract was determined by means of a Probit analysis. The 2000 ppm concentration presented the highest percentage of inhibition (27.24 ± 0.01%, Tukey P ≤ 0.05) and an LC_{50} of 33,515 ppm. This result suggests the use of the aqueous extract of *A. mexicana* L. as a preventive alternative for the control of *Sclerotinia sclerotiorum*.

Keywords:

Green chemistry, phytopathogen, chicalote, vegetable extracts

Resumen:

Sclerotinia sclerotiorum es un hongo fitopatógeno que causa la enfermedad conocida como podredumbre algodonosa que infecta cultivos de interés agronómico. El objetivo de este estudio fue evaluar la actividad antifúngica del extracto acuoso de *Argemone mexicana* L. conocida comúnmente como chicalote contra *Sclerotinia sclerotiorum*, el extracto se obtuvo de las hojas de chicalote y se probaron tres concentraciones: 500, 1000, 2000 ppm, utilizando el método de dilución del extracto en agar, además se determinó la concentración letal media (CL_{50}) del extracto mediante un análisis Probit. La concentración de 2000 ppm presentó el mayor porcentaje de inhibición (27.24±0.01 %, Tukey P≤ 0.05) y una CL_{50} de 33,515 ppm. Este resultado sugiere el uso del extracto acuoso de *A. mexicana* L. como una alternativa preventiva para el control de *Sclerotinia sclerotiorum.*

Palabras Clave:

Química verde, Fitopatógeno, chicalote, extractos vegetales

Introduction

Sclerotinia sclerotiorum is a major plant pathogen causing significant losses in crop production worldwide, the symptoms of the disease are variable, but often include watery soft rot in leaf and stem tissue [1]. Since the green revolution, the control of diseases in crops has largely depended on agrochemicals [2], but these have caused harm to consumers of food treated with these substances, because the residues persist in high concentrations, toxic to humans. The chemical resistance of these compounds to degradation has caused contamination in soils, plants, animals and in man himself, which causes serious diseases [3]. In the framework of sustainable agriculture, it has led researchers to search for

[a*] Autor de Correspondencia, Universidad Autónoma del Estado de Hidalgo, Instituto de Ciencias Agropecuarias, Rancho Universitario, Av. Universidad km.1, Ex-Hacienda de Aquetzalpa AP 32, C.P 43600 Tulancingo, Hidalgo, México. hfad@hotmail.com; https://orcid.org/0000-0003-2592-6689

[a] Universidad Autónoma del Estado de Hidalgo, Instituto de Ciencias Agropecuarias, Rancho Universitario, Av. Universidad km.1, Ex-Hacienda de Aquetzalpa AP 32, C.P 43600 Tulancingo, Hidalgo, México: iridiamsoto@gmail.com , https://orcid.org/0000-0002-0307-1651; jprieto@uaeh.edu.mx, https://orcid.org/0000-0001-5705-1704; alfredomadariaga60@gmail.com, https://orcid.org/0000-0001-6812-2221; ragcamposm@gmail.com, https://orcid.org/0000-0001-7382-5538; rjalvarado@gmail.com, https://orcid.org/0000-0003-2538-6061.

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new compounds for the control of diseases whose activity and environmental security are appropriate in current times [4]. In this sense, natural alternatives have been developed, among which are plant extracts, since they have various advantages: biological, degradable and have a minimal negative impact on human health and the environment [5]. The use of plants that are considered weeds, such as *Argemone mexicana* L that competes with crops of economic importance for nutrients, sunlight and space, is an important and economical source of obtaining plant extracts with antifungal activity. For this reason, the objective of this work is to determine the antifungal activity of the aqueous extract of *Argemone mexicana* L. on *Sclerotinia sclerotiorum* and to propose an alternative for its control or prevention.

Materials and methods

Obtaining the aqueous extract of chicalote

The collection of the plant was carried out in the municipality of Cuautepec de Hinojosa, Hidalgo, which is located at 20º 09'00" N 98'00" W. The plant was dried under shade; the extract was obtained from the leaves dry by macerating water for 15 days. Later it was concentrated by means of a rotavapor (Buchi model R-215) [6].

Antifungal effect in vitro by the dilution method of the extract in agar

In order to evaluate the antifungal effect of the extract of chicalote leaves against the fungus *S. sclerotiorum*, the tests were carried out with three concentrations, 500 ppm, 1000 ppm, 2000 ppm, as well as a control consisting of PDA medium, the tests were carried out carried out in Petri dishes and the dilution method of the agar extract was applied, which consists of making a homogeneous mixture between the medium and the extract, once the mixture gelled, the fungus explants boxes with a radius of 5 mm were placed in the center and 7 days of replanting. The petri dishes were incubated at 28ºC for 168 hours. The results were expressed as a percentage of inhibition of the growth of the fungus as reported by [7].

$$\%\ growth = [\varphi h/\varphi b] \times 100\% \quad (1)$$

$$\%\ GI = 100 - \%\ growth \quad (2)$$

Where: φh represents the fungal growth (mm), φb is the diameter of fungal growth of the corresponding negative control in each replicate, and % GI is the percentage of growth inhibition.

Statistical analysis

A completely randomized design was used, with four treatments and three repetitions per treatment. The data was analyzed by means of an analysis of variance (ANOVA) and a Tukey multiple comparison test of means ($P \leq 0.05$) was used, with the SAS-PC system software (version 9.1.3) for Windows. Probit analysis was performed to calculate the LC_{50} of the extract and was performed with the SAS program (version 9.1).

Results and Discussion

Treatment differences between treatments were observed (Table 1). The aqueous extract at 2000 ppm presented the highest percentage of inhibition (27.24 ± 0.01%) at 24 hours, while at 168 hours the percentage of fuel (8.26 ± 0.02%), The lowest concentration used was 500 ppm and 20.74 ± 0.02 percent inhibition at 24 hours. These results show the fungistatic effect of the aqueous extract of *A. mexicana* L. that may possibly be due to the presence of phenolic compounds present in this polarity [8], it is known that phenolic compounds act on the cell membrane and the potential of the mitochondrial membrane by inhibiting the formation of ATP [9].

It is worth mentioning that more bioassays are required to elucidate precisely what secondary metabolites have biological activity against this fungus. In another investigation [10] the extract of *Heliopsis longipes* was tested against this same phytopathogen where an inhibition of 15.99% is reported at a concentration of 15,000 ppm, and an average lethal dose of 57,900 ppm, unlike this research, the concentrations used were lower and the percentage of inhibition was higher, which is why the whippet turns out to be a favorable alternative for the control of *S. sclerotiorum*.

Table 1. *S. sclerotiorum* growth inhibition percentages following treatment with aqueous extracts of *A. mexicana* L. at various concentrations.

Sclerotinia sclerotiorum	% Inhibition	Treatment (ppm)		
		500	1000	2000
	24 h	20.74±0.02^{a}	23.31±0.01^{a}	27.24±0.01^{a}
	48 h	12.29±0.03^{c}	17.91±0.01^{b}	20.42±0.01^{a}
	72 h	7.97±0.02^{b}	15.41±0.04^{a}	16.39±0.01^{a}
	96 h	6.68±0.02^{c}	14.00±0.01^{b}	15.29±0.01^{a}
	120 h	5.44±0.60^{b}	11.46±0.01^{a}	12.06±0.01^{a}
	144 h	4.31±0.16^{c}	10.26±0.02^{b}	11.05±0.04^{a}
	168 h	2.30±0.04^{c}	6.96±0.01^{b}	8.26±0.02^{a}

Results are presented with a standard deviation of three replicates. The different letters indicate significant differences (Tukey $P \leq 0.05$) between hourly treatments for *Sclerotinia sclerotiorum*.

Table 2. *A. mexicana* L. extracts concentrations LC_{50} to inhibit *S. sclerotiorum* mycelial growth at 168 h of incubation.

	Extract	95% Fiducial limits			
		LC_{50} (ppm)	Lower	Upper	X^2
Sclerotinia sclerotiorum	Aqueous	33,515	15,304	134,386	39.97

X^2-square value, significant at $p<0.05$ level.

Conclusions

The aqueous extract in different concentrations shows fungistatic activity, although in concentrations of 2000 ppm it has greater effectiveness in *S. sclerotiorum*. The inhibition of mycelial growth shown by the extract may be due to the presence of some secondary metabolites acting synergistically. Therefore, the use of *A. mexicana* L. can be considered as an alternative for the prevention of cotton rot.

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