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Actividad antifúngica de compuestos fitoquímicos de extractos de plantas del semidesierto mexicano contra *Fusarium oxysporum* del tomate por el método de micro dilución en placa

Antifungal activity of phytochemical compounds of extracts from Mexican semi-desert plants against *Fusarium oxysporum* from tomato by microdilution in plate method

Marco Antonio Tucuch-Pérez[1], Roberto Arredondo-Valdés[2] & Francisco Daniel Hernández-Castillo[1]

Palabras clave: semidesierto mexicano; extractos de plantas; *Fusarium oxysporum*; fungicidas botánicos; concentración inhibitoria; compuestos fitoquímicos; tomate
Keywords: mexican semi-desert; plant extracts; *Fusarium oxysporum*; botanical fungicides; inhibitory concentration; phytochemical compounds; tomato

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Resumen

Introducción: En muchas regiones del mundo *Fusarium oxysporum* causa pérdidas en el cultivo del tomate; para su control, los fungicidas químicos son los más utilizados, sin embargo, estos fungicidas causan problemas ambientales y de resistencia; por lo tanto, alternativas ecológicas como extractos de plantas han sido desarrollados. Los objetivos de este trabajo fueron identificar cualitativamente fitoquímicos presentes en extractos etanólicos y acuosos de extractos de *Agave lechuguilla*, *Carya illinoinensis*, *Jatropha dioica*, *Larrea tridentata* y *Lippia graveolens*, y determinar su actividad antifúngica contra *F. oxysporum*.

Método: Las plantas fueron colectadas del noreste de México; se obtuvieron extractos de plantas crudos y concentrados; el porcentaje de inhibición y la concentración inhibitoria al 50 % (CI_{50}) de *F. oxysporum* de cada extracto de plantas fueron determinados a través de método de microdilución en placa.

Resultados: Se identificaron flavonoides, saponinas, taninos y quinonas. La actividad antifúngica mostró inhibición de 40 a 60 % a 1000 mg/L, por el extracto crudo acuoso de hojas de *L. graveolens*, y por el extracto concentrado acuoso de tallo de *L. graveolens* respectivamente. En cuanto a los extractos etanólicos se presentó 100 % de inhibición para el extracto crudo de ruezno de *C. illinoinensis*; en hojas y tallo de *L. graveolens* la inhibición comenzó a 250 mg/L; para los extractos resuspendidos la inhibición empezó a 125 mg/L con tallo y hojas de *L. graveolens*; y finalmente en raíces de *A. lechuguilla* y hojas de *L. graveolens* la inhibición comenzó a 250 mg/L

[1] Universidad Autónoma Agraria Antonio Narro, Departamento de Parasitología. E-mail: fdanielhc@hotmail.com
[2] Universidad Autónoma de Coahuila, Departamento de Nanobiociencia. Facultad de Ciencias Químicas

y 500 mg/L respectivamente. El mejor CI_{50} fue de 8.02 mg/L del extracto resupendido etanólico de *L. graveolens*.

Conclusión: Los extractos de *L. graveolens, A. lechuguilla* y *C. illinoinensis*, mostraron 100 % de actividad inhibitoria contra el desarrollo de *F. oxysporum*, representando una alternativa para el control de *F. oxysporum*.

Abstract

Introduction: In several regions of the world, *Fusarium oxysporum* causes losses on tomato crops; for control it, chemical fungicides are used. Nevertheless, these fungicides causing environmental and resistance problems; therefore, ecological alternatives as plant extracts have been developed. Due to the aim of this work, identify phytochemicals present in ethanolic and aqueous extracts from *Agave lechuguilla* qualitatively, *Carya illinoinensis*, *Jatropha dioica*, *Larrea tridentata*, and *Lippia graveolens* and determine their antifungal activity against *F. oxysporum.*

Method: The plants collected from the northeast of Mexico; crudes and concentrated plant extracts obtained; the inhibition percentage and inhibitory concentration to 50 % (IC_{50}) of *F. oxysporum* for each plant extract were determinate trough microdilution in the plate method

Results: The essential phytochemicals were flavonoids, saponins, tannins, and quinones. The antifungal activity showed at 1000 mg/L inhibition around 40 to 60% by aqueous crude extracts from leaves of *L. graveolens* and concentrated aqueous extracts from the stem of *L. graveolens,* respectively. The ethanolic extracts presented 100 % of inhibition for crude extracts of husk from *C. illinoinensis*; in leaves and stem from *L. graveolens* the inhibition started from 250 mg/L; for resuspended extracts, the inhibition started from 125 mg/L with *L. graveolens* stem and leaves; and finally in roots of *A. lechuguilla* and leaves from *L. graveolens* the inhibition started from to 250 and 500 mg/L respectively. The best IC_{50} was of 8.02 mg/L from the ethanolic resuspended extract of *L. graveolens* stem.

Conclusion: The ethanolic plant extracts from *L. graveolens*, *A. lechuguilla,* and *C. illinoinensis*, showed 100 % of inhibiting activity against the development of *F. oxysporum*, representing an alternative for control of *F. oxysporum*.

Introduction

Tomato is the fourth vegetable more cultivated in the world with three millions of hectares, just behind rice, wheat, and soy (FAOSTAT, 2018). Nevertheless, the tomato crop is affected by several fungal diseases as *Botrytis cinerea*, *Leveillula taurica*, *Alternaria solani*, and *F. oxysporum* (Villasanti & Pantoja, 2013). *F. oxysporum* causes vascular wilt that is one of the most destructive diseases on tomato crop, and it can cause yield losses until of the 80 % (Marlatt *et al*., 1996; Hernández- Martínez *et al*., 2014; González *et al*., 2012). At present exist several methods to control the *F. oxysporum*; chemical control is the most used; nevertheless, several reports indicate that farmers use intensive applications of synthetics products, triggering on phytopathogenic microorganisms resistance (Bautista-Baños, 2006). For these reasons, at present, there is a great necessity to develop alternatives methods for the control of plant diseases (Jeong *et al.*, 2017).

The plants from the Mexican semi-desert present a large number of phytochemicals with antifungal activity. Some of these plants are *A. lechuguilla* that exhibit the presence of steroidal saponins; *C. illinoinensis* with a high content of total phenolics compounds; that causes enzymatic inhibition by compound oxidation; *J. dioica* with a considerable amount of phytochemicals; *L. tridentata* with flavonoids, triterpenes, and triterpenoids; and *L. graveolens* that present phytochemical compounds like essential oils, iridoids, flavonoids and naphthoquinones (Blunden *et al*., 1980; Do Prado *et al*., 2009; García-Bores *et al*., 2017; Martínez *et al*., 2014; Martins *et al*. 2013).

The action mode of the phytochemical compounds present in plants are diverse, in case of terpenes and essential oils there is a membrane rupture by lipophilic compounds, the alkaloids intercalate their self with DNA, and the lectins and polypeptides create Ion channels in the microbial membrane or cause the competitive inhibition by adhesion of microbial proteins to the polysaccharide receptors from the host. (Masson, 1987; Cowan, 1999; Hernández–Lauzardo *et al*., 2007). In this sense, Jasso de Rodríguez *et al*. (2011) reported 100 % of inhibition with extracts from *L. graveolens* and *A. lechuguilla* on *Rhizopus stolonifer*. In the same way, the antifungal activity of *L. tridentata* was reported by Osorio *et al*. (2009) with extracts which presented fungicidal effect on the growth of *Phytium* sp., *Colletotrichum coccodes*, *Colletotrichum truncatum*, *Alternaria alternata*, *Fusarium solani*, and *Rhizoctonia solani*; and fungistatic impact on *Fusarium verticilloides*.

The objectives of this study were identified some phytochemicals compounds present in plant extracts from *A. lechuguilla*, *C. illinoinensis*, *J. dioica*, *L. tridentata*, and *L. graveolens*; and determinate the antifungal activity against *F. oxysporum* and their IC_{50} concentration of each plant.

Methods

Plant collection. The plant collection was in January 2016. This collect was in General Cepeda, Coahuila, México (25°21'40.55'' N y 101°28'08.68'' W). Samples stored in black plastic bags for their transfer at Laboratorio de Micología y Biotecnología from Universidad Autonoma Agraria Antonio Narro. Once in the laboratory, the samples washed with water and let it dry, then cut in small pieces around 1 cm. Samples placed in a drying stove to 60 °C until constant weight, finally each plant pulverized and sieve with a pore of 0.2 mm, this for the particle homogenization. Samplers stored in dark flasks to environment temperature (Castillo *et al*., 2010).

Plant extracts preparation. The plant extracts prepared following the Shami *et al*. (2013) methodology with some modifications. Water and ethanol used as solvents and two types of extracts developed, crudes, and concentrated. The first step was adding 14 g of the plant powder in 200 mL of solution, and then the flask was placed in a stirring grill during 72 h at 50°C (Jasso de Rodríguez *et al*., 2015). Lapsed 72 h, the obtained extract filtered with a filter paper Whatman No.1; after the purified extract separated in two parts, one of these parts was placed in the Eppendorf tube and stored to -20 °C, thus were obtained crude extracts. To get the concentrated extracts, the solvent was separated by rotary evaporation (IKA RV 10) to 150 RPM to 60 °C, after the rotary evaporation the extracts obtained were placed in a drying stove until the extracts presented constant weight and then were pulverized (Martins *et al*., 2013), the obtained powder from the pulverization stored to -20 °C. In **Table 1**, there are all the plant extracts.

Table 1. Mexican desert plant extracts used against *F. oxysporum* strain.
Tabla 1. Extractos de plantas del desierto mexicanos utilizados contra *F. oxysporum*.

Plant extracts			
Aqueous		Ethanolic	
Crudes	Concentrated	Crudes	Concentrated
A. lechuguilla leaves (AlLAC)	*A. lechuguilla* leaves (AlLAP)	*A. lechuguilla* leaves (AlLEC)	*A. lechuguilla* leaves (AlLEP)
A. lechuguilla roots (AlRAC)	*A. lechuguilla* roots (AlRAP)	*A. lechuguilla* roots (AlREC)	*A. lechuguilla* roots (AlREP)
C. illinoinensis husk (CiHAC)	*C. illinoinensis* husk (CiHAP)	*C. illinoinensis* husk (CiHEC)	*C. illinoinensis* husk (CiHEP)
J. dioica stem (JdSAC)	*J. dioica* stem (JdSAP)	*J. dioica* stem (JdSEC)	**
J. dioica roots (JdRAC)	*J. dioica* roots (JdRAP)	*J. dioica* roots (JdREC)	*J. dioica* roots (JdREP)
L. tridentata leaves (LtLAC)	*L. tridentata* leaves (LtLAP)	*L. tridentata* leaves (LtLEC)	*L. tridentata* leaves (LtLEP)
L. graveolens leaves (LgLAC)	*L. graveolens* leaves (LgLAP)	*L. graveolens* leaves (LgLEC)	*L. graveolens* leaves (LgLEP)
L. graveolens steams (LgSAC)	*L. graveolens* steams (LgSAP)	*L. graveolens* steams (LgSEC)	*L. graveolens* steams (LgSEP)

** = It was not determined.
** = No se determinó.

Phytochemical analysis of plant extracts. The phytochemicals identification used qualitative techniques, in these techniques the presence or absence of phytochemicals were determinate by colorimetry where the extract reacted and change of color; for these tests concentrated extracts prepared to 1000 mg/L; the identified phytochemicals were alkaloids (Dragendorff and Sonnenschein reaction), cyanogenic glycosides (Grignard reaction), reducing sugars (Feeling and Benedict reaction), saponins (Liberman Bouchnard reaction), tannins (Jelly and $FeCl_3$), quinones (ammonium hydroxide and Bontrager reaction), coumarins (Erlich reaction and ammonium hydroxide), purines and carotenoids (Sahgal *et al*., 2009; Usman *et al*., 2009).

Antifungal activity extract in the microdilution plate method.

***F. oxysporum* strain.** The *F. oxysporum* strain isolated from tomato plants provided from the microbiological collection of Laboratorio de Micología y Biotecnología belongs to the Departamento de Parasitología from Universidad Autonoma Agraria Antonio Narro located in Saltillo, Coahuila, México; the *F. oxysporum* strain identified with the code FoC1, with the access key in GenBank: KU533843.1.

Antifungal activity tests of the extracts against *F. oxysporum in vitro*. Microplates used, all wells filled with 100 µL of Sabouraud liquid medium. The antifungal test starts from column number four with 100 µL of the extracts; 100 µL of mixture took and added into the next column, thus successively until column 12. Getting concentrations from 1000 to 3.9 mg/L; this procedure performed for all the extracts using each row for each extract; next step added 2,3,5-Triphenyl

tetrazolium chloride as growth indicator; finally, a solution of spores from *F. oxysporum* added in all wells except in column number one, the plate incubated to 28 °C during 48 h, the absorbance lecture performed at 490 nm. For test three repetitions completed, the inhibition percentage, calculated in the same way by Moreno-Limon *et al*. (2011) formulas adopted:

$$\text{Growth percentage} = (A-B)/C\ (100)$$

A=Treatment absorbance B=Negative witness absorbance C=Positive witness absorbance

$$\text{Inhibition percentage} = 100-\text{Growth percentage}$$

Statistical analysis. Probit analysis performed to determine the inhibitory concentration to 50% (IC_{50}) of each extract. With the results obtained, variance analysis performed with the inhibitory concentrations; in the study, the treatments evaluated with three repetitions and a Tukey's range tests performed ($p<0.05$).

Results

Identified phytochemicals in aqueous and ethanolic plant extracts. The identified phytochemicals from ethanolic extracts are in **Table 2**; from *A. lechuguilla* both leaves and roots were identified compounds like carbohydrates, reducing sugars, saponins, and tannins; in the case of *C. illinoinensis* husk extracts alkaloids, flavonoids, cyanogenic glycosides, reducing sugars, saponins, tannins, quinones, coumarins, purines and carotenoids presented; for *J. dioica* root extract observed saponins, tannins, quinones, and purines; while *L. tridentata* extract presented alkaloids, flavonoids, cyanogenic glycosides, reducing sugars, saponins, tannins, quinones, coumarins, and carotenoids; finally in *L. graveolens* extracts both leaves and steam alkaloids, flavonoids, reducing sugars, saponins, tannins, quinones, coumarins, and carotenoids identified.

Phytochemicals detected from aqueous extracts presented in **Table 3**. From *A. lechuguilla*, both leaves and roots presented the same compounds as reducing sugars, saponins, tannins, and quinones, only carbohydrates, and purines were different compounds being present in leaves, for *C. illinoinensis* husk identified all the phytochemicals listed in **Table 3** except alkaloids.

Table 2. Identified phytochemicals in concentrated ethanolic extracts.
Tabla 2. Fitoquímicos identificados en extractos etanólicos concentrados.

Extract	A	C	F				GC	AZ		S		T				Q			Cu	P	Ca
			F1	F2	F3	F4		Az1	Az2	S1	S2	T1	T2	T3	T4	Q1	Q2	Q3			
AlLAP	-	+	-	-	-	-	-	-	+	+	-	+	-	+	+	+	+	-	-	+	-
AlRAP	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	+	-	-	-	-
CiHAP	-	+	-	-	-	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+
JdSAP	-	+	-	-	-	-	+	+	+	-	-	-	+	-	+	-	+	-	+	-	-
JdRAP	-	+	-	-	-	-	-	-	+	+	-	-	-	+	+	+	+	-	+	+	-
LtLAP	-	-	-	-	-	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+
LgLAP	-	+	+	+	+	+	-	-	+	-	+	+	+	-	+	-	+	-	-	-	-
LgSAP.	-	+	-	+	+	+	-	+	+	-	+	-	+	-	+	-	+	-	-	+	-

+ = Phytochemical present; - = Phytochemical no present; A = Alkaloids; C = Carbohydrates; F = Flavonoids; GC = Cyanogenic glycosides; AZ = Reducing sugars; S = Saponins; T = Tannins; Q = Quinones; Cu = Coumarins; P = Purines; Ca = Carotenoids; F1 = Flavonones; F2 = Flavones; F3 = Flavononas; F4 = Chalcones; Az1 = Reducing sugars Fehling reaction; Az2 = Reducing sugars Benedict reaction; S1 = Triterpenoids; S2 = Steroidal; T1 = Jelly; T2 = Derivatives Gallic Acid; T3 = Catechol Derivatives; T4 = Phenols; Q1 = Anthraquinones; Q2 = Benzoquinones; Q3 = Antronas. ** = It was not determined.
+ = Fitoquímico presente; - = Fitoquímico no presente; A = Alcaloides; C = Carbohidratos; F = Flavonoides; GC = Glucósidos cianogénicos; AZ = Azúcares reductores; S = Saponinas; T = Taninos; Q = Quinonas; Cu = Cumarinas; P = Purinas; Ca = Carotenoides; F1 = Flavononas; F2 = Flavonas; F3 = Flavononas; F4 = Chalconas; Az1 = Azúcares reductores reacción Fehling; Az2 = Azúcares reductores reacción Benedict; S1 = Triterpenoides; S2 = Esteroidales; T1 = Reaccion gelatina; T2 = Derivados de ácido gálico; T3 = Derivados de catecol; T4 = Fenoles; Q1 = Antraquinonas; Q2 = Benzoquinonas; Q3 = Antronas. ** = No se determinó.

In the case of *J. dioica* observed in stems and roots carbohydrates, reducing sugars, saponins, tannins, quinones, and coumarins, while cyanogenic glycosides and purines were only in stems. *L. tridentata* presented all the phytochemicals except alkaloids and carbohydrates; finally, in *L. graveolens* leaves were observed carbohydrates, flavonoids, reducing sugars, saponins, tannins, and quinones, whereas in the stems were presented the same compounds that in leaves except for purines.

Table 3. Identified phytochemicals in concentrated aqueous extracts.
Tabla 3. Fitoquímicos identificados en extractos acuosos concentrados.

Extract	A	C	F				GC	AZ		S		T				Q			Cu	P	Ca
			F1	F2	F3	F4		Az1	Az2	S1	S2	T1	T2	T3	T4	Q1	Q2	Q3			
AlLEP	-	+	-	-	-	-	-	+	-	+	-	-	-	+	+	-	-	-	-	-	-
AlREP	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-	-	-	-
CiHEP	+	-	-	-	-	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+
JdSEP	**																				
JdREP	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	-	-	-	+	-
LtLEP	+	-	+	+	+		+	+	+	+	-	+	+	-	+	+	+	+	+	-	+
LgLEP	+	-	+	+	+	+	-	-	+	-	+	+	+	-	+	+	+	-	+	-	-
LgSEP	-	-	+	+	+	+	-	-	+	+	-	-	+	-	+	-	+	-	+	-	+

+ = Phytochemical present; - = Phytochemical no present; A = Alkaloids; C = Carbohydrates; F = Flavonoids; GC = Cyanogenic glycosides; AZ = Reducing sugars; S = Saponins; T = Tannins; Q = Quinones; Cu = Coumarins; P = Purines; Ca = Carotenoids; F1 = Flavonones; F2 = Flavones; F3 = Flavononas, F4= Chalcones, Az1 = Reducing sugars Fehling reaction; Az2 = Reducing sugars Benedict reaction; S1 = Triterpenoids; S2 = Steroidal; T1 = Jelly; T2 = Derivatives Gallic Acid; T3 = Catechol Derivatives; T4 = Phenols; Q1 = Anthraquinones; Q2 = Benzoquinones; Q3 = Antronas. ** = It was not determined.
+ = Fitoquímico presente; - = Fitoquímico no presente; A = Alcaloides; C = Carbohidratos; F = Flavonoides; GC = Glucósidos cianogénicos; AZ = Azúcares reductores; S = Saponinas; T = Taninos; Q = Quinonas; Cu = Cumarinas; P = Purinas; Ca = Carotenoides; F1 = Flavononas; F2 = Flavonas; F3 = Flavononas; F4 = Chalconas; Az1 = Azúcares reductores reacción Fehling; Az2 = Azúcares reductores reacción Benedict; S1 = Triterpenoides; S2 = Esteroidales; T1 = Reaccion gelatina; T2 = Derivados de ácido gálico; T3 = Derivados de catecol; T4 = Fenoles; Q1 = Antraquinonas; Q2 = Benzoquinonas; Q3 = Antronas. ** = No se determinó

Antifungal activity tests of the extracts against *F. oxysporum in vitro*

Antifungal activity of aqueous plant extracts against *F. oxysporum*. In general, in aqueous extracts the antifungal activity was less compared with the ethanolic extracts; in the case of crude aqueous extracts, it can see in **Fig. 1**, that the inhibition percentage increased when the concentration increased, even so, the higher inhibition percentage reached it did not overcome the 50 %. The treatments with the better antifungal activity were AlLAC, JdSAC, and LgLAC; these treatments presented higher antifungal activity when they achieved to the concentration of 31.2 mg/L and kept the same inhibition percentage until the strength of 1000 mg/L with 46, 37, and 45 % respectively. In the concentrated aqueous extracts, it observed a higher inhibition percentage; nevertheless, it did not overcome the 60 %, presented in **Fig. 2**. In this case, found that the extracts with higher antifungal activity were AlRAP with 53 and LgSAP with 60 %, both to 1000 mg/L.

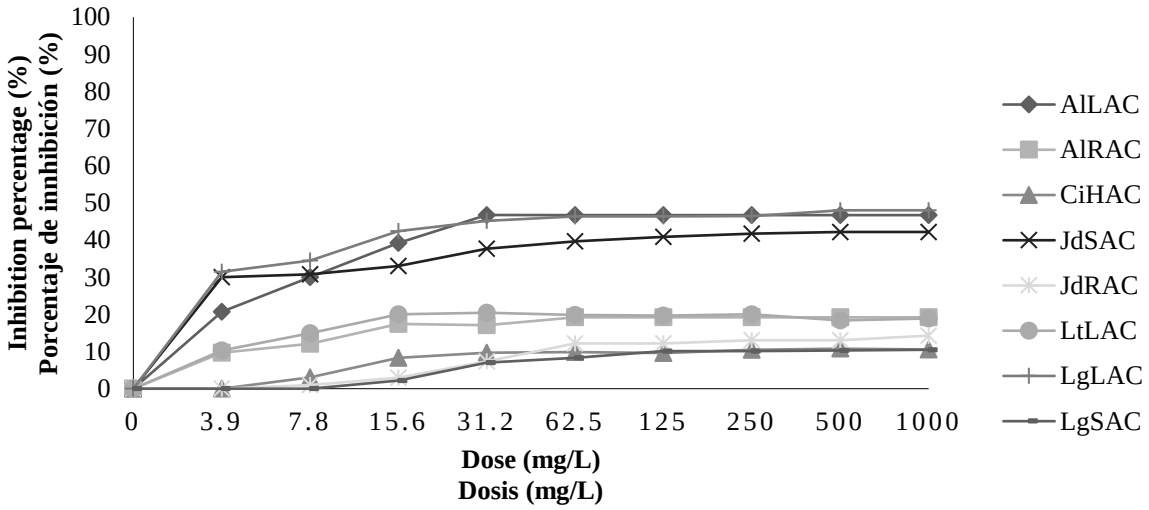

Fig. 1. Inhibition percentage of aqueous crude extracts on *F. oxysporum*.
LtLAC: *L. tridentata* leaves. AlLAC: *A. lechuguilla* leaves. AlRAC: *A. lechuguilla* roots. JdRAC: *J. dioica* roots. JdSAC: *J. dioica* stem. CiHAC: *C. illinoinensis* husk. LgSAC: *L. graveolens* stem and LgLAC: *L. graveolens* leaves.

Fig. 1. Porcentaje de inhibición de extractos crudos acuosos sobre *F. oxysporum*.
LtLAC: *L. tridentata* hojas. AlLAC: *A. lechuguilla* hojas. AlRAC: *A. lechuguilla* raíces. JdRAC: *J. dioica* raíces. JdSAC: *J. dioica* tallo. CiHAC: *C. illinoinensis* ruezno. LgSAC: *L. graveolens* tallo and LgLAC: *L. graveolens* hojas.

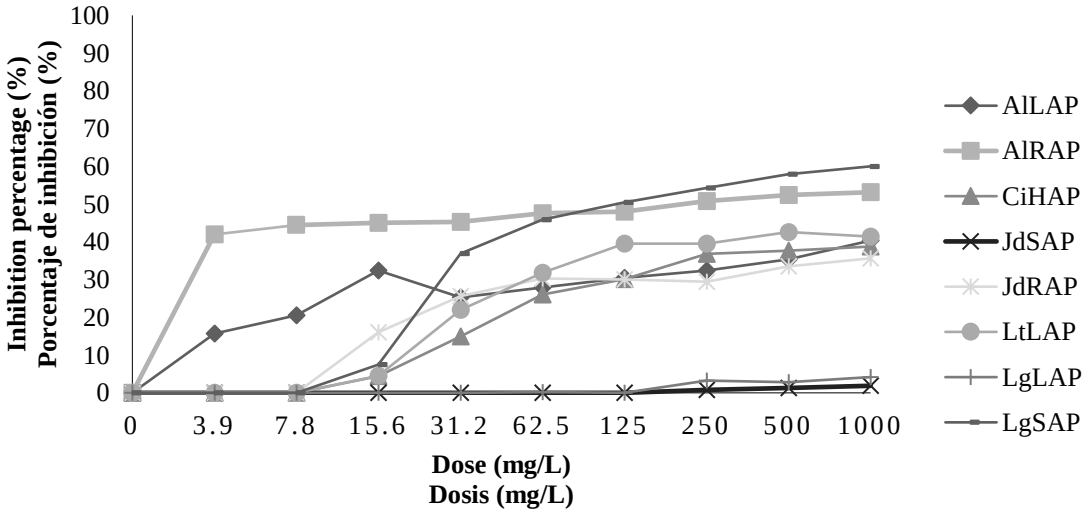

Fig. 2. Inhibition percentage of concentrated aqueous extracts on *F. oxysporum*.
LtLAP*: L. tridentata* leaves. AlLAP*: A. lechuguilla* leaves. AlRAP: *A. lechuguilla* roots. JdRAP*: J. dioica* roots. JdSAP*: J. dioica* stem. CiHAP: *C. illinoinensis* husk. LgLAP: *L. graveolens* leaves and LgSAP: *L. graveolens* stem.

Fig. 2. Porcentaje de inhibición de extractos concentrados acuosos sobre *F. oxysporum*.
LtLAP*: L. tridentata* hojas. AlLAP*: A. lechuguilla* hojas. AlRAP: *A. lechuguilla* raíces. JdRAP*: J. dioica* raíces. JdSAP*: J. dioica* tallo. CiHAP: *C. illinoinensis* ruezno. LgLAP: *L. graveolens* hojas and LgSAP: *L. graveolens* tallo.

Antifungal activity of plant ethanolic extracts against *F. oxysporum*. In the case of the ethanolic extracts, in crude extracts and concentrated extracts, it was observed an increase in antifungal activity. In **Fig. 3** it is found that all ethanolic crude extracts except LtLEC extract, presented inhibition percentage higher to 90 % in the concentration of 500 mg/L, nevertheless the treatments CiHEC, LgLEC and LgSEC were the best because there was inhibition of the pathogen to 100 % in the concentration of 250 mg/L. About concentrated ethanolic extracts, in **Fig. 4**, it is observed that the extracts LgSEP, AlLEP, AlREP, and LgLEP inhibited the pathogen development to 100 % to concentrations of 125 mg/L, 250 mg/L and 500 mg/L respectively.

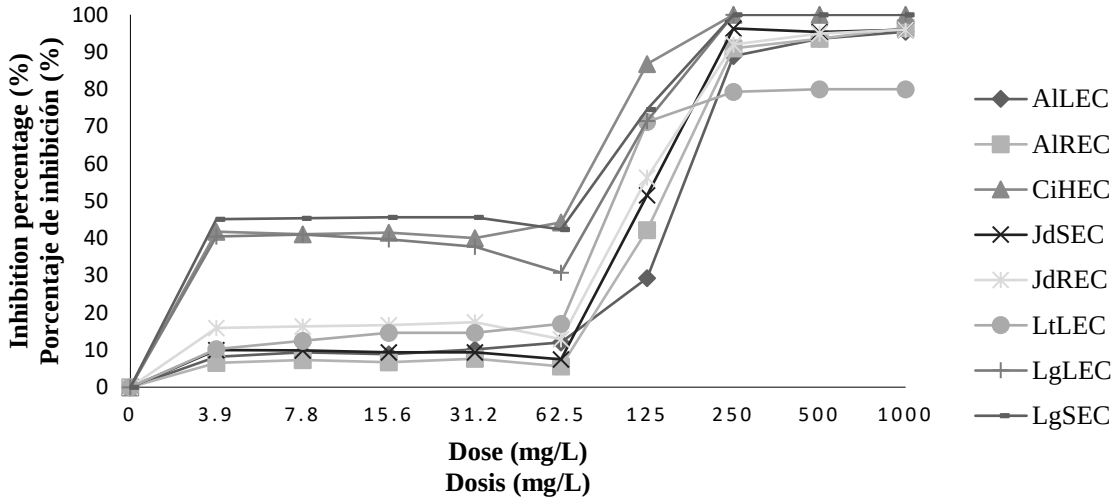

Fig. 3. Inhibition percentage of ethanolic crude extracts on *F. oxysporum*.
LtLEC: *L. tridentata* leaves. AlLEC: *A. lechuguilla* leaves. AlREC: *A. lechuguilla* roots. JdREC: *J. dioica* roots. JdSEC: *J. dioica* stem. CiHEC: *C. illinoinensis* husk. LgLEC: *L. graveolens* leaves and LgSEC: *L. graveolens* stem.

Fig. 3. Porcentaje de inhibición de extractos crudos etanólicos sobre *F. oxysporum*.
LtLEC: *L. tridentata* hojas. AlLEC: *A. lechuguilla* hojas. AlREC: *A. lechuguilla* raíces. JdREC: *J. dioica* raíces. JdSEC: *J. dioica* tallo. CiHEC: *C. illinoinensis* ruezno LgLEC: *L. graveolens* hojas and LgSEC: *L. graveolens* tallo.

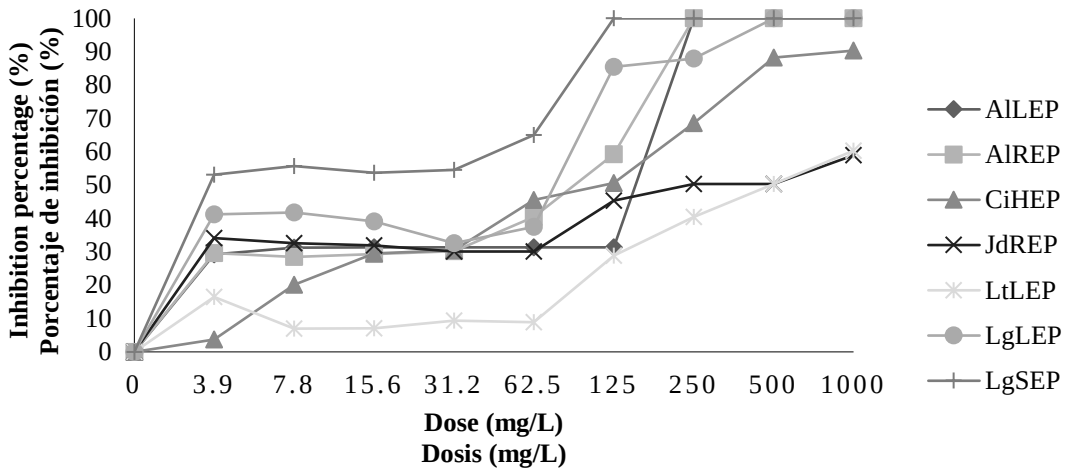

Fig. 4. Inhibition percentage of concentrated ethanolic extracts on *F. oxysporum*. LtLEP: *L. tridentata* leaves. AlLEP*: A. lechuguilla* leaves. AlREP: *A. lechuguilla* roots. JdREP: *J. dioica* roots. CiHEP: *C. illinoinensis* husk. LgLEP: *L. graveolens* leaves and LgSEP: *L. graveolens* stem.

Fig. 4. Porcentaje de inhibición de extractos concentrados etanólicos sobre *F. oxysporum*. LtLEP: *L. tridentata* hojas. AlLEP*: A. lechuguilla* hojas. AlREP: *A. lechuguilla* raíces. JdREP: *J. dioica* raices. CiHEP: *C. illinoinensis* ruezno. LgLEP: *L. graveolens* hojas and LgSEP: *L. graveolens* tallo.

IC_{50} of plant extracts. The variance analysis showed that existed significant differences in the IC_{50} (**Table 4**). For aqueous crude extracts, the lower IC_{50} was by the extract LgLAC with 535.19 mg/L, and in concentrated aqueous extracts, the lower IC_{50} were by the extracts LgSAP and AlRAP with 218 and 203 mg/L respectively. The ethanolic extracts had better IC_{50} than the aqueous extracts; this observed in **Table 4**. In this case, the crude extracts with the lower IC_{50} were the extracts CiHEC and LgSEC with 18 and 16 mg/L, respectively. By the concentrated extracts, the best IC_{50} was by the extracts LgLEP and LgSEP with 22 and 8 mg/L, respectively.

Table 4. Inhibitory concentration to 50 % (IC_{50}) of aqueous and ethanolic extracts for inhibition of *F. oxysporum*.
Tabla 4. Concentración inhibitoria al 50 % (CI_{50}) de extractos acuosos y etanólicos para inhibición de *F. oxysporum*.

Extract	IC_{50*}	Extract	IC_{50}	Extract	IC_{50}	Extract
AlLAC	535.30±0.06g	AlLAP	13109.00±0.06c	AlLEC	115.00±0.12a	AlLEP
AlRAC	253613034.00 ± 0.03a	AlRAP	203.74±0.06h	AlREC	113.72±0.16b	AlREP
CiHAC	9508417.00±0.03b	CiHAP	983.51±0.09e	CiHEC	18.03±0.19g	CiHEP
JdSAC	7875.00±0.03f	JdSAP	83361.00±0.14a	JdSEC	92.00±0.20d	**
JdRAC	69721.00±0.04e	JdRAP	1821.00±0.06d	JdREC	75.00±0.20e	JdREP
LtLAC	88075.00±0.02d	LtLAP	673.55±0.09f	LtLEC	108.50±0.21c	LtLEP
LgLAC	535.19±0.03h	LgLAP	53047.00±0.02b	LgLEC	23.09±0.22f	LgLEP
LgSAC	195805.00±0.07c	LgSAP	218.91±0.31g	LgSEC	16.24±0.15h	LgSEP

Discussion

Because all the plants are natives from the Mexican semi-desert, the phytochemicals identified are similar; thus identified compounds in leaves and roots from *A. lechuguilla* coincide with the reported by Sidana *et al*. (2016) who reported 141 steroidal saponins of pharmacologic interest; about the tannins, Castillo *et al*. (2010) mentioned them in his work. *C. illinoinensis* husk, according to Bottari *et al*. (2017), contains compounds like tannins and flavonoids. Nevertheless, it also has been identified as the presence of quinones by Xiang-ming (2010), which is similar to the compounds identified in this work. Martínez *et al*. (2014) mention quinones, coumarins, flavonoids, saponins, tannins, carbohydrates, and reducing sugars for extracts from the root of *J. dioica*. The extracts from leaves of *L. tridentata* coinciding with Martins *et al*. (2012), who identified flavonoids, saponins, and cyanogenic glycosides. Finally in extracts from *L. graveolens* was identify tannins, coinciding with the report of Hernandez-Castillo *et al*. (2010); coumarins similar to the mentioned by Cruz *et al*. (2011); and flavonoids as flavonols and flavons being this results analogous to the obtained by Güereca *et al*. (2007). Many studies have revealed the efficacy of Mexican semi-desert plant extracts to inhibit the growth of phytopathogenic fungi; previously, several works have been reported these plants as a source of compounds with antifungal activity. Carvalho *et al*. (2011) tested aqueous extracts of *Jatropha curcas*, against *Alternaria alternata* and

presented 46 % of control; Mendez *et al*. (2012) mentioned inhibition percentage of 60 % with aqueous extracts of *L. graveolens* and *A. lechuguilla* over different food bacteria. Also, these results are similar to obtained by Hernandez-Castillo *et al*. (2010), who reported 100 % of inhibition in *R. solani* with ethanolic extracts of *C. illinoinensis*, and coincide with the results of Jasso de Rodriguez *et al*. (2011) who used ethanolic extracts of *A. lechuguilla* and they observed 100 % of inhibition on *C. gloeosporioides*. Nevertheless, our results differ with the results reported by Mendez *et al*. (2012), who observed an inhibition percentage of 60 % on food bacteria using ethanolic extracts of *L. graveolens* and *A. lechuguilla*.

The phytochemical analysis showed that the extracts from these species have compounds with antifungal activity as alkaloids, flavonoids, tannins, and saponins. These compounds have different ways of effect on the pathogens; in the case of the alkaloids could be for the presence of nitrogen in their structure as amine or amide. By flavonoids presence, their composition of phenolic hydroxyls can penetrate the cellular membrane, so these hydroxyls combine, precipitate, and denature the protoplasmic proteins (Ruiton *et al*., 1998). From tannins form complexes with enzymes and other proteins provoking the inhibition of the enzymes, also they can inhibit the electrons transport through the membranes, and they can alter ions like iron and copper inhibiting the activity of some essential enzymes for microorganisms life (Scalbert & Williamson, 2000). The saponins form complexes with sterols, can affect proteins and membranes phospholipids (Stuardo & San Martin, 2008).

IC_{50} is the most widely used and informative measure of substance efficacy. It indicates how much a compound is needed to inhibit a biological process by half, thus providing a means of the potency of an antagonist substance in research. This measure is essential because a low IC_{50} indicates that a plant extract is an excellent candidate to control phytopathogenic fungi (Aykul & Martinez-Hackert, 2016). The results obtained in this work are comparable to reported by Caceres-Rueda de Leon *et al*. (2013) who observed an IC_{50} of 200 mg/L with aqueous extracts of *L. graveolens* on *F. oxysporum*, but differ with the report of Hernandez-Castillo *et al*. (2010) who reported IC_{50} of 1930 and 4340 mg/L with ethanolic extracts of *L. graveolens* and *C. illinoinensis* on *R. solani*.

The above result suggests that the low IC_{50} of *L. graveolens* could be due to the presence of compounds as thymol and carvacrol; these compounds are the most common volatile components in the Labiatae family, and distributed in plants as *L. graveolens*; these compounds

are capable of inhibiting the growth of many fungus species (Chen *et al*., 2019). In the case of the *A. lechuguilla* extracts, the antifungal activity attributed to compounds like flavonoids, phenylpropanoids, and polyphenols (López-Romero *et al*., 2018). Finally, the *C. illinoinensis* extract could be an essential source of non-polar compounds with antimicrobial activity, even though these extracts contain an unknown number of molecules (Cruz-Vega *et al*., 2008). Thus all compounds present in plant extracts award antifungal activity disrupting the cell membrane, affecting the mitochondrial function, arresting cell cycle processes at the S-phase, and provoking leakage of intercellular components due to the deterrent effect as in case of saponins (López-Romero *et al*., 2018).

Conclusion

The ethanolic plant extracts presented higher antifungal activity against *F. oxysporum* compared with the aqueous extracts, being the extracts of *A. lechuguilla* leaves and roots, *C. illinoinensis* husk and *L. graveolens* leaves and stem, the extracts with better antifungal activity. Therefore, it can be concluding that the solvent used to produce Mexican semi-desert plant extracts affects the phytochemical compounds presents in the extracts. For this reason, the use of plant extracts proposed as an alternative for control vascular wilt caused by *F. oxysporum*.

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