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Plant extracts to control *Fusarium oxisporum, Fusarium solani and Rhizoctonia solani*: a sustainable alternative for agriculture

Extractos vegetales para el control de *Fusarium oxysporum, Fusarium solani y Rhizoctonia solani*, una alternativa sostenible para la agricultura

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ABSTRACT

Agriculture currently requires alternatives to the use of pesticides to control plant pathogens, such as plant extracts that can help minimize losses from plant pathogens, without causing harm to human health. In this work, the effect of plant extracts on *Fusarium oxysporum*, *Fusarium solani* and *Rhizoctonia solani* was evaluated *in vitro*. The methanolic extracts (ME) of: *Moringa oleifera* (Moringa, leaves), *Persea americana* (Avocado), *Equisetum hymale* (Horsetail), *Larrea tridentata* (Gobernadora), *Gnaphalium semiamplexicaule* (Gordolobo), *Peumus boldus* (Boldo), *Brickellia squarrosa* (Prodigiosa), *Rosmarinus officinalis* (Rosemary) and *Physalis coztomatl* (Costomate), were obtained using a Soxhlet kit at a concentration of 10% (w/V). Using the statistical software MInitab 16® México, an analysis of variance (ANDEVA) and comparison of Tukey means ($p \leq 0.05$) were performed. The mycelial growth inhibition percentage was determined separately. The ME of *Larrea tridentata* (Gobernadora) 100% inhibited the growth of *Fusarium solani* and *Rhizoctonia solani* for up to 144 h, and of *F. oxysporum* for up to 240 h. The ME of *Brickellia squarrosa* (Prodigiosa) and *Rosmarinus officinalis* (Rosemary) also inhibited mycelial growth. These extracts represent an excellent alternative to the conventional control and management of plant pathogens.

Keywords: phytopathogens, *Fusarium oxysporum, F. solani, Rhizoctonia solani,* plant extracts and biocontrol.

RESUMEN

Actualmente la agricultura requiere alternativas al uso de agrotóxicos para controlar fitopatógenos, los extractos vegetales pueden contribuir a minimizar pérdidas por fitopatógenos sin causar daños a la salud humana. El objetivo de este trabajo fue evaluar *in vitro,* el efecto de extractos de plantas sobre *Fusarium oxysporum, Fusarium solani* y *Rhizoctonia solani.* Se evaluaron los extractos metanólicos (EM) de: *Moringa oleifera* (Moringa), *Persea americana* (Aguacate), *Equisetum hymale* (Cola de caballo), *Larrea tridentata* (Gobernadora), *Gnaphalium semiamplexicaule* (Gordolobo), *Peumus boldus* (Boldo), *Brickellia squarrosa* (Prodigiosa), *Rosmarinus officinalis* (Romero) y *Physalis coztomatl* (Costomate), que se obtuvieron utilizando un equipo Soxhlet a una concentración del 10% (p/V). Mediante el software estadístico MInitab 16® México, se hizo un análisis de varianza (ANDEVA) y comparación de medias de Tukey ($p \leq 0.05$). Por separado se determinó el porcentaje de inhibición del crecimiento micelial. El EM de *Larrea tridentata*

(Gobernadora) inhibió al 100% el crecimiento de *Fusarium solani* y de *Rhizoctonia solani* hasta por 144 h, y de *F. oxysporum* hasta por 240 h. Los EM de *Brickellia squarrosa* (Prodigiosa) y *Rosmarinus officinalis* (Romero) también inhibieron el crecimiento micelial. Estos extractos representan una excelente alternativa al control y manejo convencional de fitopatógenos.
Palabras clave: fitopatógenos, *Fusarium oxysporum, F. solani, Rhizoctonia solani,* extractos vegetales y biocontrol.

INTRODUCTION

Scientific and technological advances manage to increase the productivity of agriculture. In general, the increase is due to the incorporation of synthetic products such as fertilizers and pesticides that generate environmental problems (Samsidar *et al.,* 2018). Currently agriculture requires ecological, economic and environmentally friendly alternatives to manage diseases and minimize the use of synthetic agrochemicals (Tamilselvi and Arumugam, 2017), which do not harm the health of agricultural laborers and consumers (Samsidar *et al*., 2018).

Root diseases in crops are one of the most difficult problems to control, because in the soil there are very particular conditions that provide root phytopathogens (RP) with elements and optimal conditions for their establishment and development (García, 2010). Given the need to have ecological solutions and to reduce the negative impact of pesticides on ecosystems, plant extracts have been found to be a sustainable option to mitigate phytosanitary problems and reduce the economic losses they cause (Cerqueira *et al.,* 2016).

Traditionally the control and management of phytosanitary problems is done with pesticides, this management has adverse consequences on health and the ecosystem; in addition to generating resistance in phytopathogens to some chemical synthesis compounds used in agriculture (Del Puerto *et al.,* 2014). However, the feasibility of controlling phytopathogens with other options such as plant extracts should be explored, which can be just as effective for the control and management of phytopathogens. The extracts can be obtained through different methods: steam distillation-solvent extraction, supercritical fluid extraction (Stashenko *et al.,* 2003); soxhlet and cold leaching or percolation (Henao *et al.,* 2009).

There are research works where plant extracts are used for the management of phytopathogens. There are those that have been done for; inhibition of *Phytophthora infestans* (Gamboa-Alvarado *et al.,* 2003), *Pythium control* (Lira-Saldívar *et al.,* 2003; Osorio *et al.,* 2010), *Verticillium dahliae* control (López-Benítez *et al.,* 2005) , *Sclerotinia sclerotiorum* (Al-Reza *et al.,* 2010), *Fusarium oxysporum* (Rodríguez *et al.,* 2012; Cáceres Rueda de León *et al.,* 2013; Vásquez *et al.,* 2014; Dania *et al.,* 2014; Ferdes *et al.,* 2017), control of *Fusarium solani* (Zaker, 2014; Vásquez *et al.,* 2014) and *Rhizoctonia solani*

(López-Benítez *et al.,* 2005; Jasso de Rodríguez *et al.,* 2007; Zamora-Natera *et al.,* 2008; Dania *et al*., 2014).

The effect of plant extracts on some pathogens, whether of medical or agricultural interest, is due to the fact that they contain secondary metabolites with a fungicidal and/or bactericidal effect, including phenolic compounds, coumarins, flavonoids, tannins, quinones, among others. There are works where the effect of plants on various pathogens is evaluated; for example, the effect of the extracts of: *Moringa oleífera* (Canett-Romero *et al.*, 2014), *Equisetum hymale* (De Queiroz *et al.,* 2015), *Larrea tridentata* (Bañuelos-Valenzuela *et al.,* 2018), *Peumus boldus* (Mazutti *et al.,* 2008) and *Rosmarinus officinalis* (Rozman and Jersek, 2009).
Therefore, the objective of this work was to evaluate *in vitro*, the effect of nine methanolic extracts on the phytopathogens, *Fusarium oxysporum*, *F. solani* and *Rhizoctonia solani*. With the hypothesis that plant extracts are capable of controlling and inhibiting the growth of some phytopathogenic microorganisms.

MATERIAL AND METHODS

The study was carried out at the the Superior Technological Institute of Rioverde (ITSR) facilities, located on the Rioverde-San Ciro de Acosta Highway, Km. 4.5, Col. María del Rosario, Rioverde, San Luis Potosí, Mexico. For the present study, extracts from nine recognized plants were used; which were *Moringa oleifera* (Moringa, leaves), *Persea americana* (Avocado, leaves), *Equisetum hymale* (Horsetail, sprouts), *Larrea tridentata* (Gobernadora, leaves), *Gnaphalium semiamplexicaule* (Gordolobo, sprouts), *Peumus boldus* (Boldo, sprouts), *Brickellia squarrosa* (Prodigiosa, sprouts), *Rosmarinus officinalis* (Rosemary, sprouts) and *Physalis coztomatl* (Costomate, roots).
The extracts at 10% w/V (weight/volume) were obtained with the Soxhlet equipment (Cornig-Pyrex Model 3840-XL®), for five cycles using methanol (Merck®) as a stripping solvent. The extracts were packed in amber glass bottles and they were stored at 4 °C; Soxhlet extraction was done eight days before the boxes were prepared (Gamboa-Alvarado *et al*., 2003).

The phytopathogenic fungi used in this work were *Fusarium oxysporum*, *F. solani* and *Rhizoctonia solani*. These microorganisms were isolated from local cultures of Tomato (*Solanum lycopersicum*), and identified by molecular techniques. DNA extraction was done following the protocol described by Reader and Broda (1989). The sequence of the internal region of the 18S Rdna transcript was amplified, using the universal oligos ITS1 (5´TCC GTA GGT GAA CCT GCG G 3´) and ITS4 (5´TCC TCC GCT TAT TGA TAT GC 3´), designed by White *et al.,* (1990). The PCR products were cloned into the pGEM-Teasy vector, following the manufacturer's instructions (Promega 2015); were sequenced by the Sanger method in the National Laboratory of Agricultural, Medical and Environmental

Biotechnology of the Instituto Potosino de Investigación Científica y Tecnológica A.C. Once, ITS were sequenced, they were analyzed with BLAST (Basic Local Alignment Search Tool) (Morgulis *et al.,* 2008) at the NCBI (National Center for Biotechnology Information) http://www.ncbi.nlm.nih.gov/BLAST/.

In vitro evaluation of plant extracts against phytopathogens

The extract effect evaluation (25 ppm) was made by means of the poisoned culture method (Grover, 1962; Gamboa-Alvarado *et al.,* 2003). This technique consists of incorporating the plant extract in Potato Dextrose Agar culture medium (PDA BD Bioxon® México) and measuring the mycelial growth of the phytopathogens *F. solani*, *F. oxysporum* and *R. solani*. The treatments of moringa, gobernadora, avocado, horsetail, boldo, gordolobo, prodigiosa, rosemary and costomate extracts were evaluated by placing 500 µL (25ppm) of the extracts per Petri dish (20 mL). Agar without any extract (only PDA) was included as a control. Each of the extracts was evaluated in triplicate against each of the phytopathogens (*R. solani*, *F. solani* and *F. oxysporum*), measuring mycelial growth every 24 h with a precision digital vernier +/-. 001", +/-.02 mm (Mitutoyo; 500-196-30C®). To cultivate the microorganisms, PDA medium was used, 39 g per liter of sterile distilled water. 20 mL of a PDA: ME (Potato Dextrose Agar: Methanolic Extract) mixture were poured into Petri dishes of 90 mm diameter, in a ratio of 1: 0.05; that is, 500 µL of extract per box. All of the above in a laminar flow hood (Brand LABCONCO® LABCO07283).
After this time, the boxes were inoculated with a PDA 5 mm diameter explant, with growth of the phytopathogen and they were incubated in a bioclimatic chamber at 25 °C (Thermo Scientific® Mod 3949 brand). All tests were done in triplicate in a completely randomized experimental design.

Data analysis

With the data obtained in the experimental phase, the percentage of mycelial growth inhibition was calculated with the following formula:

$$\text{I. C. M.} = [(\text{Dc} - \text{Dt})/\text{Dc}]x100$$

where: I.C.M. is the percentage of mycelial growth inhibition, Dc is the diameter of the mycelium in the control and Dt = diameter of the mycelium in the treatment.
Likewise, the analysis of variance (ANDEVA) and the Tukey multiple mean comparison ($p \leq 0.05$) were performed. The analysis was done with the statistical software Minitab 16® Mexico.

RESULTS AND DISCUSSION

When evaluating the effect of Methanolic Extracts (ME) on *Fusarium oxysporum*, a statistical difference was observed between the treatments ($p \leq 0.05$). The ME of *L. tridentata* inhibited 100% the growth of the pathogen for up to 144 h, and 90% for up to

240 h; while that of *R. officinalis* inhibited 50.7% up to 72 h, and 42.2% for 144 h (Table 1). López-Benítez *et al.,* in 2005, presented similar results. They indicate that the extracts of *Syzygium aromaticum* and *L. tridentata* at 10% inhibit *F. oxysporum* f sp, *Lycopersici* growth, up to 144 h. Dania *et al.,* in 2014, documented that the aqueous extracts (AE) of *Oryza sativa* and *Quercus phillyraeoides* at 1.5 and 2.5% can totally inhibit *in vitro* the growth of six phytopathogens, among them *F. oxysporum*.

On the other hand, the ethanolic extracts (EE) of *Fluorensia cernua* (Leavesén), *F. microphylla* and *F. retinophylla* were shown to be an effective alternative for the control of *Fusarium oxysporum*, inhibiting its growth by 100 %, with a concentration of 1500 µL L^{-1} (Jasso de Rodríguez *et al.,* 2007).

There are studies where Essential Oils (EO) have also been evaluated for the management of *F. oxysporum.* There is the work of Ferdes *et al.,* in 2017, with AE of *R. officinalis*, at a concentration of 20-µg mL^{-1} 93% of the *F. oxysporum* growth is inhibited; while with 10-µg mL^{-1} of the AE of *P. anisum* and *S. hortensis* the growth of *F. oxysporum* is totally inhibited. Likewise, Vásquez *et al.,* in 2014, concluded that the EOs of *Chenopodium ambrosioides* at 2% and *Chenopodium album* at 0.03% completely inhibit the growth of *F. oxysporum* f. sp. *Lycopersici*, up to 11 days.

Table 1. Inhibition of mycelial growth (ICM, %) of *Fusarium oxysporum* with different methanolic extracts at 72, 144 and 240 h, after experiment start

Plant extract	72 h		144 h		240 h	
L. tridentata	100.0 ± 0.0	a	100.0 ± 0.0	a	89.4 ± 0.3	a
B. squarrosa	39.8 ± 4.4	bc	38.6 ± 2.3	bc	31.7 ± 2.7	b
G. semiamplexicaule	24.6 ± 2.3	cd	28.3 ± 1.2	cd	24.2 ± 2.4	bc
P. americana	25.1 ± 5.4	cd	26.3 ± 2.2	d	12.9 ± 4.9	cd
P. boldus	26.0 ± 3.9	cd	24.5 ± 4.1	d	11.7 ± 2.7	cd
P. coztomatl	24.8 ± 4.2	cd	27.4 ± 1.5	cd	9.2 ± 4.0	bc
M. oleifera	19.7 ± 3.4	d	17.1 ± 2.9	d	4.0 ± 1.4	d
R. officinalis	50.7 ± 2.9	b	42.2 ± 1.5	b	29.6 ± 2.2	b

Means with different letters indicate statistical difference (Tukey, $p \leq 0.05$).

In this study, the ME of *L. tridentata* inhibited *F. oxysporum* growth, up to 144 h, followed in effectiveness by that of *R. officinalis* and *B. squarrosa*. With these treatments, mycelial growth is inhibited up to 80%, compared to the negative control (Table 2). These results coincide with those reported by Osorio *et al.,* in 2010, they conclude that with a concentration of 0.7 mg kg^{-1} *L. tridentata* inhibits 100% *F. oxisporum* growth *in vitro*.

In the results of the extracts on *Fusarium solani*, by means of the ANDEVA and the comparison of Tukey means ($p \leq 0.05$), a statistically significant difference was observed between treatments. The ME of *L. tridentata* inhibited 100% of *F. solani* growth, up to ten days; showing an important inhibitory effect on this phytopathogen. The foregoing is relevant if it is considered that there are chemical products that are applied one or more times a week and do not achieve these results. Even under laboratory conditions, they

cannot inhibit the mycelium growth 100%, as reported by Yossen and Conles in 2016, which in their work with commercial molecules reached an inhibition of 60 to 97%. The *R. officinalis* ME evaluated in this work inhibited mycelium growth by 70%, compared to the control for up to 144 h. This data is similar to that obtained with aqueous extracts of *Prosopis juliflora* and *Lantana camara*, which achieve 80 and 69% mycelial inhibition, respectively (Seetha *et al.,* 2010). Likewise, studies by David *et al.,* in 2013, report that the ME of flower buds of *Calotropis gigantea* at 25% reduces *F. solani* growth by 68%.

Table 2. Average mycelial growth (mm) of *Fusarium oxysporum* with different methanolic extracts at 72, 144 and 240 h, after experiment start

Plant extract	72 h		144 h		240 h	
Agar	22.3 ± 0.4	a	44.4 ± 0.7	a	65.9 ± 0.7	a
L. tridentata	0.0 ± 0.0	e	0.0 ± 0.0	e	7.0 ± 0.2	e
B. squarrosa	13.4 ± 1.0	cd	27.3 ± 1.0	cd	45.0 ± 1.7	d
R. officinalis	11.0 ± 0.6	d	25.7 ± 0.6	d	46.4 ± 1.4	d
G. semiamplexicaule	16.8 ± 0.5	bc	31.8 ± 0.5	bc	50.0 ± 1.6	cd
P. coztomatl	16.8 ± 0.9	bc	32.3 ± 0.7	bc	53.3 ± 2.6	cd
E. hymale	18.2 ± 0.1	b	35.4 ± 1.5	b	56.7 ± 1.7	bc
P. americana	16.7 ± 1.2	bc	32.7 ± 1.0	b	57.5 ± 3.2	abc
P. boldus	16.5 ± 0.9	bc	33.5 ± 1.8	b	58.3 ± 1.8	abc
M. oleifera	17.9 ± 0.8	b	36.8 ± 1.3	b	63.3 ± 0.9	ab

Means with different letters indicate statistical difference (Tukey, p ≤ 0.05)

Table 3. Inhibition of mycelial growth (ICM, %) of *Fusarium solani* with different methanolic extracts at 72, 144 and 240 h, after the experiment start

Plant extract	72 h		44 h		240 h	
L. tridentata	100.0 ± 0.0	a	100.0 ± 0.0	a	100.0 ± 0.0	a
R. officinalis	64.7 ± 0.9	b	70.2 ± 0.3	b	58.5 ± 1.7	b
B. squarrosa	37.9 ± 0.4	c	37.2 ± 1.3	c	11.8 ± 0.9	c
P. coztomatl	26.5 ± 1.2	de	28.5 ± 1.8	d	5.7 ± 0.4	d
P. americana	26.9 ± 2.5	de	28.4 ± 1.2	d	0.5 ± 0.5	e
P. boldus	31.2 ± 1.2	cd	30.8 ± 0.3	d	0.0 ± 0.0	e
G. semiamplexicaule	26.3 ± 2.0	de	28.1 ± 0.3	d	0.0 ± 0.0	e
M. oleifera	22.6 ± 3.4	de	15.5 ± 0.9	f	0.0 ± 0.0	e
E. hymale	21.5 ± 2.4	e	22.2 ± 0.6	e	0.0 ± 0.0	e

Means with different letters indicate statistical difference (Tukey, p ≤ 0.05)

It is important to have ecological options for phytopathogenic fungi control, such as those explored in this work. In this research, the results with the ME of *L. tridentata* coincided with those of Osorio *et al.,* in 2010, who used *L. tridentata* polyphenolic extracts, and managed to inhibit 100% of *F. solani* growth, with a concentration of 0.70 mg L^{-1}.

In a study conducted by Vásquez *et al.,* (2014), the fungistatic effect of aqueous extracts (AE) and essential oils (EO) of species of *Chenopodium* genus on *F. solani* was evaluated. The authors concluded that the EO of *Chenopodium ambrosioides* at 2% and

Chenopodium album at 0.03% completely inhibit *F. solani* growth of, up to 11 days; while in this work *L. tridentata* inhibited it 100% for up to 10 days. Another case with similar results on *Fusarium* spp. was the work of Duarte *et al.,* en el 2013, who with essential oils from *Piper aduncum* subsp. *ossanum* and Piper aurintum totally inhibit the growth of this fungus.

On the other hand, Zaker (2014) concluded that the ME of *Artemisia annua* leaves at 15%, is capable of inhibiting *F. solani* growth; while in the present work in ME of *L. tridentata* it inhibited *F. solani* growth to 100%, followed by *R. officinalis* extracts and *B. squarrosa*; which, although to a lesser extent, also inhibit it (Table 4).

Table 4. Average mycelial growth (mm) of *Fusarium solani*, with different methanolic extracts at 72, 144 and 240 h, after the experiment start

Plant extract	72 h		144 h		240 h	
Agar	30.1 ± 0.4	a	66.6 ± 0.7	a	80.0 ± 0.0	a
L. tridentata	0.0 ± 0.0	f	0.0 ± 0.0	g	0.0 ± 0.0	e
R. officinalis	10.6 ± 0.3	e	19.8 ± 0.2	f	33.2 ± 1.3	d
B. squarrosa	18.7 ± 0.1	d	41.9 ± 0.9	e	70.5 ± 0.8	c
P. coztomatl	22.1 ± 0.3	bc	47.6 ± 1.2	d	75.5 ± 0.3	b
P. americana	22.0 ± 0.8	bc	47.7 ± 0.8	d	79.6 ± 0.4	a
P. boldus	20.7 ± 0.4	cd	46.1 ± 0.2	d	80.0 ± 0.0	a
G. semiamplexicaule	22.2 ± 0.6	bc	47.9 ± 0.2	d	80.0 ± 0.0	a
M. oleifera	23.3 ± 1.0	bc	56.3 ± 0.6	b	80.0 ± 0.0	a
E. hymale	23.7 ± 0.7	b	51.8 ± 0.4	c	80.0 ± 0.0	a

Means with different letters indicate statistical difference (Tukey, $p \leq 0.05$)

L. tridentata methanolic extract (ME), inhibits up to 100% of *Rhizoctonia solani* growth, during the first ten days. The above indicates that this extract is an effective fungistatic. With the ANDEVA and the comparison of Tukey means with a significance of 95%, a significant statistical difference was observed between treatments, since in addition to *L. tridentata*, the ME of *R. officinalis* also presents a remarkable inhibition percentage, causes a fungistatic effect 56 and 48% at 144 h and 240 h, respectively (Table 5).

Controlling phytopathogenic fungi growth with plant extracts represents a great advance in plant protection. In the case of *Rhizoctonia solani*; Gamboa-Alvarado *et al.,* in 2003 reported results similar to those obtained in this research work; found that the ME of *Fluorensia cernua* (Hojasén) at a concentration of 20,000 mg L^{-1}, inhibits 85% the growth of this fungus for up to 96 hours, compared to the control. López-Benítez *et al.,* in 2005, with aqueous extracts of *L. tridentata* and *Cinnamomum zeylanicum* at 10%; of Syzygium *aromaticum* at 5%, were able to inhibit growth *in vitro* for up to 144 h, and with the aqueous extract of *Quercus phillyraeoides* at 3.5% they inhibited the growth of the phytopathogen by 94%. Similarly, the alkaloid extract of *Lupinus mexicanus* against *R. solani* in a concentration of 5 mg mL^{-1} inhibits its growth 100% (Zamora-Natera *et al.,* 2008). Jasso de Rodríguez et al., (2007) mention that the ethanolic extracts of *Flourensia cernua* and *F. retinophylla* at a concentration of 1000 µL L^{-1} inhibit their growth. Similarly, Al-Reza *et*

al., en el 2010, determined that the EO of *Cestrum nocturnum* has a high fungistatic power capable of inhibiting the growth of *R. solani* up to 80%. Also Touba *et al.,* in 2012, found that *Kaempferia galanga* AE could totally inhibit it. On the other hand, Dania *et al.,* in 2014 demonstrated that the 1% aqueous extract of *Oryza sativa* Husk, *in vitro*, totally inhibits the growth of this phytopathogen.

Table 5. Inhibition of mycelial growth (ICM, %) in time of *Rhizoctonia solani* with different methanolic extracts at 72, 144 and 240 h, after experiment start

Plant extract	72 h		144 h		240 h	
L. tridentata	100.0 ± 0.0	a	100.0 ± 0.0	a	100.0 ± 0.0	a
R. officinalis	56.8 ± 0.9	b	56.2 ± 1.9	b	48.0 ± 3.0	b
B. squarrosa	32.7 ± 1.7	c	23.2 ± 2.2	c	17.8 ± 2.2	c
P. americana	24.5 ± 1.6	c	19.1 ± 1.2	cd	8.1 ± 1.3	d
P. coztomatl	26.9 ± 3.9	c	21.6 ± 1.4	cd	7.5 ± 1.1	d
P. boldus	28.7 ± 2.2	c	21.3 ± 1.1	cd	7.5 ± 2.3	d
E. hymale	25.9 ± 1.9	c	18.5 ± 0.2	cd	2.7 ± 0.9	d
M. oleifera	24.4 ± 3.1	c	17.0 ± 3.9	cd	1.8 ± 0.9	d
G. semiamplexicaule	23.8 ± 0.7	c	14.1 ± 1.3	d	1.1 ± 0.5	d

Means with different letters indicate statistical difference (Tukey, $p \leq 0.05$

Table 6. Average mycelial growth (mm) of *Rhizoctonia solani*, with different methanolic extracts at 72, 144 and 240 h, after experiment start

Plant extract	72 h		144 h		240h	
Agar	19.9 ± 0.2	a	45.8 ± 0.2	a	80.0 ± 0.0	a
L. tridentata	0.0 ± 0.0	d	0.0 ± 0.0	e	0.0 ± 0.0	e
R. officinalis	9.6 ± 0.2	c	19.5 ± 0.8	d	34.3 ± 2.0	d
B. squarrosa	15 ± 0.4	b	34.1 ± 1.0	c	54.2 ± 1.5	c
E. hymale	16.5 ± 0.4	b	36.2 ± 0.1	bc	64.2 ± 0.6	b
G. semiamplexicaule	17 ± 0.1	b	38.1 ± 0.6	b	65.2 ± 0.3	b
M. oleifera	16.9 ± 0.7	b	36.9 ± 1.7	bc	64.7 ± 0.6	b
P. americana	16.8 ± 0.3	b	35.9 ± 0.5	bc	60.6 ± 0.9	b
P. boldus	15.9 ± 0.5	b	34.9 ± 0.5	bc	61.0 ± 1.5	b
P. coztomatl	16.3 ± 0.9	b	34.8 ± 0.6	bc	61.0 ± 0.7	b

Means with different letters indicate statistical difference (Tukey, $p \leq 0.05$)

CONCLUSION

The methanolic extract of gobernadora (*Larrea tridentata*), is effective to inhibit the growth of *Fusarium oxysporum*, *Fusarium solani* and *Rhizoctonia solani*, up to ten days. Similarly, it is concluded that the methanolic extract of *Rosamarinus officinalis* (Rosemary) can be used for the management of *Fusarium oxysporum, Fusarium solani* and *Rhizoctonia solani*, with less effectiveness than that of *Larrea tridentate*.

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CITED LITERATURE

AL-REZA S, Rahman A, Ahmed Y, Kang S. 2010. Inhibition of plant pathogens *in vivo* and *in vitro* with essential oil and organic extracts of *Cestrum nocturnum* L. *Pestic. Biochem. Physiol.* 96: 86-92. https://doi.org/10.1016/j.pestbp.2009.09.005

BAÑUELOS-VALENZUELA R, Delgadillo-Ruiz L, Echavarría-Cháirez F, Delgadillo-Ruiz O, Meza-López C. 2018. Composición química y FTIR de extractos etanólicos de *Larrea tridentata, Origanum vulgare, Artemisa ludoviciana y Ruta graveolens*. *Agrociencia*. 52(3): 309-321. http://www.colpos.mx/agrocien/Bimestral/2018/abr-may/art-2.pdf

CÁCERES RUEDA DE LEON I, Colorado R, Salas E, Muñoz L, Hernández L. 2013. Actividad Antifúngica *in vitro* de Extractos Acuosos de especias contra *Fusarium oxysporum*, *Alternaria alternata*, *Geotrichum candidum*, *Trichoderma* spp., *Penicillum digitatum* y *Aspergillus niger*. *Rev. Mex. Fitopatol*. 31:105-112. ISSN 0185-3309. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0185-33092013000200003

CANETT-ROMERO R, Arvayo-Mata KL, Ruvalcaba-Garfias NV. 2014. Aspectos tóxicos más relevantes de *Moringa oleifera* y sus posibles daños. *Biotecnia.* 16(2):36-43. https://biotecnia.unison.mx/index.php/biotecnia/article/view/45/41

CERQUEIRA M, Barcellos H, Bueno P, Aires J, Dummer M. 2016. Antifungal activity of plants extracts with potential to control plant pathogens in pineapple. *Asian Pac J Trop Biomed.* 6:26-31. https://doi.org/10.1016/j.apjtb.2015.09.026

DANIA V, Fadnia O, Ayodele M, Kumar L. 2014. Efficacy of *Oryza sativa* husk and *Quercus phillyraeoides* extracts for the *in vitro* and *in vivo* control of fungal rot disease of white yam (*Discorea rotundata* Poir). *SpingerPlus*. 3:711. https://doi.org/10.1186/2193-1801-3-711

DAVID M, Kumar R, Bhavani M. 2013. Experimental studies on active metabolites of *Calotropis gigantea* for evaluation of possible antifungal properties. *Int. Res. J. Pharm.* 4:250-254. https://www.researchgate.net/publication/239524862_Experimental_Studies_on_active_metabolites_of_Calotropis_gigantea_for_evaluation_of_possible_antifungal_properties

DE QUEIROZ GM, Politi FA, Rodrigues ER, Souza-Moreira TM, Moreira RR, Cardoso CR, Pietro RC. 2015. Phytochemical characterization, antimicrobial activity, and antioxidant potential of *Equisetum hyemale* L.(Equisetaceae) extracts. *Journal of medicinal food.* 18(7):830-834. https://www.liebertpub.com/doi/abs/10.1089/jmf.2014.0089

DEL PUERTO RODRIGUEZ AM, Suárez Tamayo S, Palacio Estrada DE. 2014. Efectos de los plaguicidas sobre el ambiente y la salud. *Revista Cubana de Higiene y Epidemiología. 52*(3): 372-387. https://www.researchgate.net/publication/317518438_Efectos_de_los_plaguicidas_sobre_el_ambiente_y_la_salud

DUARTE Y, Pino O, Martínez B. 2013. Efecto de cuatro aceites esenciales sobre *Fusarium* spp. *Rev. Prot. Veg*. 28:232-235. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1010-27522013000300013

FERDES M, Al Juhaimi F, Özcan M, Ghafoor K. 2017. Inhibitory effect of some plant essential oils on growth of *Aspergillus niger*, *Aspergillus oryzae*, *Mucor pusillus* and *Fusarium oxysporum*. *S. Afr. J. Bot*. 113: 457-460.https://doi.org/10.1016/j.sajb.2017.09.020

GAMBOA-ALVARADO R, Hernández-Castillo F, Guerrero-Rodríguez E, Sánchez-Arizpe A. 2003. Inhibición del crecimiento Micelial de *Rhizoctonia solani* Kühn y *Phytophthora infestans* Mont. (De Bary) con extractos vegetales Metanólicos de Hojasén (*Flourensia cernua* D.C.), mejorana (*Origanum majorana* L.) y trompetilla [*Bouvardia ternifolia (Ca.)* Shlecht.]. *Rev. Mex. Fitopatol*. 21:13-18. https://www.redalyc.org/pdf/612/61221102.pdf

GARCÍA R. 2010. Agroecología y enfermedades de la raíz en cultivos agrícolas. Editorial COLPOS. México. Pp. 130. ISBN: 9786077699088.

GROVER RK, More JD. 1962. Toxicometric studies of fungicides against the brown rot organism *Sclerotinia fructivola* and *Sclerotinia laxa. Phytopathology*. 52:876-880.

HENAO J, Muñoz LJ, Ríos E, Padilla L, Giraldo GA. 2009. Evaluación de la actividad antimicrobiana de los extractos de la planta *Lippia origanoides* HBK cultivada en el Departamento del Quindío. *Rev Invest Univ Quindio* 19:159-64. http://blade1.uniquindio.edu.co/uniquindio/revistainvestigaciones/adjuntos/pdf/f4d9_n1918.pdf

JASSO DE RODRIGUEZ D, Hernández-Castillo D, Angulo-Sánchez JL, Rodríguez-García R, Villarreal-Quintanilla JA, Lira-Saldívar RH. 2007. Antifungal activity in vitro of *Flourensia* spp. extracts on *Alternaria* sp., *Rhizoctonia solani*, and *Fusarium oxysporum*. *Ind. Crop. Prod.* 25(2):111-116. https://doi.org/10.1016/j.indcrop.2006.08.007

LIRA-SALDÍVAR R, Balvantín-García G, Hernández-Castillo F, Gamboa-Alvarado R, Jasso-de-Rodríguez D, Jiménez-Díaz F. 2003. Evaluation of Resin Content and the Antifungal Effect of *Larrea tridentata* (Sesse and Moc. Ex D.C.) Coville Extracts from Two Mexican Deserts Aganist *Phythium* sp. Pringsh. *Rev. Mex. Fitopatol*. 21:97-101. https://www.redalyc.org/articulo.oa?id=61221201

LÓPEZ-BENÍTEZ A, López-Betancourt S, Vázquez-Badillo M, Rodríguez-Herrera S, Mendoza-Elos M, Padrón-Corral E. 2005. Inhibición del crecimiento micelial de *Fusarium oxysporum* schlechtend. F. sp. *lycopersici* (Sacc.) Snyder y Hansen, *Rhizoctonia Solani* Kühn y *Verticullium dahliae* kleb mediante extractos vegetales acuosos. *Rev. Mex. Fitopatol.* 23:183-190. https://www.redalyc.org/articulo.oa?id=61223212

MAZUTTI M, Mossi AJ, Cansian RL, Corazza ML, Dariva C, Oliveira JV. 2008. Chemical profile and antimicrobial activity of Boldo (*Peumus boldus* Molina) extracts obtained by compressed carbon dioxide extraction. *Brazilian Journal of Chemical Engineering*. 25(2): 427-434. https://www.scielo.br/scielo.php?pid=S0104-63220080002000 20&script=sci_arttext

MORGULIS A, Coulourios G, Raytselis Y, Madden TL, Agarwala R, Schäffe AA. 2008. Database indexing for production MegaBLAST searches. *Bioinformatics*. 24(16):1757-1764. https://academic.oup.com/bioinformatics/article/24/16/1757/202524

OSORIO E, Flores M, Hernández D, Ventura J, Rodríguez R, Aguilar C. 2010. Biological efficiency of polyphenolic extracts from pecan nuts Shell (*Carya illinoensis*), pomegranate husk (*Pnica granatum*) and creosote bush leaves (*Larrea tridentata* Cov.) against plant pathogenic fungi. *Ind. Crop. Prod*. 31:153-157. https://doi.org/10.1016/j.indcrop.2009.09.017

PROMEGA 2015. Technical manual. pGEM®-T y pGEM®- T Easy Vector Systems. Instructions for use of products. https://worldwide.promega.com/products/pcr/pcr-cloning/pgem-t-easy-vector-systems/?catNum=A1360

RAEDER U, Broda P. 1985. Rapid preparation of DNA from filamentous fungi. *Letters in Applied Microbiology*. 1(1):17-20. https://doi.org/10.1111/j.1472-765X.1985.tb01479.x

RODRÍGUEZ A, Ramírez M, Bautista S, Cruz A, Rivero D. 2012. Actividad antifúngica de extractos de *Acacia Farnesiana* sobre el crecimiento *in vitro* de *Fusarium oxysporum* f. sp. *lycopersici. Revista Científica UDO Agrícola*. 12:91-96. http://www.bioline.org.br/pdf?cg12011

ROZMAN T, Jersek B. 2009. Antimicrobial activity of rosemary extracts *(Rosmarinus officinalis* L.) against different species of Listeria. *Acta agriculturae Slovenica*, 93(1):51. https://pdfs.semanticscholar.org/0ad5/a7c3ece5e98fa57738406ae2c819923cf3f0.pdf

SÁNCHEZ LG, Vargas-Rincón A, Jiménez P. 2015. Evaluación de la actividad antifúngica de extractos etanólicos de dos morfotipos de *Raphanus raphanistrum* L. sobre tres hongos fitopatógenos. *Bioagro.* 27(1):3-10. https://www.redalyc.org/pdf/857/85741584002.pdf

SAMSIDAR A, Siddiquee S, Shaarani MS. 2018. A review of extraction, analytical and advanced methods for determination of pesticides in environment and foodstuffs. Trends. *Food. Sci. Technol*. 71:188-201. https://doi.org/10.1016/j.tifs.2017.11.011

SEETHA J, Reddy R, Ramanjaneyulu R. 2010. Evaluation of certain plant extracts and antagonist against *Fusarium solani* and *Alternaria tenuissima,* the incitants od root rot and die-back diseases of Mulberry. *Int. J. Indust. Entomol*. 20:1-5. http://www.koreascience.or.kr/article/JAKO201019451499104.page

STASHENKO EE, Jaramillo BE, Martínez JR. 2003. Comparación de la composición química y de la actividad antioxidante in vitro de los metabolitos secundarios volátiles de plantas de la familia Verbenaceae. *Rev. Acad. Colomb. Cienc*. 27(105): 579-597. ISSN 0370-3908. http://www.accefyn.com/revista/Vol_27/105/8-COMPARACION.pdf

TAMILSELVI N, Arumugam T. 2017. Breeding Approaches for Sustainable Vegetable Production–A Review. *Int. J. Curr. Microbiol. App*. 6:2845-2860. https://doi.org/10.20546/ijcmas.2017.611.336

TOUBA E, Zakaria M, Tahereh E. 2012. Anti-fungal activity of cold and hot water extracts of spices against fungal pathogens of Roselle (*Hibiscus sabdariffa*) *in vitro*. *Microb. Pathog*. 52:125-129. https://doi.org/10.1016/j.micpath.2011.11.001

VÁSQUEZ D, Montes R, Jiménez A, Flores HE. 2014. Aceites esenciales y extractos acuosos para el manejo *in vitro* de *Fusarium oxysporum* f. sp. *lycopersici* and *F. solani*. *Rev. Mex. Fitopatol*. 31:170-179. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0185-33092013000200008

WHITE TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. pp. 315-322 In: Innis, M. A.; Gelfand, D. H.; Sninsky, J. J.; White, T. J. (Eds.). PCR Protocols; A Guide to Methods and Applications, Academic Press, Inc., New York. http://blade1.uniquindio.edu.co/uniquindio/revistainvestigaciones/adjuntos/pdf/f4d9_n1918.pdf

YOSSEN VE, Conles MY. 2016. Eficacia de fungicidas in vitro para el control de *Fusarium oxysporum* y *F. proliferatum*, agentes causales de marchitamiento en el cultivo de orégano en la Argentina. *Revista industrial y agrícola de Tucumán*. 91(1)19-25. https://riat.eeaoc.org.ar/ojs/index.php/riat/article/view/v91n1a03/33

ZAKER M. 2014. Antifungal evaluation of some plant extracts in controlling *Fusarium solani*, the causal agent of potato dry rot *in vitro* and *in vivo*. *Inter. J. Agri. Biosci*. 3:190-195. www.ijagbio.com

ZAMORA-NATERA F, García-López P, Ruíz-López M, Salcedo-Pérez E. 2008. Composición de alcaloides en semilla de *Lupinus mexicanus* (Fabaceae) y evaluación antifúngica y alelopática del extracto alcaloideo. *Agrociencia.* 42:185-192. http://www.scielo.org.mx/scielo.php?script=sci_abstract&pid=S1405-31952008000200006&lng=es&nrm=iso