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Investigation note

In vitro antifungal activity of nanoformulations for the control of *Fusarium solani*

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Abstract

The objective of the research was to evaluate the *in vitro* antifungal activity of two extracts, creosote bush (*Larrea tridentata* L.) and mustard (*Sinapis alba* L.), nanoformulated with silicon oxide and zinc oxide nanoparticles (200 to 400 µ globular) on the mycelial growth and sporulation of *Fusarium solani*, one of the plant pathogens causing vascular wilt and root rot in at least 100 economically important crops. Inhibitory concentrations and the number of conidia were determined using the poisoned media method. Data were analyzed using probit analysis, Anova, and Tukey's test ($p \leq 0.05$). The results showed that mustard-only treatments have the most effective ID50 with 920.57 ppm; nevertheless, the mixture of mustard and creosote bush with SO_2 presented significant results on sporulation, with a formation of 0.35 and 0.48 million conidia ml^{-1} for creosote bush 3% SO_2 and mustard 5% SO_2 compared to the control (7.78).

Keywords:

mycelial growth, nanoparticles, sporulation.

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In Mexico, fruit and vegetable production is an important source of economic resources since, in addition to being a profitable agricultural activity, it requires labor for the traceability of the product as these are the most exported. They are also important for their nutritional value since they contain vitamins, fibers, minerals, and antioxidants that complement the daily diet of people (Cedillo-Portugal, 2017). For export, phytosanitary requirements established by each of the countries with which Mexico has a trade agreement must be complied with, which means that they have to be free of pathogens that cause different diseases in these crops; however, the most important diseases that cause the most production losses are fungi, which affect the quality of the product and lead to entry rejection for the exporting country (SENASICA, 2020).

The list of plant diseases presented by the American Phytopathological Society reports that more than 81 of the 101 economically important plants have at least one disease caused by *Fusarium* spp. (Leslie and Summerell, 2006). Within the genus *Fusarium* is the species *F. solani*, one of the phytopathogens that cause vascular wilt and root rot in at least 100 agricultural crops (Šiši#, 2018).

For its control, the intensive application of fungicides is mainly used, which generates health risks and damage to the environment; in addition, it is unfavorable and inadequate to control the disease, so it is important to have alternatives for its control (El-Mohamedy *et al*., 2014).

Some of the alternatives for controlling fungal diseases are using plant extracts and essential oils. Research has shown that they are safe for the environment and consumers and effective for the control of diseases caused by *Fusarium*; tests have also been carried out with commercial botanical formulations in mixtures with plant extracts and biological control microorganisms to analyze interactions (Villa-Martínez *et al*., 2015).

On the other hand, there is a technology with a remarkable impact in different sectors; in agriculture, it has diverse applications, mainly in the production of nanopesticides, the production of macro and micronutrients at the nano level, making them more efficient and sustainable (Lira *et al*., 2018). This study aimed to evaluate creosote bush and mustard extracts, nanoformulated with silicon oxide (SO_2) and zinc oxide (ZnO), against the mycelial growth and spore production of *F. solani*.

The fungus *F. solani* was reactivated in potato dextrose agar (PDA) medium after being identified by morphological characteristics according to Lesley and Summer (2006); the strain was obtained from the Toxicology Laboratory of the Department of Parasitology at the Antonio Narro Autonomous Agrarian University (UAAAN), for its acronym in Spanish. The extracts and nanoparticles (200 to 400 µ globular) were acquired from the company Culta SA de CV. Fourteen treatments were obtained, which included pure extracts and nanoformulations with SiO_2 and ZnO at 1, 3, and 5% per 100 ml of the extract.

To prepare the concentrations (0, 10, 100, 50, 1 000, 3 000, and 5 000 ppm), the extract at 100% was considered. To carry out the bioassays, the methodology followed was PDA poisoned medium in Petri dishes, leaving 24 h for solidification; a fragment of 0.5 cm of *F. solani* was placed in each Petri dish, they were incubated at 26 °C, and the measurement of the growth of the mycelium was taken with a vernier at 24 h until the control reached the dimensions of the Petri dish.

Mycelial growth was considered to obtain the percentage of inhibition using the formula: % inhibition= mycelial growth of the control-mycelial growth of the treatment/mycelial growth of the control x 100 (Ezziyyani *et al*., 2004). Spore counting was performed with a Neubauer chamber based on quantifying spore suspension (Bustillo, 2010).

To obtain the medium inhibitory dose (ID_{50}), a probit analysis was performed with the results of percentage of inhibition; on the other hand, for spore formation, a completely randomized analysis of variance was performed with four repetitions. Each repetition consisted of three dishes per evaluation and a comparison of means test with the Tukey method (*p*# 0.05), using the SAS System version 9.0 statistical program.

The values of the creosote bush and mustard treatments, alone and with the SO_2 and ZnO nanoparticles, are shown in Table 1, where it was observed that in the case of the treatments with creosote bush and SO_2, the one with 5% showed the lowest value of ID_{50} with 3 140 ppm, followed by creosote bush 1% SO_2 with 3 204 ppm. The highest ID_{50} values were creosote bush 3% SO_2

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and creosote bush without nanoparticles, with values of 3 351 and 3 238 ppm, respectively. For the mixture of creosote bush and ZnO, they presented values higher than those with SO_2, with creosote bush 1% ZnO (ID_{50} of 4 739 ppm) being the highest result.

Table 1. Medium inhibitory dose (ID_{50}) on the mycelial growth of *Fusarium solani* by the extracts of creosote bush (*Larrea tridentata*) and mustard (*Sinapis alba*) at different percentages of silicon and zinc oxide nanoparticles.

Treatment	Fiducial limit					
	ID_{50} (ppm)	Lower	Upper	ID_{90} (ppm)	*p*-value	Prediction equation
Creosote bush	3 238	2 106	4 551	21 024	<0.0001	y= (-5.536873217 ±1.5773482429)
Creosote bush/ Silicon 1%	3 204	2 661	3 773	12 065	<0.0001	y= (-7.801241684 ±2.225340777)
Creosote bush/ Silicon 3%	3 351	2 811	3 921	11 840	<0.0001	y= (-8.241009222 ±2.3377709636)
Creosote bush/ Silicon 5%	3 140	2 612	3 692	11 897	<0.0001	y= (-7.7458 ±2.215055154)
Creosote bush/Zinc 1%	4 739	4 168	5 292	9 668	<0.0001	y= (-15.2126571 ±4.1387039234)
Creosote bush/Zinc 3%	4 061	3 391	4 640	9 664	<0.0001	y= (-12.28192208 ±3.4035029437)
Creosote bush/Zinc 5%	3 988	3 139	4 684	10 228	<0.0001	y= (-11.2798788 ±3.1326844079)
Mustard	920.57	827.41	1 010	2 573	<0.0001	y= (-8.51048383 ±2.8712269152)
Mustard/ Silicon 1%	1 281	1 127	1 414	2 429	<0.0001	y= (-14.31996916 ±4.60831284193)
Mustard/ Silicon 3%	1 308	1 241	1 373	2 278	<0.0001	y= (-16.58320233 ±5.3206547718)
Mustard/ Silicon 5%	1 298	1 240	1 353	2 188	<0.0001	y= (-17.60324779 ±5.6541638072)
Mustard/Zinc 1%	1 231	1 064	1 368	2 611	<0.0001	y= (-12.11889233 ±3.9218341752)
Mustard/Zinc 3%	1 329	1 204	1 442	2 501	<0.0001	y= (-14.57031787 ±4.6649226381)
Mustard/Zinc 5%	1 244	1 168	1 314	2 191	<0.0001	y= (-16.14253382 ±5.215779407)

In this regard, the results of Tequida-Meneses *et al*. (2002), when evaluating the antifungal activity of extracts (creosote bush), showed inhibition of *Fusarium* spp., up to 100%, while Huang (2011),

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when evaluating silicon oxide, shows that the severity of the disease by *F. oxysporum* f. sp. *radicis-lycopersici* decreased considerably when transplanting tomato plants in nutrient solution added with this element. For mustard treatments, the lowest values occurred in mustard without nanoparticles, with an ID_{50} of 920.47 ppm; Drakopoulos *et al*. (2010) showed that mustard helps control diseases caused by *Fusarium*.

On the other hand, mustard with 3% ZnO had the highest ID_{50}, with 1329 ppm, followed by mustard with SO_2 at 1, 3, and 5% (1 281, 1 308, and 1 298 ppm, respectively), while mustard with ZnO at 1% and 5% obtained an ID_{50} of 1 231 and 1 244 ppm. This coincides with what was reported by Siddiqui *et al*. (2019) in a study on the inhibitory effect of zinc nanoparticles, where it was found to be the best treatment on *F. solani*. Regarding sporulation activity, mustard treatments at the highest dose (500 ppm) showed total inhibition (Table 2).

Table 2. Effect of silicon and zinc oxide nanoparticles on the sporulation of *Fusarium solani*.

Extracts	Treatments							
	Concentration	Control	1% SO_2	3% SO_2	5% SO_2	1% Zn	3% Zn	5% Zn
Creosote bush	0	7.78 b	7.78 a	7.78 a	7.78 a	19.78 b	19.78 b	19.78 a
	1 000	15.84 a	1.38 b	1.89 b	0.747 b	25.54 ab	15.41 b	26.93 a
	3 000	18.29 a	1.17 b	1.22 b	0.48 b	35.25 a	17.38 b	27.04 a
	5 000	13.38 ab	1.12 b	0.35 b	0.64 b	17.28 b	45.40 a	27.20 a
Mustard	0	7.78 a	7.78 a	7.78 a	7.78 a	19.78 a	19.78 a	19.78 a
	1 000	0.85 b	5.81 ab	2.45 b	3.68 b	15.38 ab	8.64 b	8.90 b
	3 000	0.37 b	4.58 b	1.49 b	0.48 c	5.22 bc	5.44 bc	2.18 bc
	5 000	0 b	0 c	0 b	0 c	0 c	0 c	0 c

Means with the same letter in columns are statistically equal Tukey ($p \leq 0.05$).

For the extract of creosote bush with 3% SO_2 at the dose of 5 000 ppm, it presented the lowest number of spores, 0.35 million conidia ml^{-1}, followed by 5% SO_2 at the dose of 3 000 ppm (0.48); the highest values were shown by creosote bush with 3% ZnO at the dose of 5 000 ppm, with 45.40 million ml^{-1}, followed by 1% ZnO at the dose of 3 000 ppm (35.25), surpassing the control, which presented the highest value with 7.8 million conidia ml^{-1}.

For mustard extract, the concentration of 5 000 ppm, either alone or in mixture with nanoparticles, inhibited the production of spores by 100%; other results with low values were shown by mustard alone at the dose of 3 000 ppm, with 0.37 million ml^{-1}, followed by mustard plus 5% SO_2 at the dose of 3 000 ppm with 0.48, and mustard with 3% SO_2 at the dose of 3 000 ppm with 1.49.

These results are consistent with those reported by Singh *et al*. (2017), who tested 12 botanicals against *Fusarium oxysporum*, where mustard inhibited 93.75% of the fungus. Extracts of creosote bush have been used as an ecological strategy and have been shown as an alternative for integrated management for *Fusarium* (Peñuelas-Rubio) and these, being enriched with nanoparticles, increase their effectiveness for the control of *F. solani*.

The treatments win ZnO showed the highest values; with creosote bush, 70% of the results are higher than the control, where the lowest spore production was presented by creosote bush with 3% Zn at a concentration of 1 000 ppm, with 15.41 million conidia ml^{-1}, while in those of mustard, the highest number of spores was observed at the concentration of 1 000 ppm, after the control, with 15.38 million conidia ml^{-1}; Labiadh *et al*. (2016) reported that ZnO nanoparticles have a antibacterial effect better than an antifungal effect.

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Conclusions

The mustard extract inhibited the mycelial growth of *F. Solani*, but the mixture of mustard added with SO_2 nanoparticles and creosote bush with SO_2 showed inhibition of sporulation.

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