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Antifungal Activity of Mexican Oregano (*Lippia graveolens* Kunth) Extracts from Industrial Waste Residues on *Fusarium* spp. in Bean Seeds (*Phaseolus vulgaris* L.)

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Abstract: Food security is essential to ensure everyone can access sufficient nutritious food. Cereals and legumes are fundamental foods worldwide. *Phaseolus vulgaris* L., the common bean, is an essential staple food in many nations worldwide. However, it is vulnerable to fusariosis, a disease caused by the fungus *Fusarium* spp. that can significantly decrease crop quality and yield. To combat plant diseases, industrial residues and plant residues are valuable due to their bioactive compounds with biotechnological applications. This study proposes using ethanolic extracts with phytochemical compounds, such as flavonoids, different from those reported in essential oils, to reduce the growth of *Fusarium* species both in vitro and in vivo. Industrial residues that are produced after extracting essential oils offer a promising alternative to develop organic biopesticides, promoting more sustainable and environmentally friendly agriculture.

Keywords: fusariosis; industrial residues; phytocontrollers; biofungicides

1. Introduction

Fusarium is a genus of ascomycetes filamentous fungi that grows on soils and organic substrates [1]. Some species of this genus can cause plant diseases such as wilt, blight, root rot, and cankers, producing mycotoxins in food that can harm the health of humans and animals [2]. Globally, *Fusarium* wilt can cause losses in various crops that reflect in crop yields with between a 70% and 100% reduction [3].

Fusarium wilt is a relevant disease that affects common beans *(Phaseolus vulgaris* L.), an essential food in many countries. In 2019, Asia became the largest producer, with Myanmar leading the way. Africa was next on the list, with Tanzania as a significant producer. In America, the primary producers were Brazil, the United States, and Mexico. Europe ranked fourth in the production of this legume, while in Oceania, the cultivation and feeding culture does not favor its production [4]. In the same year, the global area dedicated to bean cultivation reached 33.1 million hectares, producing 28.9 million metric tons [5,6].

In Mexico, there are many traditional creole bean varieties, such as Bayito, Bayo Blanco, Bayo grueso, Blanquito, Borroso, Canario Garbancillo, Sangre de toro, Flor de mayo, Negro Brillante, Ojo de cabra, Ojo de pato, Pinto, Punta Villa, and Querétaro [7].

These varieties stand out for their precocity, prostrate growth, adaptation to water stress, and phenological plasticity, allowing them to adjust their life cycle according to water availability [8]. Acosta-Gallegos et al. [9] mentioned the development of Pinto Bayacora by the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP). This variety combines various genetic materials, including criollos, which are crucial since they provide resistance genes. This emphasizes the importance of recovering traditional bean varieties. However, *Fusarium* spp. remains one of the main problems in Mexican crops, which requires comprehensive control programs that include genetic improvement, crop rotation, and other agroecological control methods [10].

To treat these phytopathogenic fungi, various fungicides are recommended to reduce the incidence and severity of root rot. The chemicals recommended for the treatment of common bean seeds are Metacaptan® (Captan + Methoxychlor), Thiram (Tebuconazole + Thiram), Terrazán [(Quintozene-(pentachloronitrobenzene)], vitavax (Carboxin + Captan), Ridomil (Metalaxyl-M), and Benlate (Benomil) [10]. Studies have also been carried out with the fungus *Trichoderma* sp. [11] and biopesticides as control agents for *Fusarium* spp. [12]. Natural products based on plant extracts have generated significant interest in managing phytopathogens, with the advantage that they do not negatively impact the environment or human or animal health [13]. Plants are an invaluable source of new biologically active molecules. They produce various secondary metabolites, many of which have antifungal activity [14]. Among the different antifungal compounds contained in plant extracts, alkaloids, tannins, quinones, coumarins, phenolic compounds, and phytoalexins stand out [15], which have efficiently controlled various phytopathogenic fungi [16–19].

Plant-based residues and by-products are of significant interest to industry and research sectors because they contain high-value compounds, such as secondary metabolites [20]. The scientific community has focused on valorizing these industrial and agricultural by-products and residues [21]. These biological matrices are especially attractive for their diverse bioactive properties, including antihypertensive, antimicrobial, and antioxidant activities, benefiting biotechnological use and application [22]. The versatility of the compounds of interest can vary in different by-products' vegetative remnants [23].

Oregano includes aromatic species from the *Lamiaceae* and *Verbenaceae* families. Its cultivation is classified into four main types: Turkish oregano (*Origanum onites*), Spanish oregano (*Coridohymus capitatus*), Greek oregano *(Origanum vulgare*), and Mexican oregano (*Lippia graveolens*) [24]. The oregano plant contains an essential oil fraction that is the primary source of its economic value, used as a flavoring and additive in foods. The predominant compounds in oregano essential oils are p-cymene, carvacrol, β-pinene, caryophyllene, camphene, and α-pinene [25]. After the essential oil is extracted from oregano, the leaves and stems no longer have a commercial value; however, these residues derived from industrial processes contain flavonoids and phenolic acids with antifungal potential [26]. In the search for new antifungal compounds on phytopathogenic fungi of the *Fusarium* genus, together with the use of resources that are considered waste in the industry, this work aims to evaluate the in vitro and in vivo antifungal effect for the control of *Fusarium* spp. in common bean seeds with ethanolic extracts of the leaf and stem residues of Mexican oregano to develop organic biopesticides for sustainable agriculture.

2. Materials and Methods

2.1. Plant Material and Extraction

The leaves and stems of Mexican oregano (*Lippia graveolens* Kunth) were collected as residues of the industrial process after obtaining essential oils at the company Unión de Cooperativas Oro Verde del Semidesierto S.C.R.L.C.V. The plant material was dehydrated up to 7%. The extracts were obtained through maceration using a 70:30 *v*/*v* aqueous ethanol solution (EtOH70); a mass:solvent ratio of 1:10 was used for extraction, with 100 g of plant material and 1 L of EtOH70. It was kept under agitation at 200 rpm for one hour at 25 °C. It was left to macerate for 48 h at 25 °C. The extract was then placed in an ultrasound bath at 40 °C for 10 min and filtered with Whatman paper number 1. The extracts were placed in a

rotary evaporator at 45 °C with vacuum applied, obtaining a concentrated aqueous extract that was dried by lyophilization.

One of our research's key aspects was the extraction yield calculation. This crucial metric corresponds to the amount of extract obtained (extracted or solubilized by the action of the solvent) from the raw material used. It is calculated using the following formula.

$$\%\ extract = \frac{g\ dry\ extract}{g\ plant\ used\ (Leaf\ or\ Stem)} \times 100$$

2.2. Determination of Chemical Composition by UPLC-MS

For the ultra-high-performance liquid chromatography–mass spectrometry (UPLC-MS) analysis, the stem and leaf extract were re-dissolved in 70% ethanol at 1000 mg/L (Sigma-Aldrich, Merck, Darmstadt, Germany). The analysis was carried out according to the method proposed by Villegas-Novoa et al. [27]. An Acquit UPLC-MS system (Waters, USA) was coupled to a Xevo TQ-S tandem triple quadrupole mass spectrometer (Waters Corp., Milford, MA, USA). The phenolic compounds were separated on a C18 column (100 × 2.1 mm, 1.7 µm) using a mobile phase of 7.5 mM formic acid (phase A) and acetonitrile (phase B) with a flow of 210 µL/min. The compounds were identified by interpreting their mass spectra through the MS/MS array and using standards (Sigma-Aldrich, Merck, Darmstadt, Germany) of several phenolic compounds (phenolic acids and flavonoids) using the negative ionization mode. Using standards in the analysis ensures the accuracy and reliability of the results. Quantification was achieved from the standard curve of each of the compounds (standards), and the results were reported as a relative percentage of the response of each compound relative to the total response in a UPLC chromatogram.

2.3. Fungal Strains and Culture Conditions

The fungal strains used in this study were *Fusarium graminearum* PH-1 (NRRL 31084) [28], *Fusarium oxysporum f.* sp. *lycopersici* strain 4287 (race 2, VCG 0030) [29], and *Fusarium solani* [30]. To obtain *F. graminearum* conidia, 1 cm^2 of mycelium was inoculated in a wheat germ medium for five days at 26–28 °C, shaking at 150 rpm [31]. *F. oxysporum* and *F. solani* conidia were obtained by inoculating 1 cm^2 of mycelium in potato dextrose medium (PDB) and incubating for 4–5 days at 26–28 °C, shaking at 150 rpm. The conidia were filtered, washed with sterile distilled water, and counted in a Neubauer chamber.

2.4. In Vitro Antifungal Activity of Oregano Leaf and Stem Residue Extracts on Fusarium spp. Using the Agar Diffusion Method

The effect of ethanolic extracts of oregano on the growth inhibition of three *Fusarium* species (*F. graminearum*, *F. solani*, and *F. oxysporum*) was evaluated using the agar diffusion method [32], with modifications for this study. The required volume of PDA medium (Difco Laboratories, Detroit, MI, USA) to dispense Petri plates was prepared and sterilized. Once the temperature reached 40 °C, the required volume of the ethanolic extract stock (prepared by dissolving the lyophilized extract in distilled water and sterilizing it through 0.22 µm filters) was added. We evaluated eight conditions: sterile distilled water as a negative control (CN), synthetic fungicide tebuconazole 5.0 µg/mL (Folicur 250 Ew, Bayer de Mexico S.A de C.V.) as a positive control (CP), leaf extract 4.0 mg/mL (H04), stem extract 4.0 mg/mL (TA04), leaf extract 8.0 mg/mL (H08), stem extract 8.0 mg/mL (TA08), leaf extract 16.0 mg/mL (H16), and stem extract 16.0 mg/mL (TA16); subsequently, we added 3 µL of a conidial suspension of 1 × 10^6 c/mL of the fungus. The plates were incubated at 28 °C for four days. The diameter (mm) of the radial growth of the fungus was determined and expressed as a percentage (%) of inhibition using the formula proposed by [33]: IM (%) = 100 × (C − R)/C, where IM is the radial inhibition of the phytopathogen growth in percentage, C is the diameter of the colony of the control treatment, and R is the diameter of the colony of the phytopathogen of each treatment. All tests were performed in triplicate.

2.5. In Vivo Evaluation of the Antifungal Activity of Ethanolic Extracts on Fusarium spp. in Common Bean Seeds (Phaseolus vulgaris L.)

The seeds of common bean (*Phaseolus vulgaris* L.) of the Pinto Bayacora variety were sterilized in 95% (*v*/*v*) ethanol for 5 min and 20% (*v*/*v*) sodium hypochlorite for 5 min and washed five times with sterile distilled water. Subsequently, the seeds were immersed in a solution of *Fusarium* spp. conidia (1×10^6 conidia/mL) for 20 min. Four seeds were placed in a Petri plate with a wet sterile filter paper in interaction with the conidia of the fungus to evaluate five conditions: bean seeds (SFN), bean seeds + Conidium (SFC), bean seeds + Conidium + leaf extract 32 mg (SFH), bean seeds + Conidium + stem extract 32 mg (SFTA), bean seeds + Conidium + tebuconazole fungicide 5 µg/mL (SFTEB). The plates were incubated at 28 °C for 120 h. All tests were performed in triplicate.

2.6. Experimental Design and Statistical Analysis

A completely randomized experimental design was used, and the data were processed using a one-way analysis of variance (ANOVA) and the Bonferroni–Holm post hoc test. Significance cutoffs: ns (not significant) $p > 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$ vs. CP. The statistical analysis was performed using three experimental replicates.

3. Results

3.1. Yield of Ethanolic Extracts from the Leaf and Stem Residues of Mexican Oregano (Lippia graveolens Kunth)

The extract yields were as follows: (a) the yield of the stem extract was 16 to 18%, which means that, from every 100 g of the processed stem, between 16 and 18 g of dry extract were obtained; (b) the yield of the leaf extract was 22 to 24%, obtaining between 22 and 24 g of dry extract for every 100 g of processed leaf.

3.2. Analysis and Determination of the Chemical Composition of the Extracts

Table 1 presents the main compounds identified in the leaves and stems and the chemical diversity obtained with the residues of Mexican oregano (*Lippia graveolens* Kunth).

Table 1. Main compounds identified in stem and leaf extracts of Mexican oregano.

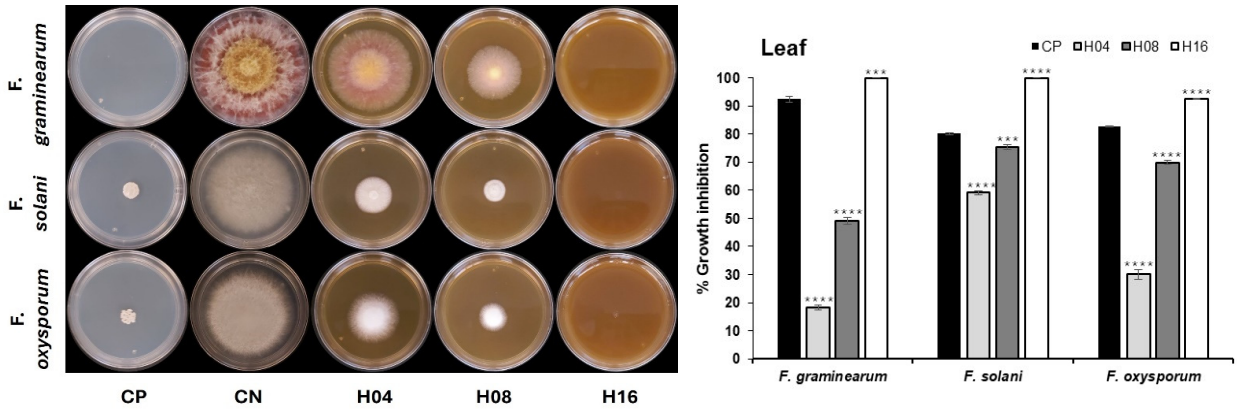
Compound	Stem Extract (%)	Leaf Extract (%)
Naringenin	27.0	22.8
Taxofolin	21.1	17.3
Caffeic acid	10.6	14.5
Erioditol	18.1	14.3
Acacetin	0.7	8.5
Luteolin	8.6	5.1
Quercetin 3-O-Glucoside	2.1	3.2
Coumaric acid	3.8	2.1
2-hydroxybenzoic acid	1.7	1.5
Apigenin	1.5	1.2
Phrolidin	0.8	1.1
Quercetin	0.7	0.7
Protocatechuic acid	0.9	0.5
Neohesperidin	0.7	0.5
Rutosidum	0.2	0.4
Quinic acid	0.3	0.3
4-hydroxybenzoix acid	0.5	0.3

In this study, the ethanolic extracts analyzed revealed the presence of 17 principal components. We identified seven compounds of the flavonoid family (Naringenin, Taxifolin, Eriodictyol, Luteolin, Quercetin 3-O-glucoside, Apigenin, Acacetin, Phloridzin, Quercetin, Neohesperidin, and Rutosidum), which constitute 64.7% of the total. Other compounds identified were phenolic acids with a group of compounds such as coumaric acid, pro-

tocatechuic acid, 4-hydroxybenzoic acid, and two ester compounds (caffeic acid, quinic acid) and a keratolytic agent (2-hydroxybenzoic acid) were also detected. This diversity of compounds reflects a wide range of products derived from the industrial by-products of Mexican oregano (*Lippia graveolens* Kunth), supported by a notable variability in the main components of each subclass.

3.3. Antifungal Effects of Mexican Oregano (Lippia graveolens Kunth) Extracts Against Phytopathogenic Fungi

During the evaluation of the in vitro antifungal activity of ethanolic extracts from the industrial waste residues of Mexican oregano leaves against *F. graminearum* (Figure 1a), we observed an inhibitory effect starting at a concentration of 4 mg/mL, reaching an inhibition of 18%. On the other hand, we determined a significant inhibitory effect of 59% starting at a concentration of 4 mg/mL in *F. solani* with leaf extract, which is more pronounced than that observed for *F. graminearum*. Finally, the results obtained for *F. oxysporum* showed an inhibitory effect starting at a concentration of 4 mg/mL, reaching an inhibition of 30%. Generally, the inhibitory effect started at concentrations of 4 mg/mL, with complete inhibition of mycelial growth at 16 mg/mL in the tested *Fusarium* spp. (Figure 1b). These results suggest that Mexican oregano extracts have great potential as natural antifungal agents, which could be beneficial for the biological control of these phytopathogens in agricultural applications. Extracts obtained from the stem of Mexican oregano (*Lippia graveolens* Kunth) showed limited efficacy against *Fusarium* spp. The growth inhibition of *F. graminearum* started from a concentration of 8 mg/mL, reaching an inhibition percentage of 18%. However, by doubling the concentration, a total inhibition (100%) of the development of *F. graminearum* was achieved, as illustrated in Figure 1c. In the case of *F. solani*, the stem extracts were more effective, inhibiting growth by 43% from a concentration of 4 mg/mL. Figure 1d clearly shows that increasing the extract concentration reduces the development of this species. Finally, the effects of the stem extracts on *F. oxysporum* were evident from a concentration of 4 mg/mL. As described in Figure 1d, at low concentrations, stem extracts were found to be more efficient in stopping the development of *F. oxysporum* compared to leaf extracts, reducing its development by up to 87% at a concentration of 16 mg/mL. Extracts from the stems of Mexican oregano (*Lippia graveolens* Kunth) exhibit varying levels of effectiveness in inhibiting the growth of *Fusarium* spp. As the concentration of the ethanolic stem extract is increased, the complete inhibition of *Fusarium* species is achieved. However, *F. graminearum* is not inhibited at an 8 mg/mL concentration, requiring twice this concentration for total inhibition. On the other hand, *F. solani* and *F. oxysporum* respond to applying 8 mg/mL of the extract, although doubling the concentration does not achieve 100% inhibition.



(**a**) (**b**)

Figure 1. *Cont.*

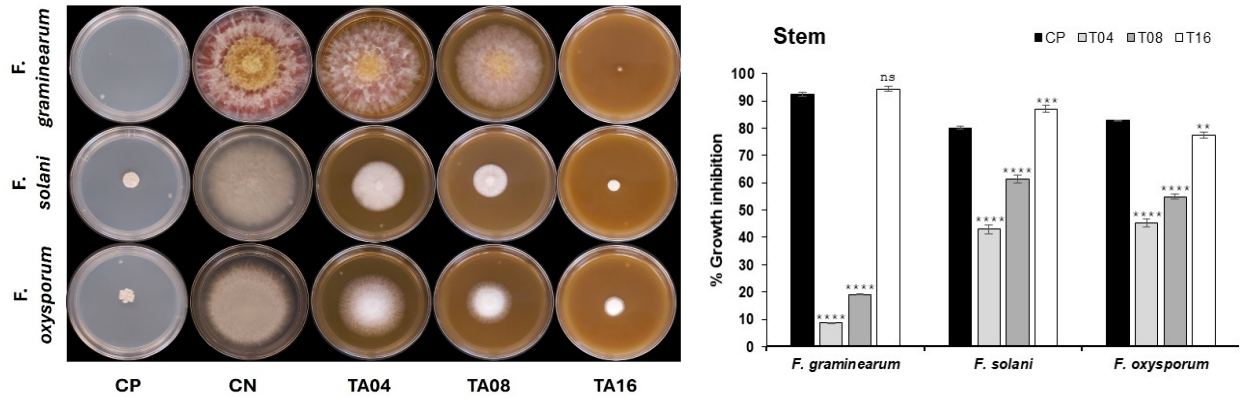


Figure 1. An evaluation of the antifungal effect of the extract from leaf and stem residue extracts from Mexican oregano at 120 h. (**a**) Fungal growth on plate PDA with leaf extract (HA04 = 4 mg/mL, HA08 = 8 mg/mL, HA16 = 16 mg/mL). (**b**) Percentage of fungal growth inhibition with leaf extract. (**c**) Fungal growth on a plate with stem extract (TA04 = 4 mg/mL, TA08 = 8 mg/mL, TA16 = 16 mg/mL). (**d**) Percentage of fungal growth inhibition with stem extract. Inhibition with tebuconazole 5 µg/mL was used as a positive control (CP). The addition of sterile water was used as negative control (CN). The statistical analysis was calculated regarding CP, using one-way Anova and the Bonferroni–Holm post hoc test. Significance cutoffs: ns (not significant) $p > 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$ vs. CP.

3.4. Effects of Mexican Oregano (Lippia graveolens Kunth) Against Phytopathogenic Fungal Infection in Bean (Phaseolus vulgaris L.) Seeds

We determined the biofungicide effect of Mexican oregano leaf and stem residue extracts from the industrial process on *Fusarium* species infecting bean seeds (Figure 2, Table 2). For *F. graminearum*, the leaf extract stands out, reducing infection by 25%, a significantly more effective treatment than the stem extract and the synthetic fungicide tebuconazole. This impressive result highlights the potential of the leaf extract of Mexican oregano as a powerful biofungicide.

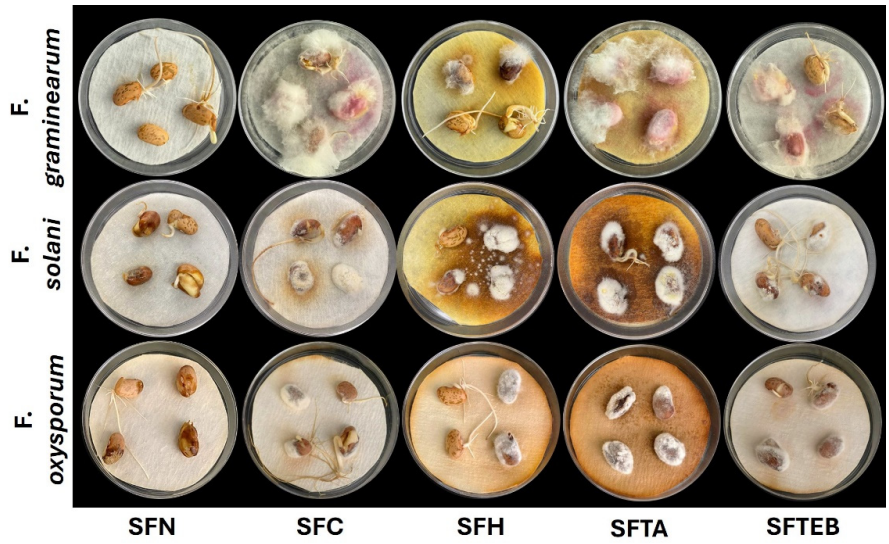


Figure 2. Evaluation of the antifungal activity of ethanolic extracts of Mexican oregano (*Lippia graveolens* Kunth) on the seed of the Bayacora bean variety infected with *Fusarium* spp. bean seeds (SFN), bean seeds + Conidium (SFC), bean seeds + Conidium + leaf extract 32 mg (SFH), bean seeds + Conidium + stem extract 32 mg (SFTA), bean seeds + Conidium + tebuconazole fungicide 5 µg/mL (SFTEB).

Table 2. Antifungal activity of ethanolic extracts of Mexican oregano residues (*Lippia graveolens* Kunth) on Pinto Bayacora bean seeds infected with *Fusarium* spp.

Conidia Treatment	*Fusarium* spp.	Beans (n)	Sprouted	Infected	Infection Level		
					Mild	Moderate	Severe
SFN	Without conidia	12 (100%)	10 (83.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
SFC	*F. graminearum*	12 (100%)	6 (50%)	12 (100%)	1 (8.3%)	3 (25%)	8 (66.6%)
	F. solani	12 (100%)	9 (75%)	5 (41.6%)	1 (8.3%)	2 (16.6%)	2 (16.6%)
	F. oxysporum	12 (100%)	5 (41.6%)	12 (100%)	8 (66.6%)	2 (16.6%)	2 (16.6%)
SFH	*F. graminearum*	12 (100%)	3 (25%)	9 (75%)	2 (16.6%)	4 (33.3%)	3 (25%)
	F. solani	12 (100%)	2 (16.6%)	12 (100%)	2 (16.6%)	2 (16.6%)	8 (66.6%)
	F. oxysporum	12 (100%)	3 (25%)	10 (83.3%)	0 (0%)	0 (0%)	10 (83.3%)
SFTA	*F. graminearum*	12 (100%)	4 (36.3%)	11 (91.6%)	3 (27.2%)	2 (16.6%)	6 (54.5%)
	F. solani	12 (100%)	1 (8.3%)	12 (100%)	0 (0%)	1 (8.3%)	11 (91.6%)
	F. oxysporum	12 (100%)	0 (0%)	12 (100%)	4 (33.3%)	0 (0%)	8 (66.6%)
SFTEB	*F. graminearum*	12 (100%)	5 (41.6%)	12 (100%)	4 (33.3%)	3 (25%)	5 (41.6%)
	F. solani	12 (100%)	8 (66.6%)	4 (33.3%)	2 (16.6%)	0 (0%)	2 (16.6%)
	F. oxysporum	12 (100%)	6 (50%)	8 (66.6%)	1 (8.3%)	0 (0%)	5 (41.6%)

Ethanolic extracts of Mexican oregano leaves and stems did not effectively control the development of *F. solani*. In vivo, we observed no inhibition at 32 mg/mL, with unfavorable results for this species. On the contrary, the synthetic fungicide tebuconazole reduced the fungus's development by 66.6%.

The percentage inhibition values on *F. oxysporum* infecting bean seeds vary between 16.7% and 33.4% for the SFH and SFTEBU treatments, respectively. In both cases, we observed a predominance of severe infection levels. On the other hand, the inhibitory activity of the stem extracts (SFTA) is ineffective for this species. The inhibition of mycelial growth of *Fusarium* spp. in an in vivo model using bioactive phytochemical extracts is presented in Table 2. These results suggest that, although Mexican oregano leaf and stem extracts have some inhibitory effects, their efficacy is limited and insufficient to control severe *F. oxysporum* infections. Higher concentrations or combinations with other biofungicidal agents may be required to improve effectiveness.

4. Discussion

The extraction of active compounds from the essential oil residues of Mexican oregano (*Lippia graveolens* Kunth) with a mixture of ethanol and water (70:30 *v*/*v*) shows variation in yields. Leaf extracts yield 22–24%, while stem extracts are 16–18%, due to the high concentration of bioactive compounds in the residual leaves. Frías-Zepeda et al. [26] reported a maximum yield of 31.18 g per 100 g of leaves and 19.95 g per 100 g of stem using a mixture of ethanol and water (50:50). Increasing the amount of ethanol improves the extraction yield. Both studies agree that leaves have a higher yield than stems due to their high concentration of bioactive compounds, even after industrial extraction. The analysis of ethanolic extracts of the industrial residues of Mexican oregano (*Lippia graveolens* Kunth) using UPLC techniques revealed a diverse and complex chemical composition. Flavonoids constitute 64.7% of the identified components, highlighting compounds such as Naringenin, Taxifolin, and Quercetin 3-O-glucoside. In addition, we identified phenolic acids, esters, and a keratolytic agent, revealing the wide range of secondary metabolites in oregano by-products. Chang et al. [34] extracted the components of *Origanum vulgare* oil from New Directions Aromatics (Ontario, Canada); the abundant components were thymol, carvacrol, p -cymene, γ-terpinene, linalool, β-caryophyllene, β-myrcene, α-terpinene, α-pinene, α-thujene, and terpinene-4-ol. Thymol and carvacrol are predominant in white thyme oil and oregano oil; they have been identified as the main responsible components for the antifungal properties of these essential oils [35].

Our study discovered that oregano leaf extracts are more potent than stem extracts in completely inhibiting *F. graminearum*, *F. solani*, and *F. oxysporum* for in vitro growth at 16 mg/mL. On the other hand, stem extracts were less effective against *F. graminearum*. De Almeida et al. [36] identified components of oregano essential oils as carvacrol (68.72%), para-cymene (12.47%), trans-caryophyllene (5.20%), and gamma-terpinene (4.70%). Pilozo et al. [37] found λ-terpinene (12.79%), trans-β-ocimene (8.85%), thymol (7.32%), and thymol methyl ether (7.04%). In our study, we mainly identified the following flavonoids: naringenin (27%), taxifolin (21.1%), eriodictyol (18.1%), luteolin (8.6%), quercetin 3-O-glucoside (2.1%), apigenin (1.5%), acacetin (0.7%), phloridzin (0.8%), quercetin (0.7%), neohesperidin (0.7%), and rutoside (0.2%). The predominant chemical compounds in our ethanolic extracts are naringenin, taxifolin, caffeic acid, and eriodictyol. These compounds could be attributed to the observed antifungal effects. Despite the lower amounts of these metabolites in leaf extracts compared to stem extracts, we propose that the components derived from Mexican oregano leaves are effective for *Fusarium* biocontrol. This discovery opens new possibilities for developing biofungicides, especially those obtained from industrial waste residues after the extraction of essential oils.

Chemical fungicides have risen, but their residues are hazardous to health and the environment. The process of bioaccumulation in organisms, as highlighted by Li et al. [38], underscores the need for safer and more effective biofungicides. These biofungicides are crucial in repressing gene expression at critical points in the metabolic pathway of the virulence factors of phytopathogenic fungi. A prime example is the mode of action of *F. graminearum*, which expresses and secretes a mixture of virulence factors and metabolites, both enzymatic and non-enzymatic, to cause diseases [32]. These include the sesquiterpenoid [39], the Fgl family lipase [40], the siderophore TAFC, and CAZymes enzymes that penetrate plant cell walls and induce host cell death [41,42]. The need for safer and more effective biofungicides should motivate us to find better solutions.

The way to control the pathogenesis molecular mechanism in phytopathogenic fungi is mainly to reduce the activity of oxidase enzymes, and such is the case of extracts containing flavonoids that combat phytopathogenic fungi by acting as chelators of metals such as iron (Fe) [43]. Furthermore, they inhibit several enzymes, such as lipoxygenase [44], cyclooxygenase [45], myeloperoxidase [46], NADPH oxidase [47], and xanthine oxidase [48], preventing the function of reactive oxygen species [49]. It is essential to determine molecules that mitigate the molecular mechanisms of pathogenesis. In 2020, a study using RNAseq and metabolomic techniques investigated changes in gene expression and metabolomic processes in beans infected with *F. solani f.* sp. *Phaseoli* [50]. They identified 29,722 genes and 300 metabolites that showed differential regulation. They found that acquired systemic resistance, mediated by hormones, is activated after the detection of the pathogen, providing an additional layer of defense in plants. Among the hormones involved in these signaling pathways are abscisic acid, auxin, brassinosteroids, cytokinins, ethylene, gibberellin, jasmonic acid, nitric oxide, and salicylic acid. In the present work, the chromatographic analysis of the study identified two isoforms of salicylic acid, 2-hydroxybenzoic acid and 4-hydroxybenzoic acid, which is in agreement with Li et al. [38], where exogenous salicylic acid inhibits the mycelial growth and pathogenicity of *F. oxysporum*. Scanning electron microscopy shows distorted and swollen hyphae and shorter hyphal septa, resulting in poor growth phenotypes. Together with the initial perception of *Fusarium* spp. by bean plant receptors, these compounds rapidly trigger signal transduction and gene expression at multiple levels [51,52]; defining that, defenses included cell wall changes, ROS production, and acquired systemic resistance by hormones. Different *Fusarium* spp. activated metabolic pathways such as energy metabolism, nitrogen mobilization, sugar increase, secondary metabolite biosynthesis, and arginine and proline metabolism. The bioactive properties of plant residues improve efficiency and specificity in pathogen control. This strategy promotes the circular economy, reduces waste, and benefits agriculture and plant health. The versatility of the compounds in different residues offers broad applications for fungal disease control.

5. Conclusions

The plant extracts are positioned as an ecological, sustainable, and viable alternative for combating plant pathogens. In this study, we demonstrated that extracts of industrial residues of Mexican oregano (*Lippia graveolens* Kunth) have an inhibitory effect on the in vitro growth of *Fusarium* spp. The predominant chemical compounds in our ethanolic extracts were naringenin, taxifolin, caffeic acid, and eriodictyol. Leaf extracts showed a more significant effect than stem extracts in inhibiting the growth of *Fusarium* spp. Furthermore, the growth inhibition of *Fusarium* spp. infecting bean seeds of a native variety showed that these extracts or some of their components could become a good alternative for sustainable control against fungi of this genus before their spread.

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