

***In vitro* antifungal activity of plant extracts and *Bacillus subtilis* (Ehrenberg) filtrates against *Macrophomina phaseolina* (Tassi) Goid**

Actividad antifúngica *in vitro* de extractos vegetales y filtrados de *Bacillus subtilis* (Ehrenberg) sobre *Macrophomina phaseolina* (Tassi) Goid

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ABSTRACT

This research objective was to characterize *Euphorbia antisiphilitica*, *Helianthus annuus* and *Thymus vulgaris* extracts, and to determine their antifungal activity against *Macrophomina phaseolina*. Additionally, the capacity of filtrates from a *Bacillus subtilis* (FBS) strain against the same pathogen was evaluated. Phytochemical characterization was performed using HPLC-MS, while the antifungal activity of the extracts was determined through poisoned medium assays at concentrations ranging from 1 to 80 %. From these results, the inhibitory concentrations at 50 % and 90 % (IC₅₀ and IC₉₀) were obtained. Subsequently, the Detached Leaf Assay (DLA) was used to evaluate the antifungal activity of the extracts and FBS IC₉₀ through the Area Under the Disease Progress Curve (AUDPC). Phenolic compounds from various chemical families were identified. Regarding the antifungal activity, 100 % inhibition was observed using *H. annuus* and *T. vulgaris* at a concentration of 20 %. In the DLA assay, FBS and the *T. vulgaris* and *H. annuus* extracts were highly effective, limiting the growth of *M. phaseolina* to 7.14 %, 12.57 %, and 13.86 %, respectively. The results demonstrate that plant extracts and FBS effectively control *M. phaseolina*.

Keywords: Biological control, bean crop, charcoal rot, *Euphorbia antisiphilitica*, *Helianthus annuus*, *Thymus vulgaris*.

RESUMEN

El objetivo del presente trabajo fue caracterizar extractos de *Euphorbia antisiphilitica*, *Helianthus annuus*, y *Thymus vulgaris*, y determinar su actividad antifúngica sobre *Macrophomina phaseolina*. Además, se evaluó la capacidad de filtrados de una cepa de *Bacillus subtilis* (FBS) sobre este mismo patógeno. La caracterización fitoquímica se realizó mediante HPLC-MS, y la actividad antifúngica de los extractos se determinó por medio envenenado con dosis del 1 al 80 %. Se obtuvieron las concentraciones inhibitorias al 50 y 90 % (CI₅₀ y CI₉₀). Posteriormente, mediante la técnica de hoja desprendida (THD) se evaluó la actividad antifúngica de la CI₉₀ de los extractos y FBS a través del área bajo la curva de progreso de la enfermedad (AUDPC). Se identificaron compuestos fenólicos de diversas

familias químicas En relación con la actividad antifúngica, se observó inhibición del 100 % de *H. annuus* y *T. vulgaris* a dosis de 20 %. En cuanto a los datos obtenidos en la THD, los FBS y los extractos de *T. vulgaris* y *H. annuus* resultaron altamente efectivos, limitando el crecimiento de *M. phaseolina* a un 7.14 %, 12.57 % y 13.86 %, respectivamente. Los resultados muestran que los extractos y FBS son efectivos para controlar *M. phaseolina*.

Palabras clave: Control biológico, cultivo de frijol, podredumbre gris, *Euphorbia antisiphilitica*, *Helianthus annuus*, *Thymus vulgaris*.

INTRODUCTION

In Mexico, one of the main crops consumed is corn (*Zea mays* L.), sugarcane (*Saccharum officinarum* L.), tomato (*Solanum lycopersicum* L.), and beans (*Phaseolus vulgaris* L.), which represent one of the country's most consumed and sold crops. In this regard, it is estimated that the national production of beans reached 584,645 t in 2023, in a cultivated area of 1,018,769 ha. Zacatecas (141, 846 ha), Sinaloa (92, 747 ha), Nayarit (69, 246 ha), Chiapas (65, 182 ha) and Guanajuato (35, 495 ha) were the main producing states (SIAP, 2024). According to the FAOSTAT (2023), global bean production reached approximately 28 million tons in 2022, with India being the leading producer. In Latin America, Mexico ranks as one of the top producers, second only to Brazil. Despite this crop's relevance, beans face many diseases that affect their output and quality nowadays. One of the principal ones is *Xanthomonas axonopodis* pv. *phaseoli* (Smith), which causes "bacterial blight", followed by *Rhizoctonia solani* (J.G. Kühn), which causes "web blight", and then *Macrophomina phaseolina* (Tassi) Goid, also known as "charcoal rot", which, if not controlled, may cause grain yield loss of up to 100 % (Mayek-Pérez et al., 2009; Mayek-Pérez and Hernández-Delgado, 2010).

This is because *M. phaseolina* can infect susceptible hosts under various environmental conditions. Its main signs include mycelium production, microsclerotia, and pycnidia in host tissues (Leyva-Mir et al., 2015; Khan and Javaid, 2023). At present, its primary source of control in bean crops is the use of chemically synthesized products; however, such has

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Received: March 21, 2025

Accepted: November 11, 2025

Published: January 28, 2026

resulted in low efficacy due to the low persistence of the active ingredients, the ample host spectrum, and the swift propagation and production of the pathogen's microsclerotia (Hernández-Pérez *et al.*, 2017).

Bean production in Mexico faces charcoal rot, for which fungicides such as carboxin, carbendazim, thiophanate-methyl and tebuconazole are used (Franco-Ortellado and Orrego Fuente, 2013). These fungicides act by inhibiting the growth and development of the pathogen by interfering with key cellular processes, such as microtubule synthesis, chitin biosynthesis, and cellular respiration. Tebuconazole belongs to the group of demethylation inhibitors (DMIs) and disrupts the biosynthesis of ergosterol in fungi. It functions by blocking the enzyme 14 α -demethylase, which plays a crucial role in the cytochrome P450-mediated oxidative demethylation of 24-methylene-24,25-dihydrolanosterol—an intermediate in the synthesis of ergosterol, a vital component of the cell membrane in filamentous fungi (Odintsova *et al.*, 2020). However, their use must be controlled to avoid negative environmental impacts and pathogen resistance, which has led to the investigation of alternative methods and the use of integrated pest management (Mendez *et al.*, 2019).

For all the reasons mentioned above, exploring biorational alternatives such as plant extracts and biological control agents under Integrated management is necessary, given their antifungal potential against phytopathogenic fungi (Ribera and Zuñiga, 2012). *Euphorbia antisiphilitica* (Bernhard), *Helianthus annuus* (Linnaeus) and *Thymus vulgaris* (Linnaeus), have shown antifungal activity against diverse phytopathogens (Ascacio-Valdés *et al.*, 2013; Lawson *et al.*, 2019; Aksit *et al.*, 2022), including *M. phaseolina* in the case of *T. vulgaris* (Khaledi *et al.*, 2015; Moghaddam and Mehdizadeh, 2020), as in that of *Bacillus subtilis* (Ehrenberg) (Singh *et al.*, 2008; Hernández-Pérez *et al.*, 2017). Thus, the objective of this study was to identify the phytochemical compounds present in the *E. antisiphilitica*, *H. annuus* and *T. vulgaris* extracts, and to assess their antifungal activity along with the filtrates of a *B. subtilis* strain, against *M. phaseolina*, the causal agent of the charcoal rot in beans *in vitro*.

MATERIAL AND METHODS

Microorganism used

The fungi *M. phaseolina* encoded MPF1 and the strain of *B. subtilis* encoded BAN2, were provided by the Mycology and Biotechnology Lab through the Department of Parasitology of Universidad Autónoma Agraria Antonio Narro (UAAAN). *M. phaseolina* was reactivated on Potato Dextrose Agar (PDA) medium. Regarding the *B. subtilis* strain, it was reactivated in a nutrient agar (NA).

Plant extracts used

The plant extracts were provided by the corporation Green-Corp Biorganiks de México S.A. de C.V., located in the city of Saltillo, Coahuila de Zaragoza, Mexico. These were a ketone extract of *E. antisiphilitica*, and ethanolic extracts of *H. annuus* and *T. vulgaris*.

Characterization of phytochemicals present in plant extracts through HPLC-MS

The HPLC-MS analysis was carried out according to the protocol posed by Ascacio-Valdés *et al.* (2016), utilizing a Varian High-Performance Liquid Chromatography System. This system included an autosampler (Varian ProStar 410, USA.), a ternary pump (Varian ProStar 2310, USA.) and a photodiode array detector (PDA) (Varian ProStar 330, USA.). A device with an ion trap was utilized for the mass spectrometry (Varian 500 – MS IT, USA.), which had an electrospray source. The samples (5 μ L) were injected into a Denali C18 reversed-phase column (150 mm x 2.1 mm, 3 μ m, Grace, USA.), and the column temperature was kept at 30 °C. 0.2 % formic acid (solvent A) and acetonitrile (solvent B) were used as eluents, while a progressive elution gradient was applied starting at 3% of B, and increasing linearly up to 9 % in 5 min, then at 16 % in 15 min, and concluding at 50 % in 45 min. After the elution procedure, the column was properly washed and reconditioned. The fluid was maintained at a constant rate of 0.2 mL/min, and the 245, 280, 320 and 550 nm wavelengths were monitored. The total effluent (0.2 mL/min) was sent undivided to the mass spectrometer source, operated in negative mode $[M-H]^{-1}$, where nitrogen was used as nebulizer gas and helium as carrier gas. The resulting ion source parameters were: spray voltage 5.0 kV, capillary voltage 90.0 V, and 350 °C temperature. The resulting data was analysed using the MS Workstation software (V 6.9), and a full scan was performed over a 50 to 2000 m/z range.

Antifungal activity of plant extracts against *M. phaseolina*

The antifungal activity of the plant extracts was determined by the poisoned medium method proposed by Ramírez-Méndez *et al.* (2024). The treatments used included the ketonic extract of *Euphorbia antisiphilitica* and the ethanolic extracts of *Helianthus annuus* and *Thymus vulgaris*, applied at concentrations of 1, 5, 7.5, 10, 20, 40, 60, and 80 %. As a positive control, the fungus was used without any treatment. A PDA medium was used to add plant extracts. Subsequently, 0.5 mm discs containing active mycelium of *M. phaseolina* were placed and incubated at 28 ± 2 °C, the radial growth of the pathogen was measured until the positive control covered the plaque completely. The inhibition percentage was determined by the formula: $\text{Inhibition percentage} = \left(\frac{DC-DT}{DC} \right) \times 100$, where DC is the diameter of the control treatment, and DT is the diameter of the different concentrations.

Preparation of *B. subtilis* filtrates

The *B. subtilis* filtrates (BSF) were prepared according the protocol by Abdelmoteleb *et al.* (2017) with some modifications. *B. subtilis* was inoculated into 250 mL of Luria-Bertani (LB) broth and incubated in an orbital shaker at 150 rpm at 30 °C for 36 h. Afterward, the bacterial suspension was centrifuged at 10,000 rpm for 10 min at 4 °C, and the resulting supernatant was filtered using 0.22 μ m nylon filters.

Antifungal activity of plant extracts from *E. antisiphilitica*, *H. annuus*, *T. vulgaris* and *B. subtilis* filtrates against *M. phaseolina* in bean leaves

The biological effectiveness of the ketone extract of *E. antisiphilitica*, and the ethanolic extracts of *H. annuus* and *T. vulgaris*, as well as of the BSF, was determined following the methodology described by Bañuelos-Baladrán and Mayek-Pérez (2008), with some modifications. Pinto Saltillo bean seeds were used, and after 30 days of development, their fully expanded leaves were collected, having an approximate size of 7 cm in length and 3 cm in width. The leaves were disinfected with 2 % sodium hypochlorite for 2 min, then rinsed with sterile distilled water, dried on sterile blotting paper, and then placed on sterile filter paper in a Petri dish, keeping it moist. The treatments used were the IC₉₀ of the plant extracts obtained from the poisoning experiment and the BSF. They were distributed as follows: T1: *E. antisiphilitica* (84.18 %), T2: *H. annuus* (50.20 %), T3: *T. vulgaris* (62.08 %), T4: BSF (100 %), T5: chemical control (Tebuconazole, 1 L/ha), T6: positive control (*M. phaseolina* inoculation without treatment) and T7: negative control (PDA medium application without the phytopathogen). Leaf inoculation was performed by placing two 0.5-cm-diameter discs with active *M. phaseolina* mycelium on the adaxial surface of each leaf, at both ends, starting from the midrib. The evaluation was carried out by measuring the necrotic leaf area on the leaves daily until the leaf corresponding to the positive control showed complete necrosis. The data obtained were used to calculate the severity of growth over time using the Area Under the Disease-Progress Curve (AUDPC). The severity curve of the fungal growth was determined using the following formula (Shaner and Finney, 1977):

$$AUDPC = \sum_{i=1}^n \left(\frac{Y_i + Y_{i+1}}{2} \right) \times t_{i+1} - t_i$$

Where: n= number of evaluations; y= percentage of affected foliage; t= number of days assessed

Statistical Analysis

A Probit analysis was performed to determine the IC₅₀₋₉₀ (50 and 90 % inhibitory concentrations) of the extracts. A completely randomized design with six replicates per treatment was utilized for the study on bean leaves. Both the IC₅₀ and IC₉₀ obtained, as well as the data collected from the leaf bioassay, were subjected to an analysis of variance and Tukey test ($p \leq 0.05$), using the Statistical AnalysisSys program version 9.0, in compliance with the assumptions of normality.

RESULTS AND DISCUSSION

Characterization of phytochemicals present in *Euphorbia antisiphilitica*, *Helianthus annuus* and *Thymus vulgaris*, through HPLC-MS

The HPLC-MS analysis detected various phytochemicals (Table 1). The *E. antisiphilitica* extract presented compounds of the families of hydroxybenzoic acids (gallic acid 4-O-glu-

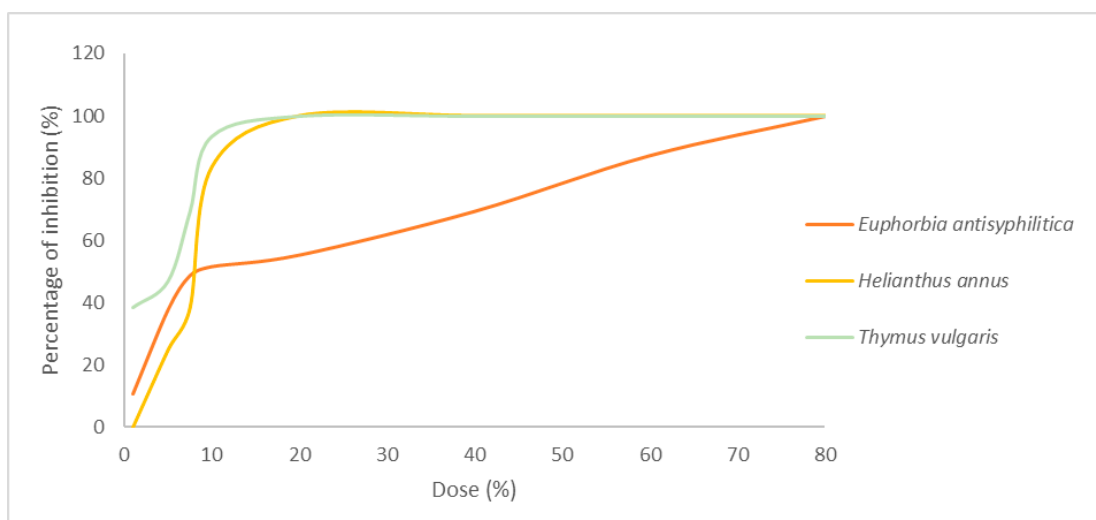
coside and gallic acid 3-O-gallate), hydroxycinnamic acids (caffeic acid 4-O-glucoside) and methoxy flavonols (patuletin 3-O-glucosyl-(1->6)-[apioiosyl(1->2)]-glucoside). Further, the extract of *H. annuus* showed compounds of the families of the hydroxycinnamic acids (1,3-dicafeoylquinic acid, 1-caffeoylquinic acid, and 1,5-dicafeoylquinic acid), tyrosols (hydroxytyrosol 4-O-glucoside, 3,4-DHPEA-EA), hydroxybenzoic acids (protocatechuic acid 4-O-glucoside), flavones (apigenin 7-O-glucoside), methoxyflavins (5,6-dihydroxy-7,8,3,4-tetramethoxyflavone) and dihydrochalcones (phloretin). Lastly, compounds from the families of the hydroxycinnamic acids (caffeic acid 4-O-glucoside), methoxyflavonols (isorhamnetin 3-O-glucoside 7-O-rhamnoside), methoxyflavones (diosmin) and phenolic terpenes (rosmanol), were identified in the *T. vulgaris* extract. Phenolic compounds are produced in plants with diverse functions, such as stress resistance, structure and support, attraction of pollinators, and defense against phytopathogens (Schöneberg *et al.*, 2018). In this regard, patuletin, found in *E. antisiphilitica*, is a phenolic compound produced by plants that can disrupt the cell membranes of phytopathogenic fungi (Ruiz Quiroz, 2013). On the other hand, it has been shown that caffeic acid, present in *E. antisiphilitica* and *T. vulgaris*, possesses antifungal activity by inhibiting the development of various species of fungi. Its mechanism of action involves weakening the cell wall and altering the antioxidant defense system (Ma and Ma, 2015), including *Candida albicans* (Candidus) (Joya *et al.*, 2017). In addition, this compound can act as a precursor to other more complex bioactives such as coumarins, lignins, tannins, flavonoids and isoflavonoids (Ávalos-García y Pérez-Urria, 2009). Diosmin and apigenin, flavonoids present in *T. vulgaris* and *H. annuus*, respectively, together with rosmanol, a phenolic terpene from *T. vulgaris*, possess relevant antifungal properties (Singh *et al.*, 2014; Aamer *et al.*, 2023). Flavonoids act by inhibiting enzymes that fungi need for cellular metabolism, which affects their development and resistance. In the case of rosmanol, its antioxidant activity increases oxidative stress in fungi, causing irreversible damage to their internal components. The joint action of these phytochemicals and different cell attack mechanisms offers a multifaceted and effective approach to combat fungal infections. For instance, propolis has been shown to inhibit the growth of *C. albicans* through compounds such as caffeic acid, flavonoids and phenolic esters, which alter the germ tube and cell wall, therefore generating deformations at the budding sites (Londoño-Orozco *et al.*, 2008).

Antifungal activity of *Euphorbia antisiphilitica*, *Helianthus annuus* and *Thymus vulgaris*, against *Macrophomina phaseolina*

The results obtained from the plant extracts antifungal activity, demonstrate that those of *H. annuus* and *T. vulgaris* inhibited the growth of *M. phaseolina* completely at 20 %, and so did the extract of *E. antisiphilitica* at 80 % (Figure 1). Regarding the IC₅₀₋₉₀, statistical differences were observed between the *H. annuus* and *T. vulgaris* extracts, and that of *E.*

Table 1. Phytochemical compounds identified in extracts of *Euphorbia antisiphilitica*, *Helianthus annuus* and *Thymus vulgaris* by HPLC-MS.**Tabla 1.** Compuestos fitoquímicos identificados en extractos de *Euphorbia antisiphilitica*, *Helianthus annuus* y *Thymus vulgaris* por HPLC-MS.

Species	Retention time (min)	Mass (m/z)	Phytochemical	Family
<i>Euphorbia antisiphilitica</i>	5.565	341.1	Caffeic acid 4-O-glucoside	Hydroxycinnamic acids
	18.346	330.9	Gallic acid 4-O-glucoside	Hydroxybenzoic acids
	37.602	786.7	Patuletin 3-O-glucosyl-(1->6)-[apiosyl(1->2)]-glucoside	Methoxyflavonols
	39.802	321	Gallic acid 3-O-gallate	Hydroxybenzoic acids
<i>Helianthus annuus</i>	4.992	316.8	Hydroxytyrosol 4-O-glucoside	Tyrosols
	5.535	376.8	3,4-DHPEA-EA	Tyrosols
	18.157	314.9	Protocatechuic acid 4-O-glucoside	Hydroxybenzoic acids
	22.02	372.9	5,6-Dihydroxy-7,8,3',4'-tetramethyl flavone	Methoxyflavones
	34.1	420.9	Apigenin 7-O-glucoside	Flavones
	34.754	514.8	1,3-Dicafeoylquinic acid	Hydroxycinnamic acids
	35.35	352.8	1-Caffeoylquinic acid	Hydroxycinnamic acids
	35.36	514.8	1,5-Dicafeoylquinic acid	Hydroxycinnamic acids
	44.121	272.8	Floretina	Dihydrochalcones
<i>Thymus vulgaris</i>	37.411	341	Caffeic acid 4-O-glucoside	Hydroxycinnamic acids
	38.964	623	Isorhamnetin 3-O-glucoside 7-O-rhamnoside	Methoxyflavonols
	41.295	607	Diosmin	Methoxyflavones
	44.886	345	Rosmanol	Phenolic terpenes

**Figure 1.** Inhibition percentage of *Euphorbia antisiphilitica*, *Helianthus annuus*, and *Thymus vulgaris* against *Macrophomina phaseolina* using the poisoned medium method.**Figura 1.** Porcentaje de inhibición de *Euphorbia antisiphilitica*, *Helianthus annuus* y *Thymus vulgaris* sobre *Macrophomina phaseolina* por el método de medio envenenado.

antisiphilitica, with a 17.79 % IC_{50} observed in the case of *T. vulgaris*, and 19.38 % for *H. annuus*. Meanwhile, the observed IC_{90} were 62.08 and 50.20 % for *T. vulgaris* and *H. annuus* respectively (Table 2). Some phytochemicals identified in species such as *E. antisiphilitica*, *H. annuus* and *T. vulgaris* have shown significant antifungal properties, mainly due to their ability to interfere with pathogenic fungi's cellular structure and function. Plant extracts are currently being considered an alternative for managing *M. phaseolina*, since several studies have demonstrated their ability to inhibit the development of this phytopathogenic fungus. For example, Vort *et al.* (2013) used *Larrea divaricata* (Cav.) extract to inhibit *M. phaseolina* with a MIC of 250 $\mu\text{g/mL}$. In this regard, it has been reported that extracts of *Moringa oleifera* (Lam.), *Morinda citrifolia* (L.), *Eucalyptus*, *Ricinus communis* (L.), *Melia azedarach* (L.), and *Azadirachta indica* (A. Juss) have presented antifungal activity against *M. phaseolina*, inhibiting its development by up to 60 %, and consequently stopping its growth (Águila-

Table 2. Inhibitory concentration at 50% and 90% of extracts of *Euphorbia antisiphilitica*, *Helianthus annuus*, and *Thymus vulgaris* against *Macrophomina phaseolina*.

Tabla 2. Concentración inhibitoria al 50 y 90 % de extractos de *Euphorbia antisiphilitica*, *Helianthus annuus*, and *Thymus vulgaris* sobre *Macrophomina phaseolina*.

Treatments	CI_{50}	CI_{90}
<i>Euphorbia antisiphilitica</i>	28.05 ^a	84.18 ^a
<i>Helianthus annuus</i>	19.38 ^b	50.20 ^b
<i>Thymus vulgaris</i>	17.78 ^b	62.08 ^b

*Values with the same letter are statistically similar (Tukey, $p < 0.05$).

*Valores con la misma letra son estadísticamente similares (Tukey, $p < 0.05$).

Abreu *et al.*, 2015). Meanwhile, Khaledi *et al.* (2015) reported that *T. vulgaris* essential oils (EOs) showed high antifungal activity against *M. phaseolina*, with a Minimum Inhibitory Concentration (MIC) of 1150 ppm. The exposed hyphae showed reduced structural alterations and the activity of the cellulase and pectinase enzymes, which are essential for pathogenicity. In turn, Moghaddam and Mehdizadeh (2020) determined that the EOs of *T. vulgaris* completely inhibited the growth of *M. phaseolina* at a concentration of 1600 $\mu\text{L/L}^{-1}$.

Antifungal activity of plant extracts from *Euphorbia antisiphilitica*, *Helianthus annuus*, *Thymus vulgaris* and *Bacillus subtilis* filtrates, against *Macrophomina phaseolina* in bean leaves

The results showed that both plant extracts and BSF inhibited the development of *M. phaseolina* on bean leaves (Figure 2). In this regard, statistical differences were observed between the treatments throughout the experiment. On the first day, statistical differences were observed between all treatments concerning the positive control, with the treatments corresponding to BSF, *T. vulgaris* and *H. annuus* as those showing the best control of the pathogen with a necrotic area of 1.43, 1.43 and 0 %, respectively. This trend continued throughout the days of the experiment. Finally, on the fifth day, a statistical difference was again observed between the treatments about the positive control, resulting in the treatments corresponding to BFS, *T. vulgaris* and *H. annuus* the ones showing the best control of leaf necrosis, with necrotic areas of 7.14, 12.57 and 13.86 %, respectively. The severity curve of fungal growth revealed a greater capacity of BSF, *T. vulgaris* and *H.*

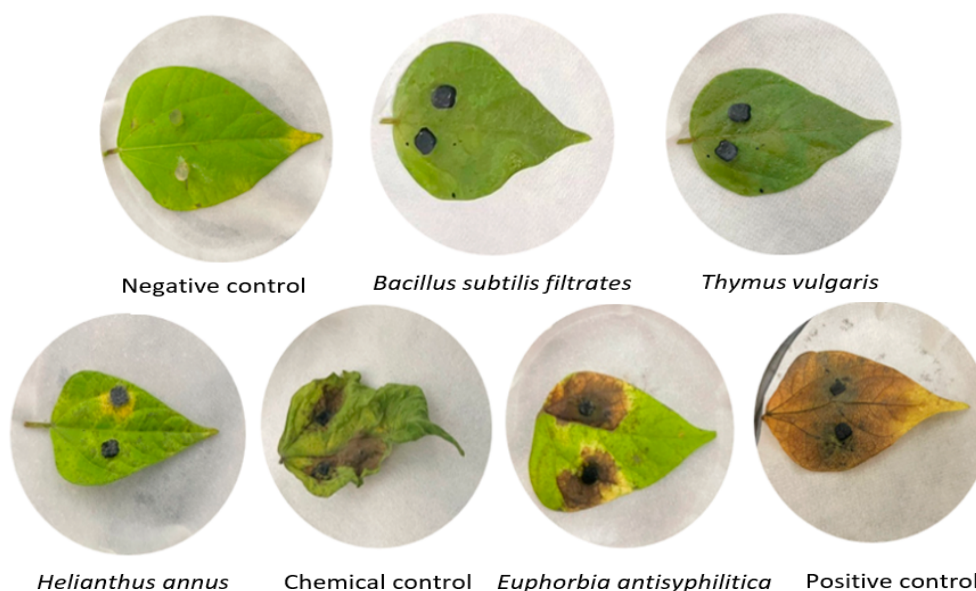


Figure 2. Antifungal activity of plant extracts (*Euphorbia antisiphilitica*, *Helianthus annuus*, *Thymus vulgaris*) and *Bacillus subtilis* filtrates, and a chemical control (Tebuconazole) against *Macrophomina phaseolina* in bean leaves, evaluated using the Detached Leaf Assay. The positive control corresponds to leaves inoculated with *M. phaseolina* without treatment, and the negative control to leaves treated with PDA medium without the pathogen.

Figura 2. Actividad antifúngica de extractos vegetales (*Euphorbia antisiphilitica*, *Helianthus annuus*, *Thymus vulgaris*) y filtrados de *Bacillus subtilis*, y control químico (Tebuconazole) contra *Macrophomina phaseolina* en hojas de frijol, evaluada mediante el ensayo de hoja desprendida. El control positivo corresponde a hojas inoculadas con *M. phaseolina* sin tratamiento, y el control negativo a hojas tratadas con medio PDA sin el fitopatógeno.

annuus to inhibit the development of the pathogen (Figure 3). These results can be attributed to the phytochemicals with antifungal activity in the extracts evaluated. Caffeic acid, which weakens the cell wall and alters the antioxidant system of fungi, was identified in *T. vulgaris* and *E. antisiphilitica* (Ma and Ma, 2015; Joya *et al.*, 2017). In addition, other compounds with antifungal properties, such as diosmin and rosmarinol in *T. vulgaris*, and apigenin in *H. annuus*, were detected. These compounds contributed to the effective inhibition of *M. phaseolina* growth observed in the treatments. The DLA technique offers an alternative to evaluate fungal diseases, allowing a controlled diagnosis under controlled temperature and humidity conditions, which favors the development of the phytopathogen. This technique also facilitates a wide range of investigations related to disease control and study of the susceptibility of diverse crops.

In a research work conducted by Han *et al.* (2017), the *in vivo* antifungal activity of purified fractions of *Curcuma zedoaria* in plants inoculated with five phytopathogenic fungi (*Rhizoctonia solani*, *Botrytis cinerea*, *Phytophthora infestans*, *Puccinia triticina* and *Blumeria graminis*) was evaluated. The plants were exposed by spraying or directly applying the inoculum, and then incubated in a humidified chamber at temperatures between 20 and 25 °C for 1 and up to 7 d, depending on the pathogen. The n-hexane and methylene chloride extracts were highly effective (80-100 %) against wheat leaf rust, like the flusilazole fungicide. At the same time, their activity against the rice sheath blight and tomato late blight was moderate and null against the tomato gray mold and the barley powdery mildew. In addition, sesquiterpene keto-lactone was identified with partial efficacy (50-65

%) against the three diseases and without phytotoxic effects on the crops evaluated. In turn, Lee *et al.* (2022) performed a pathogenicity test on rice plants inoculated with a *Magnaporthe oryzae* spore suspension. After 24 h in humid conditions, the plants were incubated at 25 °C for 7 d. The butanol extract of *Actinostemma lobatum* effectively suppressed disease symptoms on the rice leaves. At high concentrations (300 µg/mL and 150 µg/mL), its activity was comparable to the fungicide benomyl, without causing significant negative effects on the plants.

CONCLUSIONS

Plants produce many phytochemical compounds with antifungal activity as part of their metabolism, which can be extracted and used through plant extracts. In this regard, in the present study the phytochemical characterization of the extracts allowed the identification of various compounds in the extracts of *E. antisiphilitica*, *H. annuus* and *T. vulgaris*, such as caffeic acid, diosmin, apigenin, and patuletin, which show antifungal properties. These compounds might have given the extracts antifungal activity, being those of *H. annuus* and *T. vulgaris* the most effective when inhibiting the fungus growth with concentrations starting at 20 %. Regarding to the DLA procedure, it was confirmed that these same extracts and the BFS presented a high efficacy in reducing the damage caused by *M. phaseolina* in bean leaves. These results support use of plant extracts and cell filtrates from antagonistic microorganisms as alternatives for the sustainable management of *M. phaseolina*. Further research is needed to optimize and assess formulations under greenhouse and field conditions.

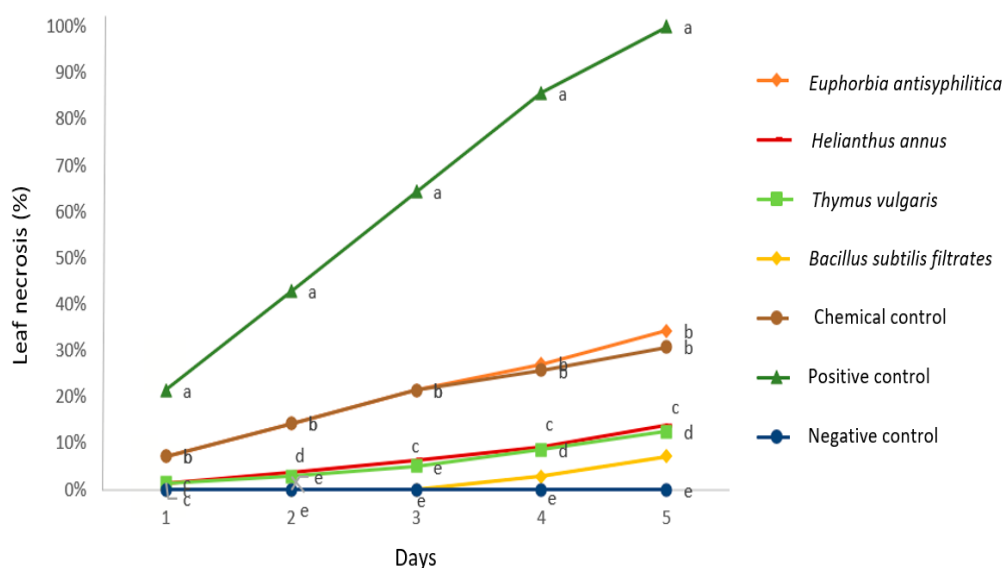


Figure 3. Area under the disease progress curve of extracts from *Euphorbia antisiphilitica*, *Helianthus annuus*, *Thymus vulgaris*, and filtrates of *Bacillus subtilis* against *Macrophomina phaseolina*. Different letters indicate significant differences between treatments according to Tukey's test ($p \leq 0.05$).

Figura 3. Área bajo la curva de progreso de la enfermedad de extractos de *Euphorbia antisiphilitica*, *Helianthus annuus*, *Thymus vulgaris* y filtrados de *Bacillus subtilis* sobre *Macrophomina phaseolina*. Letras diferentes indican diferencias significativas entre tratamientos de acuerdo con la prueba de Tukey ($p \leq 0.05$).

ACKNOWLEDGMENT

The authors wish to thank the Universidad Autónoma Agraria Antonio Narro for their support and assistance during the development of this research.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest related to the preparation and publication of this manuscript.

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