



In vitro antifungal activity and sensitivity of *Neopestalotiopsis rosae* isolates to *Baccharis salicifolia* and *Fouquieria splendens* extracts

Jesús Eduardo Ramírez-Méndez¹, Meralida Bartolón-Pérez², Francisco Daniel Hernández-Castillo³, Yisa María Ochoa-Fuentes³, Laura Castro-Rosalez⁴, Marco Antonio Tucuch-Pérez^{1*}. ¹Departamento de Botánica. ²Programa de Posgrado de Parasitología Agrícola. ³Departamento de Parasitología Agrícola. ⁴Programa de Posgrado en Agricultura Protegida. Universidad Autónoma Agraria Antonio Narro. Calzada Antonio Narro 1923, Buenavista. C. P. 25315. Saltillo, Coahuila, México.

*Corresponding author:

Marco Antonio Tucuch-Pérez
marco.tucuch_p@uaan.edu.mx

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ABSTRACT

Background/Objective. *Neopestalotiopsis rosae* is the causal agent of crown and root rot in strawberry, resulting in significant yield losses. The aim of this study was to evaluate the sensitivity of four *N. rosae* isolates (466, 323, FREC, and 372) to plant extracts (PEs) obtained from *Baccharis salicifolia* and *Fouquieria splendens*, and to determine their *in vitro* antifungal activity.

Experimental development. A qualitative phytochemical screening was performed to identify the major bioactive compounds present in the PEs. Antifungal activity was evaluated using *in vitro* microdilution assays at concentrations ranging from 3.9 to 2 000 mg L⁻¹ (10 concentrations). The 50 and 90% inhibitory concentrations (IC₅₀ and IC₉₀) were estimated, and the data were analyzed using analysis of variance (ANOVA) followed by Tukey's multiple comparison test ($p \leq 0.05$). The effects of the PEs on fungal cellular structures were also assessed.

Results. Phytochemical screening revealed the presence of phenolic compounds, saponins, and tannins in both plant extracts. Among the *N. rosae* isolates, isolates 323 and FREC were the most sensitive, exhibiting complete inhibition with both PEs at 250 mg L⁻¹. In contrast, isolates 372 and 466 required higher concentrations to achieve complete inhibition. *B. salicifolia* was more effective against isolates 466 and 372, achieving complete inhibition at 1 000 mg L⁻¹, whereas *F. splendens* required 2 000 mg L⁻¹ to completely inhibit isolate 466. Structural analyses revealed that isolate 323 was most susceptible to extract-induced damage at 250 mg L⁻¹ and above, as evidenced by inhibition of conidial germination and severe morphological alterations, including cellular collapse and dehydration. Isolates FREC and 372 exhibited mycelial thickening at 500 mg L⁻¹, whereas isolate 466 was the least sensitive, showing no evident structural damage even at this concentration.

Conclusions. Plant extracts from *B. salicifolia* and *F. splendens* contain bioactive phytochemicals with antifungal activity against *N. rosae*. Isolates 323 and FREC were the most sensitive, exhibiting 100% inhibition and pronounced structural damage at 250 mg L⁻¹, highlighting the potential of these extracts as biorational alternatives for the management of *N. rosae*. Nevertheless, further greenhouse and field studies are required to validate their efficacy under commercial production conditions.

Keywords: Biorational control, Phytochemicals, Antifungal activity, Phytopathogenic, Sustainable agriculture



INTRODUCTION

The berry industry has shown significant growth in Mexico's agricultural sector, establishing itself as one of the highest-value agricultural export products, primarily to the United States, the Middle East, Southeast Asia, and the European Union (SADER, 2023). By 2024, according to the Agri-Food and Fisheries Information System (SIAP), berry production exceeded one million tonnes, with strawberry (59.9%), blackberry (23.8%), and raspberry (16.3%) standing out as the highest-yielding species. This increase in production has given rise to major phytosanitary challenges. Among the principal phytopathogens responsible for yield and economic losses in strawberry cultivation are *Botrytis cinerea*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Rhizoctonia* spp., and, more recently, *Neopestalotiopsis rosae* (Castellanos-González *et al.*, 2020; Rebollar-Alviter *et al.*, 2020; Dholi *et al.*, 2023).

Strawberry plants infected with *N. rosae* develop symptoms as root rot, as well as the development of lesions on leaves and fruit, which can cause losses of up to 70%. The infection triggers wilting, chlorosis, and foliar necrosis, which may ultimately result in plant death. The disease occurs in strawberry transplants grown both in open fields and under tunnels, at temperatures of 23–27 °C, and its development is favored by rainfall and high leaf wetness (Rebollar-Alviter *et al.*, 2020; Olivares-Rodríguez *et al.*, 2024). As this is a relatively recent disease in strawberry cultivation, available information on its management remains limited.

Currently, the control of *N. rosae* relies primarily on the preventive and curative application of synthetic chemical fungicides, such as thiram, hymexazol, prochloraz, cyprodinil + fludioxonil, and pydiflumetofen + fludioxonil (Essa *et al.*, 2018; Acosta-González *et al.*, 2024). However, the effectiveness of these fungicides is limited by residuality, environmental impact, and pathogenic resistance (Dukare *et al.*, 2019; Islam *et al.*, 2024). Considering this situation, the implementation of integrated management using environmentally friendly methods is being promoted, such as the use of plant extracts (PEs) as a biorational control strategy (Dènè and Valiuškaitė, 2021; Cenobio-Galindo *et al.*, 2024).

In this context, plants of the genus *Baccharis* and the species *Fouquieria splendens*, distributed across the Chihuahuan and Sonoran deserts, have shown antifungal potential. In particular, *F. splendens* has demonstrated activity against *F. oxysporum*, *Rhizoctonia solani*, and the oomycete *Pythium aphanidermatum*, an effect attributed primarily to its phytochemical composition, which is rich in polyphenols (Pinto *et al.*, 2022; Ramírez-Méndez *et al.*, 2024; Ramírez-Méndez *et al.*, 2025). However, to date, only antibacterial activity has been reported for *Baccharis salicifolia*, primarily against the genus *Staphylococcus* (Escalante-Hernández, 2022), with no studies on its antifungal activity. Therefore, the objective of the present study was to identify phytochemical compounds present in extracts of *B. salicifolia* and *F. splendens*, and to evaluate their antifungal activity and *in vitro* effect on cellular structures of *N. rosae*.

EXPERIMENTAL DEVELOPMENT

Collection and processing of plant material. In August 2024, physiologically mature plants of *B. salicifolia* and *F. splendens* were collected in Saltillo, Coahuila (25°27'28.6"N, 101°06'44.4"W; 1780 m a. s. l.), and transported to the Mycology and Biotechnology Laboratory of the Universidad Autónoma Agraria Antonio Narro (UAAAN). The samples

were washed, oven-dried at 60 °C to constant weight, ground, sieved (0.2 mm), and stored in amber glass vials.

Plant extracts. Plant extracts (PEs) were obtained following the methodology proposed by Jasso de Rodríguez *et al.* (2015), with some adaptations. Briefly, 42 g of dry powder from *B. salicifolia* leaves and *F. splendens* stems were placed in 500 mL flasks and were extracted with 375 mL of 96% methanol as solvent. The mixtures were kept under continuous agitation for 72 h at 60 °C on a stirring hotplate (Thermo Scientific Cimarec, USA). Subsequently, the solvent was removed by rotary evaporation (IKA RV 10 digital V, USA) operated at 150 rpm and 70 °C. The resulting methanolic fraction was transferred to glass containers and dried in an oven (Arsa AR-130D, México) at 50 °C for 72 h. Finally, the PEs were stored in amber vials at 4 °C under refrigeration until use.

Qualitative phytochemical characterization by colorimetry. The PEs were prepared at a concentration of 2 000 mg L⁻¹ and evaluated using specific qualitative tests to detect various groups of secondary metabolites. The following tests were applied: Dragendorff and Sonnenschein for alkaloids; Fehling and Benedict for reducing sugars; Molisch for carbohydrates; H₂SO₄ and FeCl₃ for carotenoids; Ehrlich for coumarins; Liebermann-Burchard and Salkowski for sterols and terpenes; Shinoda and NaOH for flavonoids; Grignard for cyanogenic glycosides; HCl for purines; Bornträger (H₂SO₄, NH₄OH) for quinones; foam test for saponins; and tests with gelatin, FeCl₃, and K₃[Fe(CN)₆] for tannins (Sahgal *et al.*, 2009; Usman *et al.*, 2009).

Isolation of *Neopestalotiopsis rosae*. The *N. rosae* isolates were provided by the Centro Universitario Regional Centro Occidente of the Universidad Autónoma Chapingo, and are coded 466, 323, FREC, and 372. The isolates were obtained from strawberry plants showing symptoms in cultivars 'Albion' and 'Festival', from commercial plots in the municipalities of Zamora and Jacona, Michoacán. These isolates were molecularly characterized in the study conducted by Rebollar-Alviter *et al.* (2020). For preservation, the isolates were maintained on potato dextrose agar (PDA) and refrigerated at 4 °C until use.

Antifungal activity of *Baccharis salicifolia* and *Fouquieria splendens* extracts against *Neopestalotiopsis rosae*. To evaluate the antifungal activity of the PEs against *N. rosae*, the broth microdilution method was used in 96-well polystyrene microplates following the methodology of Tucuch-Pérez *et al.* (2020), with some modifications. The first column was designated as the negative control (medium without fungal cells or PEs) and the second column as the positive control (medium with fungal cell suspension without PEs). To each well, 110 µL of liquid Sabouraud medium and 40 µL of indicator solution (2,3,5-triphenyltetrazolium chloride) were added. Starting from the second column, a fungal suspension of *N. rosae* (1×10⁶ conidia mL⁻¹) was incorporated. The four isolates were evaluated individually in separate microplates, with both PEs (*B. salicifolia* and *F. splendens*) used in each case. From columns 3 to 12, PE treatments were added at concentrations ranging from 2 000 to 3.9 mg L⁻¹. Each microplate was considered one replicate with eight experimental units. The microplates were incubated at 28 ± 2 °C for 72 h, after which absorbance was measured at 490 nm using a microplate reader (Thermo Scientific Multiskan Go 51119200, USA). The inhibition percentage was calculated using the following formula:

Inhibition percentage = $((A - B) / A) \times 100$

Where: A = Absorbance of the positive control; B = Absorbance of the treatment.

Determination of the effect of *B. salicifolia* and *F. splendens* extracts on cellular structures of *N. rosae*. The effects of the PEs on fungal cellular structures were evaluated using the methodology of Ramírez-Méndez *et al.* (2024), with modifications. The PE doses consisted of 10 two-fold serial dilutions, with concentrations ranging from 2 000 to 3.9 mg L⁻¹. The fungal suspensions contained 1×10⁶ conidia mL⁻¹ of *N. rosae*. For the bioassays, 1.5 mL Eppendorf tubes were used, adjusting a total volume of 1 000 µL composed of liquid Sabouraud medium and the corresponding dose of each plant extract; subsequently, 10 µL of the fungal suspension was added. The cultures were incubated for seven days at 24 ± 2 °C, and morphological alterations were evaluated using a light microscope (10×, 40×, and 100×) equipped with a Dino-Lite® digital camera. Image capture was performed using DinoCapture 2.0 software. Each concentration was applied in triplicate, and an untreated control consisting solely of Sabouraud medium and the fungal suspension, without PEs, was included.

Statistical analysis. Probit analysis was applied to the *in vitro* assays to determine the 50% and 90% inhibitory concentrations (IC₅₀₋₉₀) for each treatment. In addition, an ANOVA was performed followed by Tukey's mean comparison test ($p \leq 0.05$). All procedures were carried out using SAS software version 9.0.

Qualitative phytochemical characterization of plant extracts. Qualitative phytochemical characterization was performed by colorimetric tests (Table 1). The results indicated that both extracts *B. salicifolia* and *F. splendens* contained the presence of alkaloids, carbohydrates, flavonoids (xanthenes), cyanogenic glycosides, saponins (triterpenoids), tannins (gallic acid and catechol derivatives), and phenols. However, *B. salicifolia* also contained purines and carotenoids, whereas in *F. splendens* the presence of quinones (benzoquinones) were detected. These results are consistent with previous studies reporting the presence of some of these phytochemicals in these plant species.

In *Baccharis* species, the main phytochemicals reported are phenolic compounds, diterpenoids, and triterpenoids, as well as volatile constituents and coumarins (Abad and Bermejo, 2007). For *B. salicifolia* in particular, several studies support this composition, such as that of Corral *et al.* (2012), who isolated halimane-type diterpenoids (salicifolic acid and 5-hydroxy-6-hydrosalicifolic acid) from the ethanolic extract of *B. salicifolia*, as well as the compounds sakuranetin and apigenin, belonging to the flavonoid group, and scopoletin, belonging to the coumarin family. Valdez-Palomino (2014), in turn, reported that hydroalcoholic extracts of *B. salicifolia* presented phenols and/or tannins, flavonoids, lactones and/or coumarins, catechins, and alkaloids. These results were obtained through colorimetric tests, which are consistent with the findings of the present study.

On the other hand, the phytochemicals of *F. splendens* have been extensively studied. For example, in the study conducted by Ramírez-Méndez *et al.* (2023), the methanolic extract of *F. splendens* stems presented alkaloids, carbohydrates, flavonoids (flavanones, flavones, flavonols, and chalcones), reducing sugars, triterpenoid-type saponins, tannins (gallic acid and catechol derivatives), as well as quinones (anthraquinones and benzoquinones). Finally, Román-Casiano *et al.* (2025) identified phenolic compounds in the ethanolic extract of *F. splendens* leaves, including flavonoids, phenolic acids, tannins, and quinic acid derivatives.

Table 1. Identification of phytochemicals present in methanolic extracts of *Baccharis salicifolia* and *Fouquieria splendens* using colorimetric assays.

	A	C	F				GC	AZ	S		T				Q			CU	P	Ca
			F1	F2	F3	F4		AZ1	S1	S2	T1	T2	T3	T4	Q1	Q2	Q3			
<i>B. salicifolia</i>	-	+	-	-	-	+	+	-	+	+	+	+	+	+	-	-	-	-	+	+
<i>F. splendens</i>	+	+	-	-	-	+	+	-	+	+	+	+	+	+	-	+	-	-	-	-

+ = Phytochemical present, - = Phytochemical absent, A = Alkaloids; C = Carbohydrates; F = Flavonoids; F1 = Aurones or chalcones; F2 = Flavanones; F3 = Flavones; F4 = Xanthones; GC = Cyanogenic glycosides; RS = Reducing sugars; S = Saponins; S1 = Triterpenoid saponins; S2 = Sterols and terpenes; T = Tannins; T1 = Tannins; T2 = Gallic acid derivatives; T3 = Catechol derivatives; T4 = Phenolic compounds; Q = Quinones; Q1 = Anthraquinones; Q2 = Benzoquinones; Q3 = Anthrone derivatives; CU = Coumarins; P = Purines; Ca = Carotenoids.

Antifungal activity of *B. salicifolia* and *F. splendens* extracts against *N. rosae* (Microdilution method). Regarding the inhibition of the different *N. rosae* isolates with the *B. salicifolia* extract (Figure 1), isolates 323 and FREC completely inhibited fungal growth at 250 mg L⁻¹, while isolates 466 and 372 required a higher concentration (1 000 mg L⁻¹). As for the IC₅₀₋₉₀ results, isolates 323 (IC₅₀ = 39.91 mg L⁻¹, IC₉₀ = 188.79 mg L⁻¹) and FREC (IC₅₀ = 44.69 mg L⁻¹, IC₉₀ = 211.82 mg L⁻¹) were statistically similar (Table 2). On the other hand, isolates 466 (IC₅₀ = 130.01 mg L⁻¹, IC₉₀ = 608.34 mg L⁻¹) and 372 (IC₅₀ = 101.59 mg L⁻¹, IC₉₀ = 480.21 mg L⁻¹) formed distinct groups, showing higher inhibitory concentrations (Tukey, p < 0.05).

Regarding the inhibition levels of the *F. splendens* extract (Figure 2), isolate 323 was the most sensitive, reaching 100% inhibition at 250 mg L⁻¹, followed by FREC and 372, which were inhibited 100% at doses of 1 000 mg L⁻¹. Isolate 466 showed the lowest sensitivity, achieving 100% inhibition only at 2 000 mg L⁻¹. These differences reflect marked heterogeneity in the isolates' responses to the evaluated extract. Regarding the IC₅₀₋₉₀, the *F. splendens* extract showed a heterogeneous response among the *N. rosae* isolates, with significant statistical differences (Tukey, p < 0.05) (Table 2). Isolate 323 was the most sensitive (IC₅₀ = 65.76 mg L⁻¹, IC₉₀ = 231.11 mg L⁻¹), followed by FREC (IC₅₀ = 118.08 mg L⁻¹, IC₉₀ = 506.69 mg L⁻¹), 372 (IC₅₀ = 185.48, IC₉₀ = 821.08 mg L⁻¹), while 466 was the most resistant (IC₅₀ = 307.37, IC₉₀ = 1,940 mg L⁻¹).

Table 2. Fifty percent and ninety percent inhibitory concentrations (IC₅₀ and IC₉₀) of *Neopestalotiopsis rosae* isolates treated with *Baccharis salicifolia* and *Fouquieria splendens* extracts.

Isolate	<i>B. salicifolia</i>		<i>F. splendens</i>	
	IC ₅₀	IC ₉₀	IC ₅₀	IC ₉₀
466	130.01 ^a	608.34 ^a	130.01 ^b	608.34 ^b
323	39.91 ^c	188.79 ^c	65.76 ^d	231.11 ^d
FREC	44.69 ^c	211.82 ^c	118.08 ^c	506.69 ^c
372	101.59 ^b	480.21 ^b	185.48 ^a	821.08 ^a

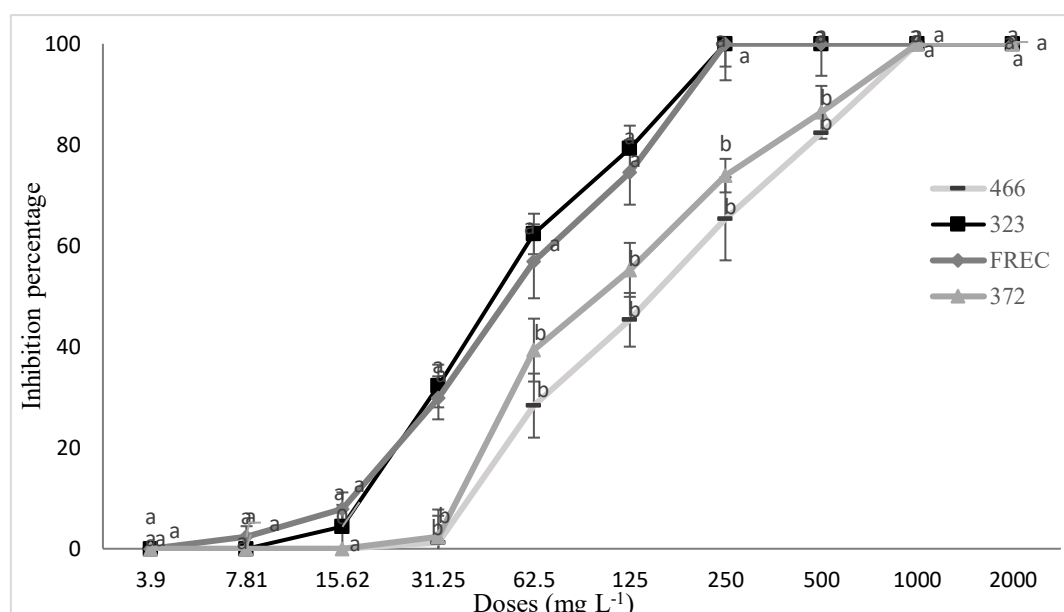


Figure 1. Inhibition percentage of *Neopestalotiopsis rosae* isolates treated with *Baccharis salicifolia* extract.
*Data followed by the same letter are not significantly different

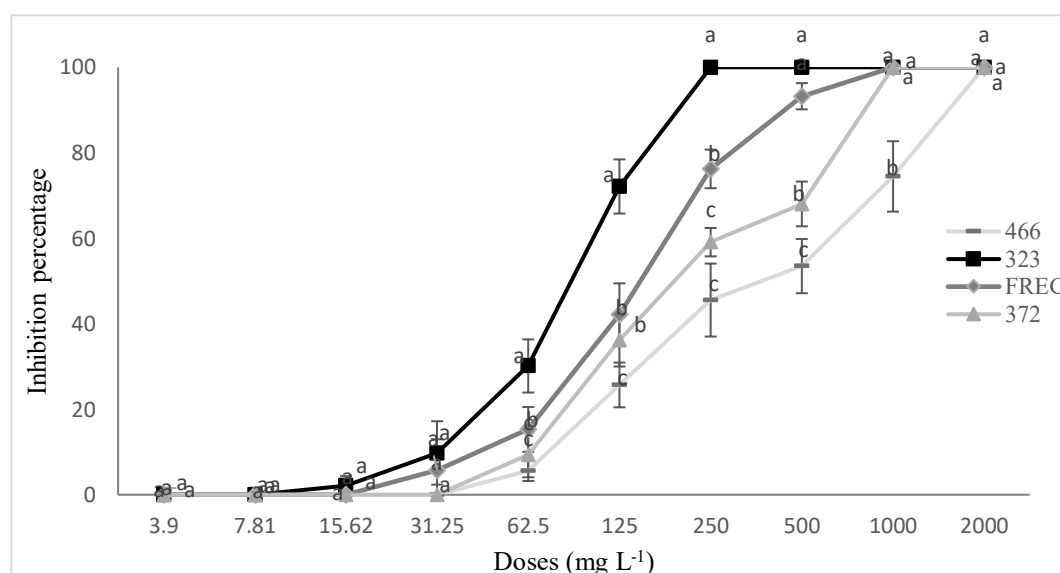


Figure 2. Inhibition percentage of *Neopestalotiopsis rosae* isolates treated with *Fouquieria splendens* extract.
*Data followed by the same letter are not significantly different.

Regarding the comparison of isolate sensitivity to the different doses evaluated between the PEs, as well as the IC₅₀₋₉₀ values, isolates 323 and FREC were the most susceptible to both PEs, followed by isolate 372, which showed a moderate response in both cases. In contrast, isolate 466 exhibited lower sensitivity, evident only against the *F. splendens* extract. The antifungal activity observed for the PEs against *N. rosae* isolates is attributed primarily to the presence of phytochemicals such as phenols, tannins, and flavonoids, identified through qualitative tests, as the involvement of these compounds in antifungal activity has been widely reported in various studies (Tucuch-Pérez *et al.*, 2020; Kengne *et al.*, 2023).

Likewise, various studies have demonstrated the efficacy of phytochemicals in controlling *Neopestalotiopsis* species. For example, Darapanit *et al.* (2021) evaluated crude

PEs against *Neopestalotiopsis* spp. and *Pseudopestalotiopsis* sp., observing that PEs of clove (*Syzygium aromaticum*) and turmeric (*Curcuma longa*) completely inhibited the growth of all isolates after 14 days of incubation at a concentration of 10 000 mg L⁻¹, values higher than those obtained in the present study. In another study, Tran *et al.* (2023) reported that the encapsulated formulation with cinnamon essential oil (*Cinnamomum verum*) showed the greatest antifungal activity against *N. rosae*, reaching 95.6% inhibition *in vitro* at a dilution of 1:500. Ye *et al.* (2023) evaluated the *in vitro* effect of thymol on *Neopestalotiopsis clavispora*, and the results showed that it limited mycelial growth with a minimum inhibitory concentration (MIC) of 100 mg L⁻¹. Zheng *et al.* (2025) demonstrated that carvacrol exhibited the greatest *in vitro* antifungal activity (EC₅₀ = 19.72 mg L⁻¹) against *Neopestalotiopsis saprophytica*.

Taken together, these results highlight the potential of phytochemicals as alternatives for managing *Neopestalotiopsis* species. In this regard, additional studies are needed to identify the bioactive compounds responsible for the antifungal activity, evaluate their efficacy under *in vivo* conditions and assess their potential integration into sustainable disease management strategies for agriculturally important crops.

Evaluation of the effect of extracts on cellular structures of *Neopestalotiopsis rosae*.

Both the *B. salicifolia* extract (Figure 3) and the *F. splendens* extract (Figure 4) induced structural alterations in *N. rosae*. However, this effect varied between the PEs and the isolates, being observed primarily at high concentrations (2 000, 1 000, or 500 mg L⁻¹) and, in some cases, down to 250 mg L⁻¹. At a concentration of 2 000 mg L⁻¹, all isolates treated with both PEs exhibited inhibition of *N. rosae* conidial germination, as well as evident structural alterations. Loss of appendages was observed in all isolates treated with the *B. salicifolia* extract (Figure 3: B1, C1, D1, and E1). Additionally, signs of dehydration and cell wall alterations were recorded, particularly in isolates 323, FREC, and 372 treated with the *F. splendens* extract (Figure 4: C1, D1, and E1). At a dose of 1 000 mg L⁻¹, the *B. salicifolia* extract induced vacuolization in the conidia of all *N. rosae* isolates (Figure 3: B2, C2, D2, and E2). The *F. splendens* extract also showed structural damage in the conidia, likewise in isolates 323, FREC, and 372 (Figure 4: B2, C2, D2, and E2).

At concentrations of 500 and 250 mg L⁻¹ of the *B. salicifolia* extract, isolate 323 remained the most sensitive (Figure 3: C3–C4), as no conidial germination was observed and the conidia exhibited morphological damage. Isolates FREC and 372 showed evident thickening (Figure 3: D3–D4 and E3–E4). In contrast, isolate 466 exhibited germination without evident structural damage (Figure 3: B3–B4). In the case of *F. splendens* at doses of 500 and 250 mg L⁻¹, isolate 323 was the most sensitive (Figure 4: C3–C4), showing inhibition of *N. rosae* conidial germination. In the conidia of isolates 372 and FREC, germinating conidia showed evidence of mycelial thickening (Figure 4: D3–D4 and E3–E4), while in isolate 466 the mycelium showed no evident damage (Figure 4: B3–B4). Finally, at a dose of 125 mg L⁻¹, germination was evident and more developed in all isolates, with conidia showing no evidence of structural malformations with either the *B. salicifolia* extract (Figure 3: B5, C5, D5, and E5) or the *F. splendens* extract (Figure 4: B5, C5, D5, and E5). These effects suggest direct antifungal action of the PEs on the integrity and development of the fungus.

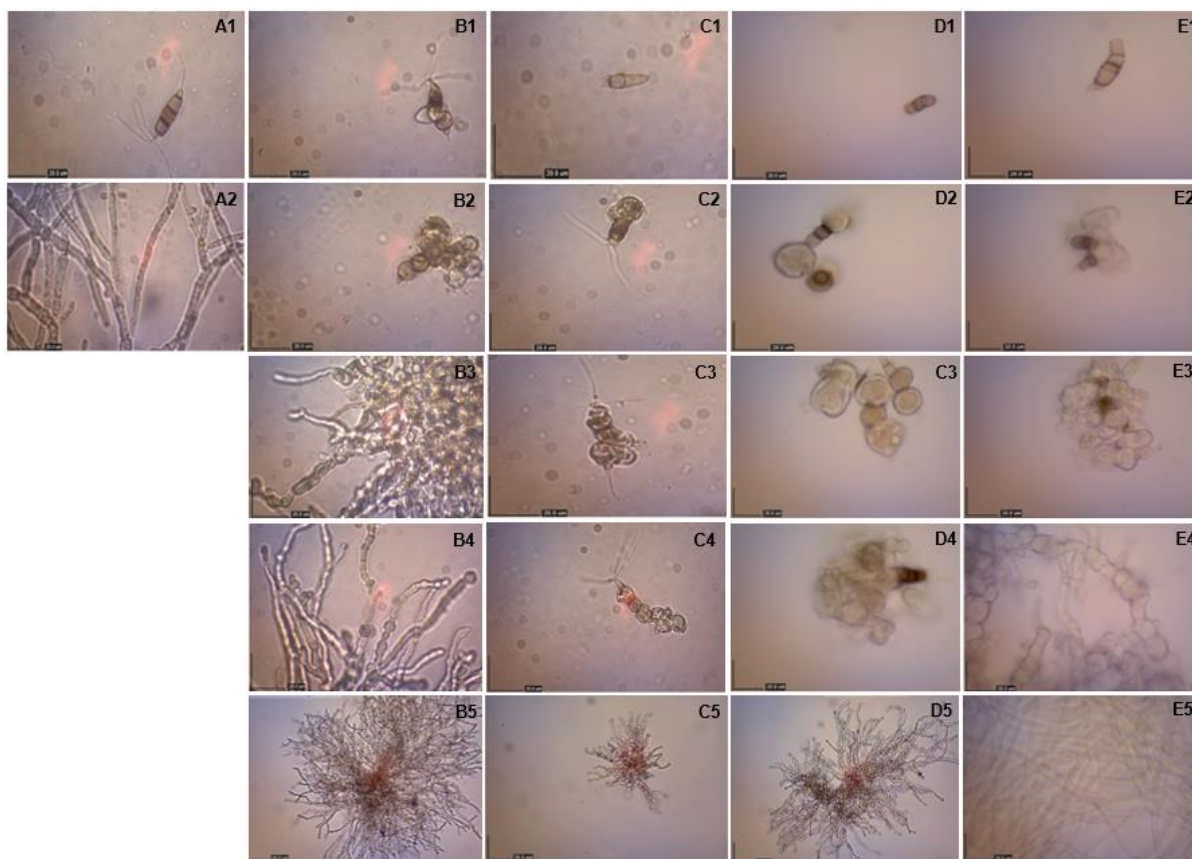


Figure 3. Effect of *Baccharis salicifolia* extract on the fungal structures of *Neopestalotiopsis rosae* isolates. A1–A2: untreated control. B1–B5: isolate 466 (2 000, 1 000, 500, 250, and 125 mg L⁻¹); C1–C5: isolate 323 (2 000, 1 000, 500, 250, and 125 mg L⁻¹); D1–D5: isolate FREC (2 000, 1 000, 500, 250, and 125 mg L⁻¹); E1–E5: isolate 372 (2 000, 1 000, 500, 250, and 125 mg L⁻¹). Microscopic observations were performed at 10× and 40× magnification.

The effect observed of the PEs on the structures of the different *N. rosae* isolates can be attributed primarily to the identified phytochemicals, as evidence exists for the mechanisms by which these compounds act on fungal cells. Various studies have demonstrated that secondary metabolites of plant origin produce similar effects in other phytopathogenic fungi. Polyphenols induce ROS production and cause lipid peroxidation damage in the fungal cell membrane, leading to increased membrane permeability, leakage of cellular components, and alterations in hyphal structure (Jiang *et al.*, 2015; Davidova *et al.*, 2025).

In this regard, the thickening observed in the fungal structures could be associated primarily with processes of cytoplasmic vacuolization induced by these compounds (Agredo-Hoyos *et al.*, 2025). Although various studies have reported the antifungal activity of isolated phytochemical compounds, such as carvacrol and thymol, against *Neopestalotiopsis* species, the available evidence has focused primarily on individual molecules (Ye *et al.*, 2023; Zheng *et al.*, 2025). To date, no studies have described structural effects induced by complex plant extracts, containing multiple phytochemicals, on the structures of *N. rosae*.

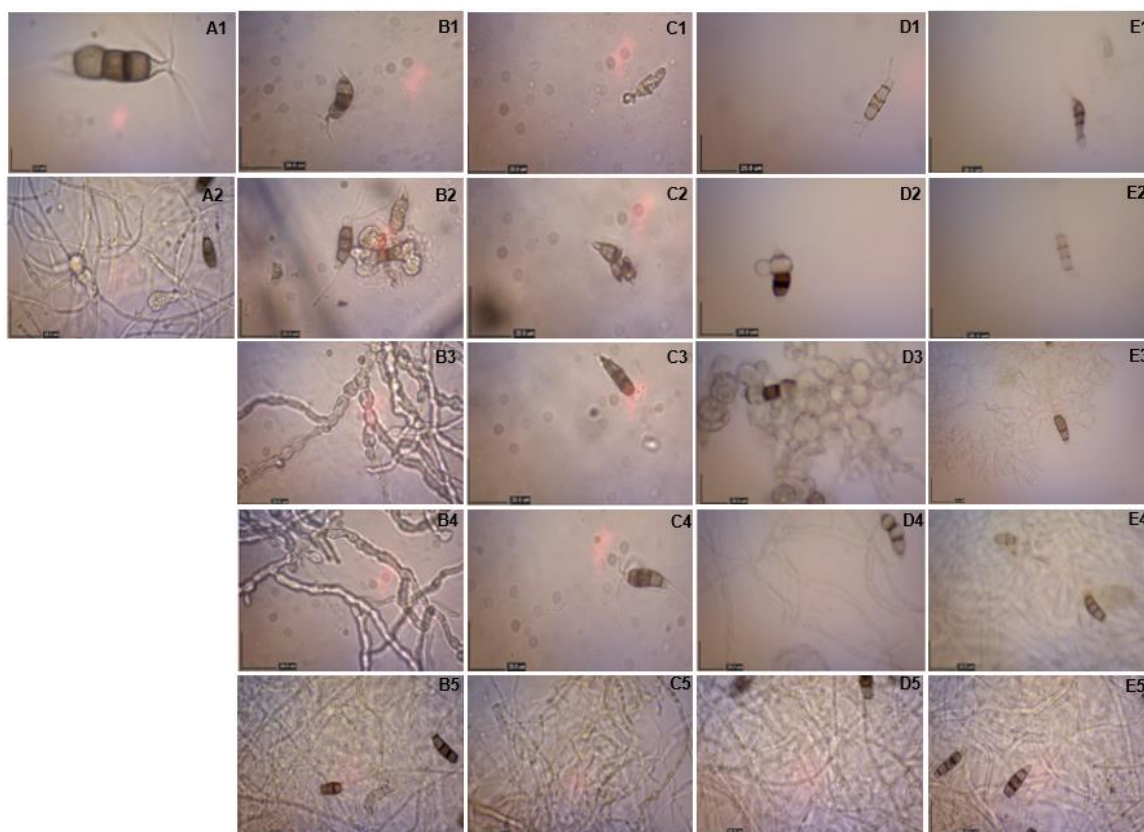


Figure 4. Effect of *Fouquieria splendens* extract on the fungal structures of *Neopestalotiopsis rosae* isolates. A1–A2: untreated control. B1–B5: isolate 466 (2 000, 1 000, 500, 250, and 125 mg L⁻¹); C1–C5: isolate 323 (2 000, 1 000, 500, 250, and 125 mg L⁻¹); D1–D5: isolate FREC (2 000, 1 000, 500, 250, and 125 mg L⁻¹); E1–E5: isolate 372 (2 000, 1 000, 500, 250, and 125 mg L⁻¹). Microscopic observations were performed using 10×, 40×, and 100× objective lenses

CONCLUSIONS

Both PEs contained alkaloids, flavonoids, saponins, tannins, and phenols; additionally, *B. salicifolia* contained purines and carotenoids, while *F. splendens* contained quinones. In the evaluation of *in vitro* antifungal activity against *N. rosae* isolates, a clearly differential sensitivity was observed among the isolates. Isolates 323 and FREC were highly sensitive, showing total inhibition and structural damage at low concentrations (250 mg L⁻¹), while isolates 372 and 466 showed lower sensitivity and required higher doses (1 000 and 2 000 mg L⁻¹), with *B. salicifolia* being the most effective extract. Taken together, these results highlight the potential of both extracts as biorational alternatives for the management of *N. rosae*, whose efficacy should be confirmed in studies under greenhouse and field conditions.

Limitations. One of the most relevant limitations of the study is the insufficient chemical analysis, as no quantitative characterization of the plant extracts was performed. Given the characteristics of the experiment conducted, it would be advisable to evaluate a greater number of fungal isolates and to incorporate the use of stains that would allow more precise visualization of damage to the structures of the phytopathogenic fungus.

Conflict of interest. The authors declare that they have no conflicts of interest regarding the research, authorship, and/or publication of this Note.

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REFERENCES

- Acosta-González U, Leyva-Mir SG, Silva-Rojas HV and Rebollar-Alviter Á. 2024. Preventive and curative effects of treatments to control strawberry root and crown rot caused by *Neopestalotiopsis rosae*. Plant Disease 108: 1278–1288. <https://doi.org/10.1094/PDIS-05-23-0958-RE>
- Abad MJ and Bermejo P. 2007. *Baccharis* (Compositae): A review update. ARKIVOC 2007(vii), 76–96. <https://doi.org/10.3998/ark.5550190.0008.707>
- Agredo-Hoyos JM, Dorigan AF, da Silveira PR, Labory CR, *et al.* 2025. Antifungal activity of essential oils in *Colletotrichum lindemuthianum* and alternative control of bean anthracnose. European Journal of Plant Pathology 172(2): 1–15. <https://doi.org/10.1007/s10658-025-03010-7>
- Castellanos-González L, Céspedes-Novoa N and Baldovino-Sanjuán A. 2020. Alternativas orgánicas para el logro de producciones más limpias de la fresa en Pamplona, Norte de Santander. INGE CUC 16(1): 193–204. <https://doi.org/10.17981/ingecuc.1612.2020.14>
- Cenobio-Galindo AJ, Hernández-Fuentes AD, González-Lemus U, Zaldívar-Ortega AK, *et al.* 2024. Biofungicides based on plant extracts: On the road to organic farming. International Journal of Molecular Sciences 25(13): 6879. <https://doi.org/10.3390/ijms25136879>
- Corral S, Cuffini SL, Cardoso SG, Bortoluzzi AJ, *et al.* 2012. Phytotoxic halimanes isolated from *Baccharis salicifolia* (Ruiz and Pav.) Pers. Phytochemistry Letters 5, Article PHYTOL-329, 4 pp. <https://doi.org/10.1016/j.phytol.2012.02.001>
- Darapanit A, Boonyuen N, Leesutthiphonchai W, Nuankaew S, *et al.* 2021. Identification, pathogenicity and effects of plant extracts on *Neopestalotiopsis* and *Pseudopestalotiopsis* causing fruit diseases. Scientific reports 11(1): 22606. <https://doi.org/10.1038/s41598-021-02113-5>
- Davidova S, Galabov AS and Satchanska GS. 2024. Antibacterial, antifungal, antiviral activity, and mechanisms of action of plant polyphenols. Microorganisms 12(12): 2502. <https://doi.org/10.3390/microorganisms12122502>
- Dènè L and Valiushkaitė A. 2021. Sensitivity of *Botrytis cinerea* isolates complex to plant extracts. Molecules 26(15): 4595. <https://doi.org/10.3390/molecules26154595>
- Dholi PK, Khatiwada P, Basnet B and Bhandari S. 2023. An Extensive Review of Strawberry (*Fragaria × ananassa*) Diseases and Integrated Management Approaches: Current Understanding and Future Directions. Fundamental and Applied Agriculture 8(4): 655. <https://doi.org/10.5455/faa.136385>
- Dukare AS, Paul S, Nambi VE, Gupta RK, *et al.* 2019. Exploitation of microbial antagonists for the control of postharvest diseases of fruits: a review. Critical reviews in food science and nutrition 59(9): 1498–1513. <https://doi.org/10.1080/10408398.2017.1417235>
- Escalante Hernández E. 2022. Identificación fitoquímica y evaluación *in vitro* del extracto de *Baccharis salicifolia* sobre bacterias de importancia clínica. Benemérita Universidad Autónoma de Puebla <https://hdl.handle.net/20.500.12371/18487>
- Essa TA, Kamel SM, Ismail AM and El-Ganainy S. 2018. Characterization and chemical control of *Neopestalotiopsis rosae* the causal agent of strawberry root and crown rot in Egypt. Egyptian Journal of Phytopathology 46(1): 1–19. <https://doi.org/10.21608/ejp.2018.87411>
- Jasso de Rodríguez D, Trejo-González FA, Rodríguez-García R, Díaz-Jiménez ML, *et al.* 2015. Antifungal activity *in vitro* of *Rhus muelleri* against *Fusarium oxysporum* f. sp. *lycopersici*. Industrial Crops and Products 75: 150–158. <https://doi.org/10.1016/j.indcrop.2015.05.048>

- Jiang XD, Feng K and Yang X. 2015. *In vitro* antifungal activity and mechanism of action of tea polyphenols and tea saponin against *Rhizopus stolonifer*. Journal of Molecular Microbiology and Biotechnology 25(4): 269–276. <https://doi.org/10.1159/00043086>
- Islam T, Danishuddin NT, Tamanna NT, Matin MN, *et al.* 2024. Resistance mechanisms of plant pathogenic fungi to fungicides, environmental impacts of fungicides, and sustainable solutions. Plants 13(19): 2737. <https://doi.org/10.3390/plants13192737>
- Kengne IC, Fankam AG, Yamako EK and Tamokou JD. 2023. Phytochemical analysis, antifungal and antioxidant properties of two herb species (*Tristemma mauritianum* and *Crassocephalum bougheyianum*) and one tree (*Lavigeria macrocarpa*). Advances in Pharmacological and Pharmaceutical Sciences 2565857. <https://doi.org/10.1155/2023/2565857>
- Olivares-Rodríguez G, Angeles-Núñez JG, Mondragón-Rojas F, Rivas-Valencia P, *et al.* 2024. Control *in vitro* de *Neopestalotiopsis* sp. aislada de fresa empleando *Trichoderma* y fungicidas comerciales. Revista Mexicana de Fitopatología 42(4): 52. <https://doi.org/10.18781/R.MEX.FIT.2024-28>
- Pinto AA, Ruano-González A, Ezzanad A, Pinedo-Rivilla C, *et al.* 2022. Bio-Guided Isolation of New Compounds from *Baccharis* spp. as Antifungal against *Botrytis cinerea*. Metabolites 12(12): 1292. <https://doi.org/10.3390/metabo12121292>
- Ramírez-Méndez JE, Hernández-Castillo FD, Tucuch-Pérez MA, Camacho-Aguilar II, *et al.* 2023. Efectividad biológica de extractos de *Agave striata* y *Fouquieria splendens* contra *Clavibacter michiganensis* subsp. *michiganensis*. Biotecnia 25(1): 34–42. <https://doi.org/10.18633/biotecnia.v25i1.1751>
- Ramírez-Méndez JE, Hernández-Castillo FD, Gallegos-Morales G, Jasso-Cantú D, *et al.* 2024. Biological effectiveness of *Agave striata* and *Fouquieria splendens* extracts against *Pythium aphanidermatum* and *Rhizoctonia solani* *in vitro*. Revista Mexicana de Fitopatología 42(4) 40. <https://doi.org/10.18781/r.mex.fit.2024-23>
- Ramírez-Méndez JE, Hernández-Castillo FD, Jasso de Rodríguez D, Jiménez-Pérez O, *et al.* 2025. Evaluación de extractos vegetales del Desierto Chihuahuense contra *Fusarium oxysporum* en el cultivo de chile. Revista Chapingo Serie Zonas Áridas 24. e2503. <https://doi.org/10.5154/r.rhsza.2025.04.03>
- Román-Casiano KM, Bermudes-Contreras JD, Cornejo-Garrido J, Delgado-Alvarado EA, Uribe-Soto JN, *et al.* 2025. Phytochemical profile, toxicological safety, and antihyperglycemic effects of the leaf extract of *Fouquieria splendens* Engelm in streptozotocin-induced diabetic rats. Future Pharmacology 5(4): 64. <https://doi.org/10.3390/futurepharmacol5040064>
- Rebollar-Alviter A, Silva-Rojas HV, Fuentes-Aragón D, Acosta-González, *et al.* 2020. An emerging strawberry fungal disease associated with root rot, crown rot and leaf spot caused by *Neopestalotiopsis rosae* in Mexico. Plant Disease 104(8):2054–2059. <https://doi.org/10.1094/PDIS-11-19-2493-SC>
- Sahgal G, Ramanathan S, Sasidharan S, Mordi MN, *et al.* 2009. Phytochemical and antimicrobial activity of *Swietenia mahagoni* crude methanolic seed extract. Tropical Biomedicine 26(3): 274-279.
- SADER (Secretaría de Agricultura y Desarrollo Rural). 2023. Berries, segundo producto del campo con mayor valor de exportación: Agricultura. Gobierno de México. <https://www.gob.mx/agricultura/prensa/berries-segundo-producto-del-campo-con-mayor-valor-de-exportacion-agricultura?idiom=esadutmn>
- SIAP (Servicio de Información Agroalimentaria y Pesquera). 2024. Avance de siembras y cosechas: cultivos perennes, año agrícola 2024 [Base de datos]. Secretaría de Agricultura y Desarrollo Rural. https://nube.agricultura.gob.mx/avance_agricola/
- Tran TNM, Vu NBD and Nguyen MH. 2023. Antifungal activity of essential oil-encapsulated lipid nanoemulsions against *Neopestalotiopsis rosae* causing leaf spot on strawberry. Journal of Plant Diseases and Protection 130(4): 823-832. <https://doi.org/10.1007/s41348-023-00760-6>
- Tucuch-Pérez MA, Arredondo-Valdés R and Hernández-Castillo FD. 2020. Antifungal activity of phytochemical compounds of extracts from Mexican semi-desert plants against *Fusarium oxysporum* from tomato by microdilution in plate method. Nova Scientia 12(25): 1-19. <https://doi.org/10.21640/ns.v12i25.2345>
- Usman H, Abdulrahman FI and Usman A. 2009. Qualitative phytochemical screening and *in vitro* antimicrobial effects of methanol stem bark extract of *Ficus thonningii* (Moraceae). African Jurnal Traditional Alternative Medicine 6(3): 289-295. <https://www.ajol.info/index.php/ajtcam/article/view/57178>
- Valdez-Palomino R del P. 2014. Contenido de fenoles totales y flavonoides totales en *Oenothera rosea* Ait. (“yawar suqu”), *Baccharis salicifolia* R. and P. (“chilca”) y *Piper elongatum* Vahl (“matico”) [Tesis de licenciatura, Universidad Nacional de San Cristóbal de Huamanga]. Universidad Nacional de San Cristóbal de Huamanga, Perú. 19-29 pp.
- Ye S, Shuai L, Luo D and Ba L. 2023. Inhibitory activity and mechanism of action with thymol against the blueberry pathogenic fungi caused by *Neopestalotiopsis clavispora*. Horticulturae 9(9): 983. <https://doi.org/10.3390/horticulturae9090983>
- Zheng H, Li JH, Guo Z, Smaghe G, *et al.* 2025. Antifungal activities and underlying mechanisms of carvacrol against *Neopestalotiopsis saprophytica* causing brown leaf spot in *Rosa roxburghii*. Pest Management Science 81(9): 5848–5863. <https://doi.org/10.1002/ps.8940>