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Determination of the phytochemical and biofungicide characteristics of Peruvian pepper tree (*Schinus molle*) against *Neofusicoccum* sp. that damages post-harvest avocados (*Persea americana* Mill)

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ABSTRACT

Objective: To evaluate the effect of leaf, stem, and fruit plant extracts of Peruvian pepper tree (*Schinus molle*) on *Neofusicoccum* sp.

Design/Methodology/Approach: Methanol extractions of Peruvian pepper tree were used to determine its phytochemical profile. The inhibition caused by the plant extracts was evaluated at a 0.01, 0.03, and 0.07% concentration, using the in vitro poisoned agar technique against the phytopathogen isolated from post-harvest avocados.

Results: Saponins, coumarins, total phenols, flavonoids, and antioxidant activity recorded positive results. Stem obtained better ABTS inhibition results (92.7±0.8%), while the leaves recorded a better DDPH inhibition result (88.2±0.24%). The *in vitro* assays showed the effects of the extracts on *Neofusicoccum* sp., higher concentrations (0.07%) had a better mitigation effect. The inhibition of the pathogen fluctuated between 43.8 and 49.5% for all the plant extracts.

Study Limitations/Implications: Methanol was used as a solvent to obtain *S. molle* extracts.

Findings/Conclusions: The resulting inhibition was associated with a pseudo-quantitative approach that helped to understand the effect of bioactives against this phytopathogen and to include *Schinus molle* as an alternative within a biorational plan for the phytosanitary protection of crops.

Keywords: extracts, bioactives, phytopathogens, biological control, diseases.

INTRODUCTION

Mexico is the main producer and exporter of avocado (*Persea americana* Mill.) worldwide. Most of the avocados produced in Mexico come from the Pacific central region (Jalisco, Michoacán, and Nayarit), which accounts for 96% of the domestic production (Herrera-González *et al.*, 2024). Avocado is a climacteric fruit and, consequently, its preservation and commercialization to distant markets is difficult, because its consumption quality, shelf life, and commercial value decrease (Ramírez-Gil *et al.*, 2020). Post-harvest deterioration of avocado is mainly caused by fungal diseases, such as anthracnose and peduncle ringing. The symptoms of these diseases particularly appear during the ripening process and increase due to mechanical damage, physiological disorders, unappropriated storing temperatures, long refrigeration periods, harvest season, and fruitage (Arpaia *et al.*, 2018; Bowen *et al.*, 2018). *Neofusicoccum* sp. was previously reported in avocados by Molina-Gayosso *et al.* (2012). For their part, Sasia *et al.* (2023) isolated *Neofusicoccum* sp. from plants with bacterial canker symptoms in stems, which impacted 60% of avocado plantations. *Neofusicoccum* sp. is the third most frequently found fungi in avocado plantations. Modern agriculture depends on the use of chemical pesticides to control phytopathogens. This situation has gradually resulted in pest resistance, changes in soil microbial diversity, and environmental pollution (Jin-Lian *et al.*, 2016). Custode *et al.* (2023) pointed out that, in order to tackle this problem, natural solutions such as plant extracts are being researched.

Currently, new technological initiatives and trends have resulted in bioproducts such as bioinsecticides, bioherbicides, bioacaricides, bionematicides, and biofungicides. These alternatives include stimulants of the plant defenses, biological control techniques, and natural byproducts obtained from plants and microorganisms (Sharma and Malik, 2012; Isman and Grieneisen, 2014; Ordanza-Beneitez, 2017). Biological control involves the use of beneficial organisms and their products, such as secondary metabolites that reduce the effects of pathogens on plants and promote a favorable response (García-Espejo *et al.*, 2016).

Plants synthetize variable concentrations of different secondary metabolites and perhaps use them as defense mechanism. They belong to different groups, including essential oils, alkaloids, coumarins, steroids, phenols, flavonoids, glycoside, rubber, iridoids, lignans, mucilage, pectin, quinones, saponins, tannins, and terpenoids (Paumier *et al.*, 2018 and Pinard *et al.*, 2019).

These substances are synthetized as a plant response to fungi, bacteria, and pest attacks, as well as allelopathic effect, pollution, and adverse effects resulting from climatic factors, and herbivores. These chemical defenses can be found in different concentrations in nature and depend on the type of metabolite, the characteristics of the plant, and the conditions to which the plant is subjected (Reyes-Silva *et al.*, 2020). The species *Schinus molle* L. is variously known by people of the Americas as pepper, Peruvian pepper, American pepper, false pepper, molle, molli, anacahuita, or pirul (Enersis, 2014). This plant has several biological effects, including antioxidant, anti-inflammatory, hypotensor, analgesic, antispasmodic, antifungal, and antitumor properties (Zamora, 2007, and Rebolledo, 2020). The phytochemical profile of the leaves, stems, and fruit of *Schinus molle* and its effect on *Neofusicoccum* sp. were identified under *in vitro* conditions. Consequently, the aim was to include this plant as a biorational management alternative.

MATERIALS AND METHODS

Production of the Peruvian pepper tree (*Schinus molle*) extract

The first stage of this research was to obtain the Peruvian pepper tree extract from different parts of the tree (leaves, stems, and fruits); consequently, the research team collected samples from the nearby plots of La Barca, Jalisco (20° 21' 20.5" N, 102° 26' 13.3" W), prioritizing undamaged and pest- and disease-free plant material (Figure 1). This plant material was processed in the Phytopathology Lab of the Centro Universitario de la Cienéga, La Barca, Jalisco, University of Guadalajara.

The plant material was collected from the nearby plots of La Ciénega region, Jalisco, Mexico. The plant material was carefully divided into leaves, stems, and fruits. Half a kilogram of each plant material was collected. The plant material was washed with running water. Subsequently, it was washed with distilled water. Finally, it was left to dry under natural abiotic conditions until the appropriate drying was achieved (temperature and moisture, sun or shade).

Each sample was lyophilized in a Nutrebule equipment to obtain fine particles. The technique proposed by Del Toro-Sánchez *et al.* (2015) was used in the extraction process. Three-point-zero g of a given lyophilized plant part (leaves, stems, and fruits) were poured into Corning® tubes. Afterwards, 30 mL of methanol were added to the tubes and the mixture was allowed to rest for 24 h. Subsequently, it was homogenized for 30 s in an ULTRA-TURRAX® (T 25 DS1 digital homogenizer). Then, the mixture was sonicated for 15 minutes, at 4 °C and at 4,000 rpm, using a centrifuge machine (Heraeus Megafuge 16R, Thermo Fisher Scientific,Waltham, MA, USA). The supernatant was filtered in a round-bottom flask, restricting the light. The process was repeated with the precipitate. Once the process concluded, the yield of the extract was determined during the methanolic phase; the solvent was evaporated using the rotavapor system (Heidolph Rotavapor, 4003 VAC Senso T), reporting extract grams per dry sample grams (gE/gms). The total extract was recovered adding 10 mL of methanol and, subsequently, it was refrigerated at 5 °C.

Figure 1. *Schinus molle* plant material used to obtain the extracts: leaf (A); stem (B); fruit (C).

Phytochemical profile based on qualitative tests

Saponin identification (foam testing)

The method proposed by García *et al.* (2009) and Sánchez *et al.* (2010) was used to determine saponin content. A 1:9 dilution rate (1 mL of crude extract and 9 mL of distilled water) was poured into a test tube. The mixture was vigorously shaken by hand for 30 s. Afterwards, the mixture rested for 15 min. The results depended on the height of the foam: <5 mm: ($-$), negative content; 5-10 mm: ($+$), low content; 10-15 mm: ($+$), moderate content; >15 mm: ($++$), high content.

Coumarin identification

A 1:9 dilution from the crude extract (1 mL of crude extract and 9 mL of distilled water) was used to identify coumarins. Two mL of this solution were poured into a test tube with a cap. Afterwards, a strip of filter paper soaked in NaOH (0.06 g mL^{-1}) was placed inside the tube, avoiding contact with the crude extract dilution. Subsequently, the tube was heated in a Bunsen burner until it produced enough vapors. To verify the accumulation of coumarins, the paper was observed under a transilluminator UV chamber (Labnet, model: TM-26). If the filter paper showed fluorescent dots, the sample was positive (García *et al.*, 2009; Sánchez *et al.*, 2010).

Alkaloid identification

To identify the alkaloid content, 6 mL of extract and 6 mL of 10% HCL were poured into a test tube and boiled for 5 minutes. Afterwards, the mixture was cooled, filtered, and transferred to two tubes. A drop of Dragendorff's reagent was added to one of the tubes. The result was positive when the Dragendorff's reagent formed an orange precipitate.

Tannin identification

To identified the tannin content, 0.7 g of the lyophilized sample were added to a flask, along with 200 mL of potassium ferrocyanide $K_4[Fe(CN)_6]$. The final mixture had a concentration of 0.004 M. The mixture was kept at 100 rpm during 15 minutes, without light. Subsequently, 20 mL of 0.008 M ferric chloride ($FeCl_3$) (García *et al.*, 2009; Sánchez *et al.*, 2010) were added. Changes in color were considered as a positive result: dark green indicated condensed tannins, while blue meant hydrolysable tannins.

Total phenol identification

The Folin Ciocalteu assay was used to determine the phenol content (Prior *et al.*, 2005, Mullen *et al.*, 2007). Twenty-five μL of the Folin 1 N solution were added to 10 μL of crude extract. Afterwards, the mixture rested in the refrigerator for 5 minutes. Subsequently, 25 μL of 20% Na_2CO_3 and 140 μL of distilled water were added to obtain a final volume of 200 μL. The mixture rested for half an hour and, then, absorbance was calculated at 760 nm with a Thermo Fisher Scientific™ FI-01620 Microplate Reader. The standard gallic acid in methanol was used to draw a curve (0-1 mg/mL). The results were expressed as mg of gallic acid per g of dry sample (mg EAG/gms). All the measurements were carried out in triplicate.

Total flavonoid determination

The colorimetric method proposed by Venu *et al.* (2012) was used to determine the total flavonoid content. Eighty μL of an aluminum trichloride - ethanol solution (20 g L^{-1}) were added to 80 μL of the extract. The mixture was shaken for 30 s and, afterwards, it was covered with Parafilm and left in the dark for one hour, at 25 °C. Subsequently, the mixture was shaken for 30 s and absorbance was measured at 415 nm with a Thermo Fisher Scientific™ FI-01620Microplate Reader. Standard quercetin in methanol was used to draw a curve. The results were expressed as mg of quercetin per g of fresh sample (mg EQ/gmf).

Antioxidant capacity determined by ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid)

The ABTS radical was prepared following the methodology described by Re *et al.* (1999). Nineteen-point-three mg of ABTS were dissolved in 5 mL of distilled water. Meanwhile, 0.0378 g of potassium persulphate were weighted and mixed with 1 mL of water. Afterwards, 88 μL of the persulphate solution were added to the ABTS. This mixture was left in the dark for 12 hours. An adjusted solution was prepared at a 0.7 ± 0.01 absorbance and a wavelength of 734 nm in a Thermo Fisher Scientific™ FI-01620Microplate Reader. Twenty μL of the sample were added to 270 μL of the prepared cationic radical solution. After a 30 minutes rest, absorbance reached 734 nm. The assay was carried out in triplicate and the results were expressed in inhibition percentages.

$$Inhibition\% = \frac{control\ absorbance - sample\ absorbance}{control\ absorbance} \times 100 \qquad (1)$$

Antioxidant capacity determined by DPPH (2,2-Diphenyl-1-Picrylhydrazy)

One-point-five mg of the DPPH radical was weighted and dissolved in 50 mL of methanol, which was adjusted to a 0.7 ± 0.01 absorbance, in a 515 nm wavelength (Molyneux, 2004). Two-hundred μL of the radical were taken from this mixture, along with 20 μL of sample. Subsequently, the mixture rested for 30 minutes in the dark and was measured at a 515 nm wavelength, using a Thermo Fisher Scientific™ FI-01620 Microplate Reader (Molyneux, 2004). The assay was carried out in triplicate and the results were expressed in inhibition percentages, using the ABTS equation.

Isolation of *Neofusicoccum* sp. from post-harvest avocados (*Persea Americana* Mill)

The methodology proposed by Ceja *et al.* (2000) was used to isolate the pathogen from post-harvest avocados. The fruits were subjected to a disinfestation process with 2% sodium hypochlorite for 3 minutes. Afterwards, they were washed with sterile distilled water and, finally, approximately 1 cm cuts were made in the epicarp. The cuts were place in Petri dishes with potato dextrose agar (PDA) and chloramphenicol (1 mL of antibiotic/L of the PDA medium, obtained from a 50 mg antibiotic/mL of ethanol). The Petri dishes were incubated at 25±1 °C until the mycelium appeared (2-4 days). The growths were purified in new Petri dishes with PDA, isolating individual colonies with the hyphal head technique. The *Neofusicoccum* sp. isolated strains were identified with an optical microscope (model CX311RTSF, Olympus, Tokyo, Japan) with a 40X augmentation.

In vitro antifungal analysis of the extract of leaves, stems, and fruits of *Schinus molle* used against *Neofusicoccum* sp.

The method proposed by Jiménez *et al.* (2006) was used in this analysis. The PDA culture medium was poisoned at different concentrations (0.07, 0.03, and 0.01%). Each dose was applied in the previously sterilized and melted PDA medium, at 30-45 °C. Once the medium was jellified, an active growth of *Neofusicoccum* sp. was inoculated in the center of the dish with a plug. Three replicates per treatment were made. The dishes were incubated at 25±1 °C for seven days. Subsequently, the diameters of the treated and control colonies were measured to calculate the inhibition percentage using the following formula:

$$\% = \frac{DCC - DTC}{DCC} \times 100 \qquad (2)$$

where: *DCC*: diameter of the control colony; *DCT*: diameter of the treated colony.

Statistical analysis

All the experiments had three repetitions. Data were evaluated using a multifactorial analysis of variance (ANOVA), followed by the Least Significant Difference (LSD) test, with a 95% reliability. All the analysis were conducted with the Statgraphics Centurion XVI software (StatPoint Technologies, Inc., Warrenton, VA, USA).

RESULTS AND DISCUSSION

Collection and yield determination of leaves, stems, and fruits extract of *Schinus molle*

The yield from the extracts of each plant section was not very diverse; however, they were diverse enough to conduct the in vitro assay. The amount of plant material followed the fruit>stem>leaf order (Table 1). The fruit extract recorded the highest content (43%), while the leaf extract recorded the lower value (34%). Alfaro-Pérez *et al.* (2018) pointed out that the quality of the plant extracts depends on the collection site. The growing conditions of the crops have a significant influence on the production of secondary metabolites

Table 1. Yield of the *Schinus molle* extract.

Vegatable sample	Initial weight (g)	Extract (g)	Yield gE/gms	Yield (%)
Leaf	3	1.02	0.34	34
Stem	3	1.16	0.38	38
Fruit	3	1.3	0.43	43

g: grams; gE/gms: extract grams per dry sample grams; %: percentage.

—*i.e.*, the bioactive compounds obtained in the plant extracts. In addition, they can have antibacterial and antifungal properties.

For their part, Holopainen *et al.* (2018) and Estrada-Jiménez *et al.* (2019) pointed out that the presence of secondary compounds is related with the age of the plant material, the defense mechanisms, the content of primary nutrients, and the influence of the soil and weather. The complexity of the ecosystem —particularly weather and soil— in which the plants develop has a significant influence on the active principles and the secondary compounds, which have a marked effect on the plants: the lack and excess of these elements can cause stress, leading to the synthesis of these substances.

In this context, Amyrgialakiu *et al.* (2014) and Caprioti *et al.* (2014) mentioned that the quantitative and qualitative yield of the extraction mainly depends on the polarity of the solvent used in the process. However, no standardized method or solvent has been defined for this process. They both depend on the chemical composition of the extracted compounds, amount and position of the hydroxyl group, and molecular size. In addition, other factors such as solvent concentration, temperature, contact time, particle size, and mass-solvent ratio must be taken into account. The Peruvian pepper tree yield obtained in this research was good.

Phytochemical profile of methanol extracts of leaves, stems, and fruits of Peruvian pepper tree

The analysis conducted to determine the chemical bioactive compounds (Table 2) in the leaf, stem, and fruit methanol extracts showed that stems were the only extract that recorded saponin content. The low content of saponins is likely the result of the absence of fungal or bacterial agents in the tree during the sampling stage (February-March); consequently, the tree was not subjected to stress. Gongóra *et al.* (2022) pointed out that saponins are secondary metabolites naturally produced by plants as a consequence of biotic stress. In addition, saponins can potentially be used in different areas of the industry, particularly in food technology, health, and agriculture.

Güçlü-Üstündağ and Mazza (2007) reported that extracts with saponins are used to improve agricultural productivity because they stimulate plant growth and function as insecticides and fungicides.

Table 2. Phytochemical profile of the methanol extracts of leaves, stems, and fruits of Peruvian pepper tree.

secondary metabolite	Plant fraction		
	Sheet	**Stem**	**Fruit**
Saponins	(−)	(+)	(−)
Coumarins	(+)	(+)	(+)
Alkaloids	(−)	(−)	(−)
Tannins	(+); condensable	(+); hidrolyzable	(+); hidrolyzable
Total phenols	(+)	(+)	(+)
Flavonoids	(+)	(+)	(+)

(+): positive; (−): negative.

Meanwhile, the three different leaf, stem, and fruit methanol extracts recorded a positive coumarin content. Oliveros *et al.* (2011) recorded coumarin levels in three extraction processes, using the infusion method. Although this process is not very aggressive, they reported significant coumarin quantities.

For its part, no alkaloids were found in any of the Peruvian pepper extracts. These results match the findings of Ferreira *et al.* (2023), who did not find alkaloids in all the tests conducted with ethanol extracts of *S. weinmanniifolia* leaves. Nevertheless, these results do not mean that alkaloids were not present in the plant.

Each extract obtained a positive tannin content. Leaf extract recorded condensed tannins, while stem and fruit extracts recorded hydrolysable tannins (Table 2). Jin *et al.* (2013) pointed out that, on the one hand, condensed tannins have different physical and chemical properties. In addition, once they are consumed, they have different biological properties: antioxidant, chemotherapeutic, anti-inflammatory, and anti-microbial. On the other hand, water-soluble tannins have a carbohydrate nucleus (mainly glucose). They are susceptible to hydrolysis under physiological conditions, enabling the gradual release of primary components.

For their part, Okuda *et al.* (2011) mentioned that tannins can be produced in higher amounts under adverse environmental conditions —*i.e.*, these secondary metabolites defend the plants against predators (herbivores) and perform antimicrobial activities.

Meanwhile, the total phenol contents were 6.8, 17.1, and 30.1 mg EAG/g for leaf, stem, and fruit extracts, respectively. Polyphenols and phenolic compounds are widely distributed among plants. These are natural molecules of the secondary metabolism of the plants that include >8,000 different compounds. They are a large group of chemical substances, with different structures, chemical properties, and biological activities (Valencia *et al.*, 2017). Phenols are usually researched due to their different functions such as nutrient assimilation, protein synthesis, enzymatic activity, photosynthesis, formation of structural components, and defense against adverse environmental factors (pathogens and insects) (Manach *et al.*, 2004). In addition, phenols are known for their outstanding antioxidant capacity (Gomez *et al.*, 2016).

The flavonoid contents recorded 4.6, 3.2, and 1.8 mg/EQ/g for leaf, stem, and fruit extracts, respectively. Agudelo *et al.* (2013) reported flavonoids in the extract of *S. weinmanifolia* leaves. Their results match the reports of other studies about similar species, which recorded flavonoids found in methanol extracts of *Schinus longifolius* leaves and *Schinus molle* leaves and fruits (López *et al.*, 2017). Wimalaratne *et al.* (1996) pointed out that Peruvian pepper tree leaves have tannins, alkaloids, flavonoids, spheroidal saponins, sterols, terpenoids, rubber, resin, and >20 essential oils. Likewise, Lannacone and Alvariño (2010) reported that *S. molle* ethnobotanical extracts subjected to a blank test include a mixture of active substances of *S. molle* leaves that could have synergy and antagonist effects. The metabolites found in this study proved that the edaphoclimatic conditions favored the methanol expression of these secondary bioactives, as a result of the interaction between genotype and environment. Growing Peruvian pepper tree —a natural extract source for the industry, agriculture, and pharmacopoeia sectors— plays a key role in the determination of its beneficial potential.

Antioxidant capacity

The antioxidant capacity was determined using the ABTS and DPPH (synthetic radicals) methods. The results were expressed in inhibition percentages. Secondary metabolites produced by plants include a wide range of antioxidants (Siddartha *et al.*, 2022). The analyzed extracts of Peruvian pepper tree recorded high inhibition percentages, revealing the bioactive content that provides their antioxidant capacity to the extracts (Figure 2). The inhibition of the methanol extracts in the radical ABTS reached 90.8±0.4, 91.8±0.04, and 92.7±0.8% for leaf, fruit, and stem extracts, respectively. Meanwhile, the highest inhibition resulting from the radical DPPH reached 88.2±0.24% (leaf extract), followed by 87.7±0.15% (fruit), and 84.9±0.30% (stem). According to the results of the ANOVA, extract, radical, and dilution factors, as well as other interactions, recorded significant effects on inhibition ($p \leq 0.05$). Mendoza (2011) recorded 69.44% (leaves), 56.23% (stems), and 32.84% (fruits) antioxidant capacity applying the free radical capture DPPH test to the hydroalcoholic extract of *Schinus molle*.

The different inhibition percentages obtained with the free radical DPPH method applied by Mendoza (2011) and this research team could be the result of the solvents used to develop the extracts. Meanwhile, ABTS recorded the highest inhibition percentages out of the two free radical methods (ABTS and DPPH) used in this research to determine the antioxidant capacity of the extracts. According to Villaño *et al.* (2007), the inhibition percentage difference between the two free radical methods could be the result of the affinity of the compounds of the extracts. Consequently, ABTS had the highest affinity, because the leaf, stem, and fruit extracts recorded a >91% inhibition. These results match the findings of Ibarra *et al.* (2018), who subjected the leaf extract of *P. auriculata* to the same free radical methods (ABTS and DPPH).

In vitro **antifungal analysis of leaf, stem, and fruit extracts from *Schinus molle* against *Neofusicoccum* sp.**

Isolation of the *Neofusicoccum* sp. phytopathogen

Post-harvest avocados with necrotic lesions were used to obtain the pathogen. The method consisted in repeatedly extracting the rotten mesocarp (Figure 3A). The microscopic (Figure

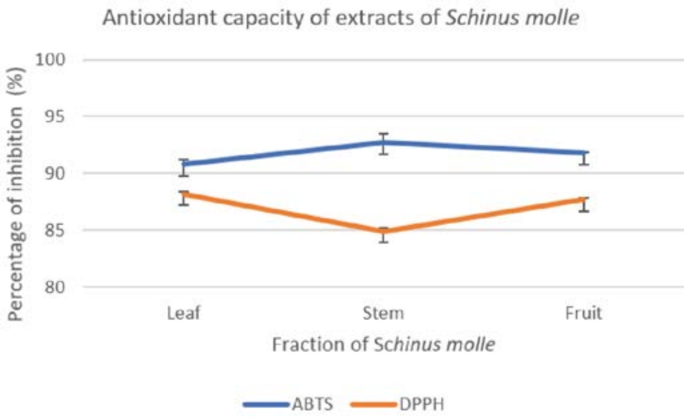

Figure 2. Antioxidant capacity of the Peruvian pepper tree leaf, stem, and fruit extracts.

3C) and macroscopic (Figure 3B) characteristics of the selected colonies were analyzed. In order to confirm its presence, the fungus was grown in potato dextrose broth and pine needles (Figure 3D). The aim was to observe the conidia (Figure 3E). The colonies with similar morphology to the colonies found in the bibliography were chosen. Consequently, the axenic strain (Figure 3B) that was used in the subsequent in vitro experiments was obtained.

In vitro evaluation of the Peruvian pepper tree (*Schinus molle*) extracts against *Neofusicoccum* sp.

The *in vitro* poisoned agar technique showed that the biofungicide effect depends on the content of the bioactives (Figure 4) and, consequently, the crude extract effect on *Neofusicoccum* sp. was mitigated with a higher plant extract concentration (0.07%).

López-López *et al.* (2022a) inoculated post-harvest avocados with *Neofusicoccum* sp. and reported necrotic lesions in 83.3% of the fruit. In addition, these authors pointed out that the confrontation of *Neofusicoccum* sp. and an antagonist during the *in vitro* tests resulted in a more aggressive colonization of the pulp, accelerating ripening and increasing colonization in conspicuous areas. Figure 5 shows that leaf, stem, and fruit extracts recorded the higher inhibition percentages (43.8-49.5%) with a 0.07% concentration. These results can indicate a quantitative association with the high phenol content in the leaf extract and, simultaneously, in the stem extract.

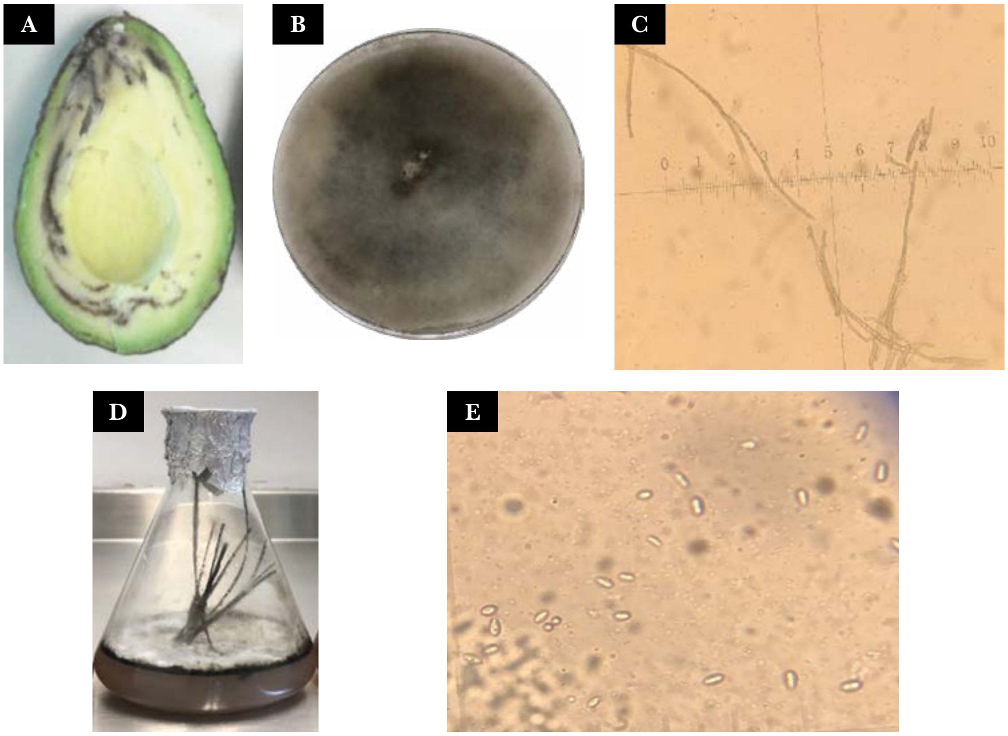

Figure 3. A) Avocado colonized by *Neofusicoccum* sp.; B) *Neofusicoccum* sp. development after 10 growing days in PDA; C) *Neofusicoccum* sp. hyphae at 40 X; D) Active growth of *Neofusicoccum* sp. in potato dextrose broth with pine needles; E) *Neofusicoccum* sp. conidia at 40 X.

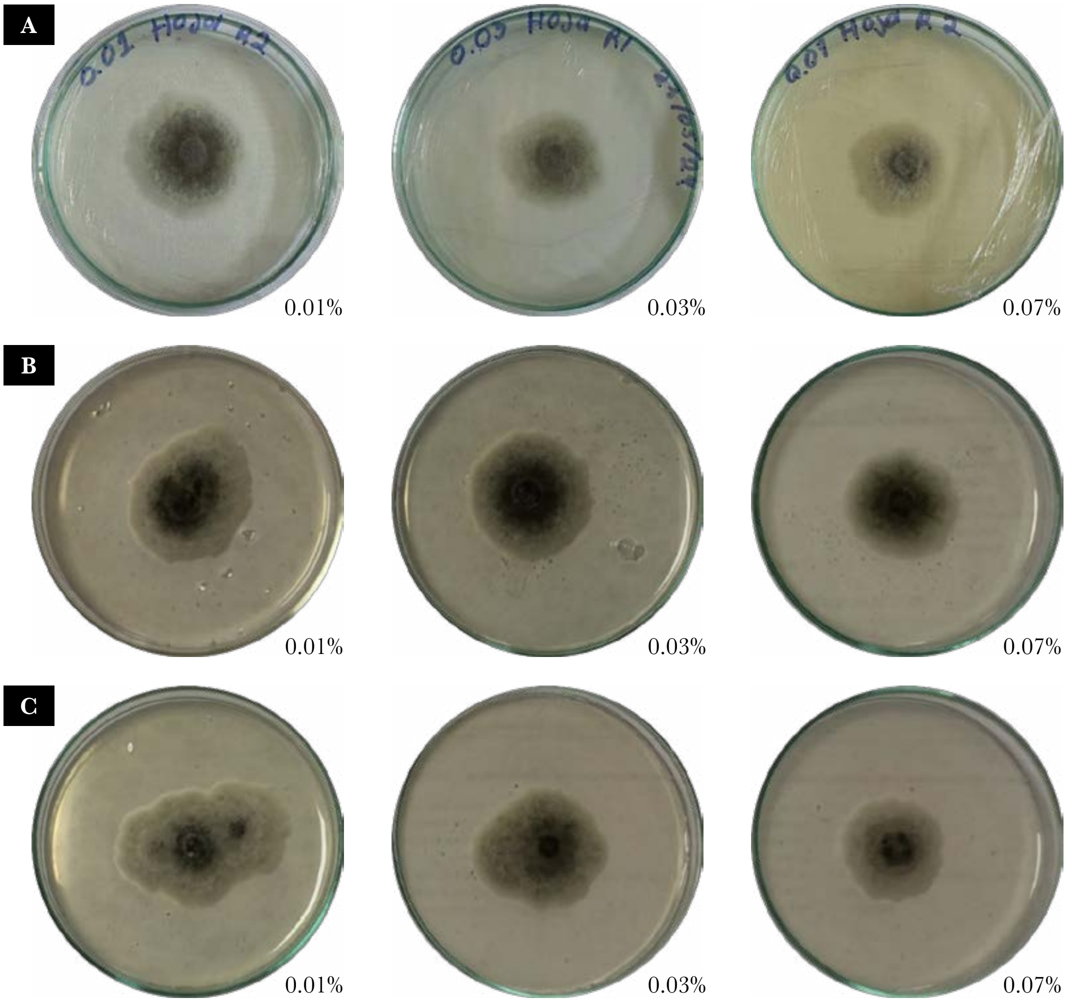

Figure 4. *In vitro* evaluation of the Peruvian pepper tree extract inhibition against *Neofusicoccum* sp., carried out on a poisoned PDA medium, at 0.01, 0.03, and 0.07% concentrations. A: leaves; B: stems; C: fruits.

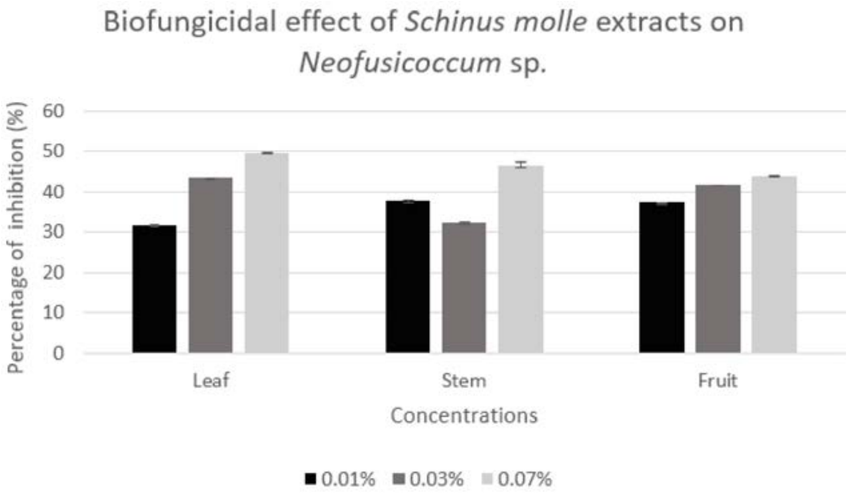

Figure 5. Evaluation of the effect of three concentrations of the bioactive content of *Schinus molle* leaf, stem, and fruit extracts. Different letters show significant differences ($p<0.05$).

Meanwhile, lower concentrations of flavonoids were mostly found in both leaf and stem extracts. Nevertheless, they provide an understanding of the bioactive effects on *Neofusicoccum* sp. The stem extract mainly recorded positive qualitative results, increasing the potential production of an in vitro inhibition boost against the phytopathogen. The inhibition recorded by the three extracts, at a 0.07% concentration, was statistically significant ($p<0.05$). The phytopathogen inhibition has a high potential to control diseases caused by fungi in post-harvest avocados (López-López *et al*., 2022b). In addition, Hernandez *et al*. (2019) pointed out the importance of conducting a biological effectiveness evaluation with plants grown under different conditions. This recommendation will validate their application under field conditions.

Meanwhile, the 0.03% concentration recorded a 43.3, 41.6, and 32.2% inhibition for leaf, fruit, and stem extracts, respectively. Finally, the 0.01% concentration obtained results ranging from 31 to 37% for the three extracts. The inhibition statistical analysis of both the 0.03 and 0.01% concentrations was statistically significant ($p<0.05$).

Aguilar *et al*. (2013) reported that the use of ethanol and oil extracts of Peruvian pepper tree against *C. gloeosporioides* resulted in a 300 mg L^{-1} inhibition. Meanwhile, Ávila-Sosa *et al*. (2011) used a lower concentration of ethanol extracts and obtained 400 mg L^{-1}, showing that Peruvian pepper tree had a higher inhibitory effect with lower concentrations. Despite their low inhibition percentages, all the extracts used in this research undeniably had secondary metabolites with high antimicrobial activity in their chemical composition. The bioactive expression of each extract of Peruvian pepper tree had a changing behavior. Storing time could be taken into account to determine the phytochemical profile, in order to establish its influence on the qualitative and quantitative reduction of the bioactive.

CONCLUSIONS

Traditional methods were used to isolate and identify *Neofusicoccum* sp. in post-harvest avocados. *Schinus molle* produces phytochemical compounds with a 49.5% antifungal inhibition, at a 0.07% concentration, against *Neofusicoccum* sp. based on the phytochemical quantitative profile, the fruit extract recoded the highest phenol content and the leaf extract obtained the highest flavonoid content, while the stem extract recorded the highest quality. The ABTS synthetic radical recorded the highest antioxidant capacity (92.7% inhibition) in the stem extract. *S. molle* can be included in a biorational plan for the phytosanitary protection of crops, as a result of its bioactive content in the leaf extract. The tests conducted using the extracts have proven the existence of biological alternatives to control *Neofusicoccum* sp. under controlled conditions; however, the said extracts should be tested under open field conditions. Understanding their behavior under competition with other microorganisms, as well as the influence of environmental factors, is fundamental.

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