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IN VITRO EFFECTIVENESS OF A COMMERCIAL PINE ESSENTIAL OIL ON FOOD BACTERIA AND PHYTOPATHOGENIC FUNGI

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ABSTRACT

Pathogenic microorganisms in foods are harmful to human health and generate important economic losses. This investigation identified the main compounds in a pine essential oil (PEO), and its antimicrobial effect was evaluated in four food bacteria species and two phytopathogenic fungal species. Twenty major compounds were found in the PEO: α-terpinolene (47.1 %), eucalyptol (22 %), p-menthane (16.7 %), and p-cymene (8.43 %). The greatest antibacterial action was found on *Escherichia coli* and *Staphylococcus aureus*, with an inhibition halo measuring 2.3 and 2 cm, respectively, at a minimum inhibiting concentration of 90 %. Using the inverted box or well technique, the PEO had an effect on *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* at all concentrations evaluated, with inhibition values ranging from 40 to 60 %. For *R. stolonifer*, the 45 % PEO concentration inhibited germination completely.

Keywords: *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* spp., *Colletotrichum gloeosporioides*, *Rhizopus stolonifer*.

INTRODUCTION

Diseases transmitted to humans through the consumption of fresh fruits and vegetables due to the presence of pathogenic bacteria and the deterioration caused by fungi in pre- and post-harvest are becoming an increasingly important issue in agricultural product conservation on a national and international scale. Consumption of agricultural products contaminated with bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella* spp. causes severe economic losses, including compensation to consumers for bacterial toxicity and total loss of the product (Cortés-Higareda et al., 2021), and in the international market, the closure of borders to the trade of these products. Occasionally, the consumption of contaminated agricultural products with different serovars of *Salmonella* spp. causes severe symptoms in human beings, which can lead to the death of the consumer.

Phytopathogenic fungi of diverse genera and species cause losses of up to 40 % of the total harvest (Ramos-García *et al*., 2010). In the particular case of *Colletotrichum gloeosporioides* and *Rhizopus stolonifer*, their development causes severe rotting in a large diversity of agricultural products such as avocado (*Persea americana* Mill.), mango (*Mangifera indica* L.), soursop (*Annona muricata* L.), berries (*Rubus* spp., *Vaccinium* spp.), tomato (*Solanum lycopersicum* L.), and others (Bautista-Baños *et al*., 2014; Quintero-Mercado *et al*., 2019; Herrera-González *et al*., 2020).

The main and most common option to reduce the presence of these microorganisms is the use of synthetic compounds (sanitizers and fungicides). However, its use is currently questioned due to environmental contamination, harm to human health, and the generation of microbial resistance. Due to this, other alternatives have been evaluated, such as the use of essential oils from plants (Ramos-García *et al*., 2010; Bautista-Baños *et al*., 2020).

Pine (*Pinus* spp., Pinaceae) essential oils extracted from seeds, leaves, and flowers have made a great contribution to the pharmaceutical, cosmetics, and construction industries. Krauze-Baranowska *et al*. (2002) documented that the general composition of essential oils across the *Pinus* genus varies according to the species, although its composition lies within the group of monoterpenes (α-β pinene, germacrene, myrcene) and oxygenated monoterpenes (borneol, bornyl acetate, Δ-3-carene).

Regarding its bactericidal and fungicidal effect, there are *in vitro* investigations that verify this activity, mainly using *Pinus sylvestris* L. The essential oil, extracted from the needles of this species, presented bactericidal activity against *Bacillus cereus*, *Micrococcus luteus,* and *Alcaligenes faecalis*, although no fungicidal effect was observed (Chao *et al*., 2000). Fungicidal activity was displayed in *P. sylvestris* against *Aspergillus niger*, *Penicillium funiculosum,* and *Trichoderma viride*. The mycelia of *Penicillium funiculosum* were controlled by *P. sylvestris* extracts (Motiejūnaite and Peciulyte, 2004). Volatiles and extracts of the essential oil from *P. sylvestris* displayed a notable control in the germination of *Mucor* spp., *R. stolonifera*, and *Fusarium oxysporum* spores (Tullio *et al*., 2007). Control has also been reported on *Candida albicans* with the application of this essential oil (Salamon *et al*., 2019). The commercial product Resin Adher CE (MS Agros S.A. de C.V., Mexico City, Mexico), made with *P. sylvestris* essential oil, affects the germination of *Aspergillus flavus* (Segura-Palacios *et al*., 2021).

Based on the above, the goals of this investigation were to know the chemical composition of the commercial essential oil from *P. sylvestris* and evaluate its *in vitro* effect on four food bacteria and four phytopathogenic fungi of agricultural importance.

MATERIALS AND METHODS

Chemical profile of the *Pinus sylvestris* essential oil

The pine essential oil (PEO) was obtained from Xipe Naturals (Mexico City, Mexico). To determine its composition, the methodology by Black-Solís *et al*. (2017) was followed. A gas chromatograph-mass spectrometry (GS-MS SCION 456-GC, Bruker Daltonics Inc., Billerica, MA, USA) was used with the headspace technique. This system was operated using the software MS Workstation version 8.2 (Bruker Daltonics Inc., Billerica, MA, USA). The GC-MS detector was operated on exploration mode, between 50 and 500 mass/load. The GC oven was equipped with a BR-1MS capillary column with a film thickness of 0.25 mm, 30 m in length, and an internal diameter of 0.25 mm. The analysis was performed by direct injection, diluting the oil in chloroform, with a time of analysis of 29 min per sample, using helium (1 mL min^{-1}) as a carrier gas, at 220 °C for the injector and 250 °C for the transfer line. In 1 mL of chloroform, 5 μL of the sample were dissolved, and 1 μL of this solution was injected. The initial oven temperature was 55 °C and maintained for 1 min. Subsequently, it was raised to 155 °C at a heating speed of 5 °C min^{-1} and maintained for 2 min. Finally, a temperature of 255 °C was reached at a heating speed of 20 °C min^{-1} and maintained for 1 min.

Fungi and bacteria acquisition

Two Gram-positive strains (*Staphylococcus aureus* and *Listeria monocytogenes*) and two Gram-negative strains (*Salmonella typhi* and *Escherichia coli*) were used as trial bacteria, which were obtained from the Professional Interdisciplinary Biotechnology Unit of the National Polytechnic Institute (UPIBI-IPN, Mexico). Phytopathogenic fungi *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* were obtained from the strain bank of the Postharvest Technology Laboratory of the Biotic Products Development Center (CEPROBI-IPN, Mexico).

The bacteria were activated in agar soybean tripcasein (AST) nutrient medium at 37 °C for 24 h. A bacterial concentration of $1–2 \times 10^8$ colony-forming units (CFU) per milliliter was determined in a spectrophotometer (GENESYS 10S UV-Vis, Thermo Scientific, USA) at an absorbance of 0.08–0.098 with a wavelength of 650 nm. The activation of fungi was carried out in potato dextrose agar (PDA) nutrient medium at 25 °C for 10 and 4 days for *C. gloeosporioides* and *R. stolonifer*, respectively.

Minimum inhibiting concentration (MIC)

The MIC of the PEO in the bacteria was determined with the agar diffusion technique as indicated by Lara-Cortés *et al*. (2016). For this purpose, 10 mL of 0.8 % AST previously inoculated with 20 μL of the bacteria studied and 5 mL of semisolid 1 % AST were emptied into Petri dishes (60 × 15 mm in diameter) and left to dry for 1 h. Using a hole puncher, 10 mm-diameter holes were made. Subsequently, 20 μL of the PEO were placed serially in each hole, in concentrations of 90, 45, 22.5, 11.2, and 5.6 %. The boxes were sealed and incubated for 48 h at 37 °C. After this time, the inhibition halo was measured (clearer areas around the hole). The evaluation was carried out in six Petri dishes (repetitions) per concentration (treatment).

Growth and germination kinetics of the pathogenic fungi

To evaluate these variables, the reverse box and the well techniques were used. For the former, the methodology by Ramos-García *et al*. (2012) was followed. Sterile filter paper (Wattman No. 1), 15 mm in diameter, was placed on the lid of the Petri dish (100 × 15 mm in diameter), previously treated with 20 µL for each treatment with the different concentrations of the PEO (100, 90, 45, 22.5, 11.2, and 5.6 %). On the other hand, a 10 mm-diameter disk with fungus was placed in the center of the Petri dish. The dishes were sealed and incubated at 25 ± 2 °C and kept in an inverted position. Six repetitions were carried out per treatment.

For the well technique, the methodology by Moreno-Limón *et al*. (2012) was followed. To this end, every Petri dish (60 × 15 mm) with PDA culture medium under sterile conditions had 0.5 mL of spore suspension (10^5 spores mL^{-1}), which was spread throughout the Petri dish using a sterile metal spreader. The fungal solution was left to dry, and later, using a 10 mm-diameter borer, a well was made in the center of the dish, where 20 µL of each concentration was added (100, 90, 45, 22.5, 11.2, and 5.6 % of PEO). The dishes were sealed and incubated at 25 ± 2 °C. Six repetitions were carried out per treatment.

Mycelial growth

For the reverse box technique, culture diameter measurements were taken every day for 13 days for *C. gloeosporioides* and for 7 days for *R. stolonifer* (in centimeters) using two perpendicular directions traced in the center of the box as a reference point. The average value of the repetitions per treatment was taken.

Germination of spores

The germination of spores was determined in both techniques, according to the method described by Black-Solis *et al*. (2017). To this end, two Petri dishes with mycelial growth of the fungus from each treatment were used. Twenty milliliters of sterile distilled water were added, and the mycelium was scraped with a sterile bacteriological loop. The spore suspension was filtered and adjusted to 10^5 spores mL^{-1}. Subsequently, three disks (repetitions) of PDA culture medium with a diameter of 15 mm were placed along a slide inside a Petri dish. Twenty milliliters of the spore solution (10^5) were placed on each disk.

The Petri dishes were sealed and incubated for 2, 4, 6, 8, and 10 h for both fungal species. After incubation, one drop of lactophenol blue was added to each disk, and 100 conidia were evaluated per disk under an optic microscope at 40x (Nikon Alphaphot-2 YS2-H, Japan). The percentage of germination ($G_{(\%)}$) was determined using the following equation:

$$G_{(\%)} = \frac{E_C}{T_E}\ x\ 100$$

where E_G represents the number of germinated conidia and T_E the total of conidia. A conidium was considered germinated when a germinative tube was observed, regardless of its length. Three repetitions were carried out per treatment.

Statistical analysis

For the variables of mycelial growth and spore germination, a totally randomized design was used. The difference in the antifungal activity was established using an analysis of variance (ANOVA) and Tukey's test ($p \leq 0.05$). The data in percentages were transformed with the arcsine function. The InfoStat statistical package, version 2020 (di Rienzo *et al*., 2020), was used.

RESULTS AND DISCUSSION

Chemical profile of the essential oil

In regard to the composition of the *P. sylvestris* essential oil, 20 major compounds were obtained (Table 1). The main components identified were α-terpinolene (47.1 %),

Table 1. Chemical composition of the pine (*Pinus sylvestris* L.) essential oil used (Xipe Naturals, Mexico City, Mexico).

No.	Compound	Retention time	Total percentage
1	4-octene, 2,6-dimethyl-, [S-(E)]	5.90	0.358
2	α-tricyclene	6.09	0.421
3	camphene	6.63	6.339
4	β-myrcene	7.61	0.629
5	Terpinolene	7.86	1.618
6	α-phellandrene	7.93	0.282
7	p-mentane	8.14	16.722
8	p-cymene	8.33	8.431
9	Eucalyptol	8.53	22.022
10	Terpinene	9.30	3.279
11	α-terpinolene	10.10	47.195
12	Fenchol	10.60	1.347
13	Isoborneol	11.73	0.770
14	Iso-β-terpineol	11.85	0.252
15	Endo-borneol	11.96	0.966
16	Terpinen-4-ol	12.30	6.213
17	Terpineol	12.60	3.564
18	Isoterpinolene	12.79	1.071
19	Diphenyl ether	17.78	0.415
20	Longifolene	18.55	0.687

eucalyptol (22 %), p-menthane (16.7 %), and p-cymene (8.43 %) within a retention time of 8 to 10 min. These values mostly coincide with the chemical identification reported by Mitić *et al*. (2018) and Ibáñez and Blázquez (2019), who identified between 30 and 41 major compounds in fresh *P. sylvestris* needles within the group of oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes. The differences in the content of compounds can be explained mainly by the differences in the geographic locations of the tree (Maciag *et al*., 2007).

Antibacterial activity

In general terms, the greatest antibacterial action corresponded to the highest concentration of 90 % (Table 2). On the other hand, *P. sylvestris* exerted the highest inhibiting effect on *E. coli* and *S. aureus*. Regarding *L. monocytogenes*, only the 90 and 45 % concentrations inhibited it, and no effect was observed on *S. typhy* (Table 2).

Table 2. Effect of the minimum inhibiting concentration of the pine (*Pinus sylvestris* L.) essential oil on the growth of bacteria in foods.

	Minimum inhibiting concentration (%)				
Bacteria	90	45	22.5	11.2	5.6
	Inhibition halo (cm)†				
Escherichia coli	2.3 ± 0.23 b	2.1 ± 0.2 b	2.0 ± 0.1 b	2.0 ± 0.1 b	1.7 ± 0.3 b
Listeria monocytogenes	1.8 ± 0.29 b	1.6 ± 0.2 b	0 ± 0 a	0 ± 0 a	0 ± 0 a
Salmonella typhy	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a
Staphylococcus aureus	2.0 ± 0.12 b	1.9 ± 0.1 b	1.8 ± 0.1 b	1.6 ± 0.2 b	1.5 ± 0.2 b

†Means (± = mean standard error) with different letters in the same column are significantly different ($p < 0.05$).

Unlike other investigations, the inhibition area due to the effect of the *P. sylvestris* oil was not as large. Czerwińska and Szparaga (2015) found that applying this oil using the diffusion method (1 μg mL^{-1}) gave the largest inhibition halo in *S. aureus* (12 mm), followed by *L. monocytogenes* (10 mm) and *E. coli* (8.2 mm). In that study, other bacteria sensitive to the extract were *Micrococcus leuteus* (12 mm) and *Bacillus subtilis* (8.2 mm). Likewise, Oyewole *et al.* (2021) confirmed the bactericidal effect of the *P. sylvestris* needles using the same technique. In this case, the area of inhibition measured approximately 20 mm, whereas the CMI starting at 0.39 mg mL^{-1} significantly inhibited *E. coli* and *S. aureus*, with these two bacteria as the most affected by PEO.

Canillac and Mourey (2001) mentioned that among the factors associated with the bactericidal effect are the main components of the oil, the concentration used, and the bacteria under study. Likewise, Kim *et al.* (2013) pointed out that the antimicrobial activity against three Gram-positive bacteria (*B. subtilis*, *B. natto,* and *S. aureus*) and four Gram-negative ones (*E. coli*, *Salmonella enteritidis*, *S. typhosa,* and *Pseudomonas aeruginosa*) may be due to the changes in chemical components, with monoterpenes and sesquiterpenes the major compounds in the essential oil of the *Pinus* genus. Zhang *et al*. (2023) documented the growing interest in natural products with antioxidant and antimicrobial effects and their mechanisms of action with extracts of *Pinus densiflora* needles as a natural and efficient antimicrobial agent, aimed at the food industry with safer, fresher, and healthier components, which generates a large demand for natural and safe conservation technologies.

Regarding the fungicidal effect, the dynamics of the mycelial growth in the different *C. gloeosporioides* treatments during the 13-day incubation period (Figure 1A) and *R.*

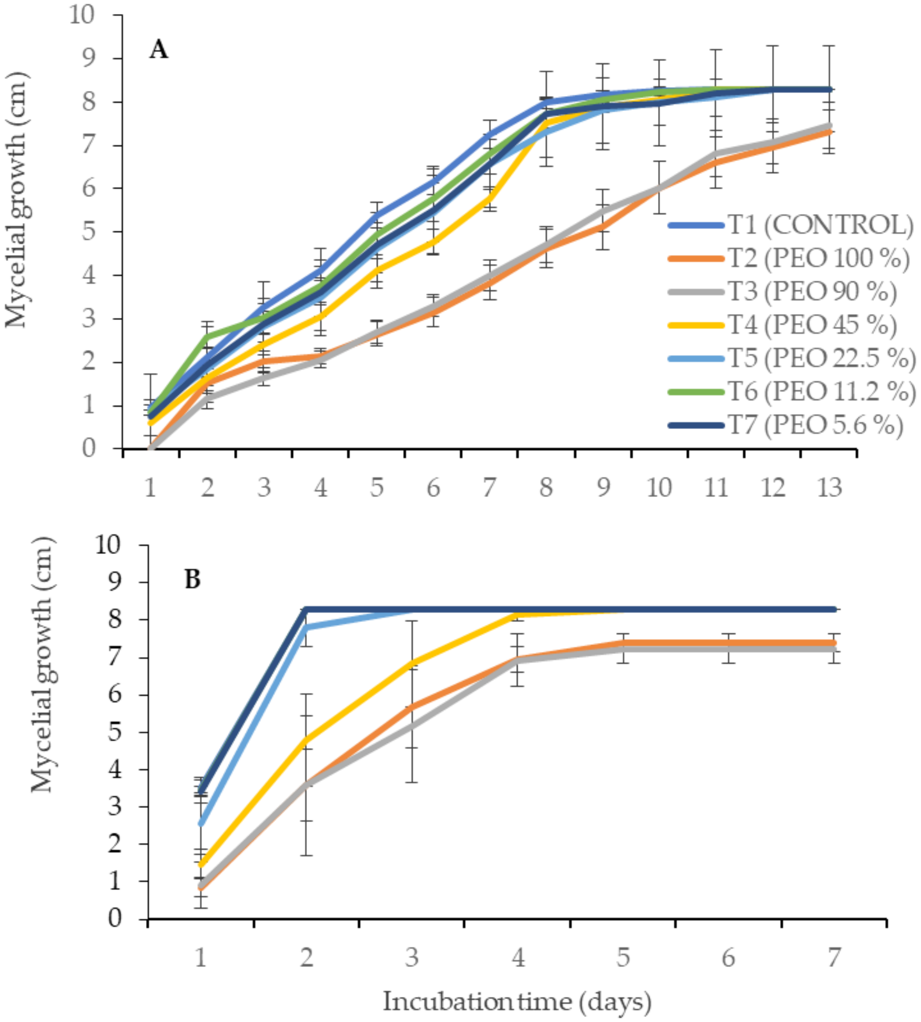

Figure 1. Mycelial growth kinetics of the fungi evaluated in the presence of different concentrations of pine (*Pinus sylvestris* L.) essential oil. A: *Colletotrichum gloeosporioides* in a 13-day incubation period; B: *Rhizopus stolonifer* in a 7-day incubation period. Vertical bars indicate the mean standard error.

stolonifer during the 7-day incubation period (Figure 1B) showed that *C. gloeosporioides* growth, in all treatments, increased gradually along the days of incubation, whereas in *R. stolonifer*, only in concentrations of 45, 90, and 100 % was gradual growth observed. In both fungi, the greatest fungicidal effect was with the application of PEO at concentrations of 90 and 100 %, with values at the end of incubation of around 6 to 7 cm.

Regarding germination, in the reverse box (Figure 2) and well (Figure 3) techniques, a rate increase was observed with incubation time. In both techniques, there was a

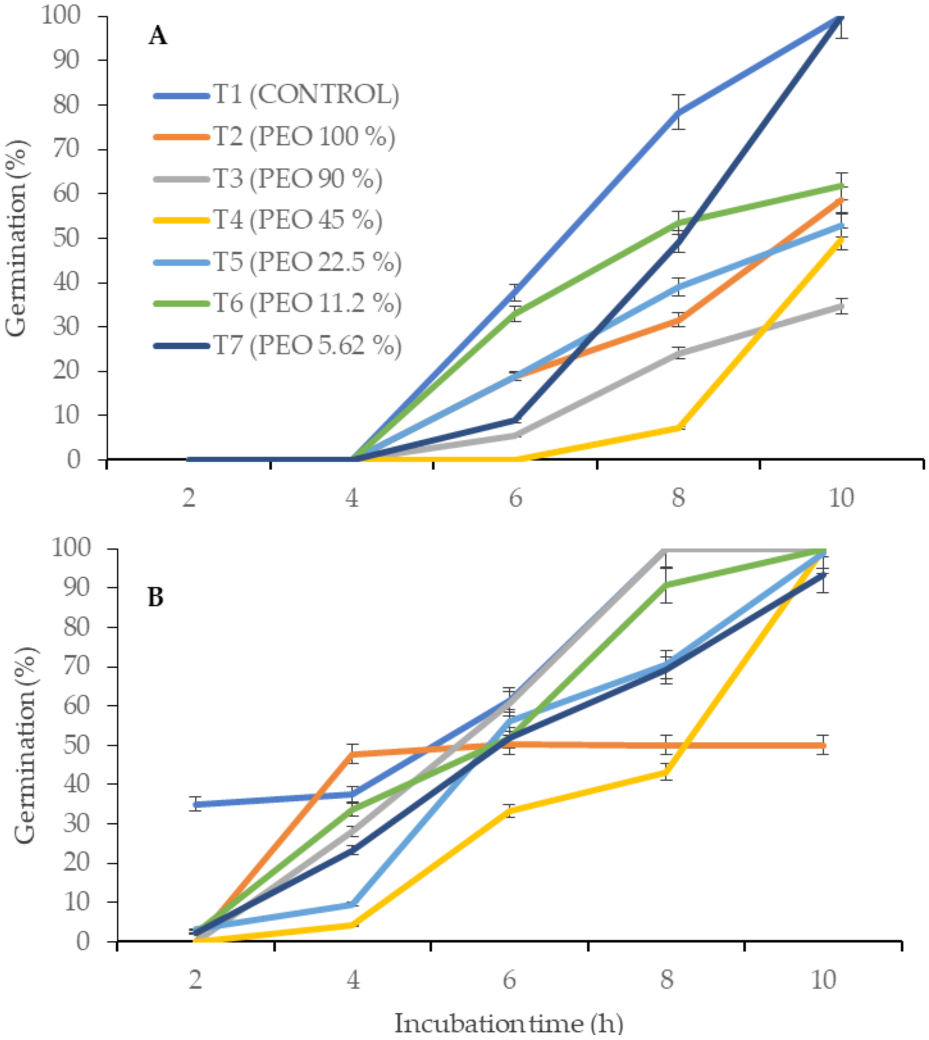

Figure 2. Germination kinetics of the fungi evaluated in the presence of different concentrations of pine (*Pinus sylvestris* L.) essential oil using the reverse box method during a 10-hour incubation period. A: *Colletotrichum gloeosporioides;* B: *Rhizopus stolonifer*. Vertical bars indicate the mean standard error. The data in percentages was transformed with the arcsine function. Kruskal-Wallis test A: (H = 0, 0, 36.79, 34.94, 32.56; p = 0.0001); B: (H = 32.63, 14.04, 9.91, 19.77, 6.94; p = 0.0001, 0.0290, 0.1282, 0.0026, 0.0526).

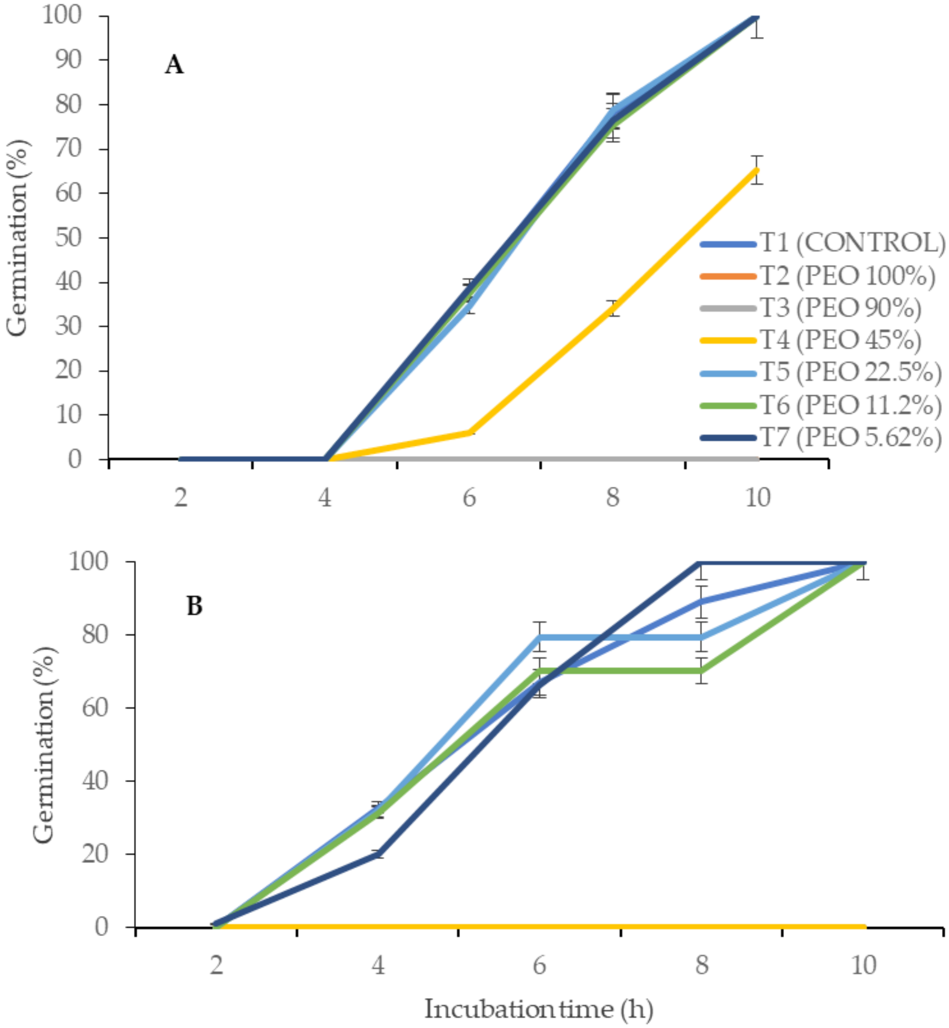

Figure 3. Germination kinetics of the fungi evaluated during a 10-hour incubation period in the presence of pine (*Pinus sylvestris* L.) essential oil using the well technique. A: *Colletotrichum gloeosporioides;* B: *Rhizopus stolonifer*. Vertical bars indicate the mean standard error. The data in percentages were transformed with the arcsine function. Kruskal-Wallis test A: (H = 0, 0, 32.38, 32.78, 32.29; *p* = 0.0001); B: (H = 10.47, 34.28, 32.03, 35.40, 30.14; *p* = 0.0001).

greater fungicidal effect of the PEO on *C. gloeosporioides*, since in both most of the concentrations applied, its germination was inhibited. The PEO concentrations from 11.2 % onwards inhibited germination between 40 and 60 %, whereas in *R. stolonifer*, only the concentrations of 45 and 100 % had an inhibiting effect. However, the inhibition with the 45 % concentration was total (Figure 3B).

In general, spores were more susceptible than the mycelia. In regard to the application technique, the PEO volatiles had a stronger inhibiting effect for both fungal species in comparison with the direct application on the medium. Coinciding with these results, Tullio *et al*. (2006) documented a much lower inhibiting CMI in the development of *Fusarium oxysporum* (0.03 %), *Rhizopus* spp. (0.05 %), and *Cladosporium cladosporioides* (0.2 %) after 3 and 7 days of incubation when applying volatiles of the *P. sylvestris* essential oil, in comparison with the integration of the essential oil in the medium, since, in this case, the concentration needed to trigger inhibition was greater than 1 %. Other *Pinus* species with a notorious fungicidal activity on phytopathogenic fungi of the *Fusarium* genus were the essential oils from the needles of *P. ponderosa*, *P. resinosa*, and *P. strobus*, in concentrations of 2 and 5 % (Krauze-Baranowska *et al*., 2002). Likewise, Motiejūnaite and Peciulyte (2004) verified, in various species of the genus *Aspergillus*, the effectiveness of *P. sylvestris* with a concentration of 2.5 % when applied in the nutrient medium (1–1.5 % v/v). Additionally, in later studies, Amri *et al.* (2013) pointed out percentages of inhibition of more than 50 % in *Giberella avenaceae* and *Rhizoctonia solani* with the concentration of *P. sylvestris* of 4 μL mL^{-1}. Coinciding with Oyewole *et al.* (2021), this study also identified the following chemicals in the PEO under study with antimicrobial activity: terpineol, isoborneol, and fenchol.

CONCLUSIONS

According to the goals presented, 20 major compounds were identified in the commercial pine essential oil, all belonging to the terpene chemical group. Regarding the antimicrobial activity of the essential oil, the most affected bacteria were *Escherichia coli* and *Staphylococcus aureus* starting at a concentration of 90 %. For the fungi *Colletotrichum gloeosporioides* and *Rhizopus stolonifer*, spore germination was the most affected developmental stage during the incubation period. In *C. gloeosporioides*, inhibition occurred at all concentrations, whereas in *R. stolonifer*, it was only observed at a concentration of 45 %.

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REFERENCES

Amri I, Hanana M, Gargouri S, Jamoussi B, Hamrouni L. 2013. Comparative study of two coniferous species (*Pinus pinaster* Aiton and *Cupressus sempervirens* L. var. *dupreziana* [A. Camus] Silba) essential oils: Chemical composition and biological activity. Chilean Journal of Agricultural Research 73 (3): 259–266. https://doi.org/10.4067/s0718-58392013000300008

Bautista-Baños S, Bosquez-Molina E, Barrera-Necha LL. 2014. *Rhizopus stolonifer* (soft rot). *In* Bautista-Baños S. (ed.), Postharvest Decay Control Strategies. Academic Press: San Diego, CA, USA, pp: 1–44. https://doi.org/10.1016/B978-0-12-411552-1.00001-6

Bautista-Baños S, Correa-Pacheco ZN, Black-Solis JD. 2020. Current status of the effectiveness of essential oils for controlling phytopathogenic fungi, a review. Acta Agrícola y Pecuaria 1 (6): E0061008. https://doi.org/10.30973/aap/2020.6.0061008

Black-Solis J, Ventura-Aguilar RI, Barrera-Necha LL, Bautista-Baños S. 2017. Chemical characterization, compositional variability, and mathematical modelling of the effect of essential oils in *Alternaria alternata*. Revista Mexicana de Fitopatología 35 (2): 204–226. https://doi.org/10.18781/r.mex.fit.1612-5

Canillac N, Mourey A. 2001. Antibacterial activity of the essential oil of *Picea excelsa* on *Listeria*, *Staphylococcus aureus* and coliform bacteria. Food Microbiology 18 (3): 261–268. https://doi.org/10.1006/fmic.2000.0397

Chao SC, Young DG, Oberg CJ. 2000. Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. Journal of Essential Oil Research 12 (5): 639–649. https://doi.org/10.1080/10412905.2000.9712177

Cortés-Higareda M, Bautista-Baños S, Ventura-Aguilar RI, Landa-Salgado P, Hernández-López M. 2021. Bacterias patógenas de los alimentos agrícolas frescos y mínimamente procesados. Estado actual en el control del género *Salmonella*. Revista Iberoamericana de Tecnología Postcosecha 22 (1): 12–28.

Czerwińska E, Szparaga A. 2015. Antibacterial and antifungal activity of plant extracts. Rocznik Ochrona Środowiska 17 (1): 209–229.

di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW. InfoStat versión 2020. Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.

Herrera-González JA, Bautista-Baños S, Salazar-García S, Gutiérrez-Martínez P. 2020. Current situation of postharvest handling and fungal diseases of avocado 'Hass' for export in Michoacán, Mexico. Revista Mexicana de Ciencias Agrícolas 11 (7): 1647–1660. https://doi.org/10.29312/remexca.v11i7.2402

Ibáñez MD, Blázquez MA. 2019. Phytotoxic effects of commercial *Eucalyptus citriodora*, *Lavandula angustifolia*, and *Pinus sylvestris* essential oils on weeds, crops, and invasive species. Molecules 24 (15): 2847. https://doi.org/10.3390/molecules24152847

Kim H, Lee B, Yun KW. 2013. Comparison of chemical composition and antimicrobial activity of essential oils from three *Pinus* species. Industrial Crop and Research 44: 323–329. https://doi.org/10.1016/j.indcrop.2012.10.026

Krauze-Baranowska M, Mardarowicz M, Wiwart M, Pobłocka L, Dynowska M. 2002. Antifungal activity of the essential oils from some species of the genus *Pinus*. Zeitschrift für Naturforschung C 57 (5–6): 478–82. https://doi.org/10.1515/znc-2002-5-613

Lara-Cortés E, Troncoso-Rojas R, Hernández-López M, Bautista-Baños S. 2016. Evaluation of the antimicrobial activity of cinnamaldehyde in the preservation of edible dahlia flowers, under different storage conditions. Revista Chapingo Serie Horticultura 22 (3): 177–189. https://doi.org/10.5154/r.rchsh.2016.02.002

Maciag A, Milakovi D, Christensen HH, Antolovi V, Kalemba D. 2007. Essential oil composition and plant-insect relations in Scots pine (*Pinus sylvestris* L.). Food Chemistry and Biotechnology 71: 71–94.

Mitić ZS, Jovanović B, Jovanović SČ, Mihajilov-Krstev T, Stojanović-Radić ZZ, Cvetković VJ, Mitrović TLj, Marin PD, Zlatković BK, Stojanović GS. 2018. Comparative study of the essential oils of four *Pinus* species: Chemical composition, antimicrobial and insect larvicidal activity. Industrial Crops and Production 111: 55–62. https://doi.org/10.1016/j.indcrop.2017.10.004

Moreno-Limón S, Salcedo-Martínez SM, Cárdenas-Ávila ML, Hernández-Piñero JL, Núñez-González MA. 2012. Efecto antifúngico de capsaicina y extractos de chile piquín (*Capsicum annuum* L. var *aviculare*) sobre el crecimiento *in vitro* de *Aspergillus flavus*. Polibotánica 34: 191–204.

Motiejūnaite O, Peciulyte D. 2004. Fungicidal properties of *Pinus sylvestris* L. for improvement of air quality. Medicina (Kaunas) 40 (8): 787–794.

Oyewole KA, Oyedara OO, Awojide SH, Olawade MO, Adetunji CO. 2021. Chemical constituents and antibacterial activity of essential oils of needles of *Pinus sylvestris* (scots pine) from South West Nigeria. Research Square 1: 1–15. https://doi.org/10.21203/rs.3.rs-635195/v1

Quintero-Mercado A, Dangon-Bernier F, Páez-Redondo A. 2019. Aislamientos endofíticos de *Colletotrichum* spp. a partir de hojas y ramas de mango (*Mangifera indica* L.) cultivar Azúcar en el municipio de Ciénaga, Magdalena, Colombia. Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales 43 (166): 65–77. https://doi.org/10.18257/raccefyn.788

Ramos-García ML, Bautista-Baños S, Barrera-Necha LL, Bosquez-Molina E, Alia-Tejacal I, Estrada-Carrillo M. 2010. Compuestos antimicrobianos adicionados en recubrimientos comestibles para uso en productos hortofrutícolas. Revista Mexicana de Fitopatología 28 (1): 44–57.

Ramos-García ML, Hernández-López M, Barrera-Necha LL, Bautista-Baños S, Troncoso-Rojas R, Bosquez-Molina E. 2012. *In vitro* response of *Fusarium oxysporum* isolates to isothiocyanates application. Revista Mexicana de Fitopatología 30 (1): 1–10.

Salamon I, Kryvtsova M, Bucko D, Tarawneh AH. 2019. Chemical characterization and antimicrobial activity of some essential oils after their industrial large-scale distillation. Journal of Microbiology Biotechnology and Food Sciences 8 (4): 984–988. https://doi.org/10.15414/jmbfs.2019.8.4.984-988

Segura-Palacios MA, Correa-Pacheco ZN, Corona-Rangel ML, Martinez-Ramirez OC, Salazar-Piña DA, Ramos-García ML, Bautista-Baños S. 2021. Use of natural products on the control of *Aspergillus flavus* and production of aflatoxins *in vitro* and on tomato fruit. Plants 10 (12): 2553. https://doi.org/10.3390/plants10122553

Tullio V, Nostro A, Mandras N, Duo P, Banche G, Cannatelli MA, Cuffini AM, Alonzo V, Carlone NA. 2007. Antifungal activity of essential oils against filamentous fungi determined by broth microdilution and vapour contact methods. Journal of Applied Microbiology 102 (6): 1544–1550. https://doi.org/10.1111/j.1365-2672.2006.03191.x

Zhang Y, Chung WK, Moon SH, Lee JG, Om AS. 2023. Comparison of antibacterial activities of Korean pine (*Pinus densiflora*) needle steam distillation extract on *Escherichia coli* and S*taphylococcus aureus* focusing on membrane fluidity and genes involved in membrane lipids and stress. Molecules 29 (1): 165. https://doi.org/10.3390/molecules29010165