PINEWOOD PROTECTION AGAINST SAPSTAIN USING CITRUS ESSENTIAL OILS

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[1] Received on 08.10.2021 accepted for publication on 09.03.2022.
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ABSTRACT – Natural color influences the decision to acquire a wood product. Sapstain is a fungal deterioration of pinewood that affects its aesthetic attributes and generally causes its rejection. The aim was to control the sapstain on pinewood with citrus essential oils. Causal agents of sapstain on pinewood were identified by molecular methods to genus level as *Alternaria* sp., *Hypocrea* sp., *Trichoderma* sp., and *Geosmithia* sp. Citrus essential oils were tested to control the fungal growth. For the treated pinewood probes, the adsorption and retention of citrus essential oils, fungal adhesion, and wood satin as fungal growth indicators were determined. The composition of the essential oils was determined by gas chromatography. Fungi were sensible to essential oils rich in D-limonene, γ-terpinene, α-terpineol, geraniol, eugenol, or β-bisabolene. Absorption and retention data suggest that 12.37±1.62 kg/m^3 is the minimal amount to inhibit the fungal adhesion and growth on pinewood. Citrus essential oils control sapstain in pinewood, but poor retention should be improved.

Keywords: Fungi; Control; Sensitivity.

PROTEÇÃO DA MADEIRA DE PINUS CONTRA MANCHAS USANDO ÓLEOS ESSENCIAIS CÍTRICOS

*RESUMO – A cor natural influencia a decisão de compra de um produto de madeira. Mancha é uma decomposição fúngica da madeira de Pinus que afeta seus atributos estéticos e geralmente causa sua rejeição. O objetivo foi controlar mancha em madeira de Pinus com óleos essenciais cítricos. Agentes causadores de mancha em madeira de Pinus foram identificados por métodos moleculares em nível de gênero como **Alternaria** sp., **Hypocrea** sp., **Trichoderma** sp. e **Geosmithia** sp. Os óleos essenciais cítricos foram testados para controlar o crescimento de fungos. Para as sondas de madeira de Pinus tratada, foram determinados a adsorção e retenção de óleos essenciais cítricos, adesão fúngica e coloração da madeira como indicadores de crescimento fúngico. A composição dos óleos essenciais foi determinada por cromatografia gasosa. Os fungos foram sensíveis aos óleos essenciais ricos em D-limoneno, γ-terpineno, α-terpineol, geraniol, eugenol ou β-bisaboleno. Os dados de absorção e retenção sugerem que 12,37±1,62 kg/m³ é a quantidade mínima para inibir a adesão fúngica e o crescimento em madeira de **Pinus**. Os óleos essenciais cítricos controlam manchas da madeira de **Pinus**, mas a retenção deficiente deve ser melhorada.*

Palavras-Chave: Fungos; Controle; Sensibilidade.

1.INTRODUCTION

More than 6,400 active ingredients are registered as pesticides. In formulation with "inert" compounds, result in more than 100,000 agrochemical products (Kegley et al., 2016). In 2013, 1.29 million ton of pesticides were applied to generate a global agricultural production of 23.34 billion ton (García Hernández et al., 2018). These pesticides support the extraction of wood from the forest and its derivatives. Since the world production and trade of forest products in 2020 was 3,912 million m^3 of roundwood, and about 1,984 million m^3 were industrialized. The difference in millions of m^3 of roundwood was put to other uses (FAO, 2021).

The durability of wood is compromised by biological agents such as xylophagous insects and fungi. Treating wood with conventional chemicals is intended to increase its durability. Hoping it subsides of timber extraction decreasing and prevent the forest from shrinking, loss of biodiversity and environmental pollution by chemicals (Folke et al., 2021; Steffen et al., 2015). On the other hand, toxic effect on biomes occurs when preserved wood is disposed or recycled either as wood or as cellulose fiber or when used as fuel (Hernández-Berriel et al., 2019). An alternative to replace the conventional preservatives of wood are plant products (Broda, 2020). Some common components of pure plant essential oils in *in vitro* tests showed antifungal effect, e.g. α-asarone (Park et al., 2020).

An aesthetic defect of wood that causes rejection in the consumer is the sapstain (Sedliačiková et al., 2021). It is caused by heterogeneous fungal consortia in which members differ from each other due to the site of wood extraction, the life history of the tree, and the tree species (Huang et al., 2021). Wood stain is caused by wood mold fungi (e.g. *Aspergillus* sp., *Penicillium* sp. and *Trichoderma* sp.) and stain fungi (e.g. *Alternaria* sp., *Botrydiplodia* sp. and *Fusarium* sp.).

Non-toxic plant antifungals are an alternative for the control of sapstain fungi. Among them, essential oils have been successfully proven to preserve wood against biodeterioration caused by termites and decay fungi (Raya González et al., 2013; Ramírez López et al., 2021). A source of these plant compound are essential oils (Akarca and Sevik, 2021). Plant essential oils for the prevention of biodeterioration of wood, such as neem oil and flaxseed, are now successfully marketed. Also, some pure components of plant essential oils in *in vitro* tests showed antifungal effect, e.g. terpineol (Ye et al., 2021). They are applied to wood via different methods, where the most successful was impregnating the wood by immersion or by heat and pressure (Ahmed et al., 2020; Hu et al., 2021).

The citrus industry is of global economic importance because citrus is among the largest and most cosmopolitan tree crops in the world. In the *Citrus* genus, the species of greatest economic importance are: *Citrus sinensis* L. (sweet orange), *C. aurantium* L. (sour orange), *C. limon* (L.) Burm. f. (lemon), *C. medica* L. (citron), *C. paradisi* Macfad. (grapefruit), *C. aurantifolia* (Christm.) Swingle (lime), *C. reticulata* Blanco (mandarin orange), and *C. grandis* (L.) Osbeck (pomelo, shaddock) (Valencia Sandoval and Duana Avila, 2019).

Citrus essential oil is a complex mixture of secondary metabolites and their content as well as composition depends on factors, such as: species, variety and cultivar, cultivation, extraction and separation methods. Its volatile constituents are a mixture of monoterpene (such as limonene) and sesquiterpene hydrocarbons and their oxygenated derivatives, including aldehydes, ketones, acids, alcohols and esters (Clery et al., 2022). On this frame, of interest are plant essential oils from citrus. Because it is an opportunity to link local production chains, such as, pinewood and plant essential oils. Therefore, the aim was to evaluate citrus essential oil as possible preservatives of pinewood against sapstain.

2. MATERIALS AND METHODS

Twenty-six essential oils represented as by-products of different stages of the pellatrici style (Briefly: cold pressing, centrifugation step, winterization, deterpenation by vacuum distillation) extraction process were tested. The aliquots were obtained from a citrus local industry. Four citrus essential oils (by duplicate) were selected with antifungal effect and correspond to lime (7-9), lemon (19) and clove (20) internal control. Both ethanol and DMSO were used to make dilutions of essential oils.

2.1 Isolation of sapstain fungi

Pinewood splinters with sapstain (15 specimens) were obtained from *Pinus* sp. beams in drying process

from sawmill in Facultad de Ingeniería y Tecnología de la Madera at Morelia, Mich. México. The splinters were stored in sterile vials until their processing. Fungal monosporic cultures were obtained by serial dilution and spread plate method from a homogenized pinewood splinter (Ben-David and Davidson, 2014). An aliquot from the dilution was streaked in petri dishes with Sabouraud dextrose agar (Bioxon®). By its different colonial morphology, primary cultures were obtained. Or, young hyphae from a *mycelia sterilia* colony was dissected and subcultivated. Axenic fungi cultures were obtained in acidic growth media with presence of cefotaxime and kanamycin. The fungal cultures obtained were kept and propagated in Sabouraud dextrose agar for 5 to 30 days at 20 °C in accord to velocity of fungal growth.

2.2 Identification of sapstain fungi

Sapstain fungi were identified based on *18S rRNA* gene sequencing. They were first identified using fungal specific primer set: NS1 (GTAGTCATATGCTTGTCTC) and NS6 (GCATCACAGACCTGTTATTGCCTC) were purchased to oPools™Oligo Pool. The DNA was obtained from mycelium of 5 to 30 days of age depending on the fungus, using protocol modified by Orozco Mosqueda et al. (2015). The sequences were obtained with capillary sequencers ABI model 3730xl and submitted to NCBI-BLAST alignment search in the Gen-Bank (Altschul et al., 1990). Sequences sharing ≥99% similarity with a partial 18S rDNA sequence (ca. 600 bp) were considered as representing identical species reported in database.

2.3 Antifungal effect of the essential oils.

Sapstain control in pinewood was carried out according to the norm ASTM D4445-10 (ASTM, 2019). Five sterile filter paper discs were moisturized with sterile deionized water inside 90 mm x 15 mm Petri dishes. Three *Pinus* sp. probes measuring 7 mm x 3 mm transversally and 70 mm longitudinally were placed inside the Petri dishes. The pinewood blocks were treated both for immersion as spraying with deionized water or 2% DMSO or citrus essential oils at different concentrations or clove oil (internal control). Chromated arsenate copper (CCA) and *o*-Phenyl Phenol (OPP) at the concentrations indicated by the providers were used as positive control. Concentration of preservers and essential oils were 1 g/ml. One fungus propagule (5 mm^3) was inoculated on each wood block, and incubated at 26 ºC for four weeks. The fungal growth was observed every week. Finally, the fungi were removed from the wood blocks with a scalpel and dried. The sapstain presence was evaluated with a TES-135A color meter.

2.4 Absorption and retention of citrus essential oils on *Pinus* sp. wood

Two batches of wooden blocks (probes) of uniform dimensions were formed to the absorption and retention of essential oils tests. Then, one of them was immersed in wood preservatives and essential oils (EO) for 24 h. While the other was sprayed with preservatives and essential oils (EO). Subsequently and separately, both batches were dried at room temperature to constant weight. The citrus essential oil retention was calculated with the following equation (Ávila-Calderón et al., 2012): $R = (A^*C)/100$. Where: **R** = Retention (kg/m^3), **A** = net absorption (kg/m^3) and **C** = preservative concentration (%). The essential oil absorption was determined with the following equation (Simsek et al., 2010): $A= (P_2-P_1)/V$. Where: **A** = Absorption (kg/m^3), $\mathbf{P_1}$= Initial weight of the block before the treatment (kg), $\mathbf{P_2}$= Final of the block after the treatment (kg) and **V** = Volume of the block after the treatment (m^3).

2.5 Natural resistance of pinewood to sapstain and color difference acceptance criteria

Color difference determinations of the wood blocks with sapstain controls and treated was carried out according to the norm ASTM D 2244-05 (ASTM, 2005), using a TES-135A color meter. Three measurements were performed in the distal and center sections of each block to homogenize the color. The CIE $L^*a^*b^*$ coordinates were used and the color difference (ΔE^*) was determined with the equation: $\Delta E = [(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$. Where: $\mathbf{\Delta E^*}$ = Color difference, $\mathbf{\Delta L^*}$ = Lightness-darkness difference, $\mathbf{\Delta a^*}$ = Red-green difference and $\mathbf{\Delta b^*}$ = Yellow-blue difference.

2.6 Gas chromatography coupled to mass spectrometry

The chemical composition of the essential oils was determined by Gas chromatography (CG Thermo Scientific model TRACE 1310). The furnace initial temperature was 50 ºC for 5 min, the temperature was

gradually increased by 20 ºC/min until 200 °C where it was maintained for 5 min, and afterwards it was increased to 250 ºC and maintained for 10 min. The interphase temperature was 260 ºC. Helium was used as mobile phase in a 15 m long column, with internal diameter of 0.25 mm and a stationary phase layer of 0.25 µm. The spectra were analyzed with the Thermo Finnigan Xcalibur™ software (TFS, 2020) and compared with internal standards and the NIST v.2.0 database. The reported compounds met the following criteria: area percentage ≥ 2% and coincidence factor ≥ 900.

2.7 Mycelial adhesion to pinewood probes treated with essential oils and conventional wood preservatives.

Mycelial adhesion to pinewood blocks treated with citrus essential oils and conventional wood preservatives was performed with *Alternaria* sp and determined by scanning electron microscopy at 1000x magnification. The probes (pine wood blocks) were treated with a) no preservative or fungal spores, b) DMSO, c) OPP, d) CCA, e) EO 8 and f) EO 20. Then, they were inoculated with *Alteraria* sp spores and incubated at 20°C for 5 to 30 days according to the growth rate of each fungus.

2.8 Statistical analysis

The statistical significance between treatments was obtained using a variance analysis and a Tukey means comparison with α = 0.5 (STATISTICA 10).

3.RESULTS

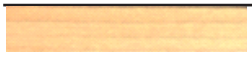
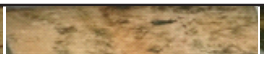
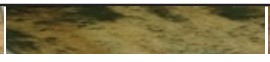
3.1 Fungal isolates from pinewood with sapstain

Fungal diversity in sapwood pine splinters was determined using the *18S rRNA* gene. The amplification of the NS1 and NS6 region of the *18S rRNA* gene allowed discrimination of the isolated fungi. In order to characterize its sensitivity to essential oils and its ability to produce stain on pine wood. All of them had identity of 99% with fungi of genres *Alternaria*, *Hypocrea*, *Trichoderma* and *Geosmithia*. Noting that the NS1 primers have amplified rRNA from a wide variety of fungi, protists, and red and green algae. NS6 has amplified all fungal rRNAs tested. Then, the identification of fungal isolates was done at the intermediate taxonomic levels genera. Because, all of them exhibited identity with other fungal genres, such as, *Actinomucor*, *Penicillium*, *Paecilomyces* and *Nomuraea*. Other barcode genes are necessary to know the fungal species of sapstain on pinewood.

3.2 Natural resistance of pinewood to sapstain and color difference acceptance criteria

The color attributes of pinewood were determined using CIE $L^*a^*b^*$ color space. Its color coordinates were light with chromatic coordinates for light red and saturated yellow. The wood color difference was determined with pine wood blocks impregnated with dimethyl sulfoxide (DMSO). Numeric differences in the absolute color coordinates for lightness and chroma were observed on inoculated pinewood blocks. The results were a reduction in lightness and chroma. The typical tolerance values for $\Delta L^*(5)$, $\Delta a^*(1)$, $\Delta b^*(4)$ and color difference $\Delta E^*(6.5)$ are the parameters used as acceptance criteria for pinewood sapstain control; Values over these parameters are rejected. Pinewood blocks without preservatives and treated with DMSO (1 mg/ml), either by immersion or spraying, were inoculated with sapstain fungi. Fungal growth on wood was registered as total color difference values. Nonetheless, pinewood did not exhibit any natural resistance and did not stand fungal

Table 1 – Dimethyl sulfoxide effect in natural resistance of pinewood to sapstain and fungal viability determined by CIELab method (color difference, ΔE^*).
Tabela 1 *– Efeito do dimetilsulfóxido sobre a resistência natural da madeira de pinus à mancha e viabilidade fúngica determinada pelo método CIELab (diferença de cor, ΔE^*).*

Treatments	ΔE^*			
	***Alternaria* sp.**	***Hypocrea* sp.**	***Trichoderma* sp.**	***Geosmithia* sp.**
				
Without	17.59±0.51a	18.03±0.55a	19.84±0.71b	179.64±0.71a
				
DMSO (1 g/ml)	9.96±0.76b	9.00±1.00b	11.67±0.92b	9.75±0.33b

Data are $\bar{x}$ ± EE, n = 6; Tukey test (α = 0.5).
Os dados são x ± EE, n = 6; Teste de Tukey (α = 0,5).

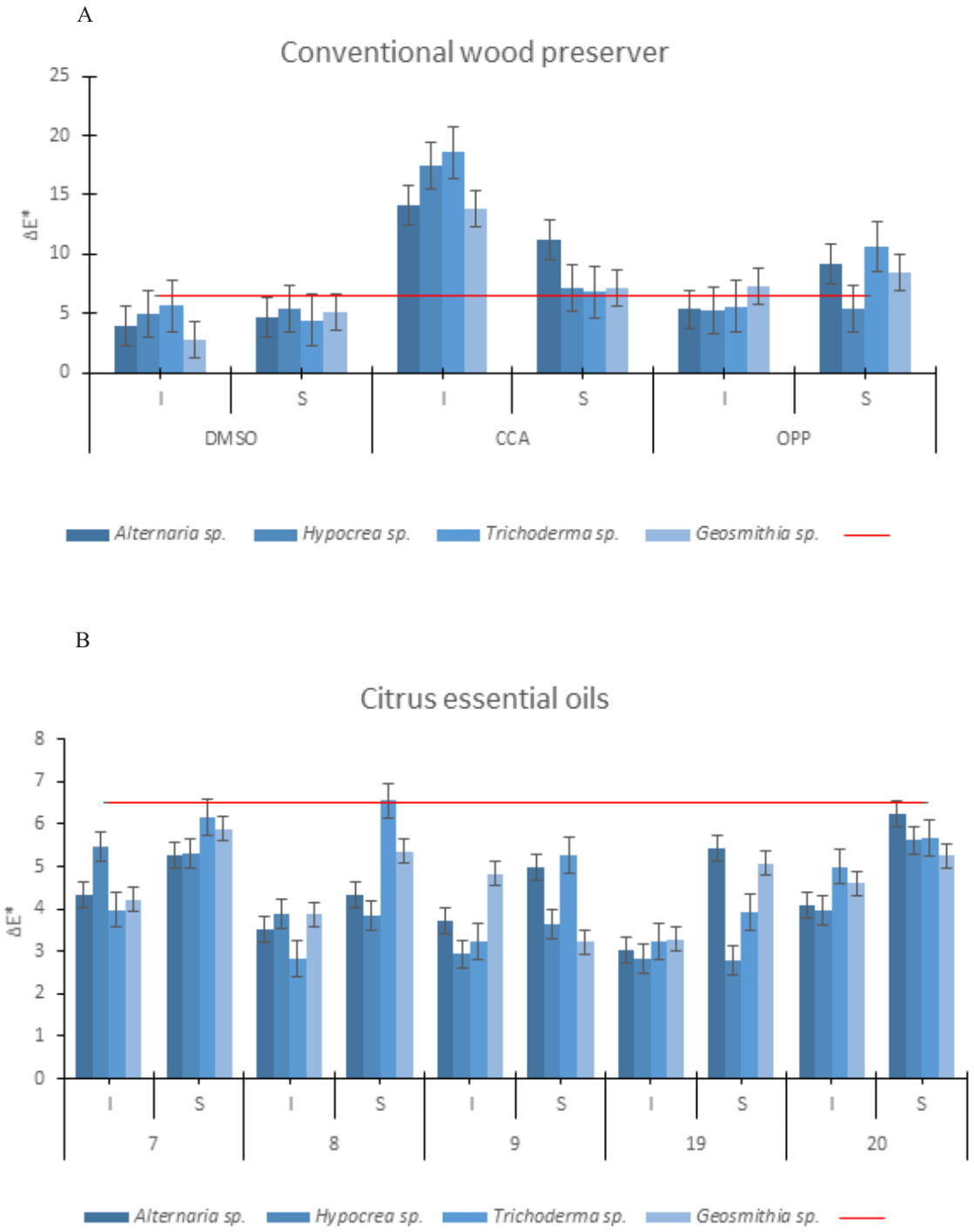


Figure 1 – Control of sapstain on pinewood. Color differences were determined in pinewood treated with conventional preserver (A). Essential oils applied (B). Application methods: immersion (I) and spraying (S). Line in red = acceptance criteria. Data are $\bar{x} \pm$ EE, n = 6; Tukey test ($\alpha = 0.5$).

Figura 1 *– Controle de mancha em* ***Pinus****. As diferenças de cor foram determinadas em madeira de* ***Pinus*** *tratada com conservante convencional (A). Óleos essenciais aplicados (B). Métodos de aplicação: imersão (I) e pulverização (S). Linha em vermelho = critério de aceitação. Os dados são* $\bar{x} \pm EE$*, n = 6; Teste de Tukey (*$\alpha = 0,5$*).*

Table 2 – Absorption and retention of essential oils and conventional preservatives on pinewood.
Tabela 2 *– Absorção e retenção de óleos essenciais e conservantes convencionais em madeira de* ***Pinus****.*

Essential oils (1 g/ml)	**Immersion** (kg/m³)		**Spraying** (kg/m³)	
	Absorption	**Retention**	**Absorption**	**Retention**
OPP*	11.71±1.35	0.23±0.02	8.24±0.73	0.16±0.01
7	231.08±15.92	34.57±6.48	61.22±4.70	17.76±2.25
8	144.48±25.80	11.67±1.35	31.32±4.76	6.82±0.76
9	198. 85±23.13	38.73±6.64	37.78±5.40	10.22±0.79
19	200.89±17.27	35.91±4.56	36.23±5.31	9.06±1.95
20	245.62±32.89	63.01±13.42	63.52±5.09	18.01±2.37

Data are $\bar{x}$ ± EE, n = 6.
*OPP 2% in accordance to manufacturer.
Os dados são x ± EE, n = 6.
**OPP 2% conforme fabricante.*

growth, according to the total color difference (ΔE^*) between the non-inoculated wood blocks and the blocks after inoculation (Table 1). The interpretation is that pinewood does not possess any natural resistance to biodeterioration.

3.3 Control of sapstain on pinewood by essential oils

The essential oil with code 7-9, 19 and 20 inhibited all four fungal isolates. The essential oil with code EO 20 (internal control) exhibited the lowest GI values, it was even more efficient than the controls. Fungal sensitivity to essential oils was diverse. With the spraying method, ΔE^* values were located between 2.78 ± 0.34 and 17.74 ± 1.37. However, wood blocks treated with EOs were significantly different, the obtained ΔE^* values were interval 2.78 ± 0.34 to 8.68 ± 0.99. A generally darkened color in the blocks was observed, except the ones treated with

Table 3 – Chemical composition of essential oils used as preservatives against sapstain on pinewood.
Tabela 3 *– Composição química dos óleos essenciais utilizados como conservantes de madeira de* ***Pinus****.*

Constituent	RT*	Essential oils (Relative abundance)					*Identification
		7	8	9	19	20	
β-pinene	5.95	2.43			3.09		MS, ST
D-limonene	6.60	3.19	14.83	12.19	53.77		MS, ST
γ-terpinene	7.02	4.29	7.49	33.6			MS, ST
Terpinolene	7.39			5.53			MS
β-linalool	7.58		2.13				MS, ST
exo-fenchol	7.72			2.30			MS
1-terpineol	7.95			5.11			MS
β-terpineol	8.06			4.15			MS, ST
Isoborneol	8.27			4.26			MS
(-)-terpinen-4-ol	8.36	3.26	8.39	3.74			MS
α-terpineol	8.51	2.97	7.82	20.07			MS
cis-geraniol	8.83			8.03			MS
β-citral	8.91	4.63	4.60		4.13		MS
Geraniol	9.04				7.93		MS, ST
α-citral	9.16	5.74	5.89		4.23		MS
δ-elemene	9.63	4.24					MS
Eugenol	9.84					52.89	MS
(-)-*β*-elemene	10.02	3.26					MS
Caryophyllene	10.23	7.13	4.77	2.49		14.17	MS, ST
α-bergamotene	10.31	8.70	5.91	3.63			MS
Humulene	10.45	3.58	2.20			5.89	MS
Germacrene D	10.62	3.63	2.40				MS
β-bisabolene	10.78	16.90	9.30	8.79			MS
Eugenol acetate	10.93					12.89	MS
Longifolene	10.98				2.29		MS
Caryophyllene oxide	11.28					3.02	MS

* MS = NIST v.2.0g library spectra and literature. ST = authentic standard compounds.
**MS = espectros e literatura da biblioteca NIST v.2.0g. ST = compostos padrão autênticos.*

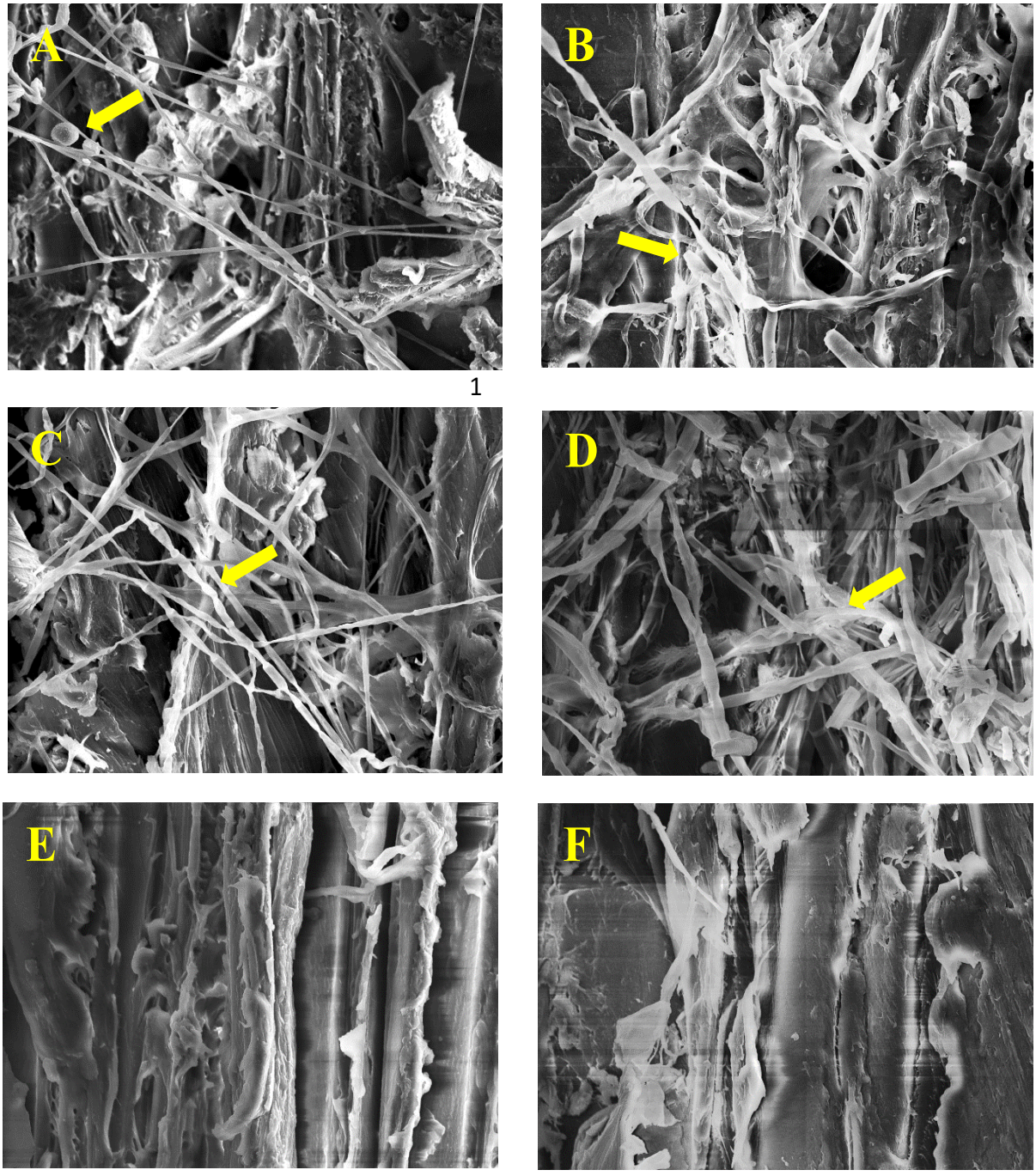


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Figure 2 – Mycelial adhesion to pinewood blocks treated with essential oils and conventional wood preservatives. Scanning electron microscopy, 1000x magnification: a) No treatment (*Alternaria* sp.), b) DMSO, c) OPP, d) CCA, e) EO 8 and f) EO 20. The yellow arrows indicate the fungal structures, in panel (A) spores are seen near the hyphae. In panels B, C and D the hyphae are indicated. In panels A and C areolate scores are observed.

***Figura 2** – Adesão micelial a blocos de madeira de **Pinus** tratados com óleos essenciais e conservantes convencionais de madeira. Microscopia eletrônica de varredura, aumento de 1000x: a) Sem tratamento (**Alternaria** sp.), b) DMSO, c) OPP, d) CCA, e) EO 8 e f) EO 20. As setas amarelas indicam as estruturas fúngicas, no painel (A) esporos são vistos perto das hifas. Nos painéis B, C e D estão indicadas as hifas. Nos painéis A e C são observadas pontuações de areolato.*

the EO 19 (ΔE^*= 2.78 ± 0.34) and *Hypocrea* sp. This notorious darkened color in the blocks was due to an excessive fungal growth. Nevertheless, ΔE^* were located between 2.78 ± 0.34 and 6.55 ± 1.12, which allowed for their acceptance (Figure 1).

3.4 Absorption and retention of essential oils and conventional preservatives on pinewood.

Wood blocks impregnated with essential oils by the immersion method showed an increased absorption compared to the spraying method. With the immersion method, absorption values were between 144.48 ± 25.80 and 245.62 ± 32.89 kg/m^3. Retention values for CCA and OPP by the spraying method were of 0.16 ± 0.03 and 0.16 ± 0.01, respectively. The essential oil retention values by the spraying method were located in the interval of 6.82 ± 0.76 and 18.0 1± 2.37. The essential oil retention value of 12.37 ± 1.62 was the minimum inhibitory concentration against fungal growth (Table 2).

3.5 Chemical composition of the essential oils

Twenty-six chemical compounds were identified (Table 3). In the EO 20 (internal control) the identified chemical compounds with the largest relative abundance were eugenol (52.89%), caryophyllene (14.17%) and eugenol acetate (12.89%). The compounds with the largest relative abundance for the EO 8 were D-limonene (14.83%), β-bisabolene (9.30%), (-)-terpinen-4-ol (8.39%), α-terpineol (7.82%) and γ-terpinene (7.49%). γ-terpinene (33.6%), α-terpineol (20.07%), D-limonene (12.19%), β-bisabolene (8.79%) and nerol (8.03%) were the chemical compounds with the biggest relative abundance in the EO 9. D-limonene (53.77%) was the chemical compound with the biggest relative abundance for the EO 19. For the EO 7 the compounds with the largest relative abundance were β-bisabolene (16.90%), α-bergamotene (8.70%) and caryophyllene (7.13%).

3.6 Mycelial adhesion to pinewood probes treated with essential oils and conventional wood preservatives.

The pinewood probes to which the essential oils were applied by spraying and which were inoculated with *Alternaria* sp. were evaluated by Scanning Electron Microscopy at 1 000x to observe the fungus penetration (Figure 2).

Probes with no treatment (Figure 2A), where the hyphae and other fungal structures (spores) can be observed. Also, it is observing some big mycelia agglomerations and areolate punctuations. In probes with DMSO (Figure 2B) the hyphae can be appreciated in the interradial spaces close to the areolate punctuations. Hyphae and areolate punctuations can also be observed (Figures 2C). Big agglomerations of fungal structures are covering the interradial spaces (Figure 2D). In the blocks impregnated with the EOs 8 and EO 20 an absence of fungal structures was observed (Figures 2E and 2F). The essential oils prevent adhesion of the fungus to the wood surface.

4. DISCUSSION

Citrus essential oils with potential antifungal activity were evaluated for the control of sapstain on pinewood. Because pinewood has no natural resistance to sapstain. With specific primers NS1 and NS6, the genres of fungal isolates from sapstain pinewood were known. For the western México forest and biome, in this research it reports that cultivable fungi isolates from sapstain pinewood were of the genres *Alternaria*, *Hypocrea*, *Trichoderma* and *Geosmithia*. However, other barcode genes are necessary to know the fungal species. Each genre has specific barcode gene but other primers from αEFT and β tubulin genes could be included. Wood stain is caused by a heterogeneous consortium of fungi that can be wood mold fungi or stain fungi. However, an explanation of its presence *e.g. Alternaria alternata*, is frequently isolated from *Pinus* sp. it might be a contamination associated to sawmill process or is a pine endophyte (Marmolejo Monciavais, 2018; Gutiérrez-Flores et al., 2020).

It is known that *Trichoderma* species displace sapstain fungi in Scots pine (Gibbs, 1999; Jankowiak et al., 2021). While *Alternaria* sp. and *Trichoderma* sp. are *Pinus mangalensis* endophyte (Wang et al., 2019). Presence of *Hypocrea* sp. in sapstain is explicable because it is the teleomorph of *Trichoderma* (anomorph). *Gesomithia* sp. presence on sapstain is attributable to its dissemination by insects from the Scolytinae subfamily reported as members of local biome (Kolarik et al., 2017; Lázaro Dzul et al., 2020; Hernández-García et al., 2020).

These fungal isolates were sensible to four essential oils evaluated. Some components of the EO exhibit antifungal activity, *e.g. Trametes hirsuta*,

Schizophillum commune, *Pycnoporus sanguineus*, *Aspergillus niger*, *A. flavus* and *Penicillium aurantiogriseum* were sensible to eugenol (Valdés-Pérez et al., 2016; Zhang et al., 2016). Another example is the sensitivity of *Alternaria alternata*, and *Trichoderma viride* to eucaliptol, α-pinene, γ-terpinene and terpinen-4-ol (Salem et al., 2016). A terpene mixture from oranges inhibited *Alternaria tenuissima* growth, where D-limonene was the most abundant secondary metabolite (Quintana-Obregón et al., 2017). This demonstrates that the EOs control sapstain on pinewood.

The efficacy on the control of sapstain is dependent on the absorption and retention of the preservative. The absorption and retention analyses were performed for the essential oils and the controls. Generally, the wood blocks treated with the essential oils by immersion or spraying, showed adequate levels of absorption and retention for sapstain control on pinewood. Where, the retention parameter from 6.82 ± 0.76 to 11.67 ± 1.35 is the indicator of the minimum inhibitory concentration necessary to inhibit fungal growth on pinewood.

Color is an aesthetic attribute of any item for its acceptance. Typical tolerance values are known as criteria for color acceptance. These values are stablished for each component $\Delta L^*(5)$, $\Delta a^*(1)$ and $\Delta b^*(4)$ to identify the coordinate that is exceeding the limit. $\Delta E^*(6.5)$ was used for the evaluation of the antifungal effect of the essential oils as an individual attribute.

The post fungal growth observation on wood preserved with essential oils is that the coordinates a^* and b^* maintained low levels compared to L^*. The coordinate a^* on heartwood maintained similar levels on different sites. Meanwhile, the coordinate b^* showed higher values than the coordinate a^*. This study demonstrated a correlation between colorimetric parameters, with increases in lightness (L^*) and yellow pigmentation (b^*). Additionally, this result is in accordance with observations in the colorimetric coordinates CIE $L^*a^*b^*$ of eucalyptus pellets and coffee industry residues (Cisneros et al., 2019; Pegoretti et al., 2019). Citrus essential oils prevented the darkening of the surface of pinewood blocks, with values of $\Delta E^* < 6.5$.

Two economically and politically important products in the regional economy are linked; wood industries and vegetable essential oils production, particularly citrus EO. Antimicrobial properties of EOs are evidenced. Meanwhile, their physicochemical properties make them favorable to be used for impregnating wood.

5. CONCLUSIONS

The conclusion is that citrus essential oils with a substantial content of limonene, *γ*-terpinene, α-terpineol, geraniol, eugenol or *β*-bisabolene show antifungal effect against *Alternaria* sp., *Hypocrea* sp., *Trichoderma* sp. and *Geosmithia* sp., fungi that cause sapstain on wood from *Pinus* sp.

AUTHOR CONTRIBUTIONS

Crisanto Velázquez-Becerra and Mauro M. Martínez-Pacheco: data collection, analyse and text written, David Raya González and José Cruz de León: methodological contributions,, search oversight. Wuilver E. García Reynoso and Alberto Flores García: data collection and experimental set up. Abril Munro Rojas: project management. All authors read and approbed the final manusript.

6. ACKNOWLEDGEMENTS

The authors acknowledge the partial financing from UMSNH for this research. WEGR holded a CONACyT scholarship.

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