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Chemical composition and *in vitro* antifungal activity of essential oils from *Lippia alba* and *Lippia turbinata*

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Abstract

Fungal diseases are one of the most critical causes of postharvest losses in agricultural production, demanding safer and more sustainable control strategies. This study evaluated the chemical composition and *in vitro* antifungal activity of essential oils (EOs) from *Lippia alba* and *Lippia turbinata* against *Botrytis cinerea*, *Monilinia fructicola*, *Rhizopus stolonifer*, *Colletotrichum nymphaeae*, *Fusarium hainanense*, and *Penicillium digitatum*. The volatile method was applied using a concentration of 1000 ppm, with carbendazim as the chemical control. The EO of *L. alba* completely inhibited the growth of all fungi except *P. digitatum* (47.6 ± 1.4%), while *L. turbinata* showed 100% inhibition for all tested species, without significant differences compared to the fungicide ($p>0.05$). Gas chromatography–mass spectrometry analysis revealed β-linalool (26.72%) and trans-dihydrocarvone (16.29%) as major components in *L. alba*, and (−)-carvone (58.23%) and D-limonene (27.72%) in *L. turbinata*. The strong inhibitory activity and distinct chemical profiles suggest the potential of both species as sources of natural biofungicides for postharvest disease management.

Keywords: Volatile compounds; antifungal activity; postharvest pathogens; native plants.

Introduction

Over the past decades, fungi have been responsible for an increasing number of epidemics affecting humans, plants, and animals[1]. Globally, phytopathogenic fungi represents a major threat to agricultural productivity. Postharvest fungal diseases constitute one of the most critical challenges to global food security due to their impact on crop losses, food quality, and safety. Economic losses due to storage diseases can exceed those caused in the field. In developed countries, this loss can represent up to 25%; while in developing countries it is often higher, exceeding 50%[2–4].

In this regard, among the wide range of postharvest pathogens responsible for these losses, several species stand out for their high prevalence and destructive capacity. *Botrytis cinerea* is one of the most devastating species, affecting numerous crops worldwide and thriving under storage and transport conditions[5,6]. Similarly, *Colletotrichum* species represent a complex genus associated with anthracnose diseases that collectively account for more than 50% of global fruit and vegetable losses[7,8]. *Fusarium* species are also of major concern due to their cosmopolitan distribution, ability to cause severe plant diseases such as root rot and vascular wilt, and production of chemically stable mycotoxins, including fumonisins, zearalenone, and trichothecenes, that contaminate up to half of harvested crops annually[9,10]. Likewise, *Rhizopus stolonifer* is among the fastest-growing postharvest pathogens, capable of penetrating uninjured fruit cuticles and causing rapid tissue liquefaction under humid conditions[11–13]. Similarly, *Penicillium digitatum*, the causal agent of green mold in citrus fruits, spreads rapidly under humid, moderate conditions and has developed resistance to common fungicides such as imazalil and thiabendazole, making its control increasingly difficult[14,15]. Collectively, these pathogens represent a major challenge for the development of effective and sustainable postharvest disease management strategies. Finally, *Monilinia fructicola*, the causal agent of brown rot in stone and pome fruits, leads to significant pre- and postharvest losses and has shown a moderate risk of developing resistance to conventional fungicides[16].

Given the growing prevalence of resistant strains and the environmental concerns associated with synthetic fungicides, there is an urgent need for sustainable alternatives that align with the United Nations' Sustainable Development Goals (SDG 2 and SDG 12), while meeting consumer and market demands for safer, low-residue produce[17–22]. Environmental impacts, including soil and water contamination, effects on non-target organisms, and disruption of ecological balance, have also raised serious concerns[23,24].

One of the most important plant defenses is the production of secondary metabolites that possess a wide range of biological activities. Natural plant products offer a set of structurally different antifungal agents that could represent an alternative to synthetic fungicides for the control of phytopathogenic fungi[3,25]. These compounds not only inhibit fungal growth but also enhance plant defense mechanisms, thereby contributing to sustainable postharvest disease management both *in vitro* and *in vivo*, and extending the overall quality and shelf life of fresh produce[26,27]. Within this framework, several plant-derived essential oils have shown remarkable antifungal potential. These compounds are selectively active against various fungal species, biodegradable, and potentially suitable for use as agrochemicals in integrated disease management programs[23,24]. Within this context, species of the genus *Lippia* have attracted particular attention due to their chemical diversity coupled with strong biological activity.

Lippia alba (Mill.) N.E.Br. ex Britton & P. Wilson and *Lippia turbinata* Griseb. (Verbenaceae) have emerged as promising botanical candidates for the postharvest control of phytopathogenic fungi. *L. alba*, commonly known as "salvia morada," is widely distributed throughout tropical and subtropical regions of South America, including riparian forests in Argentina[28], and has a long-standing use in traditional medicine for gastrointestinal, respiratory, and pain-related disorders[29] (**FIGURE 1 A**). Its essential oil has demonstrated strong inhibitory activity against a wide range of fungal species, including aflatoxigenic *Aspergillus flavus*[29] and several plant pathogens such as *Ustilago scitaminea*, *Cochliobolus falcatum*, and *Curvularia lunata*[26], as well as *Fusarium* spp., *Penicillium funiculosum*, and *Sclerotinia sclerotiorum*[27]. Similarly, *L. turbinata*, known as “Poleo” and native to central and western Argentina[30,31], has shown antifungal potential; exposure of peanut seeds to its essential oil vapors effectively prevented contamination by *Aspergillus* species[29] (**FIGURE 1 B**).

FIGURE 1: Inflorescences of *Lippia alba* (A) and *Lippia turbinata* (B). Images are not to scale.

This study aimed to evaluate the antifungal efficacy *in vitro* and chemical composition of *L. alba* and *L. turbinata* essential oils, with emphasis on their potential as biofungicides for postharvest disease management.

Material and Methods

Essential oil and plant material

The plants were collected from farms and roadsides in areas surrounding the Litoral region of Argentina between 2019 and 2020. Each plant sample was taxonomically identified, and a voucher specimen was deposited in the Herbarium “Arturo Ragonese” of the Facultad de Ciencias Agrarias, Universidad Nacional del Litoral (Herbarium SF), Kreder 2805, (3080HOF), Esperanza, Argentina: *Lippia alba* JFP13266 y *Lippia turbinata* JFP11609. Fresh leaves of each species were subjected to steam distillation using a Clevenger-type apparatus. The plant material was weighed to determine the essential oil yield per kilogram of processed fresh material. The obtained essential oils were stored in amber glass vials under refrigeration until further use.

Fungal strains

The phytopathogenic fungi *Botrytis cinerea*, *Monilinia fructicola*, *Rhizopus stolonifer*, *Colletotrichum nymphaeae*, *Fusarium hainanense*, and *Penicillium digitatum* were used in this study. *B. cinerea* was characterized and deposited in the Mycology Reference Center (CEREMIC) of the Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (FCByF–UNR), under the code CCC-100.39. *M. fructicola* was characterized and deposited in the Phytopathology Department of the San Pedro Experimental Station, Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina, under the code INTA-SP345. *R. stolonifer* was characterized and deposited in the Microbiology Laboratory of the Facultad de Ingeniería Química, Universidad Nacional del Litoral (FIQ–UNL), Santa Fe, Argentina, under the code LMFIQ-317. *C. nymphaeae* and *F. hainanense* were characterized by the Phytopathology Chair of the Facultad de Ciencias Agrarias, Universidad Nacional del Litoral (FCA–UNL), and deposited in the Mycology

Reference Center (CEREMIC) of FCByF–UNR under the codes CEREMIC 13-2019 and CEREMIC 01-2019, respectively. *P. digitatum* was characterized and deposited in CEREMIC, under the code CCC-102.

In vitro antifungal activity of essential oils using the volatile method

Petri dishes (60 mm in diameter) were filled with 20 mL of PDA medium. Once solidified, each plate was inoculated with a conidial suspension of 10^4 CFU/mL placed into a well located at the center[32]. After the water from the inoculated conidial suspension had evaporated, 10 µL of the essential oils of *Lippia alba* and *Lippia turbinata* were deposited on the inner side of the plate lids, while sterile water was used as a growth control (T+). A commercial dose of carbendazim was used as a chemical control (T−). The amount of essential oil applied corresponded to a concentration of 1000 ppm. The Petri dishes were incubated upside down to allow the essential oil vapors, once evaporated, to come into contact with the culture medium where the fungus was growing (methodology adapted from Álvarez-Castellanos *et al.*[33]. When the mycelium of the control plates completely covered the surface of the medium (approximately 7 days), the mycelial diameter of each plate treated with essential oil was measured by scanning the plates (LA2400 scanner, WinRHIZO 2013 software; Regent Instruments Canada Inc., 2013) for subsequent analysis using ImageJ® software[34]. All assays were performed in triplicate, and the percentage of fungal growth inhibition was calculated according to the following equation: $I\% = 100\ (C - M)/C$, where I% represents the inhibition percentage, C is the average mycelial area of the three control plates, and M is the average mycelial area of the three treated plates. Confidence intervals (95%) for the mean mycelial growth inhibition of each treatment were calculated with a significance level of $\alpha = 0.05$ using R software[35].

Identification of volatile constituents of essential oils

The essential oil was subjected to gas chromatography coupled with mass spectrometry (GC-MS) to identify its main components. The compounds were identified by comparing their mass spectra with those available in the NIST 2011 database. The sample was analyzed using a gas chromatograph (Agilent model 7890B) coupled to a mass spectrometer (Agilent model 5977) equipped with an HP-5MS UI capillary column (30 m × 0.25 mm, film thickness 0.25 µm). The operating conditions were as follows: injector column temperature 250°C; oven temperature initially set at 160°C and held for 3 min, then increased at 5°C min^{-1} up to 300°C; total run time: 31 min. Injection volume: 1 µL; split ratio: 1:20, according to the method described by Adams[36].

Results and Discussion

Yield of Essentials oils

Essential oils were obtained through steam distillation, which is recognized as the most widely used extraction method among several techniques such as enfleurage, expression, and fermentation[37–39]. In the present study, the extraction yield was 0.54% for *Lippia alba* and 0.56% for *Lippia turbinata*.

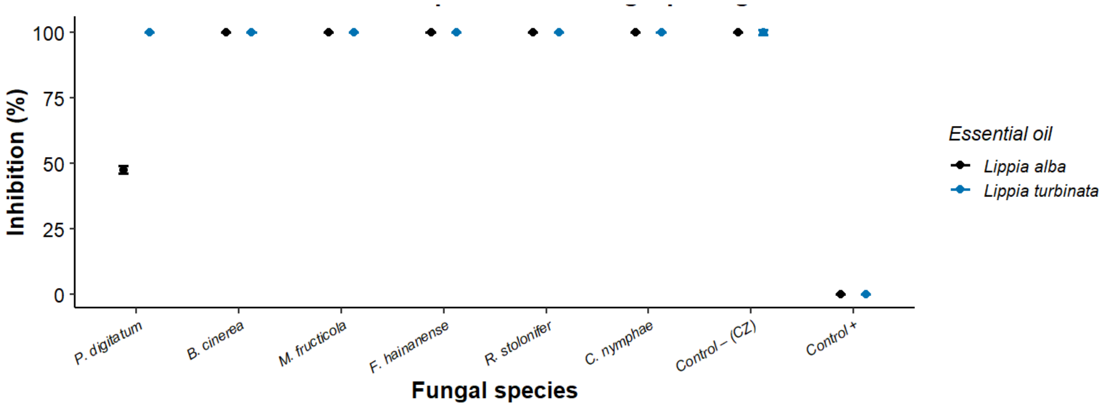
Previous studies have shown that the yield of *L. alba* varies seasonally, with higher values reported in summer (0.15-0.61%), autumn (0.47%), and spring (0.55%)[40]. In Argentina, authors have reported a range of 0.6-2% (mL/100 g of dry sample) in four chemotypes evaluated over two years[41].

In the case of *L. turbinata*, the yields obtained in this study are consistent with those previously reported in the literature[31]. Secondary metabolites responsible for biological activity are usually present in low concentrations in plant material, as observed in this work. Their extraction, purification, and characterization remain major challenges in the development of new pharmaceuticals or agrochemicals[42].

Antifungal *in vitro* activity

Complete inhibition (100%) was observed for *Botrytis cinerea*, *Fusarium hainanense*, *Monilinia fructicola*, *Rhizopus stolonifer*, and *Colletotrichum nymphaeae* by the essential oil (EO) of *Lippia alba* (**FIGURE 2**). In contrast, *Penicillium digitatum* exhibited partial sensitivity, with an average inhibition of 47.6 ± 1.4 %. Statistical analysis revealed that the inhibition of *P. digitatum* was significantly lower ($p < 0.05$) than that observed for the other tested fungi, indicating differential susceptibility among species.

FIGURE 2: *In vitro* inhibitory effect of *Lippia alba* and *Lippia turbinata* essential oils against postharvest fungal pathogens. Points represent mean percentage inhibition, and error bars indicate 95% confidence intervals. Fungal species tested include *Penicillium digitatum, Botrytis cinerea, Monilinia fructicola, Fusarium hainanense, Rhizopus stolonifer, and Colletotrichum nymphae.* Negative (Control +) and chemical (Control – CZ) controls are included.



These results indicate a broad-spectrum antifungal activity, consistent with previous reports. Peixoto *et al.*[43] described similar minimum inhibitory concentrations, particularly against *Lasiodiplodia theobromae*, *Fusarium pallidoroseum*, and *F. solani*, achieving total inhibition at 0.2 mL/100 mL. The inhibitory effect of *L. alba* EO against *Alternaria* spp., as reported by Tomazoni *et al.*[44], further supports the broad anti-fungal potential of this species. In that study, full growth inhibition was achieved at relatively low doses, highlighting the ability of monoterpenes to disrupt fungal membrane permeability and ergosterol content. Similarly, Sabali *et al.*[45] reported complete inhibition of *Aspergillus flavus* at concentrations between 100 and 1000 ppm, demonstrating strong mycelial growth reduction for *Aspergillus*-related species. This is particularly relevant for food protection, as *A. flavus* is a well-known producer of aflatoxins. Therefore, *L. alba* EO could serve as a natural antifungal alternative to prevent post-harvest contamination. Finally, Arruda *et al.*[27] showed that *L. alba* EOs from the Brazilian Chaco, dominated by linalool, were highly effective against *Sclerotinia sclerotiorum*, achieving total growth inhibition.

On the other hand, complete inhibition (100%) was observed for all tested phytopathogenic fungi using the essential oil (EO) of *L. turbinata*, showing no statistically significant differences compared to the chemical control ($p>0.05$). These results indicate that the EO matched the efficacy of the synthetic fungicide under the tested conditions. The antifungal activity observed is consistent with previous reports on *L. turbinata* and related species. For instance, Passone and Etcheverry[29] demonstrated that volatile fractions from *L.*

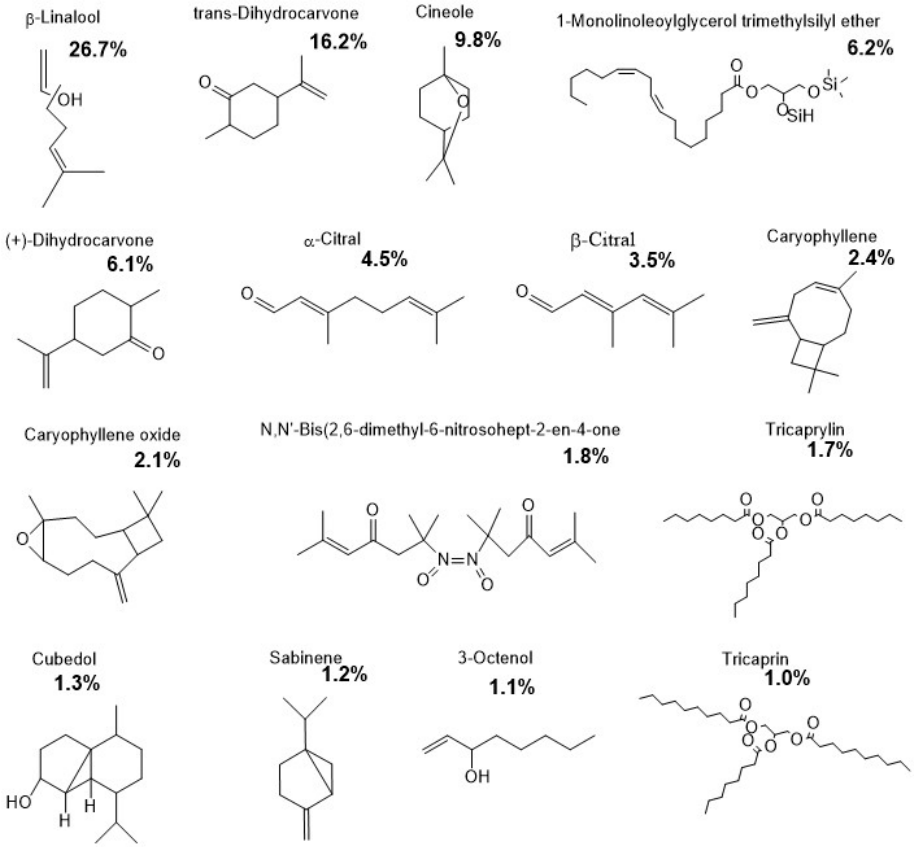
turbinata markedly inhibited *Aspergillus* section Flavi during peanut storage. Similarly, Leal *et al.*[46] confirmed the strong inhibitory capacity of *L. turbinata* EO across multiple fungal genera, supporting its broad-spectrum antifungal potential. Furthermore, ethanolic extracts of *L. turbinata* evaluated by Sayago *et al.*[47] exhibited fungicidal activity against *Verticillium dahliae*, reinforcing the potential of this species as a natural source of antifungal compounds. Likewise, Dellacassa *et al.*[48] reported variable levels of fungicidal activity of *L. turbinata* and *L. integrifolia* essential oils against *Ascosphaera apis*, an ascomycete fungus, confirming that differences in chemical profiles among *Lippia* species can influence their biological efficacy.

Chemical Composition

Essential oils (EOs), as the main components of the plant volatilome, are complex mixtures of volatile, lipophilic, and aromatic compounds whose qualitative and quantitative composition determines their biological activity[49].

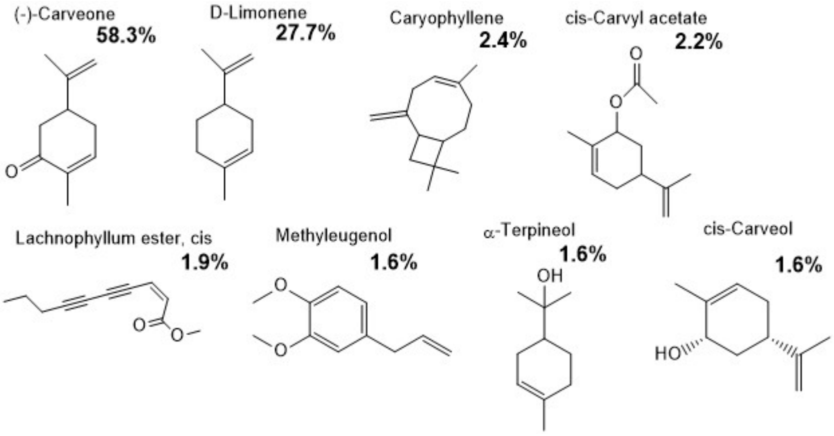
The essential oil of *Lippia alba* was mainly characterized by β-linalool (26.72%), trans-dihydrocarvone (16.29%), cineole (9.28%), dihydrocarvone (6.19%), α-citral (4.58%), β-citral (3.51%), caryophyllene (2.41%) and caryophyllene oxide (2.19%). Minor constituents, including sabinene (1.20%) and 1-octen-3-ol (1.14 %), accounted for a total of 86.2% of the oil composition (**FIGURE 3**). This chemical profile shows a predominance of oxygenated monoterpenes, particularly β-linalool and dihydrocarvone, consistent with the *L. alba* chemotype previously reported in Santa Fe, Argentina[41,50].

FIGURE 3: Chemical composition of *Lippia alba* essential oil showing the major and minor constituents identified by GC–MS. The predominant compounds were β-linalool (**26.7%**), trans-dihydrocarvone (**16.2%**), and cineole (**9.8%**), followed by 1-monolinoleoylglycerol trimethylsilyl ether (**6.2%**), (+)-dihydrocarvone (**6.1%**), α-citral (**4.5%**), β-citral (**3.5%**), and caryophyllene (**2.4%**). Other components detected in lower proportions included caryophyllene oxide (**2.1%**), N,N′-bis(2,6-dimethyl-6-nitrosohept-2-en-4-one) (**1.8%**), tricaprylin (**1.7%**), cubedol (**1.3%**), sabinene (**1.2%**), 3-octenol (**1.1%**), and tricaprin (**1.0%**).



In contrast, the essential oil of *Lippia turbinata* exhibited a composition dominated by (-)-carvone (58.23%) and D-limonene (27.72%), followed by caryophyllene (2.48%), cis-carvyl acetate (2.25%), cis-lachnophyllum ester (1.90%), methyl eugenol (1.66%), α-terpineol (1.64%) and cis-carveol (1.61%), representing 97.49% of the total oil (**FIGURE 4**). This high percentage of identified compounds reflects the purity of the oil and agrees with the findings of Corzo *et al.*[31], who performed a meta-analysis of Argentine *L. turbinata* populations and reported consistent carvone–limonene chemotypes, suggesting strong genetic control of this profile.

FIGURE 4: Chemical composition of *Lippia turbinata* essential oil showing the major and minor constituents identified by GC–MS. The predominant compounds were (–)-carvone (**58.3%**) and D-limonene (**27.7%**), followed by caryophyllene (**2.4%**) and cis-carvyl acetate (**2.2%**). Other components detected in lower proportions included lachnophyllum ester, cis (**1.9%**), methyleugenol (**1.6%**), α-terpineol (**1.6%**), and cis-carveol (**1.6%**).



The chemical comparison between the two *Lippia* species reveals clearly differentiated patterns: *L. alba* shows a predominance of β-linalool and cineole, while *L. turbinata* is rich in carvone and limonene. Such variability has been attributed to both genetic and environmental factors influencing EO composition[31,49]. In *L. alba*, β-linalool and cineole have been associated with antifungal and antimicrobial activities, supporting *in vitro* results against several fungal genera[25,26].

Carvone and limonene are the main monoterpenes responsible for their antimicrobial and repellent properties in *L. turbinata*. These compounds exhibit strong inhibitory activity against major phytopathogenic fungi such as *Aspergillus*, *Fusarium*, and *Penicillium*[46,51]. Moreover, the presence of minor constituents such as methyl eugenol and cis-carveol may contribute to synergistic effects that enhance the overall bioactivity of the oil. Girardi *et al.*[30] demonstrated that microencapsulation of *L. turbinata* EO preserved its antifungal efficacy over time, reinforcing the hypothesis that the combined action of major and minor terpenoids is crucial for the stability and persistence of its biological activity.

Conclusion

The results provide a scientific basis for the development of essential oils formulations from *Lippia* species, contributing to safer and more sustainable fungal control strategies in postharvest systems.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Contributions

Study conception: MAB; MGD
Data curation: NM; NHA; MIS
Data collection: NHA; MIS
Data analysis: MIS
Writing – original draft: NHA; MIS
Writing – review & editing: NHA; MIS.

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