Antifungal Activity of *Lonicera caprifolium* Essential Oil against Selected Phytopathogenic Fungi and its Insecticidal Activity

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Abstract
Honeysuckle (*Lonicera caprifolium*) essential oil is extensively used in aromatherapy to improve mood and well-being, along with several therapeutic health benefits. Honeysuckle essential oil is believed to possess various beneficial biological properties. Its anti-inflammatory, antioxidant, and antimicrobial properties make it a wonderful oil for the prevention of bacterial and fungal infections and for the relief of aching joints and sore muscles. In our work, the main objective was to monitor antifungal activity against plant pathogens and insecticidal activity. Among the microscopic filamentous fungi, *Monilia fructigena, Fusarium solani, Botrytis cinerea,* and *Trichoderma harzianum* were used for antimicrobial activity and *Megabruchidius dorsalis* for insecticidal activity. Fruit and vegetable models (apricot and radish) were used for *in situ* studies. For *in vitro* antifungal activity, it was found that *Lonicera caprifolium* essential oil was the most effective against *Botrytis cinerea*. On the fruit and vegetable model, the best antifungal activity was found against *M. fructigena* on both tested models. *L. caprifolium* essential oil proved to be a suitable antimycotic agent against phytopathogenic fungi and insecticidal agent against *Megabruchidius dorsalis*.

Keywords: microscopic filamentous fungi, insecticidal and antifungal potential, *in vitro* and *in situ* condition, *Lonicera caprifolium* essential oil

1. Introduction

The Chinese Pharmacopoeia formally lists Flos Lonicerae (FL), a traditional Chinese medicinal herb made from the dried flower buds of four key species of the genus *Lonicera* (Caprifoliaceae) [1]. For ages, it has been used in traditional Chinese medicine to treat sores, carbuncles, furuncles, swelling, and early-stage infections caused by exopathogenic wind-heat or epidemic febrile disorders [2,3]. These aliments, which are frequently ascribed to external pathogenic sources like bacteria and viruses, are basically inflammatory processes including heat, redness, discomfort, and swelling. Additionally, FL is frequently utilized as a raw material to make a variety of health care goods that are offered in Asian markets, including tea, wine, and cola.
Flavonoids, organic acids, iridoids, saponins, and essential oils are all found in Flos Lonicerae, according to several phytochemical investigations [4-6]. Linalool, geraniol, and α-terpineol have been found in a number of investigations on essential oils (EOs) from various sections of *Lonicera macranthoides* Hand-Mazz., including leaves and aerial parts gathered from various locations [7-9]. Additionally, it has been noted in scientific literature that essential oils exhibit a variety of antimicrobial properties [10]. When

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compared to traditional antibiotics used to treat a variety of illnesses, they have demonstrated their efficacy [11]. Additionally, essential oils have shown antibacterial efficacy against pathogenic microorganisms. Antifungal and insecticidal activity of honeysuckle *Lonicera caprifolium* Linnaeus (Dipsacales: Caprifoliaceae) were assessed in this study. The study's findings present significant novel insights that have the potential to contribute to the development of an innovative, environmentally sustainable antifungal bioproduct and insect control solution.

2. Materials and methods

2.1. Essential oil

Lonicera caprifolium essential oil was purchased from Inevita SK (Bratislava, Slovakia). The essential oil was stored at 4 °C before use.

2.2. Fungal strains

The antifungal activity of *Lonicera caprifolium* essential oil (LCEO) was evaluated against a range of microscopic filamentous fungi *Monilia fructigena* CCM F-300, *Fusarium solani* CCM 8014, *Botrytis cinerea* CCM F-314, and *Trichoderma harzianum* CCM F-470**.** All fungal strains were obtained from the Czech Collection of Microorganisms in Brno, Czech Republic. Bacterial inocula were cultured in Sabouraud Dextrose broth (SDB, Oxoid, Basingstoke, UK) for 5 days at 25 °C before analysis. The optical density of the inocula was adjusted to 0.5 McFarland standard on the day of the experiment.

2.3. Disc diffusion method

In an effort to assess the antimicrobial activity, we opted for the disc diffusion method, a technique that we have previously outlined. We proceeded with the preparation of small discs (6 mm in diameter) that were saturated with 100 % concentration of LCEO and placed them on Sabouraud Dextrose agar (SDA) for the fungal strains. The strains were then incubated at 25 °C for a duration of 48 hours. To conclude the process, we measured the inhibition zones in mm. The blank discs were used as negative controls, while the antibiotic discs (fluconazol from Oxoid, Basingstoke, UK) served as positive controls [12].

2.3. In situ antifungal activity

The present study set out to assess the *in situ* antifungal activity of LCEO. A range of substrates were tested for this purpose, and these included commercial apricot and radish, as well as specific microscopic filamentous fungi. The substrates were sliced into pieces measuring 0.5 mm, thoroughly cleaned, and placed in 60 mm Petri dishes. These dishes had previously been inoculated with microscopic filamentous fungi samples. LCEO was dispersed in ethyl acetate at concentrations of 500, 250, 125, and 62.5 μg/L. Ethyl acetate filter sheets served as controls. The plates were hermetically sealed and then placed within an incubator set at 25 °C for a period of seven days in triplicate. Assessment of fungal colony growth was facilitated using the ImageJ software to calculate fungal volume densities. This was undertaken alongside standard methods for measuring *in situ* colony development [12].

2.4. Insecticidal activity

The model organism, *Megabruchidius dorsalis* Fahreus, 1839, was used to evaluate the insecticidal activity of LCEO. *M. dorsalis* is a granivorous insect and we decided to test LCEO for insecticidal activity against this insect species. Each Petri plate was lined with sterile filter paper, and groups of fifty *M. dorsalis* insects were put within. The LCEO was diluted with a 0.1 % polysorbate solution to create a range of concentrations, including 100 %, 50 %, 25 %, 12.5 %, 6.25 %, and 3.12 %. 100 μL of each LCEO concentration was added to sterile filter paper discs, and the plates were then covered with parafilm and allowed to sit at room temperature for a full day. A volume of 100 μL of the 0.1 % polysorbate solution was given to the control group. Insects were counted both alive and dead after a whole day. In three different investigations, this experimental process has been successfully repeated.

3. Results and discussion

The best antifungal activity of LCEO (Figure 1) was found against *Botrytis cinerea* (9.67 mm). The lowest antifungal was found against *Trichoderma harzianum* (7.33%). *In situ* analyses show the best antifungal activity in lower concentration 62.5 µg/mL on both models against *Monilia fructigena* (Figure 2 and 3).

In the literature available to us, LCEO has not been tested against these types of phytopathogenic microorganisms as it was in our study.

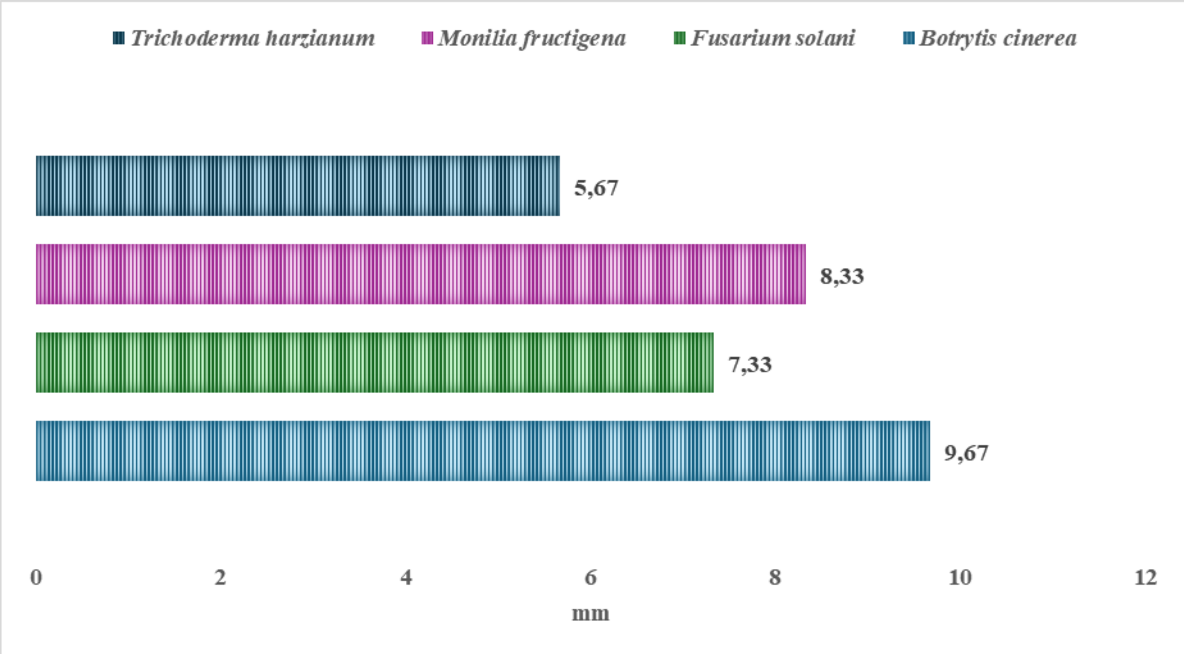

Figure 1. Antifungal activity of LCEO with disc diffusion method in mm

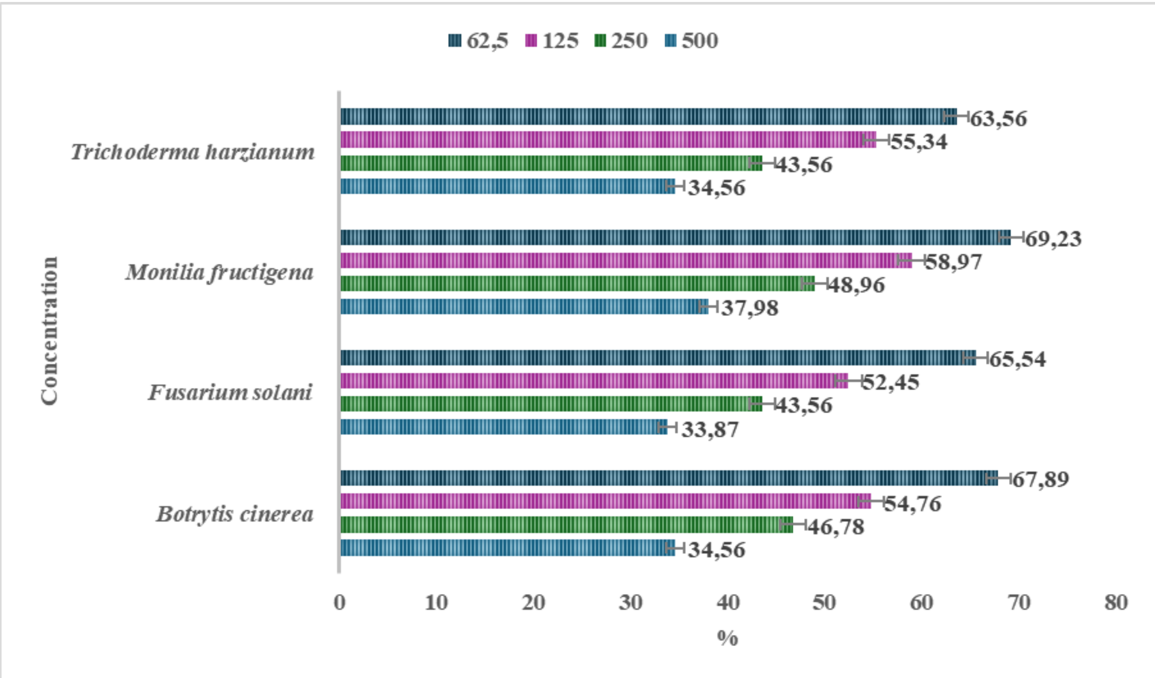

Figure 2. Antifungal activity of LCEO *in situ* on apricot model in %

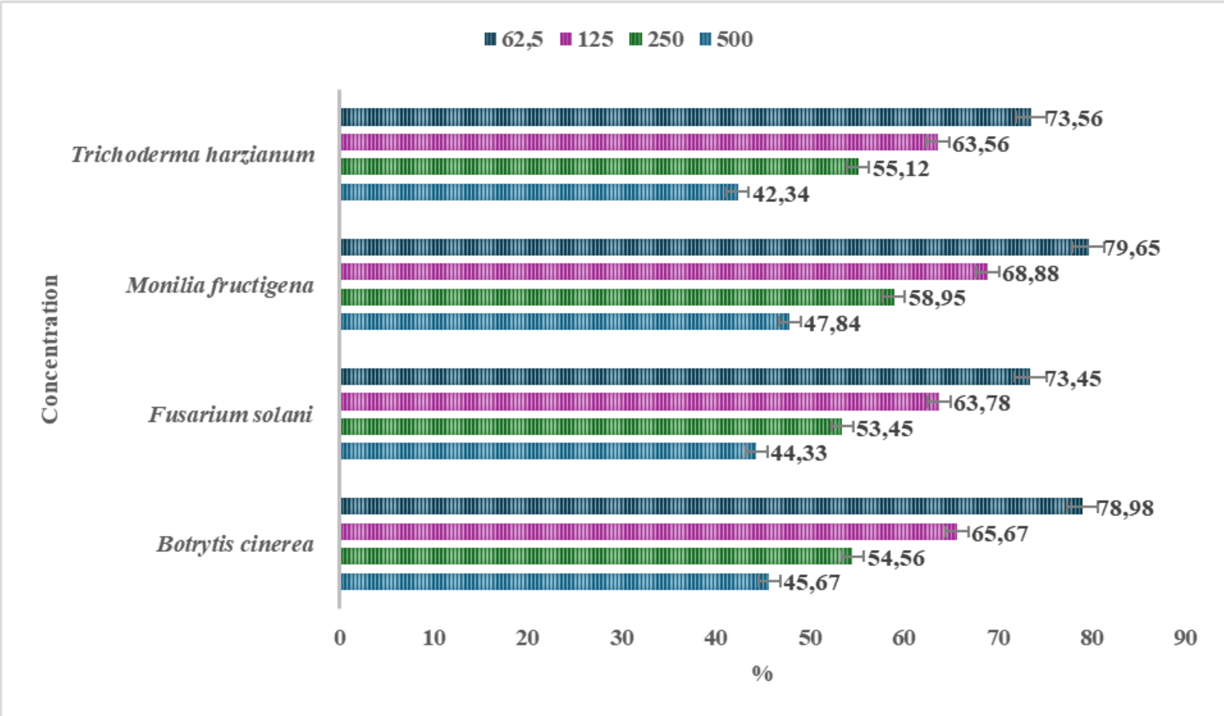

Figure 3. Antifungal activity of LCEO *in situ* on radish model in %

The present study examined the insecticidal efficacy of LCEO against *M. dorsalis*, as illustrated in Table 1. The results of the study demonstrated that the highest levels of insecticidal activity were observed in trials where 25 %, 50 % and 100 % of the LCEO solution was applied. Nevertheless, concentrations of 6.25 % and 3.125 % of the LCEO did not demonstrate a repellent effect against *M. dorsalis*. Of interest is the observation that the *M. dorsalis* population (50 %) was impacted by a concentration of 12.5 %. In contrast, the data demonstrate that 25 % of the insects were effectively killed by that concentration.

Table 1. Insecticidal activity of LCEO against *Megabruchidius dorsalis* (n=50)

Concentration (%)	Number of Living Individuals	Number of Dead Individuals	Insecticidal Activity (%)
100	0	100	100.00 ± 0.00
50	30	70	70.00 ± 0.00
25	40	60	60.00 ± 0.00
12.5	50	50	50.00 ± 0.00
6.25	80	20	20.00 ± 0.00
3.125	100	0	0.00 ± 0.00
Control group	100	0	0.00 ± 0.00

In the study by Rahman and Kang [13], the essential oil of *L. japonica* exhibited a significant antidermatophytic effect against all the tested human infectious fungal pathogens, with the exception of *T. mentagrophytes* KCTC 6085. The antidermatophytic activity of *L. japonica* essential oil is attributable to the presence of certain major components, including trans-nerolidol, caryophyllene oxide, linalool, p-cymene, hexadecanoic acid, eugenol, geraniol, trans-linalooloxide, globulol, pentadecanoic acid, veridiflorol, benzyl alcohol and phenyl ethyl alcohol. A significant number of essential oils and their constituents have been shown to possess antifungal properties. However, the high cost of production and the relatively low concentrations of active principles frequently hinder their direct utilisation in the management of fungal diseases in animals and plants. Notwithstanding this limitation, chemical investigation of antifungal compounds present in essential oils is considered important because of the possibility of synthesising these compounds or their analogues, which may be used in the control of fungal diseases [14].

Yang et al. [15] identified a novel monoterpene compound, designated 7-acetyl-8,9-dihydroxy thymol, and a known compound, 7,8-dihydroxy-9-butyryl thymol, from the dried flower buds of *Lonicera japonica* Thunb. The antibacterial properties of these compounds were tested using standard bacteriological methods against the following strains of microorganisms: *Staphylococcus aureus, Escherichia coli, Micrococcus luteus*, and *Bacillus cereus*. It is noteworthy that both compounds exhibited substantial antibacterial activity.

The findings of Muturi et al. [18] indicate the possibility of utilising *L. caprifolium* essential oil and certain of its derivatives as mosquito larvicides. However, further studies are required before the oil and its fractions can be recommended for use in integrated vector management. These studies must establish its metabolic target and effect on mammals and other non-target organisms. Further studies are required to develop formulations that exhibit enhanced persistence, given the chemical instability of essential oils and their susceptibility to oxidative deterioration when exposed to oxygen, light, moisture, and heat.

4. Conclusions

In conclusion, the floral essential oil of *L. caprifolium* exhibited antifungal properties, thus providing a potential natural remedy for fungal infections. Consequently, the investigation of the effects of essential oil of *L. caprifolium* against other significant fungi could provide a new approach to develop antifungal agents that would control severe fungal infections in humans. Therefore, *L. caprifolium* EO has the potential to serve as an alternative to synthetic fungicides in the pharmaceutical and food industries. Furthermore, it could be utilised for the screening and development of novel types of selective and natural fungicides, which could be employed to treat superficial fungal infections in plants.

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