









## Effect of Lime Species and Drying Process on Antioxidant and Antifungal Activities of Lime Essential Oils (*Citrus sp.*) in Vietnam

Vu Thi Thanh Dao <sup>1,2,3</sup>, Tuty Anggraini <sup>4</sup>, Tran Nghia Khang <sup>1,3</sup>, Ly Thi Thanh Thao <sup>1,2</sup>, Le Phan Hoai Ngoc <sup>1,2</sup> and Aswaldi Anwar <sup>5,\*</sup>

<sup>1</sup>An Giang University, Ho Chi Minh city, Vietnam

<sup>2</sup>Vietnam National University, Ho Chi Minh city, Vietnam

<sup>3</sup>Doctoral program in Agricultural Science, Faculty of Agriculture, Andalas University, Indonesia

<sup>4</sup>Faculty of Agriculture, Andalas University, Indonesia

<sup>5</sup>Faculty of Agricultural Technology, Andalas University, Indonesia

\*Corresponding author: [aswaldianwar@agr.unand.ac.id](mailto:aswaldianwar@agr.unand.ac.id)

### ABSTRACT

Essential oils of lime are now much more valuable than juice or fresh fruit due to their limited quantity and growing demand. Amid climate change, switching to lime cultivation offers greater economic efficiency. This study aimed to evaluate the effect of lime peel treatment on the extraction efficiency, composition, antioxidant, and antifungal activities of essential oils from four lime species (*Citrus sp.*) commonly grown in An Giang province, Vietnam, including *Citrus aurantifolia*, *Citrus latifolia*, *Citrus hystrix*, and *Citrus limonia*. The findings indicated that pre-distillation drying of the peel reduced the mass of raw material by 10%, thereby reducing solvent consumption, but also lowered essential oil yield. However, this treatment did not significantly affect limonene concentration or antioxidant and antifungal activities of the essential oil. Although the essential oil of *C. hystrix* produced the highest extraction yield, its limonene content was the lowest, resulting in the lowest antioxidant activity. The essential oil of *C. latifolia* yielded more extract than the other three species, exhibited the highest antioxidant activity (IC<sub>50</sub> range 134.64–144.31 µL/mL), and showed the most potent antifungal effect against *Fusarium equiseti*. There were no significant differences in antioxidant and antifungal activities between the essential oils of *C. aurantifolia* and *C. limonia*. Notably, the essential oil extracted from the fresh peel of *C. latifolia* possessed a pleasant aroma and received the highest score on the hedonic scale.

**Keywords:** Antioxidant, Antifungal effect, Essential oil, Lime species

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### INTRODUCTION

Phytopathogenic fungi can damage a wide range of crops, leading to reductions in the quality and quantity of agricultural products. Chemical fungicides are effective in controlling *Fusarium* species, including *F. exqu岸ite*, but excessive use can damage the soil, disrupt the balance of flora and fauna, induce resistance, and pollute the environment. Therefore, biological solutions are considered a safe solution to control fungal diseases (El-Morsy et al., 2023).

Lime, available in various types, is broadly categorized into sweet and acid limes, with the latter known for its

medicinal properties. Acid lime is a familiar fruit to most consumers in many countries. However, the primary use of lime is in juice and fruit juice products. Lime essential oil is exploited and traded mainly in the food, pharmaceutical, and cosmetic industry (Lota et al., 2002).

Essential oils are complex mixtures with concentrations varying from 20 to 60 natural compounds. Their composition will vary significantly depending on variety, season, geographical location, and fruit ripeness (Bora et al., 2020). The different biological activities of essential oils may be due to the characteristics of their chemical composition and lead to their different applications such as: a flavoring additive for beverage and

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confectionery products in the food industry, a masking agent for the unpleasant taste of drugs in the pharmaceutical industry, household appliances or perfume industry (Preedy, 2016; Kodagoda & Marapana, 2017); a good ingredient in aromatherapy, skin care and alternative healing methods or a natural preservative for foods due to its insecticidal, antibacterial and antifungal properties (Bora et al., 2020). Adding lime essential oil to edible films helps preserve food effectively due to its ability to inhibit the growth of bacteria that cause food poisoning, such as *E. coli*, *S. Typhimurium*, *B. cereus*, *S. aureus*, and *L. monocytogenes* (Preedy, 2016; Zanganeh et al., 2021). Besides, essential oils have potential applications in traditional medicine that are good for health as well as in disease prevention and treatment due to their properties, such as antioxidant and anti-inflammatory, immune system support, anti-obesity or anticancer (Liu et al., 2022). Lime essential oil resulted in decreased fasting blood glucose and low-density lipoprotein levels, while increasing hepatic glycogen concentration and high-density lipoprotein in hyperglycemic rats. Additionally, essential oil from *C. aurantifolia* demonstrated inhibitory effects on the proliferation of vascular smooth muscle cells (Brah et al., 2023).

Therefore, the industrial use of essential oils is considered a promising field with continued growth, requiring research into product safety and the development of new raw materials for pharmaceutical, aromatic food ingredients, and agricultural products. The essential oils extracted from lime peels are a diverse and dynamic field of research, allowing researchers to explore various aspects related to the properties and applications of lime peel essential oils. Although there have been some studies done on essential oils, including lime essential oil, some of the following problems still exist. Research has mainly focused on *C. aurantifolia* or *C. hystrix* cultivars, while there is little published information on *C. latifolia*, especially *C. limonia*, as well as other lime species. Due to its composition, essential oil from different lime species may have slightly different flavors and biological properties. Additionally, some types of lime may be more sustainable or easier to source than others, which may be something to consider for environmentally conscious consumers.

Several recent studies have demonstrated that pretreatment of raw materials prior to extraction by drying has a significant impact on the quality and chemical composition of essential oils. Abedi et al. (2020) showed that drying peppermint leaves at 45°C increased the yield of essential oil while limiting the loss of volatile compounds compared to drying at higher temperatures. Similarly, Türkmen et al. (2024) studied *Lavandula angustifolia* and found that the highest amount of essential oil was obtained when drying raw materials at 35°C, while drying at 65°C significantly reduced the yield and changed the main components of the essential oil.

The research evaluated the performance, composition, and properties of essential oils extracted from popular lemon species (*Citrus sp.*) grown in An Giang, Vietnam, in order to select the most suitable raw materials and processing methods of lemon peels for effective essential oil extraction. This contributes to increasing the added

value of agricultural products, such as lemon essential oil, helping to exploit this valuable source of raw materials effectively, and creating conditions for the development of the agricultural sector.

## MATERIALS & METHODS

### Material

Fresh limes are harvested from small farms in An Giang province, Vietnam, specifically *C. aurantifolia* (Cho Moi commune, Cho Moi district), *C. latifolia* (Binh Hoa commune, Chau Thanh district), *C. limonia* (Vinh Nhuan commune, Chau Thanh district), and *C. hystrix* (Ba Chuc commune, Tri Ton district).

### Experiment Design

The experiment was designed according to a randomized method with two factorial factors. It was conducted in three repetitions. The first factor is the lime species, including *C. aurantifolia*, *C. latifolia*, *C. limonia*, and *C. hystrix*. The second factor is the treatment, including fresh and dried samples.

After harvesting, the limes were packed in cartons to limit impact and transported immediately to the laboratory. The lime peels were separated from the fruit and dried at 40°C for 4 hours. Lime essential oil was extracted according to the method described by Lin et al. (2019) with minor modifications: the fresh or dried peel was then blended into 2cm and water was added for extraction (ratio 1:3 w/v). Hydro distillation was then performed for 3 hours by a Clevenger-type apparatus. The extracted essential oil was dehydrated with anhydrous sodium sulfate and filtered. The essential oil was stored at 4°C and protected from light until used.

### Analysis Method

**Essential oil yield (% v/w):** The yield of extraction was calculated according to the volume of essential oil and the mass of fresh material (Sandhu et al. 2021).

**Essential oil composition:** The chemical composition of the essential oil was analyzed using gas chromatography–mass spectrometry (GC–MS). Separation was performed on a DB-5MS capillary column (30m × 0.32mm i.d., film thickness 0.25µm). The injector temperature was maintained at 250°C and the injection was carried out in split mode (split ratio 20:1). Helium was employed as the carrier gas under linear velocity control at a pressure of 15.8kPa, with a total flow of 35.3mL/min and a column flow of 1.54mL/min. The oven temperature was programmed as follows: initial temperature 50°C (held for 2min), then increased at a rate of 10°C/min to 310°C and held for 10min, giving a total run time of 38min. The ion source and interface temperatures were set at 200 and 250°C, respectively. Mass spectra were acquired in electron impact (EI) mode, scanning over the range m/z 30–450 with a solvent delay of 3min. The components of the essential oil were identified by comparing their mass spectra with those available in the NIST14 mass spectral library.

**Antioxidant activity (IC<sub>50</sub>):** the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to determine the antioxidant activity of the samples as described by Sandhu

et al. (2021) with modifications. The essential oils samples were diluted at different concentrations in ethanol, namely 50, 100, 150, and 200 µL/mL for *C. aurantifolia*, *C. latifolia*, and *C. limonia*, while 100, 200, 300, and 400 µL/mL for *C. C. hystrix*. Then, 1mL of sample was immersed with 5mL of ethanol, and then 1mL of DPPH solution (0.5mM) was added. The solution was mixed well and incubated for 60min in the dark at room temperature. The whole experiment was performed in the dark. The absorbance of the incubated mixture was recorded using a UV-VIS spectrophotometer at 517 nm. The DPPH solution was used as a reference. Results of the DPPH were reported as percentage radical scavenging activity computed using the following equation:

$$\text{DPPH (\%)} = (\text{Control}_{\text{Abs}} - \text{Sample}_{\text{Abs}}) / (\text{Control}_{\text{Abs}}) \times 100$$

The  $\text{IC}_{50}$  values were used to determine the quality of the radical scavenging property.  $\text{IC}_{50}$  (concentration providing 50% inhibition) values were calculated from the percentage disappearance versus concentration plot.

### Fungal Isolation

Fungi were isolated according to the modified and supplemented procedure of Iqbal & Utera (2015): take 10g of infected/damaged fruit peel, cut into small pieces, and put into a conical flask containing 90mL of sterile distilled water at dilution level  $10^{-1}$ . Shake the conical flask until the sample is evenly dispersed. Dilute with distilled water to a concentration of  $10^{-5}$ . Pipetted 100 µL of each dilution into a petri dish containing PDA medium (Potato Dextrose Agar). Use a sterile glass rod to spread the liquid evenly over the surface of the agar. Incubate in an incubator at 30°C for 2-3 days. After 2-3 days, use a sterile round inoculating rod to spot the suspected pathogenic fungi on the previously inoculated agar plate and transfer to a new PDA agar plate (1 spot per plate), incubate the plate in an incubator at 30°C for 2-3 days, proceed sequentially until only one type of fungus appears on the petri dish. Observe fungal spores under a microscope, based on the morphological characteristics obtained, identify the fungus using molecular biology techniques (PCR) and gene sequencing with primer pairs ITS1: 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4: 5'-TCCTCCGCTTATTGATATGC-3' (White et al., 1990). PCR products were sent for sequencing at the Loci Institute for Molecular Biology Research, Ho Chi Minh City.

**Diameter of antifungal:** evaluation of antifungal activity by surface diffusion method on agar discs as described by Thompson (1989) with modifications. Add 20 µL of essential oil to a petri dish (8cm x 1.5cm), then pour 15mL of PDA medium (Potato Dextrose Agar), leave until the medium solidifies. Make a well in the middle of the dish with a diameter of 6mm. The pathogenic fungus is cultured on PDA medium after 7 days (maximum fungal growth stage), forming a mycelial ring with a diameter of 6mm at the edge of the fungal plate. Use a sterilized straight inoculation tube to transfer the mycelial ring to the well of the plate containing lemon essential oil. Incubate the mushroom plates at  $28 \pm 2^\circ\text{C}$ . Use a ruler with the most minor division of 1mm to measure the diameter of the colony every 24 hours, and record the results until the incubation time reaches 96 hours.

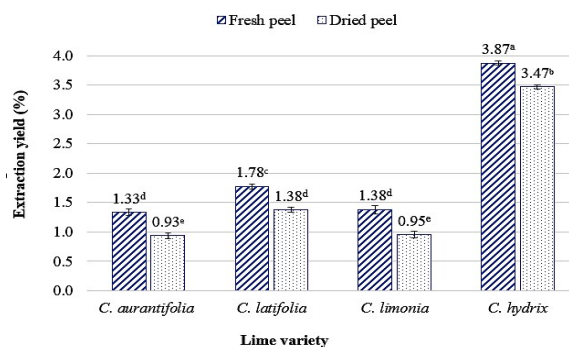
### Statistical Analysis

The evaluation data were analyzed using Microsoft Excel, and statistical analysis was performed using Portable Statgraphics Centurion software (version 15.2.11.0, USA).

## RESULTS & DISCUSSION

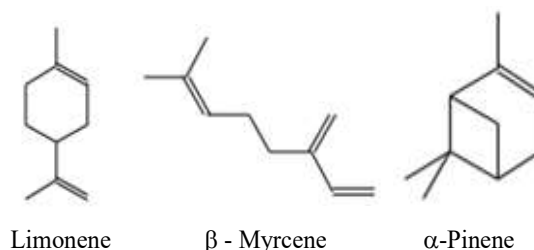
### Effect of Lime Species and Drying Process on the Extraction Efficiency of Peel Essential Oils

The essential oil extraction yield from fresh and dried peel of four lime species has been presented in Fig. 1. The results showed that *C. hystrix* had the highest extraction yield in both fresh and dried peel (3.87 and 3.47%), followed by the fresh form of *C. latifolia* (1.78%). The *C. aurantifolia* had the lowest extraction yield in both fresh and dried peel (1.33 and 0.93%) and was not statistically different from *C. limonia* (1.38% and 0.95%). Statistical analysis revealed that the extraction efficiency of essential oil from lime peel was significantly affected by lime species ( $P < 0.05$ ) and drying process before extraction ( $P < 0.05$ ), at a 5% significance level.



**Fig. 1:** Extraction efficiency of lime essential oil according to species and treatment. Mean values with the same superscript letter are not significantly different ( $P < 0.05$ ).

The yield and composition of essential oils vary greatly depending on many factors such as variety, season, geographical location, fruit ripeness, and extraction method (Bora et al., 2020). Although the drying process reduces the mass of raw materials, thereby reducing the amount of solvent used, it causes some essential oil components to evaporate, resulting in a slight decrease in extraction efficiency. According to Thamkaew et al. (2021), drying can significantly reduce the essential oil content of many materials even when they are air-dried at room temperature. The essential oil content of most herbs decreases, especially at drying temperatures above 60°C.



**Fig. 2:** Structure of some important compounds in essential oils of lime species.

**Table 1:** Composition of lime essential oil according to species and treatment

N <sup>o</sup>	Compound	Concentration (%)							
		<i>C. aurantifolia</i>		<i>C. latifolia</i>		<i>C. limonia</i>		<i>C. hystrix</i>	
		Fresh	Dried	Fresh	Dried	Fresh	Dried	Fresh	Dried
1	Limonene	60.73	60.78	47.65	45.61	64.56	63.44	18.65	18.85
2	β - Myrcene	2.84	3.16	2.15	2.36	2.92	2.95	1.70	1.93
3	α-Pinene	2.48	3.01	2.68	2.92	2.55	2.74	3.54	3.89
4	β -Pinene	6.90	-	13.89	14.44	-	-	30.13	-
5	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	15.11	15.53	-	13.14	13.49	13.77	(0.72)	(0.45)
6	3-Carene	-	-	13.18	-	-	-	-	-
7	Bicyclo[2.2.1]heptane, 7,7-dimethyl-2-methylene-	-	-	-	-	-	-	-	27.02
8	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	-	-	-	-	-	-	20.20	-
9	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	-	7.33	-	-	6.56	6.96	-	-
10	Cyclopentene, 3-isopropenyl-5,5-dimethyl-	-	-	-	-	-	-	-	20.16
11	α-Phellandrene	1.60	1.78	2.60	3.19	1.53	1.76	-	-
12	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	-	-	-	2.65	-	-	-	-
13	2,6-Octadienal, 3,7-dimethyl-, (E)-	-	-	-	2.42	(0.10)	-	-	(0.05)
14	Benzene, 1-methyl-2-(1-methylethyl)-	-	2.32	-	2.27	2.12	1.76	(0.27)	(0.14)
15	2,6-Octadienal, 3,7-dimethyl-, (Z)-	(0.14)	(0.11)	-	1.85	(0.09)	(0.09)	-	-
16	3-Cyclohexene-1-methanol, alpha.,alpha.4-trimethyl-	1.20	0.81	-	(1.01)	0.79	0.72	1.62	1.79
17	(+)-4-Carene	-	-	1.20	(0.82)	0.77	0.78	0.24	0.18
18	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	-	0.91	-	(0.74)	0.72	0.76	0.28	0.30
19	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	(0.59)	(0.33)	-	(0.57)	(0.37)	(0.27)	2.15	1.39
20	1,6-Octadien-3-ol, 3,7-dimethyl-	(0.44)	(0.28)	-	(0.48)	(0.28)	(0.26)	1.13	1.28
21	6-Octenal, 3,7-dimethyl-, (R)-	0.70	0.64	-	(0.05)	(0.48)	(0.55)	12.24	16.16
22	(E)-Citral	-	-	1.96	-	-	-	-	-
23	α-Phellandrene	0.73	-	-	-	-	-	-	-
24	6-Octen-1-ol, 3,7-dimethyl-	(0.49)	(0.23)	-	-	(0.31)	(0.19)	2.55	2.28
25	β-Citral	-	-	1.51	-	-	-	-	-
26	Cymene	2.19	-	-	-	-	-	-	-
27	Neryl acetate	-	-	2.74	-	-	-	-	-
28	Nonanal	(0.04)	(0.03)	1.79	-	(0.04)	(0.03)	-	(0.06)
	Total	96.18	97.28	91.34	94.53	97.69	97.03	95.43	95.94
	Number of compounds	32	29	29	35	29	34	28	28

Values in parentheses do not belong to the top 10 highest concentrations in each essential oil. (-): means not detected.

### Effect of Lime Species and Drying Process on Compositions of Peel Essential Oils

Table 1 shows only the top 10 highest concentrations in each essential oil extracted from the dried or fresh peels of the four lime species studied. Gas chromatography-mass spectrometry (GC-MS) analysis showed that the number of compounds in the essential oils extracted from fresh and dried lime peels of *C. aurantifolia* was 32 and 29 compounds, respectively. Meanwhile, the essential oils extracted from dried lime peels of *C. latifolia* and *C. hystrix* contained more compounds, namely 35 and 34 compounds, compared to 29 compounds in the essential oil from fresh lime peel. The number of compounds in the essential oil of *C. limonia* was 28, including the extracts from fresh or dried peels.

Limonene was the most abundant compound in the essential oils extracted from the fresh and dried peels of *C. aurantifolia* (60.73 and 60.78%), *C. limonia* (64.56 and 63.44%), and *C. latifolia* (47.65 and 45.61%). This was followed by 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- in the essential oils of *C. aurantifolia* (15.11 and 5.53%) and *C. limonia* (13.49 and 13.77%), and α-Pinene in the essential oils of *C. latifolia* (13.89 and 14.44%). Although the essential oil from *C. hystrix* contains Limonene, this compound is second only in proportion to α-Pinene in fresh peel (30.13%) or Bicyclo [2.2.1] heptane, 7,7-dimethyl-2-methylene- in dried peel (27.02%). It should be noted that the content of volatile substances in essential oils depends on the geographical origin of the fruit (Lubinska-Szczygeł et al., 2023). Although Yuniati et al. (2024) noted that the drying process had a minor impact on some of the chemical components of the

essential oil extracted from sweet orange peels, and thus resulted in little change in chemical composition. However, the results of the present study showed that pre-drying did not significantly alter the proportions of the most important compounds in the essential oils of lime species, except for *C. hystrix*.

The components found in all the essential oil samples studied were limonene, β-myrcene, and α-pinene (Fig. 2), in which the ratio of β-myrcene and α-pinene in the samples did not differ much. According to the report by Bora et al. (2020), the concentration of limonene in lime essential oil varies from 32 to 98%, depending on the variety, and exhibits many important biological activities, including inhibiting carcinogenic properties and preventing cancer.

### Effect of Lime Species and Drying Process on Antioxidant Activity (IC<sub>50</sub>) of Peel Essential Oils

The IC<sub>50</sub> (Inhibitory concentration 50%) value represents the overall antioxidant capacity of lime essential oil by measuring DPPH. The higher the antioxidant activity of the essential oil, the lower the IC<sub>50</sub> value and vice versa. The results presented in Table 2 show that the IC<sub>50</sub> values of the studied essential oils ranged from 134.64-343.06 μL/mL. The essential oil from *C. latifolia* had the lowest IC<sub>50</sub> value corresponding to the strongest antioxidant capacity, followed by *C. aurantifolia* and *C. limonia*, while *C. hystrix* had the highest value. In addition, there was no statistically significant difference in the IC<sub>50</sub> values of the essential oils extracted from fresh and dried peels ( $P > 0.05$ ). However, the value determined from dried peels was slightly higher than that from fresh peels of the

same species. Yang & Park (2025) studied the composition and antioxidant capacity of essential oils from 21 citrus cultivars. The results showed that essential oils from all studied species could eliminate DPPH free radicals, as shown by IC<sub>50</sub> values ranging from 86.17 to 3025.67mg/mL. However, the antioxidant capacity of essential oils varied by species and was lower than the activity of ascorbic acid.

**Table 2:** IC50 value of lime essential oil according to species and treatment

	Treatment		Average	P value
	Fresh peel	Dried peel		
Species				
<i>C. aurantifolia</i>	169.95±10.22	166.69±2.15	168.32 <sup>b</sup>	0.0000
<i>C. latifolia</i>	134.64±4.48	144.31±7.73	139.48 <sup>a</sup>	
<i>C. limonia</i>	166.66±8.40	167.04±7.50	166.85 <sup>b</sup>	
<i>C. hystrix</i>	343.06±11.97	319.38±15.10	331.22 <sup>c</sup>	
Average	203.58 <sup>A</sup>	199.36 <sup>A</sup>		
P value	0.3552			

Values (Mean±SD) with the same small letters in a column or the same capital letters in a row did not differ significantly (P<0.05)

Olatunya and Akintayo (2017) reported that peel drying can increase some essential oil components and has an impact on the antioxidant activity of essential oils obtained from *C. acida* (lemon), *C. mandarin* (grape) and *C. reticulata* (tangerine) peels. Specifically, *C. acida* peel essential oil had the lowest IC<sub>50</sub> value (101.10g/mL) and fresh *C. mandarin* had the highest value (387.60g/mL). Limonene, considered the main component of orange peel essential oil, has an antioxidant potential equivalent to that of a potent antioxidant. The higher free radical scavenging and DPPH discoloration ability of fresh lime essential oil may be due to some other unsaturated terpenes present.

Gangwar et al. (2024) showed that the drying process had a significant impact on the antioxidant capacity of the essential oil from *Hedychium spicatum* rhizomes, as shown by the change in IC<sub>50</sub> value, with the essential oil from material dried at 30°C in an oven giving the lowest IC<sub>50</sub>, demonstrating higher antioxidant capacity than other drying methods. This is because different drying methods can alter the chemical composition of essential oils, thereby affecting their bioactivity. However, the results of the present study did not show a statistically significant difference in the IC<sub>50</sub> values of essential oils from dried and undried materials. Thus, essential oils from three lime species, *C. latifolia*, *C. aurantifolia*, and *C. limonia*, all have good antioxidant activity and therefore can be applied in many fields such as food, pharmaceuticals, and cosmetics.

### Effect of Lime Species and Drying Process on Antifungal Activity of Peel Essential Oils

Symptomatic papaya fruit samples were analyzed to isolate the fungal species. *Fusarium equiseti* mycelia are white; the mycelia proliferate and form a thin mycelial network on the surface of the medium. The growth rate is 90mM after 10 days of incubation. Microscopic observation shows that the mycelia are smooth, branched, cylindrical, and septate. The species produces asexual spores that are rhomboid, pointed at the tip, and white. The spores are cylindrical, short, single, and septate. The spores are oval, hyaline, and septate, 0–1. The macroconidia are curved with tapered and elongated

apical cells. These findings are consistent with the preliminary description of *F. equiseti* by Hami et al. (2021). The identification results indicate that the identified fungus exhibits 100% sequence similarity to *F. equiseti*.

The identification results confirmed that the identified fungus is *F. equiseti*. According to some reports, *F. equiseti* can damage melon (*Cucumis melo*), soybean (*Glycine max*), dill (*Cuminum cyminum*), cauliflower (*Brassica oleracea*), winter rapeseed (*Brassica napus*), tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*), cabbage (*Brassica oleracea*), and squash (*Cucurbita pepo* L.) (El Hazzat et al., 2022). In addition, some strains of *F. equiseti* can produce mycotoxins such as zearalenone, which are often found in combination with toxins such as trichothecenes and fumonisins, causing life-threatening effects (El-Morsy et al., 2023).

Tropical fruit *Fusarium* rot can also endanger human health, as numerous *Fusarium* species are known to act as mycotoxin "factories" under suitable conditions, leading to contamination in processed foods. *F. equiseti* is a fungal species belonging to the *Fusarium* genus, responsible for causing *Fusarium* wilt disease in various crops and agricultural commodities. This fungus can significantly impact agricultural yield and the quality of produce. *F. equiseti* is classified as a secondary invasive species in crops, emerging after soil contamination. Its longevity is attributed to its capacity to produce a large number of resilient chlamydospores (Batt & Tortorello, 2014). *Fusarium* species typically form colonies that are woolly, cotton-like, flat, or fluffy in appearance and spread widely. The colony's color varies, with the top ranging from white to purple and the bottom exhibiting shades from tan to other hues (Nelson et al., 1994). A 2023 study explored the combined use of chitosan nanoparticles and *Trichoderma longibrachiatum* and *Penicillium polonicum* for controlling *F. equiseti*. The results indicated enhanced antifungal activity, suggesting a potential integrated approach for managing *F. equiseti*-related diseases (El-Morsy et al., 2023). Adnani et al. (2024) demonstrated that seed treatment with *Trichoderma asperellum* effectively controlled *F. equiseti* in chickpea, promoting plant growth and reducing disease incidence. De Souza Marques et al. (2024) study investigated the physiological and histopathological effects of *F. equiseti* on cotton plants. The findings revealed alterations in leaf physiology and histology, contributing to understanding the pathogen's impact on cotton. *F. equiseti* was identified as the causal agent of root rot in *Peganum harmala* (Syrian rue) in China. This report expands the known host range of the pathogen (Jia et al., 2025).

*Fusarium* is generally regarded as a weak pathogen that necessitates a stressing factor or some form of natural or artificial injury to thrive (Zakaria et al., 2013). Additionally, it is well-known as a secondary invader, indicating that its presence in lesions is crucial for the infection of papaya fruits (Zakaria et al., 2022). The spores typically disperse through water, wind, soil, pests, and human activities to infect new hosts (Dita et al., 2018). At first, papaya fruit infected by the *Fusarium* species



**Fig. 3:** Colony and spore morphology of *Fusarium equiseti* pathogenic on Papaya.

Descriptions	Graphic Summary	Alignments	Taxonomy						
Sequences producing significant alignments				Download	Select columns	Show	100		
<input checked="" type="checkbox"/> select all 100 sequences selected				GenBank	Graphics	Distance tree of results	MSA Viewer		
	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per Ident	Acc. Len	Accession
<input checked="" type="checkbox"/>	<a href="#">Fusarium equiseti</a> isolate FE/TP/UK/01/2020 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S rRNA gene, partial sequence; internal transcribed spacer 2, 5.8S rRNA gene, partial sequence	<a href="#">Fusarium equiseti</a>	989	989	100%	0.0	100.00%	548	<a href="#">MT507512.1</a>
<input checked="" type="checkbox"/>	<a href="#">Fusarium equiseti</a> isolate MO157 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S rRNA gene, partial sequence; internal transcribed spacer 2, 5.8S rRNA gene, partial sequence	<a href="#">Fusarium equiseti</a>	989	989	100%	0.0	100.00%	545	<a href="#">KX197555.1</a>

**Fig. 4:** Fungal sequences result (tested by Loci Institute for Molecular Biology Research, HCM city).

develops a small, water-soaked spot, which is then followed by a depressed area on the fruit. During the advanced stage of infection, a dense, white mycelial mat will develop in the decayed area (Rahman et al., 2008). *Fusarium* infections affecting the roots and stems of Papaya have been previously documented in India (Gupta et al., 2019) as well as in Brazil (Correia et al., 2013).

*Fusarium equiseti* is among the newly identified pathogens and is likely a significant plant pathogen affecting leafy vegetables, such as both wild and cultivated lettuce and arugula (Gilardi et al., 2018a; Gullino et al., 2019). This particular soil-borne fungus thrives as a saprophyte in soil or decaying plant matter, which enables it to easily adapt to intensive agricultural practices (Gilardi et al., 2018b; Gullino et al., 2019). *F. equiseti* can produce a species of mycotoxins, thereby presenting a possible health risk to both humans and animals (Munkvold et al., 2021).

The essential oil extracted from 4 lime species in An Giang, including *C. aurantifolia*, *C. latifolia*, *C. limonia*, and *C. hystrix*, was evaluated for its resistance to *F. equiseti* with the disc diffusion technique (Fig. 3 and Fig. 4).

The results showed that the four essential oils, including *C. aurantifolia*, *C. latifolia*, *C. limonia*, and *C. hystrix*, all exhibited antifungal activities against *Fusarium equiseti* (Table 3; Fig. 5). *C. latifolia* has the highest antifungal activity against *F. equiseti*. With *F. equiseti*, *C. latifolia* essential oil (fresh peel and dried peel) inhibited fungal growth (i.e., the fungus grew poorly on agar plates) more strongly than the other three essential oils. Each lemon species has different chemical compositions in its essential oils, and these compounds may have strong antifungal or antibacterial properties. Lemon essential oils contain many compounds, such as limonene and other terpenes. These compounds may have antifungal effects by interfering with the cell structure of fungi, reducing their growth, or killing them. *C. latifolia* contains high concentrations of compounds with potent antifungal activity, so it will be able to inhibit the growth of *F. equiseti* better than the control sample and other lemon species. The chemical makeup of essential oils determines their

antifungal efficacy (Burt, 2004). The antifungal effects of various essential oils are regulated by several mechanisms rather than just one because of the quantitative and compositional variances between them (Burt, 2004). Their hydrophobic qualities are the primary mechanism; they can attack and damage the cell membrane or interfere with the enzyme system, resulting in respiratory failure and ultimately cell death (Noshirvani et al., 2017).

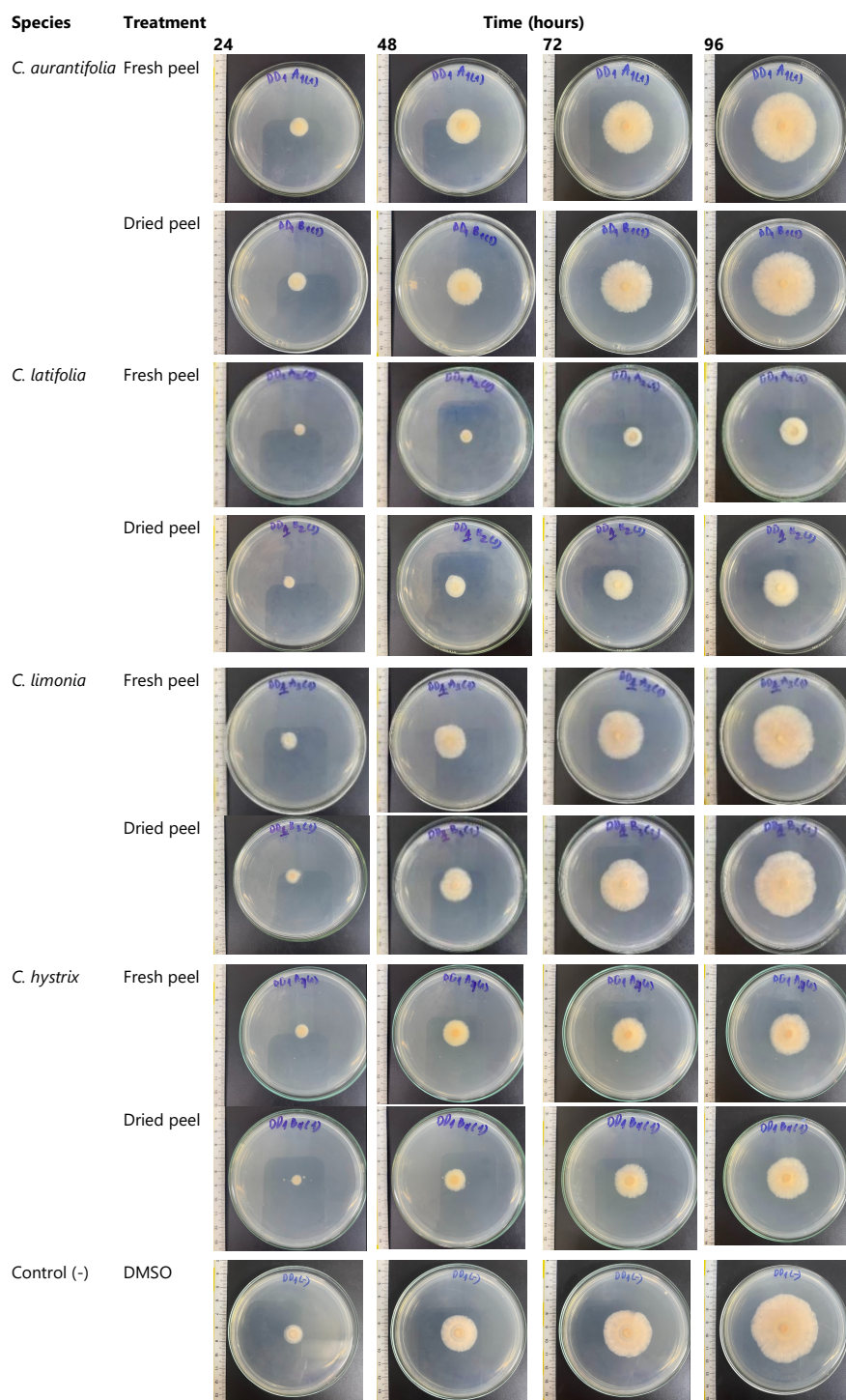
**Table 3:** Antifungal diameter on *Fusarium equiseti* of lime essential oil according to species and treatment

Species	Treatment method	Time (hours)			
		24	48	72	96
<i>C. aurantifolia</i>	Fresh peel	1.07±0.06 <sup>b</sup>	2.07±0.12 <sup>d</sup>	3.03±0.15 <sup>c</sup>	4.00±0.20 <sup>c</sup>
	Dried peel	0.93±0.06 <sup>b</sup>	1.90±0.10 <sup>c</sup>	3.00±0.30 <sup>c</sup>	3.77±0.21 <sup>c</sup>
<i>C. latifolia</i>	Fresh peel	0.63±0.06 <sup>a</sup>	1.07±0.06 <sup>a</sup>	1.73±0.12 <sup>a</sup>	2.37±0.21 <sup>a</sup>
	Dried peel	0.67±0.03 <sup>ab</sup>	1.13±0.06 <sup>a</sup>	1.63±0.15 <sup>a</sup>	2.37±0.12 <sup>a</sup>
<i>C. limonia</i>	Fresh peel	0.93±0.06 <sup>c</sup>	1.77±0.12 <sup>c</sup>	2.87±0.23 <sup>c</sup>	3.80±0.35 <sup>c</sup>
	Dried peel	1.0±0.10 <sup>cd</sup>	2.07±0.12 <sup>d</sup>	3.17±0.15 <sup>c</sup>	4.07±0.12 <sup>c</sup>
<i>C. hystrix</i>	Fresh peel	0.77±0.06 <sup>b</sup>	1.37±0.06 <sup>b</sup>	1.93±0.15 <sup>a</sup>	2.90±0.10 <sup>b</sup>
	Dried peel	0.67±0.06 <sup>ab</sup>	1.37±0.06 <sup>b</sup>	2.30±0.10 <sup>b</sup>	3.07±0.21 <sup>b</sup>
Control (-)	DMSO	1.33±0.06	2.47±0.06	3.30±0.18	4.27±0.15
P Value		0.0000	0.0000	0.0000	0.0000

Values (Mean±SD) with the same superscript letter in each column did not differ significantly (P<0.05)

Zhao et al. (2022) investigated the antifungal activity of lemon leaf extract (LLE) against *Penicillium digitatum* (a fungal pathogen). The findings revealed that LLE treatment led to an increase in cell membrane permeability, resulting in the leakage of intracellular contents. Also led to decreased levels of adenosine triphosphate (ATP), indicating disrupted energy metabolism. Moreover, altered morphological changes in fungal spores, such as sunken surfaces and malformations, were also reported. Jian et al. (2023) explored the combination of limonene (a significant component of lemon essential oil) with triazole fungicides against *F. graminearum*. The results indicated that limonene enhanced the antifungal activity of the fungicides. The combination exhibited synergistic effects, improving the efficacy of the treatment. While this study focused on a different *Fusarium* species, it highlights the potential of lemon-derived compounds in enhancing





**Fig. 5:** Antifungal properties of lime essential oils on *Fusarium equiseti*.

antifungal treatments. Gharzouli et al. (2024) investigated the antifungal properties of nanoemulsified essential oils, including those from lemon. The findings suggested that Nanoemulsification improved the stability and dispersion of essential oils. The enhanced formulation exhibited potent antifungal activity against various fungal pathogens. This approach underscores the potential of lemon essential oil in nanotechnology-based antifungal applications. Mirza et al. (2023) study evaluated the antimicrobial efficacy of *Citrus limon* leaf and peel extracts against various pathogens, including *Fusarium oxysporum*.

The findings indicated that both leaf and peel extracts exhibited antimicrobial activity, with the leaf extract showing a maximum zone of inhibition of around 40% when methanol was used as a solvent. While this study focused on *F. oxysporum*, it suggests that *C. limon* leaf extracts may possess antifungal properties against *Fusarium* species. Uwineza et al. (2022) explored the effects of lemon balm (*Melissa officinalis*) extracts on the growth and mycotoxin biosynthesis of *Fusarium* species, including *F. culmorum* and *F. proliferatum*. The findings revealed that the extracts exhibited a concentration-

dependent inhibitory effect on mycelium growth and suppressed the biosynthesis of mycotoxins. While this study did not focus on *F. equiseti*, it suggests that lemon-derived extracts may possess antifungal properties against *Fusarium* species.

The study by Ahmed et al. (2022) tested five citrus peel extracts, including *Citrus aurantifolia* (lemon), *C. sinensis* (orange), *C. maxima* (grapefruit), *C. limon* (passion fruit), and *C. reticulata* (tangerine), for antifungal activity. The results showed that grapefruit peel extract (PPE) was the most effective antifungal agent and recorded the highest inhibition diameter against *F. equiseti*, followed by orange peel extract (OPE) and lemon peel extract (LPE). In contrast, the lowest antifungal activity was recorded in mandarin peel extract (MOPE) and lime peel extract (KPE), respectively. The inhibition diameter representing the antifungal activity of the other extracts ranged from 0 to 25mm, making a significant difference between them and (PPE). GC-MS analyzed the most active extract (PPE), and the results included the detection of 50 different compounds. According to the GC-MS results, furfural (4.91%), auroptenol (1.38%), 2-methoxy-4-vinylphenol (0.92%) and hexadecanoic acid-methyl ester (0.80%) were the main components in the essential oil obtained from grapefruit peel extract (PPE), in addition to ethyl oleate (0.65%), alpha-terpineol (0.32%), osthole (0.31%), which are known to be antifungal compounds.

*Citrus sinensis* plant essential oils (PEOs) were evaluated for their ability to inhibit *F. oxysporum*. MFC was evaluated at four concentrations (25, 50, 75, and 100µL/mL), whereas MIC was evaluated at two values (25 and 50µL/mL). For both concentrations, *C. sinensis* displayed the biggest inhibition zone (47.5 and 46.3m<sup>2</sup>). The lowest illness incidence and severity were observed in treatments using *C. sinensis* PEO (Yousafi et al., 2022). The peel extracts prevented *P. ultimum* and *F. solani* from growing mycelia. Orange and lemon oils were shown to be highly vulnerable to both post-harvest infections. *Citrus* peels are abundant in a variety of phytochemicals and bioactive substances, according to the current study. They also showed encouraging promise in preventing the proliferation of these harmful fungi (Hajji-Hedfi et al., 2022). The application of *E. camaldulensis* and *C. sinensis* essential oils at 50µL/mL showed intense action against *F. culmorum*, with 100%, whereas *E. camaldulensis*/*C. sinensis* (50µL/mL) had a fungal mycelial growth inhibition (FMGI) value of 65.66%. According to the findings, EOs and their mixtures from *E. camaldulensis*, *C. aurantium*, and *C. sinensis* can be used as a biofungicide to combat mold (Elgat et al., 2020).

The pathogen was isolated from wilted bitter melon stems and was confirmed as *F. equiseti* morphologically by the presence of large triseptate, sickle-shaped conidia and small spherical conidia. Among the essential oils tested, clove essential oil showed the highest inhibitory activity against mycelial growth (100%) at 0.5% concentration, followed by peppermint, wintergreen, and tea tree essential oils. Compared to neem essential oil, neem essential oil showed the least inhibitory activity (15.54%). GC-MS analysis of clove essential oil revealed 38 compounds, of which eugenol was the predominant

compound responsible for the antifungal activity (Ragul et al., 2024).

In addition, the extraction method can significantly influence the quality and composition of the resulting essential oil. If the steam distillation method of extraction is performed optimally, the active compounds in both the fresh and dried peel can be extracted relatively efficiently. This can result in essential oils from both sources having quite similar chemical compositions, resulting in no significant difference in antifungal activity.

The essential oils from the fresh and dried peels of the studied lime species are shown in Fig. 6. All samples obtained were transparent, colorless, and had a pungent, pleasant, and aromatic odor. Sensorially, the essential oils were quite similar except for the apparent difference in odor. The odor of the extracted essential oils varied significantly depending on the lime species, and the drying process prior to extraction affected the odor of the essential oils. Therefore, the essential oil extracted from the fresh peel of *C. latifolia* had a pleasant aroma and scored highest on the Hedonic scale compared to the other essential oil samples in this study.



Fig. 6: The essential oil extracted from 4 lime species in An Giang, Vietnam.

## Conclusion

It was concluded from the studies that pre-distillation drying of the peel reduced the mass of the raw material (10%), thus reducing the amount of solvent used but decreasing the yield of essential oil. However, this treatment did not significantly affect the limonene content, antioxidant, and antifungal activity of the essential oil. Although *C. hystrix* essential oil had the highest extract yield, the Limonene content was the lowest, and therefore had the lowest antioxidant activity. The *C. latifolia* essential oil had a higher extract yield than the other two species and also had the highest antioxidant effect (IC<sub>50</sub> range 134.64-144.31µL/mL) and the best *Fusarium equiseti* antifungal activity. There was no significant difference between essential oils from *C. aurantifolia* and *C. limonia* in terms of antioxidant and antifungal activities. The essential oil extracted from fresh lime peel of *C. latifolia* had a pleasant aroma and was rated highest in terms of favorability on the Hedonic scale.

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**Conflict of Interest:** None.

**Data Availability:** Data will be available at request.

**Ethics Statement:** It is to confirm here that there was no involvement of any humans or animals in this study.

**Author's Contribution:** Vu Thi Thanh Dao performed the experiments, collected and interpreted the data. Le Phan Hoai Ngoc and Ly Thi Thanh Thao assisted in performing the experiments in the Laboratory. Aswadi Anwar, Tuty Anggraini and Tran Nghia Khang helped in planning, supervising and evaluating the experiments.

**Generative AI Statement:** The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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