International Journal of Horticultural Science and Technology
Journal homepage: https://ijhst.ut.ac.ir

# Evaluation of the Antibacterial Effect of *Cymbopogon citratus* and *Pelargonium graveolens* Essential Oils on the Growth of the Pathogenic Bacteria *Pectobacterium carotovorum* and *Pantoea stewartii*

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**ARTICLE INFO**

*Article history*:

Received: 10 August 2024,
Received in revised form: 20 December 2024,
Accepted: 29 December 2024,

*Article type:*

Research paper

**Keywords:**

Antibacterial,
Essential oils,
GC–MS,
MBC,
MIC

**ABSTRACT**

*Pectobacterium carotovorum* and *Pantoea stewartii* are bacterial pathogens. Essential oils derived from medicinal plants have shown potential in inhibiting the growth of these plant bacteria, offering a natural alternative to agrochemicals. This approach not only helps reduce environmental pollution but also lowers toxin consumption and production costs. This study aimed to evaluate the effects of *Cymbopogon* and *Pelargonium* essential oils on two pathogenic bacteria *P. carotovorum* and *P. stewartii*. The essential oils were extracted and analyzed using gas chromatography-mass spectrometry (GC-MS) to identify their chemical constituents. Their antibacterial activity was assessed through the disk diffusion method, while the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Additionally, the combined effects of the two essential oils were studied using transmission electron microscopy (TEM). GC-MS analysis identified 75.64% of *Cymbopogon*'s essential compounds and 81.12% of *Pelargonium*'s. The largest inhibitory zone was observed with *Cymbopogon* essential oil against *P. carotovorum* (28 ± 2.54 mm), while the smallest was seen with *Pelargonium* essential oil against *P. stewartii* (9 ± 0.81 mm). The MIC and MBC values of the essential oils against the pathogens ranged from 2-3 mg $mL^{-1}$. A synergistic antibacterial effect was also noted when the *Cymbopogon* and *Pelargonium* oils were combined. TEM analysis revealed that *Cymbopogon* essential oil caused significant structural damage to *P. carotovorum* bacterial cells, including complete cell destruction, damage to the cell wall, swelling of the nuclear area, and changes in cytoplasmic density.

## Introduction

The extensive use of chemical toxins in agriculture has led to a wide range of environmental problems, including the contamination of water, soil, and food; harm to animals; poisoning of farmers; elimination of non-target organisms; and the emergence of resistant plant pathogens. In some cases, resistance to these chemical compounds renders them ineffective (Alonso-Gato et al., 2021). To mitigate these adverse effects, alternative control strategies—such as biological control and the use of natural products—are being explored (Puvača et al., 2021). Iran, with its favorable climate, strategic geographical location, and long-standing tradition of medicinal plant cultivation and use, is regarded as

one of the world's key regions for the growth of medicinal plants. This rich heritage has fueled considerable interest in utilizing natural plant compounds for plant disease management. In recent years, researchers have extensively examined the antibacterial, antifungal, and insecticidal properties of essential oils and plant extracts (Rathore et al., 2023; Proto et al., 2022). Essential oils—particularly those derived from medicinal plants—have demonstrated antimicrobial activity against a broad spectrum of microorganisms, including both gram-negative (Helander et al., 1998) and gram-positive bacteria (Kim et al., 1995). Although gram-negative bacteria are generally more resistant to the antagonistic effects of essential oils due to the presence of lipopolysaccharides in their outer membranes, exceptions have been reported (Alam et al., 2022).

Potatoes, a vital and strategic crop, are highly susceptible to numerous pathogens. Among the most damaging are bacteria from the genus *Pectobacterium*, which are major contributors to global reductions in both the quantity and quality of agricultural produce. These bacteria produce pectolytic enzymes that cause soft rot in various plants, including potatoes. *Pectobacterium carotovorum* subsp. *carotovorum* (P.c.c.), the primary causative agent of soft rot, was first identified in the United States as the pathogen responsible for "carrot soft rot" (Dye, 1969). This bacterium has a broad host range and infects many agricultural and ornamental plants such as potatoes, corn, rice, sugar beets, and vegetables. In Iran, soft rot has been reported across most potato-growing regions, including Isfahan, Khuzestan, Fars, the northern provinces, Kurdistan, and Ardabil (Baghaee-Ravari et al., 2011; Shakibafard et al., 2018). The disease significantly diminishes both the quality and yield of potato crops, with the most severe infections affecting tubers, seed pieces, and growing plants. Infected tubers serve as a primary vector for the disease's spread during planting (Moraes et al., 2017).

The genus *Pantoea* comprises several important bacterial pathogens that affect corn (*Zea mays* L.), including *Pantoea agglomerans*, *P. dispersa*, *P. citrea*, *P. punctata*, *P. terrea*, *P. ananatis*, and *P. stewartii*. These species are pathogenic to a wide range of crops such as sugar beet, soap plants, eucalyptus, rice, corn, millet, pineapple, and guar gum. Notably, *P. stewartii* has two subspecies: *P. stewartii* subsp. *stewartii*, which causes Stewart's wilt of corn, and *P. stewartii* subsp. *indologenes*, which is associated with green foxtail leaf spot (*Setaria italica*), infections in corn and pearl millet (*Pennisetum americanum*), pineapple rot (*Ananas comosus*), and guar gum (*Cyamopsis tetragonolobus*). These subspecies are primarily differentiated by the indole test.

Stewart's wilt leads to the clogging of the plant's vascular system with sticky substances, resulting in yellowing, wilting, stunted growth, and ultimately, plant death (Gehring et al., 2014). The use of plant essential oils to eliminate pathogens has emerged as a promising approach in plant protection. The efficacy of essential oil formulations can be enhanced by optimizing factors such as pH, temperature, oxygen levels, and the inclusion of stabilizing additives (Proto et al., 2022). Essential oils—also known as volatile oils—are concentrated natural extracts located in specialized cells, glands, and ducts within various plant parts, including leaves, flowers, fruits, buds, and branches. These oils are often stored as spherical droplets within plant cells. Of the approximately 250,000 known species of flowering plants, only about 2,000 are reported to produce essential oils. These oils are rich in bioactive compounds and exhibit well-documented antioxidant and antimicrobial properties (Turek and Stintzing, 2013). Chemically, essential oils are complex mixtures of volatile compounds, typically containing 60 or more distinct constituents.

Lemongrass (*Cymbopogon citratus*), a plant native to Iran and commonly found in tropical regions such as India and Sri Lanka, has demonstrated strong antioxidant and antibacterial potential. A member of the Poaceae family, lemongrass is a tropical grass known for its characteristic lemon-like aroma (Bassolé et al., 2011). Its essential oil—widely used in the cosmetic and pharmaceutical industries—exhibits potent antibacterial, antifungal, and appetizing properties. Globally, around 6,000 tons of lemongrass essential oil are produced each year (Majewska et al., 2019). Another noteworthy genus, *Pelargonium*, includes a diverse group of plants within the Geraniaceae family, comprising numerous species and subspecies (Miller, 2002). The antimicrobial activity of species such as geranium (*Pelargonium graveolens*) is largely attributed to the presence of monoterpenes in their leaf chemical composition (M'hamdi et al., 2024). Aromatic geranium essential oil is considered one of the most valuable plant-derived oils, with widespread use in agriculture, cosmetics, and other industries.

The significance of natural compounds as sources of nontoxic, biodegradable agents has gained growing attention. Plant-based pesticides, in particular, offer an environmentally friendly and cost-effective alternative to synthetic chemicals—especially in developing countries, where access to commercial fungicides may be limited due to high costs. Continued research in this area holds great promise for the development of sustainable plant-derived products, providing viable alternatives to chemical pesticides that pose risks to both human health and the environment. In recent years, numerous laboratory studies have investigated the effects of plant-derived compounds on fungi and plant-

pathogenic bacteria, consistently confirming the antimicrobial potential of various essential oils. The present study aimed to assess the effects of lemongrass (*Cymbopogon citratus*) and geranium (*Pelargonium graveolens*) essential oils on the growth of *Pantoea stewartii* and *Pectobacterium carotovorum* subsp. *carotovorum*.

## Material and methods

### *Preparation and cultivation of bacterial samples*

Bacterial isolates of *Pantoea stewartii* and *Pectobacterium carotovorum* subsp. *carotovorum* were obtained from the Bacteriology Collection of the Department of Plant Protection, Shahid Chamran University, Ahvaz. The isolates were cultured on Luria–Bertani (LB) agar and kept in petri dishes at 28 °C.

### *Plant materials*

In this study, two plant species, lemongrass (*Cymbopogon citratus*) and geranium (*Pelargonium graveolens*), were collected from various habitats in Khuzestan Province. The plants were identified and authenticated by the Department of Botany at Khuzestan University of Agricultural Sciences and Natural Resources. After collection, the plants were air-dried at room temperature for 7 d, ensuring they were kept away from direct light to preserve their properties.

### *Essential oil extraction method*

The aerial parts (leaves and stems) of the tested plant species were dried at room temperature in the shade. After removing all waste materials, the samples were ground using a grinder. Essential oils were then extracted from each sample through water distillation using a Clevenger apparatus for 3 h. The extraction process was repeated three times for each sample, with 100 g of plant material used per repetition. The obtained essential oils were dried using sodium sulfate, dehydrated, and stored in dark glass containers at 4 °C in a refrigerator until further analysis and biological testing (Hasanvand et al., 2021).

### *Identification of essential oil compounds*

The identification and analysis of essential oil compounds, following dehydration with sodium sulfate, were carried out using a gas chromatograph coupled with an Agilent mass spectrometer (GC/MS: GC 7890, MS 5975 C). The mass spectra obtained were compared with those of standard reference compounds. The GC/MS system was equipped with an HP-5MS capillary column, measuring 30 m in length and 0.25 mm in internal diameter. Electron ionization (EI) was employed as the ionization mode, with an ionization energy of 70 electron volts. The oven temperature program began with an initial hold at 50 °C for 5 min, followed by a temperature ramp from 50 °C to 240 °C at a rate of 3 °C per min, and then from 240 °C to 300 °C at a rate of 15 °C per min. The injection port temperature was maintained at 290 °C. Helium was used as the carrier gas at a constant flow rate of 0.8 mL per min. Compound identification was performed by comparing the retention times and inhibition indices of the detected components with those of authentic standards, and by consulting reference data from reputable scientific sources (Adams and Sparkman, 2007).

### *Determination of antibacterial properties of essential oils using the disc diffusion method*

This method was used for the preliminary screening of the antibacterial activity of lemongrass (*Cymbopogon citratus*) and geranium (*Pelargonium graveolens*) essential oils against *Pantoea stewartii* and *Pectobacterium carotovorum* subsp. *carotovorum* isolates. A bacterial suspension of $10^8$ CFU $mL^{-1}$ was prepared, and 100 µL were evenly spread on nutrient agar (NA) medium using L-shaped glass rods. Sterile paper discs impregnated with 6 µL of essential oils (containing 10 µg each) were placed on the NA plates inoculated with the bacterial isolates. The Petri dishes were then incubated at 28 °C for 48 h, after which the diameters of the inhibition zones around the discs were measured. Ampicillin (100 mg $mL^{-1}$) and distilled water were used as positive and negative controls, respectively. All experiments were performed in triplicate (Mousavifar et al., 2023). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using both microdilution and macrodilution methods. For this, a fresh bacterial suspension ($10^8$ CFU $mL^{-1}$) was prepared from 24-h cultures in liquid medium. In a 96-well ELISA plate, 70 µL of nutrient broth (NB) were dispensed into each well. Then, 70 µL of essential oil were added to the first well in each row, and serial twofold dilutions were performed across the wells to generate a range of concentrations. Subsequently, 10 µL of the bacterial suspension were added to each well. The plates were incubated at 28 °C in a shaker incubator at 120 rpm. Bacterial growth was assessed by measuring turbidity and culturing from each well on solid medium to determine preliminary MIC and MBC values. To refine the MIC and MBC measurements, the macrodilution method was employed using 15 mL Falcon tubes. Five concentrations between the initially determined MIC and MBC were selected for more precise evaluation. This experiment was also conducted in triplicate to ensure reliability and reproducibility.

### *Examining the combined effects of essential oils*

The synergistic effects between the essential oils were determined using the fractional inhibitory concentration index (FICI) and the microdilution method on 96-well ELISA plates (Mirzaei-Najafgholi et al., 2017). In each row of the plate, 70 µL of a dilution series of one essential oil (prepared at concentrations corresponding to 2x, 1x, 1/2, 1/4, 1/8, 1/16, 1/32, and 1/64 of the MIC) was added. Similarly, in the vertical direction, 70 µL of the dilution series of another essential oil was added to create a matrix of combinations. Finally, 5 µL of a bacterial suspension at a concentration of $10^8$ CFU $mL^{-1}$ was added to each well. The plates were incubated at 28 °C in a shaker incubator at 125 rpm for 24 h. Each experiment was conducted in triplicate for accuracy and reproducibility. The FICI was calculated using the following formula:

$$FICI = FIC\ A + FIC\ B = \frac{(MIC\ A\ in\ combination)}{(MIC\ A\ alone)} + \frac{(MIC\ B\ in\ combination)}{(MIC\ B\ alone)}$$

FICI: The sum of the fractional inhibitory concentration of plant A and B essential oils
MIC A: The minimum inhibitory concentration of essential oil A
MIC B: The minimum inhibitory concentration of essential oil B

Here, the interaction of two substances is defined as a synergistic effect. If the FICI index was less than 0.5, it would be synergistic; if 0.5<FICI<1, it would be incremental; if 1<FICI≤4, it would be indifferent; and if FIC>4, it would be antagonistic.

### *Investigating the effects of essential oils on preventing disease occurrence in tubers*

The tubers were initially sterilized by immersion in a 1% solution of commercial sodium hypochlorite for 2 min, followed by thorough rinsing with sterile distilled water. A 2×2 mm wound was then made in each tuber using a sterile lance, and 30 µL of a bacterial suspension ($10^8$ CFU $mL^{-1}$) was injected into the wound to a depth of 2 mm to inoculate the tubers. Lemongrass and geranium essential oils were applied independently by spraying at concentrations of one or 2 µL $mL^{-1}$ onto the tuber surfaces. For the infection control treatment, sterilized distilled water was sprayed on the tubers after the injection of the bacterial suspension, serving as a substitute for the essential oils. In the healthy control treatment, 30 µL of sterilized distilled water were injected into the wound before spraying. To maintain adequate humidity within the storage boxes, sterile wet cotton was used. The experiment was conducted under laboratory conditions in a completely randomized design, with three replicates per treatment. After 7 d, the average percentage of tuber damage, defined by the diameter of the contamination zone, was calculated and subjected to statistical analysis. Upon the appearance of visible signs of contamination in the infected control tubers, the extent of infection was documented. Each tuber was sectioned into eight equal parts using a sterile knife, with each segment corresponding to 12.5% of the tuber's total area. The number of infected and uninfected segments was recorded. To quantify the percentage of contamination, the number of infected segments was multiplied by 12.5, yielding the contamination percentage for each tuber.

### *Investigating the antibacterial effect of lemongrass essential oil against P. carotovorum bacteria using transmission electron microscopy*

For primary fixation, bacterial cells were subjected to glutaraldehyde via Glauert's method (Glauert, 1975). After stabilizing the bacteria, the bacteria were prepared with an electron microscope.

### *Data analysis*

The experiments were performed in a completely randomized design, and statistical analyses were carried out with SAS 9.4 software. Duncan's multiple range test (DMRT) ($P \leq 0.05$) was employed to identify significant differences among mean values.

## Results

### *Compositions of essential oils*

GC–MS analysis of the essential oils revealed that 75.64% of the identified compounds were from lemongrass, while 81.12% were from geranium. In lemongrass essential oil, the primary constituents were geranyl (27.36%) and neral (18.68%). In geranium essential oil, citronellol (29.01%) and geraniol (13.8%) were the predominant components. These findings are summarized in Tables 1 and 2 and illustrated in Figures 1 and 2.

**Table 1.** Percentage of compounds in geranium leaf essential oil by the GC-MS device.

| Row | Name of compound | Composition percentage | RT | KI | Row | Name of compound | Composition percentage | RT | KI |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Thujene <α-> | 0.01 | 5.62 | 924 | 27 | Geraniol | 13.8 | 17.95 | 1249 |
| 2 | Pinene <α-> | 1.58 | 5.85 | 932 | 28 | Citronellyl formate | 0.35 | 18.92 | 1271 |
| 3 | Camphene | 0.03 | 6.26 | 946 | 29 | Geranyl formate | 0.97 | 20.11 | 1298 |
| 4 | Verbenene | 0.02 | 6.67 | 961 | 30 | Citral <dimethoxy-(Z)-> | 0.08 | 20.93 | 1316 |
| 5 | Sabinene | 0.02 | 6.91 | 969 | 31 | Methyl geranate | 0.23 | 21.19 | 1322 |
| 6 | Pinene <β -> | 0.04 | 7.04 | 974 | 32 | Citronellyl acetate | 1.3 | 22.41 | 1350 |
| 7 | Myrcene | 0.11 | 7.43 | 988 | 33 | Copaene <α-> | 0.49 | 23.49 | 1374 |
| 8 | Phellandrene <α-> | 0.04 | 7.85 | 1002 | 34 | Geranyl acetate | 0.28 | 23.70 | 1379 |
| 9 | Carene <δ-3-> | 0.01 | 8.10 | 1008 | 35 | Bourbonene <β-> | 1.59 | 24.05 | 1387 |
| 10 | Cymene <o-> | 0.21 | 8.59 | 1022 | 36 | Gurjunene <α-> | 0.06 | 25.00 | 1409 |
| 11 | Limonene | 0.65 | 8.69 | 1024 | 37 | Caryophyllene <(E)-> | 2.9 | 25.36 | 1417 |
| 12 | Cineole <1,8-> | 0.17 | 8.76 | 1026 | 38 | Citronellyl propanoate | 1.01 | 26.47 | 1444 |
| 13 | Ocimene <(Z)-β-> | 0.06 | 8.96 | 1032 | 39 | Guaiadiene <6,9-> | 0.2 | 26.39 | 1442 |
| 14 | Ocimene <(E)-β-> | 0.03 | 9.42 | 1044 | 40 | Germacrene D | 0.17 | 28.15 | 1484 |
| 15 | Terpinene <γ-> | 0.04 | 9.78 | 1054 | 41 | Valencene | 0.27 | 28.66 | 1496 |
| 16 | Linalool oxide <cis-> (furanoid) | 0.04 | 10.29 | 1067 | 42 | Geranyl isobutanoate | 0.53 | 29.41 | 1514 |
| 17 | Linalool | 1.49 | 11.32 | 1095 | 43 | Germacrene B | 0.65 | 31.24 | 1559 |
| 18 | Rose oxide <cis-> | 3.09 | 11.77 | 1106 | 44 | Naphthalene <2-acetyl-> | 0.24 | 33.22 | 1608 |
| 19 | Rose oxide <trans-> | 1.24 | 12.45 | 1122 | 45 | Citronellyl pentanoate | 2.78 | 33.82 | 1624 |
| 20 | Isopulegol | 0.09 | 13.43 | 1145 | 46 | Aromadendrene <epoxide-allo-> | 0.2 | 34.42 | 1639 |
| 21 | Menthone | 1.48 | 13.55 | 1148 | 47 | Geranyl valerate | 1.22 | 35.02 | 1655 |
| 22 | Menthone <iso-> | 4.54 | 13.99 | 1158 | 48 | Citronellyl angelate | 1.59 | 35.06 | 1656 |
| 23 | Menthan-2-one <cis-ρ-> | 0.19 | 15.55 | 1194 | 49 | Citronellyl tiglate | 1.75 | 35.46 | 1666 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 24 | Terpineol <γ-> | 0.35 | 15.75 | 1199 | 50 | Caryophyllene <14-hydroxy-9-epi-(E)-> | 0.91 | 35.53 | 1668 |
| 25 | Citronellol | 29.01 | 16.80 | 1223 | 51 | Germacrone | 1.35 | 36.48 | 1693 |
| 26 | Car-3-en-2-one | 0.71 | 17.75 | 1244 | 52 | Geranyl tiglate | 0.95 | 36.59 | 1696 |
| No. Composition | | | | | | | | | 52 |
| Total | | | | | | | | | 81.12 |
| Standard Error | | | | | | | | | 0.61904 |

**Table 2.** Percentage of compounds in lemongrass leaf essential oil by the GC-MS device.

| Row | Name of compound | Composition percentage | KI | Row | Name of compound | Composition percentage | KI |
|---|---|---|---|---|---|---|---|
| 1 | Pinene <α-> | 0.14 | 932 | 14 | Geranyl formate | 1.59 | 1298 |
| 2 | Linalool oxide <*cis*-> (furanoid) | 0.25 | 1067 | 15 | Citral <dimethoxy-(Z)-> | 1.1 | 1316 |
| 3 | Linalool oxide <*trans*-> (furanoid) | 0.2 | 1084 | 16 | Geranyl acetate | 6.11 | 1379 |
| 4 | Linalool | 1.11 | 1095 | 17 | Farnesene <(Z)-β-> | 0.18 | 1440 |
| 5 | Rose oxide <*cis*-> | 0.1 | 1106 | 18 | Caryophyllene <9-epi-(E)-> | 0.34 | 1464 |
| 6 | Citronellal | 0.14 | 1148 | 19 | Germacrene D | 0.24 | 1484 |
| 7 | Citronellol | 0.43 | 1223 | 20 | Longicamphenylone | 0.12 | 1562 |
| 8 | Neral | 18.68 | 1235 | 21 | Nerolidyl acetate <(Z)-> | 0.47 | 1676 |
| 9 | Carvone | 0.71 | 1239 | 22 | Farnesol <(2Z,6Z)-> | 0.3 | 1698 |
| 10 | Carvone oxide <*cis*-> | 1.48 | 1259 | 23 | Farnesyl acetate <(2Z,6E)-> | 1.42 | 1821 |
| 11 | Geranial | 27.36 | 1264 | 24 | Farnesyl acetate <(2E,6E)-> | 1.07 | 1845 |
| 12 | Linalool acetate <dihydro-> | 4.21 | 1272 | 25 | Farnesyl acetone <(Z,Z)-> | 1.19 | 1860 |
| 13 | Linalool oxide acetate <*trans*-> (pyranoid) | 6.7 | 1287 | | | | |
| No. Composition | | | | | | | 25 |
| Total | | | | | | | 75.64 |
| Standard Error | | | | | | | 1.32 |

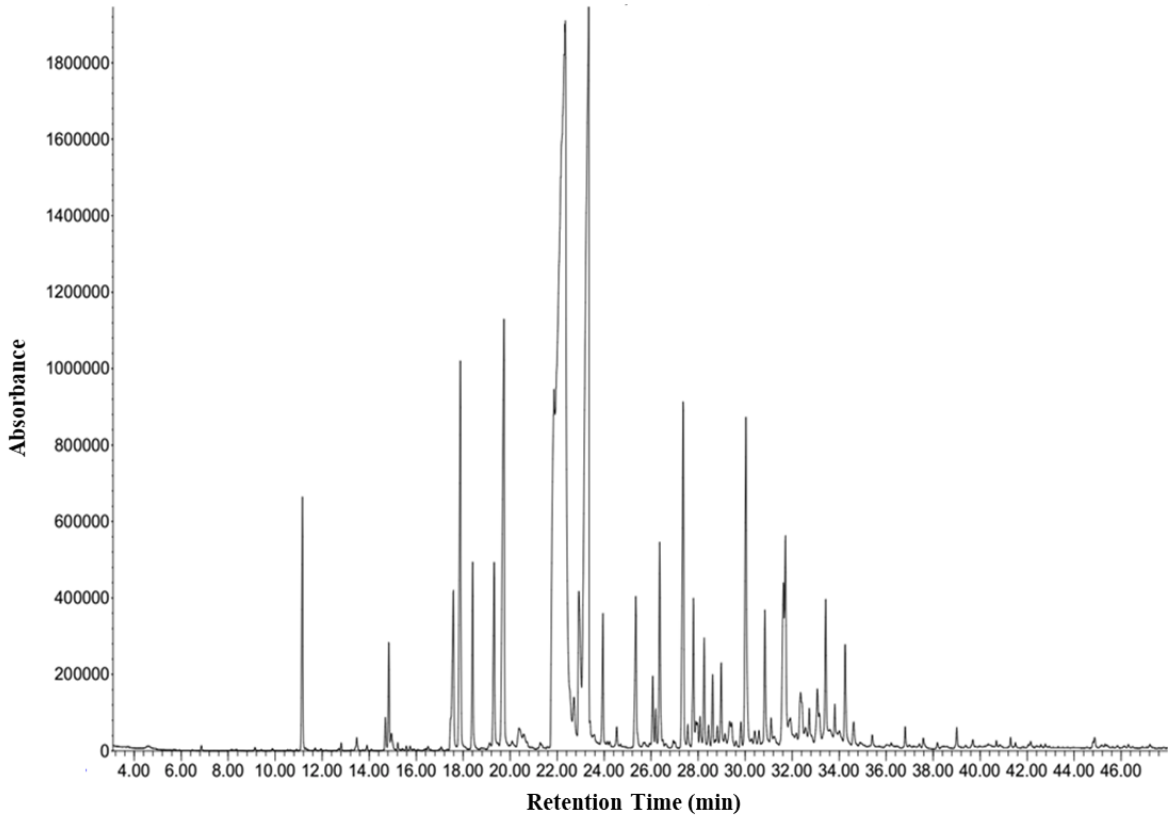


**Fig. 1.** Chromatographic diagram of compounds identified in geranium essential oil.

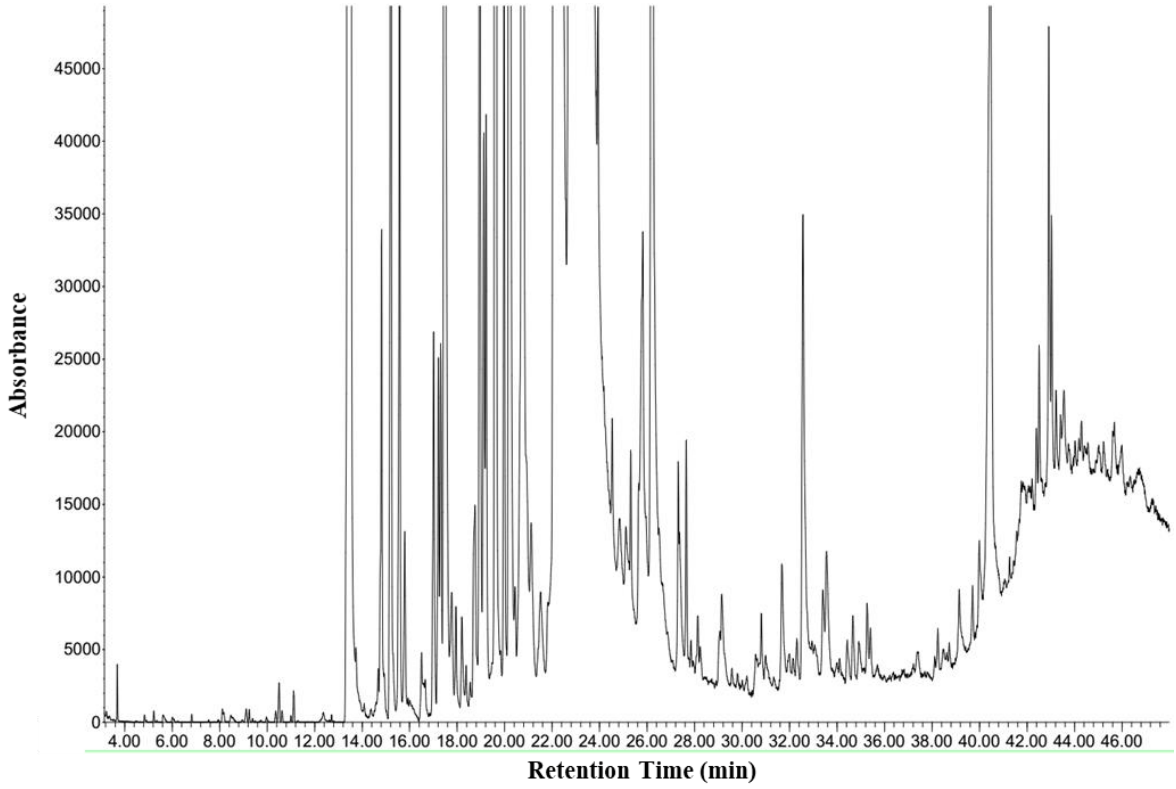


**Fig. 2.** Chromatographic diagram compounds identified in lemongrass essential oil.

### *Investigating the effect of essential oils on bacterial isolates using the disk leakage method*

The antibacterial effects of lemongrass and geranium essential oils on *Pantoea stewartii* and *Pectobacterium carotovorum* subsp. *carotovorum* were assessed using the disk diffusion method. The results indicated that both essential oils effectively inhibited the growth of the tested pathogenic bacteria. The strongest antibacterial activity was observed with lemongrass essential oil against *P. carotovorum* subsp. *carotovorum*, which produced the largest inhibition zone, measuring $28 \pm 2.54$ mm. In contrast, the weakest effect was recorded for geranium essential oil against *P. stewartii*, with an inhibition zone of $9 \pm 0.81$ mm. Overall, both essential oils demonstrated pronounced inhibitory activity, particularly against *P. carotovorum* subsp. *carotovorum* (Figs. 3 and 4).

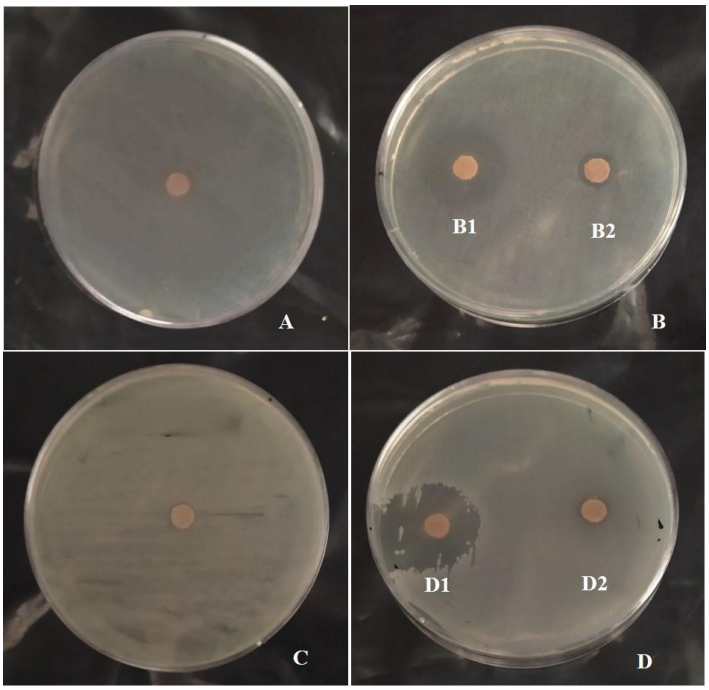


**Fig. 3.** Non-growth halo formation of cultures attributed to *Pectobacterium carotovorum* and *Pantoea stewartii* bacteria in response to two essential oil treatments of lemongrass and geranium. (**A**) control; (**B1**) lemongrass essential oil + *Pectobacterium carotovorum*; (**B2**) geranium essential oil + *Pectobacterium carotovorum*; (C) control; (**D1**) essential oil of lemongrass + *Pantoea stewartii*; (**D2**) geranium essential oil + *Pantoea stewartii*.

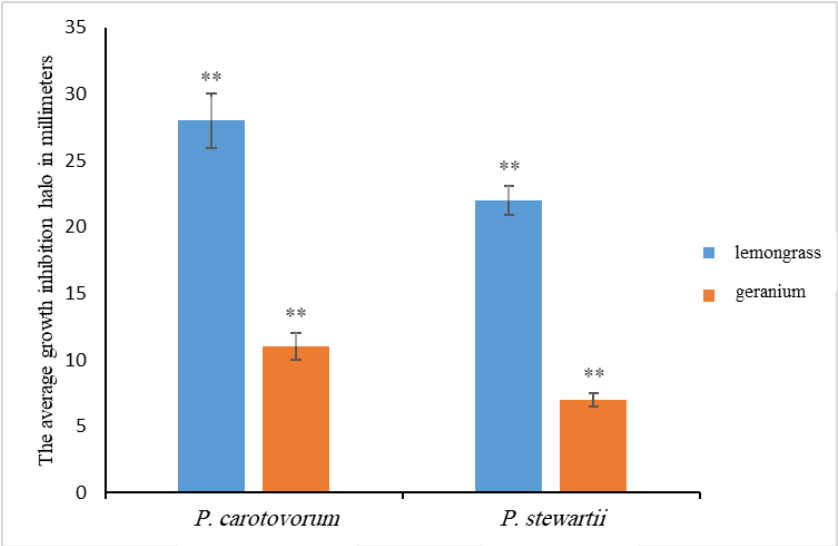


**Fig. 4.** Average diameters (mm) of inhibitory halos pertaining to *Pantoea stewartii* and *Pectobacterium carotovorum* isolates in response to lemongrass and geranium essential oils.

### *Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)*

The MIC and MBC values of the essential oils were determined using both microdilution and macrodilution methods (Fig. 4). The results revealed that lemongrass essential oil exhibited the lowest MIC and MBC values against *Pectobacterium carotovorum* subsp. *carotovorum*, with values of 2 µL mL$^{-1}$ and 3 µL mL$^{-1}$, respectively. Against *Pantoea stewartii*, lemongrass essential oil also demonstrated strong antibacterial activity, with MIC and MBC values of 1 µL mL$^{-1}$ and 2 µL mL$^{-1}$, respectively. Moreover, the combined application of lemongrass and geranium essential oils showed a synergistic effect, enhancing antibacterial efficacy against both bacterial isolates.

### *Effect of essential oils on preventing the occurrence of disease in tubers*

Analysis of variance (ANOVA) showed that the application of essential oils had a statistically significant effect in preventing tuber contamination by both pathogenic bacteria ($P \leq 0.01$). Mean comparison analysis further indicated that, compared to the negative control, both essential oils significantly reduced tuber contamination caused by *Pectobacterium carotovorum* subsp. *carotovorum* and *Pantoea stewartii* (Figs. 5 and 6).

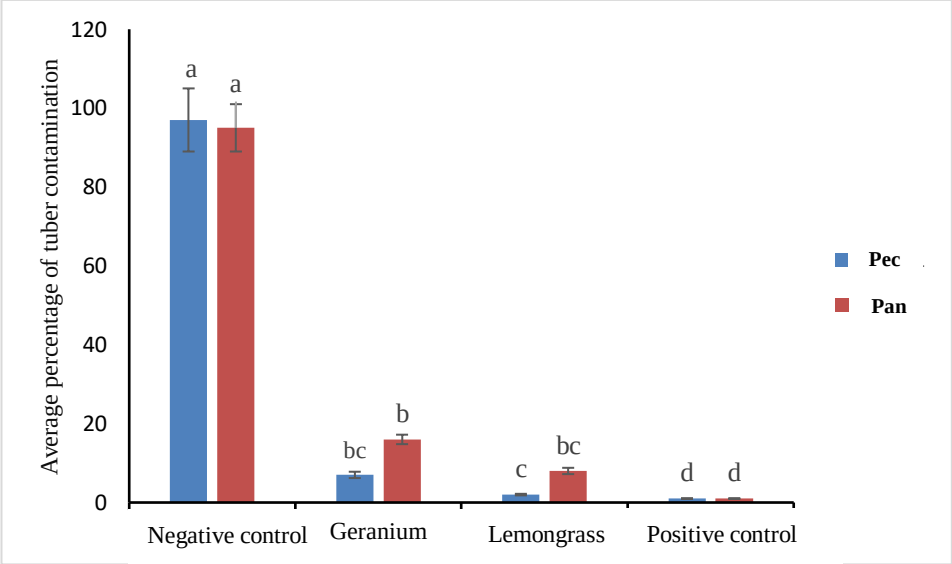


**Fig. 5.** Comparison of the average effect of lemongrass and geranium essential oils in reducing potato tuber infection caused by *Pantoea stewartii* and *Pectobacterium carotovorum* isolates**.**

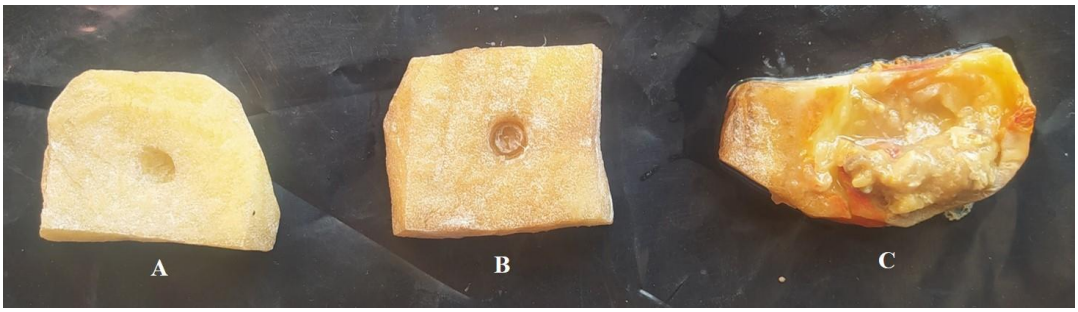


**Fig. 6.** Comparison of the effect of pathogenic bacteria and essential oils on potato tuber, (**A**) positive control; (**B**) mutual effect of lemongrass essential oil on *Pectobacterium carotovorum* bacteria; (**C**) negative control (only pathogenic bacteria).

### *Investigating the antibacterial effect of lemongrass essential oil against P. carotovorum subsp. carotovorum using transmission electron microscopy*

An investigation into the effects of lemongrass essential oil on *Pectobacterium carotovorum* subsp. *carotovorum* revealed substantial cellular damage. Notable observations included complete cell lysis, disruption of the cell wall, discoloration of the nuclear region, alterations in cytoplasmic density, and cellular swelling in treated samples (Fig. 7).

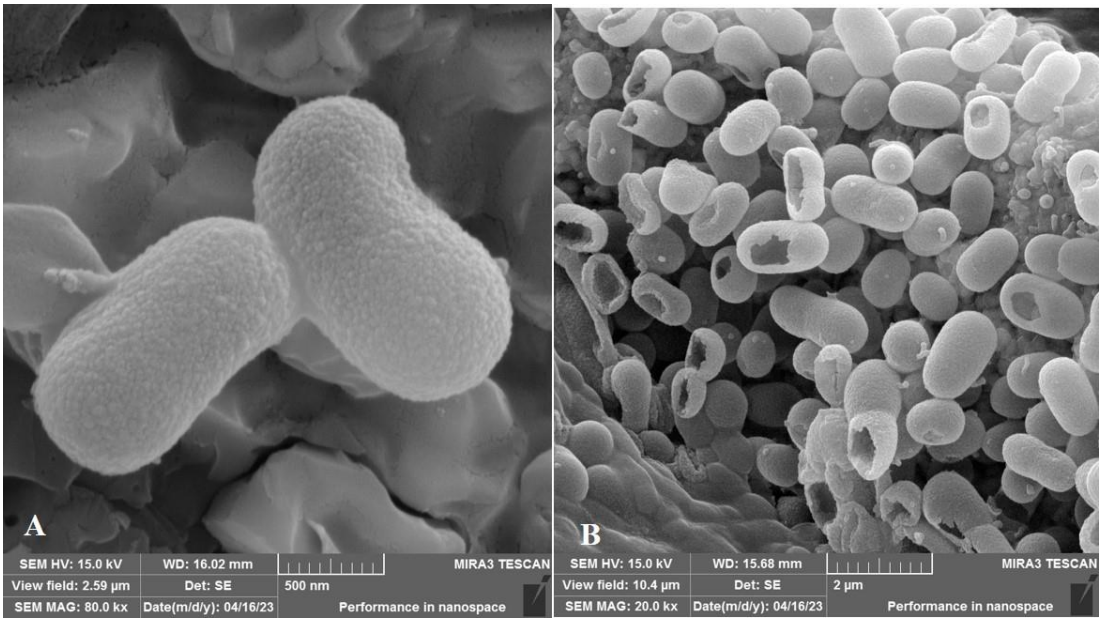


**Fig. 7.** Transmission electron microscopic photo of the effects of lemongrass essential oil at MIC concentration on *P. carotovorum* bacteria, (**A**) control sample; (**B**) complete bacterial cell destruction, bacterial cell content leakage, cytoplasm condensation and bacterial cell genome swelling.

## Discussion

The adverse environmental and health impacts associated with pesticide use have become increasingly critical, highlighting the urgent need for sustainable alternatives to chemical toxins. Among these alternatives, essential oils (EOs) and plant extracts have emerged as promising candidates. Essential oils are natural products derived from plant secondary metabolism (Murbach et al., 2016), composed of volatile molecules—primarily secondary metabolites—with a wide range of biological activities (Raveau et al., 2020). These compounds not only enhance product safety but also help reduce agricultural losses caused by pests and plant pathogens (Mangalagiri et al., 2021). The variability in antifungal activity among essential oils is largely attributed to differences in their chemical composition, which is influenced by plant species and environmental factors (Puvača et al., 2021). The antibacterial potential of essential oils against both Gram-positive and Gram-negative phytopathogenic bacteria has become a key focus of global research (Altiner and Bilal, 2023).

This study examined the antibacterial effects of lemongrass (*Cymbopogon citratus*) and geranium (*Pelargonium graveolens*) essential oils on the plant pathogens *Pantoea stewartii* and *Pectobacterium carotovorum* subsp. *carotovorum*, assessing their efficacy in both culture media and potato tubers. The findings underscore the significant antibacterial properties of key compounds such as citronellol and geraniol, highlighting their potential as cost-effective and environmentally friendly alternatives to synthetic antibiotics.

Lemongrass essential oil, in particular, demonstrated strong antibacterial activity. This is likely due to the multifaceted mechanisms by which essential oils exert their antimicrobial effects. These mechanisms include integration into the cytoplasmic membrane, leading to the disruption of enzyme-associated protein structures; inhibition of succinate- and NADH-dependent reactions; interference with electron transfer in the respiratory chain; and disruption of oxidative phosphorylation. Collectively, these effects compromise microbial viability and contribute to the oils' potent antibacterial action.

The lipophilic nature of essential oils is fundamental to their antimicrobial activity, enabling them to increase membrane permeability or cause membrane disruption—potentially through enzymatic processes such as protein kinase activity (Chouhan et al., 2017). Terpenes, a major constituent of lemongrass essential oil, are believed to be primarily responsible for its strong antimicrobial effects (Abdel-Gwad et al., 2022). In addition to terpenes, other compounds commonly found in essential oils—such as phenolic compounds, fatty acids, and esters—also contribute significantly to their antimicrobial properties.

Phenolic compounds, in particular, play a crucial role due to their hydrophobic nature, which allows them to interact with and disrupt cell membranes, compromising membrane integrity and affecting vital cellular functions. Moreover, phenolic compounds possess the unique ability to form

chelates with metal ions, thereby reducing the reactivity and bioavailability of essential metals such as iron and copper—both critical for microbial survival and growth. By sequestering these metal ions through metal-ligand complex formation, phenolic compounds hinder microbial metabolism and proliferation (Al Allan, 2024).

The antimicrobial efficacy of essential oils is influenced by both their chemical composition and the specific mechanisms of action of their bioactive constituents, particularly phenolic compounds. Numerous studies have confirmed the antibacterial and antifungal activities of essential oils under various conditions against a broad spectrum of microorganisms (Majewska et al., 2019; Hou et al., 2022). Recent research has further emphasized the effectiveness of plant-derived essential oils in combating key phytopathogens such as *Ralstonia solanacearum* and *Pectobacterium carotovorum*, reinforcing their potential as viable alternatives to synthetic agrochemicals (Jílková et al., 2024). For instance, Jeong et al. (2009) demonstrated that a 0.5% concentration of *Cymbopogon* essential oil completely inhibited the growth of *P. carotovorum*. Similarly, Ghalem et al. (2016) confirmed the antibacterial activity of thyme, lemongrass, and geranium essential oils against both *Pantoea stewartii* and *P. carotovorum*, providing further evidence of their broad-spectrum antimicrobial potential.

In another study, the EOs of *Pelargonium graveolens* and *Cymbopogon martinii*, along with their major compounds citronellol and geraniol, were evaluated in both liquid and vapor phases against Gram-positive and Gram-negative bacterial strains. Microdilution tests revealed that geraniol exhibited a minimum inhibitory concentration (MIC) of 0.05% v/v against nearly all tested strains. Transmission electron microscopy demonstrated that the antibacterial activity of *C. martinii* EO was primarily attributable to geraniol, which caused significant structural damage to bacterial cells (Murbach Teles Andrade et al., 2016).

Carvacrol, a major constituent of thyme (approximately 45%) and oregano (60–74%) essential oils, is well recognized for its broad-spectrum antibacterial activity. In Gram-positive bacteria, carvacrol disrupts membrane integrity by increasing permeability to $H^+$ and $K^+$ ions. In Gram-negative bacteria, it compromises the outer membrane, facilitating increased permeability of the cytoplasmic membrane to ATP and causing the release of lipopolysaccharides. Nevertheless, Gram-negative bacteria are generally more resistant to essential oils than Gram-positive bacteria, largely due to the presence of a lipopolysaccharide-rich outer membrane that serves as a barrier, limiting the direct interaction between essential oil components and the cytoplasmic membrane (Raveau et al., 2020).

The antimicrobial properties of various essential oils, including those derived from cumin, savory, lemongrass, and mint, have been evaluated. All tested essential oils exhibited inhibitory and antibacterial effects, although mint essential oil demonstrated superior efficacy compared to the others. While all studied plant species showed antibacterial potential, the degree of bacterial growth inhibition varied depending on the essential oil source and the specific bacterial isolate involved (Vaou et al., 2021). The antibacterial activity of essential oils is largely attributed to oxygenated monoterpenes, such as 1,8-cineole, and low-molecular-weight sesquiterpenes like α-murolol. Additional bioactive constituents found in the aerial parts of many aromatic plants, including 1,8-cineole, camphor, α-pinene, β-pinene, sabinene, geraniol, and α-terpineol, also contribute to their antimicrobial efficacy. Gas chromatography–mass spectrometry (GC–MS) analysis in the present study identified geraniol and citronellol as the dominant compounds in geranium and lemongrass essential oils, respectively. These findings underscore the potential of these natural compounds as effective antimicrobial agents. Given the increasing concerns associated with chemical antibacterial agents, including adverse side effects and the rise of bacterial resistance, the use of natural substances such as plant-derived essential oils offers a promising and practical alternative for plant disease control.

## Conclusions

Exploring antibacterial agents and synergistic combinations to enhance efficacy and mitigate bacterial resistance to bactericides is a crucial step in controlling diseases caused by phytopathogenic bacteria. Non-chemical control methods have garnered increasing attention in recent years due to their potential to reduce environmental pollution and support the principles of sustainable agriculture. Among these alternatives, natural agents, including antagonistic microorganisms and plant-derived compounds, have been proposed as viable strategies for managing bacterial plant diseases (Hou et al., 2022). The findings of this study highlight the significant antibacterial activity of lemongrass and geranium essential oils against plant-pathogenic bacteria, underscoring their potential as natural antibacterial agents for the management of destructive plant diseases without adverse environmental or health effects. However, further field-based research is essential to validate the effectiveness of these essential oils under natural growing conditions. Future studies should focus on optimizing application concentrations, evaluating long-term efficacy, and assessing the economic

feasibility of integrating these essential oils into large-scale agricultural practices.

## Acknowledgments
The authors thank the Plant Protection Department, Faculty of Agriculture, Lorestan University, Khorramabad, Iran.

## Conflict of Interest
The authors indicate no conflict of interest in this work.

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