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Unlocking the Multifunctional Properties of *Litsea cubeba* Essential Oil: Composition, Antimicrobial, Antibiofilm and Insecticidal Activities

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ABSTRACT

Litsea cubeba essential oil (LCEO) is recognised for its broad spectrum of biological activities, including antimicrobial and insecticidal effects, making it a promising natural alternative to synthetic agents. The aim of this study was to evaluate the chemical composition, antimicrobial in vitro and in situ, antibiofilm and insecticidal activities of LCEO against selected bacterial and yeast strains, as well as against the storage pest *Callosobruchus maculatus*. The antimicrobial activity was assessed by disc diffusion assay, minimum inhibitory concentrations (MIC_{50} and MIC_{90}) and in situ vapour-phase tests on fresh produce (apple, pear, potato, parsley). Antibiofilm effects were determined using the crystal violet method, and MALDI-TOF MS spectral profiling was applied to compare planktonic and biofilm-associated *Salmonella enterica* cells on different surfaces over time. Insecticidal activity was tested in fumigation assays at different concentrations. GC–MS analysis revealed that citral isomers (neral 31.5%, α-citral 30.1%) and limonene (19.6%) were the dominant constituents of LCEO, accounting for more than 80% of the total volatile fraction. LCEO exhibited significant antimicrobial activity, with Gram-positive bacteria showing larger inhibition zones (up to 15.67 mm for *S. aureus*) compared with Gram-negative strains and yeasts. MIC values confirmed strong effects against *Y. enterocolitica* (0.156 mg/mL) and *C. albicans* (0.077 mg/mL), while *E. coli* was the most resistant (2.218 mg/mL). In situ assays revealed matrix-dependent responses: on pears, inhibition exceeded 90% for both Gram-positive and Gram-negative bacteria at 500 μg/L, while on potatoes maximum bacterial inhibition was recorded at the lowest concentration (62.5 μg/L), followed by a biphasic decline. Biofilm formation of *S. enterica* was effectively suppressed (MIC_{50} = 0.33 mg/mL; MIC_{90} = 0.35 mg/mL), and MALDI-TOF MS analyses demonstrated time- and surface-dependent spectral changes between planktonic and biofilm-associated cells. Insecticidal assays confirmed complete mortality of *C. maculatus* adults at ≥ 50% LCEO. In conclusion, LCEO displays strong and multifaceted antimicrobial, antibiofilm and insecticidal activities, supporting its potential application as a natural preservative and biocontrol agent.

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1 | Introduction

Litsea cubeba (Lour.) Pers. (Lauraceae), commonly referred to as May Chang, is an important source of lemon-scented essential oil that has long been utilised in the food, cosmetic and pharmaceutical industries. Its essential oil is rich in citral, which imparts its characteristic citrus aroma, and is also a precursor for the industrial synthesis of vitamin A and aromatic compounds. In recent years, *Litsea cubeba* essential oil (LCEO) has attracted increasing attention as a natural antimicrobial and preservative agent, aligning with the growing demand for 'clean-label' solutions in food preservation and agricultural production [1, 2].

The chemical profile of LCEO is dominated by citral, a mixture of the geometric isomers geranial (E-citral) and neral (Z-citral), often accounting for more than 60% of the total oil. Other components include limonene, linalool and citronellal, though their concentrations vary depending on geographical origin, harvest season and extraction method. For instance, Borotová et al. [3] reported geranial (39.4%), neral (29.5%) and d-limonene (14.3%) as dominant compounds, whereas You et al. [4] highlighted that geranial and neral together could reach 78%–87% of the volatile fraction. Such variability underscores the need to link compositional profiles to biological activity.

The antimicrobial activity of LCEO has been extensively studied in vitro, demonstrating broad inhibitory effects against foodborne pathogens and spoilage organisms. Reported targets include *Vibrio parahaemolyticus*, *Listeria monocytogenes* and *Lactobacillus plantarum* [1, 5]. The underlying mechanisms involve disruption of cytoplasmic membranes, leakage of nucleic acids and proteins, inhibition of ATPase activity, and impairment of cellular metabolism [6]. In situ applications on fresh produce further confirmed its efficacy, where surface treatment or vapour-phase exposure significantly reduced microbial loads [7]. Encapsulation methods, such as β-cyclodextrin inclusion complexes, improved stability and mitigated sensory drawbacks, expanding the practical potential of LCEO [8].

Beyond planktonic inhibition, LCEO shows promising antibiofilm properties. Although specific studies targeting *Salmonella enterica* are limited, nanoemulsions of LCEO have been shown to reduce viability and induce membrane disruption in *Salmonella* cells, particularly when combined with ultrasound treatment [9]. Citral, the predominant constituent, is reported to interfere with quorum sensing, suppress extracellular polymeric substance (EPS) synthesis, and inhibit biofilm formation *S. typhimurium* [10]. These findings provide a rationale for evaluating LCEO as a natural antibiofilm agent against *S. enterica* in food systems.

In addition, LCEO has been explored for its insecticidal properties [11]. Although direct studies against *Callosobruchus maculatus* are scarce, citral-rich oils such as lemongrass (*Cymbopogon citratus*) demonstrate fumigant and contact toxicity, repellency and oviposition inhibition against bruchid beetles, including *C. maculatus*. Given that citral and linalool are major constituents of LCEO, there is strong mechanistic support for its insecticidal potential. Altogether, the literature positions LCEO as a multifunctional essential oil with significant potential in food preservation and stored-product protection. Nonetheless, integrated studies linking chemical composition with antimicrobial, antibiofilm and insecticidal activities remain limited. This study therefore aims to comprehensively evaluate LCEO encompassing its chemical profile, in vitro and in situ antimicrobial properties, antibiofilm activity against *S. enterica* and insecticidal efficacy against *C. maculatus* to provide new insights into its practical applications.

2 | Materials and Methods

2.1 | *Litsea cubeba* Essential Oil: Origin and Quality Specifications

The essential oil of *Litsea cubeba* (LCEO) used in this study was supplied by GuiLin Four Seasons Sunshine, TieShan Industrial Zone, Guilin, Guangxi, China. According to the quality specification certificate provided by the manufacturer, the oil is a pale yellow to yellow liquid with a characteristic citral-like aroma. The relative density at 20°C was reported in the range of 0.880–0.905, with a refractive index of 1.4800–1.4900. The optical rotation at 20°C ranged from +3° to +12°. The content of citral (sum of neral and geranial) was specified to be not less than 66%. Other constituents included limonene and linalool. The solubility test indicated that 1 mL of oil was miscible with 3 mL of 90% ethanol, yielding a clear solution. The essential oil was packed in clean, dry, galvanised iron drums and stored under cool, dry and ventilated conditions, away from contamination and ignition sources. Under these conditions, the shelf life was indicated as 36 months.

2.2 | Chemical Composition of LCEO

The volatile constituents of *Litsea cubeba* essential oil (LCEO) were analysed by gas chromatography coupled with mass spectrometry (GC–MS). The analyses were performed using a Clarus 500 instrument (Perkin Elmer, Waltham, MA, USA) equipped with both a flame ionisation detector (FID) and a mass spectrometric detector. Separation was achieved on a VF-5 ms capillary column (Varian Factor Four, 30 m × 0.25 mm, film thickness 0.25 μm). The oven temperature was initially set at 60°C, programmed to rise at 6°C/min to 220°C, and maintained at this final temperature for 20 min. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. Identification of the compounds was accomplished by comparing the obtained mass spectra with those in the Wiley 2.2 and NIST 11 libraries. Linear retention indices (LRIs) were determined by injecting a homologous series of n-alkanes (C_8–C_{25}) under the same chromatographic conditions, and the results were confirmed with published reference values [12]. The relative proportions of individual constituents were calculated from the peak areas of the total ion chromatogram and expressed as percentages. No correction factors or internal standards were employed. Each analysis was performed in triplicate to ensure accuracy and reproducibility.

2.3 | Evaluation of Antimicrobial Properties

2.3.1 | Microbial Strains and Culture Maintenance

To assess the antimicrobial potential of *Litsea cubeba* essential oil (LCEO), we employed a panel of reference strains including both bacteria and yeasts. The bacterial group encompassed

Gram-positive isolates (*Enterococcus faecalis* CCM 4224, *Listeria monocytogenes* CCM 4699 and *Staphylococcus aureus* CCM 3953) as well as Gram-negative representatives (*Escherichia coli* CCM 3953, *Salmonella enterica* subsp. *enterica* CCM 3807 and *Yersinia enterocolitica* CCM 7204T). The yeast panel consisted of *Candida albicans* CCM 8186, *C. glabrata* CCM 8270, *C. krusei* CCM 8271, *C. parapsilosis* CCM 8260 and *C. tropicalis* CCM 8223. Bacterial strains were cultivated in Mueller-Hinton broth, while yeast strains were maintained in Sabouraud Dextrose broth. All cultures were incubated under standard conditions (37°C for bacteria, 25°C for yeasts) for 24 h prior to testing. Inocula were standardised to 0.5 McFarland turbidity to guarantee consistent assay conditions.

2.3.2 | Disc Diffusion Assay for Growth Inhibition

The antimicrobial effect of *Litsea cubeba* essential oil (LCEO) was evaluated using the agar disc diffusion method. Sterile paper discs (6 mm in diameter) were impregnated with the tested oil and placed on the surface of Mueller-Hinton agar (MHA) plates previously inoculated with bacterial suspensions, or on Sabouraud Dextrose agar (SDA) plates seeded with yeast cultures. The procedure followed modified protocols described by Kačániová et al. [13]. Plates were incubated for 24 h at 37°C for bacterial isolates and at 25°C for yeasts. After incubation, inhibition zones surrounding the discs were measured. The activity was classified according to zone diameter as weak (≤ 5 mm), moderate (6–10 mm) or strong (> 10 mm). Negative controls were discs containing only the solvent, while cefoxitin served as the positive control for Gram-positive bacteria, gentamicin for Gram-negative bacteria and fluconazole for yeasts.

2.3.3 | Broth Microdilution Assay for MIC Determination

Minimum inhibitory concentrations (MIC_{50} and MIC_{90}) of LCEO were determined by the broth microdilution method in sterile 96-well microtiter plates, following modifications of the procedure described by Kačániová et al. [14]. Each well contained 50 μL of microbial suspension standardised to 0.5 McFarland and 50 μL of serial twofold dilutions of the oil. Dilution series were prepared in Mueller-Hinton broth (MHB) for bacteria and in Sabouraud Dextrose broth (SDB) for yeasts, giving final concentrations between 10 and 0.00488 mg/mL. Negative controls contained broth and essential oil without inoculum, while positive controls consisted of broth with inoculum but no oil. Plates were incubated at 37°C for bacteria and 25°C for yeasts. Growth was measured spectrophotometrically at 570 nm using a Glomax microplate reader (Promega, Madison, WI, USA). MIC_{50} and MIC_{90} were defined as the lowest concentrations of LCEO producing 50% and 90% inhibition of growth compared to untreated controls. All assays were conducted in triplicate to ensure reproducibility.

2.4 | In Situ Assessment of Antimicrobial Action in Food Matrices

To simulate natural contamination, representative Gram-positive, Gram-negative, and yeast strains were applied to fresh produce. Apple, pear, potato and parsley were purchased commercially, cut into slices of 0.5 mm thickness, and aseptically dried. Each slice was placed in a sterile 60 mm Petri dish and inoculated with microbial suspensions adjusted to 0.5 McFarland density. Solutions of LCEO were prepared in ethyl acetate at 500, 250, 125 and 62.5 μg/L. Sterile filter paper discs impregnated with these solutions were fixed to the inner surface of Petri dish lids to allow exposure of the inoculated substrates to the volatile phase of the oil. Control plates contained discs loaded only with ethyl acetate. All dishes were hermetically sealed and incubated at 37°C for 7 days. After incubation, microbial growth on the produce surfaces was assessed using conventional culture-based methods. The degree of inhibition was semi-quantitatively analysed with ImageJ software (NIH, USA) according to the modified method described by Kačániová et al. [13].

2.5 | Investigation of Antibiofilm Activity

2.5.1 | Crystal Violet Microtiter Plate Assay

The ability of *Litsea cubeba* essential oil (LCEO) to inhibit biofilm formation was assessed using the crystal violet staining method adapted from Kačániová et al. [15]. Bacterial suspensions were grown in Mueller-Hinton broth (MHB) within sterile 96-well polystyrene microplates. Serial twofold dilutions of the oil were prepared, producing a concentration range from 10 to 0.0048 mg/mL. Plates were incubated without shaking at 37°C for 24 h. Following incubation, planktonic cells were removed by rinsing the wells with sterile phosphate-buffered saline (PBS). The remaining biofilm layer was stained with 0.1% (w/v) crystal violet for 15 min, washed repeatedly with distilled water and air-dried. Bound dye was solubilised with 96% ethanol, and absorbance was measured at 570 nm using a microplate reader. The minimum biofilm inhibitory concentration (MBIC) was defined as the lowest concentration of LCEO that prevented visible biofilm formation. $MBIC_{50}$ and $MBIC_{90}$ were the concentrations reducing biofilm biomass by 50% and 90%, respectively, compared to untreated controls.

2.5.2 | MALDI-TOF MS Analysis of Biofilm Spectral Alterations

To investigate changes in the mass spectral profiles of biofilms exposed to LCEO, matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Bremen, Germany) was used. A biofilm-forming strain of *Salmonella enterica* was cultivated in 20 mL of Mueller-Hinton broth (MHB) within polypropylene tubes and inoculated with 100 μL of a standardised bacterial suspension. Glass slides and stainless-steel coupons served as solid supports for biofilm growth, following the protocol of Kačániová et al. [16]. Experimental variants contained 0.1% (v/v) LCEO, while control cultures lacked oil. Incubation was performed at 37°C with shaking at 170×g for 3, 7 and 14 days. At each sampling time, biofilm biomass from both slides and coupons was collected, and planktonic cells from parallel control tubes were also examined. Mass spectra were recorded using a Microflex LT MALDI-TOF MS instrument (Bruker Daltonics, Bremen, Germany) operated in linear positive ion mode

across an m/z range of 2000–20,000. Data analysis was carried out with Bruker Biotyper software. Comparative evaluation of spectral profiles was conducted by hierarchical clustering based on Euclidean distances to differentiate treated and untreated biofilms, as well as to compare biofilm and planktonic cell populations.

2.6 | Insecticidal Activity Against *Callosobruchus maculatus*

The fumigant toxicity of *Litsea cubeba* essential oil (LCEO) was evaluated against adults of *Callosobruchus maculatus*, a common storage pest. Groups of 50 insects were placed into sterile Petri dishes containing filter paper discs treated with 100 μL of LCEO at concentrations of 100%, 50%, 25%, 12.5%, 6.25% and 3.125%. To improve dispersion, the oil was diluted in 0.1% polysorbate 80 (Tween 80; Sigma-Aldrich, St. Louis, MO, USA). The dishes were hermetically sealed with parafilm to minimise volatilisation and maintained at room temperature for 24 h. Control sets consisted of discs impregnated only with 0.1% Tween 80 solution. After exposure, the numbers of surviving and dead beetles were recorded. Each bioassay was conducted in triplicate. Mortality rates were used to evaluate the dose-dependent insecticidal effect of LCEO under confined conditions.

2.7 | Statistical Analysis

All statistical analyses and graphical outputs were performed with JMP Student Edition version 18.2 (SAS Institute, Cary, NC, USA). Data are expressed as mean values ± standard deviation (SD), with all measurements conducted in triplicate. A one-way ANOVA was applied, followed by Tukey's HSD test at a significance level of $p \leq 0.05$.

3 | Results

3.1 | Chemical Composition of LCEO

By GC/MS analysis 21 components were detected and identified (Table 1). The terpenoids (67.4%) covered the highest percentage followed by monoterpenes (27.1%) and sesquiterpenes (2.4%). Neral (31.5%), α-citral (30.1%) and limonene (19.6%) were the principal volatiles. The other minor compounds ranging from 0.1% to 2.6%.

TABLE 1 | Chemical composition of LCEO (percentage mean values± standard deviation).

N.	Components	LRI^{calc}	LRI^{lit}	%
1	α-pinene	938	941	2.1 ± 0.08
2	camphene	941	942	0.4 ± 0.02
3	sabinene	968	972	2.6 ± 0.07
4	β-pinene	970	975	2.4 ± 0.5
5	limonene	1025	1029	19.6 ± 3.5
6	1,8-cineole	1030	1033	1.6 ± 0.06
7	linalool	1087	1092	2.0 ± 0.05
8	*cis*-limonene oxide	1115	1118	0.1 ± 0.02
9	6-octenal, 3,7-dimethyl—	1138	1146	0.7 ± 0.05
10	isoneral	1171	1165	1.4 ± 0.06
11	terpinen-4-ol	1178	1168	0.2 ± 0.02
12	3,7-dimethyl-3,6-octadienal	1180	1184	2.1 ± 0.06
13	α-terpineol	1192	1193	0.5 ± 0.02
14	neral	1218	1220	31.5 ± 5.15
15	α-citral	1278	1287	30.1 ± 4.10
16	α-cyclogeraniol	1350	1353	0.3 ± 0.03
17	β-elemene	1387	1393	0.2 ± 0.02
18	β-caryophyllene	1461	1457	1.5 ± 0.07
19	humulene	1464	1466	0.2 ± 0.02
20	β-bisabolene	1486	1495	0.1 ± 0.01
21	Caryophyllene oxide	1610	1613	0.4 ± 0.02
	Total			100.0
	Monoterpenes			27.1
	Sesquiterpenes			2.4
	Terpenoids			67.4
	Others			3.1

Note: The components are reported according to their elution order on apolar column.
Abbreviations: LRI^{calc}, linear retention indices measured on apolar column; LRI^{lit}, linear retention indices from literature.

3.2 | Antimicrobial Activity by Disc Diffusion Assay

The inhibition zones obtained for LCEO and reference antibiotics against the tested microorganisms are summarised in Table 2. In general, LCEO exhibited measurable inhibitory effects against both Gram-positive and Gram-negative bacteria, as well as against yeast isolates, although the extent of inhibition was markedly lower compared with conventional antibiotics.

Among Gram-negative bacteria, inhibition zones of LCEO ranged from 7.67 mm for *S. enterica* to 8.67 mm for *E. coli* and 8.33 mm for *Y. enterocolitica*. In contrast, antibiotic treatments produced significantly larger zones, exceeding 29 mm across all Gram-negative strains ($p \leq 0.05$). For Gram-positive bacteria, LCEO demonstrated stronger activity than against Gram-negative isolates, with inhibition zones of 13.33 mm for *L. monocytogenes*, 13.67 mm for *E. faecalis* and the highest effect of 15.67 mm for *S. aureus*. Nevertheless, antibiotic controls consistently yielded zones over 30 mm. Yeast species were the least susceptible to LCEO. Inhibition zones ranged between 5.67 mm (*C. albicans* and *C. krusei*) and 7.33 mm (*C. parapsilosis*). Antibiotics, by comparison, produced zones between 27.33 and 30.33 mm (Table 2). Notably, the biofilm-forming strain of *S.*

TABLE 2 | Antimicrobial activity assessed by the disc diffusion method.

Microorganism	Inhibition zone of LCEO (mm)	Inhibition zone of ATB (mm)
G-negative bacteria		
Escherichia coli CCM 3953	8.67 ± 0.58^{c}	29.67 ± 0.59^{bcd}
Salmonella enterica CCM 3807	7.67 ± 0.59^{cd}	32.33 ± 0.58^{a}
Yersinia enterocolitica CCM 7204T	8.33 ± 0.57^{cd}	30.33 ± 0.58^{bc}
G-positive bacteria		
Enterococcus faecalis CCM 4224	13.67 ± 0.59^{b}	31.33 ± 0.58^{ab}
Listeria monocytogenes CCM 4699	13.33 ± 0.58^{b}	30.67 ± 0.58^{abc}
Staphylococcus aureus CCM 4423	15.67 ± 0.58^{a}	31.33 ± 0.58^{ab}
Yeast		
Candida albicans CCM 8136	5.67 ± 0.58^{e}	29.67 ± 0.59^{bcd}
Candida glabrata CCM 8270	6.67 ± 0.58^{de}	29.33 ± 0.59^{cd}
Candida krusei CCM 8271	5.67 ± 0.58^{e}	28.33 ± 0.57^{de}
Candida parapsilosis CCM 8260	7.33 ± 0.59^{cde}	30.33 ± 0.58^{bc}
Candida tropicalis CCM 8264	6.67 ± 0.58^{de}	27.33 ± 0.58^{e}
BFB		
Biofilm forming *S. enterica*	8.67 ± 0.58^{c}	29.33 ± 0.59^{cd}

Note: Data represents the mean (±standard deviation) of three samples. Different letters in each column indicate significant differences ($p \leq 0.05$).
Abbreviations: ATB, Antibiotics; LCEO, *Litsea cubeba* essential oil.

enterica showed an inhibition zone (8.67 mm) like that observed for *E. coli*. However, the antibiotic control again achieved a significantly larger effect (29.33 mm). Overall, the results indicate that LCEO possesses broad-spectrum antimicrobial activity, with comparatively stronger effects against Gram-positive bacteria than against Gram-negative bacteria and yeasts. Despite being significantly less potent than standard antibiotics, the consistent inhibitory action across all tested groups supports its potential role as a natural antimicrobial agent.

3.3 | Minimum Inhibitory Concentrations (MIC_{50} and MIC_{90})

The minimum inhibitory concentrations (MIC_{50} and MIC_{90}) of *Litsea cubeba* essential oil (LCEO) against the tested microorganisms are presented in Table 3. The results revealed considerable variability in susceptibility among bacterial and yeast strains. Among Gram-negative bacteria, *Y. enterocolitica* was the most sensitive, with MIC_{50} and MIC_{90} values of 0.156 and 0.191 mg/mL, respectively. In contrast, *E. coli* exhibited the highest resistance, requiring 2.218 mg/mL (MIC_{50}) and 2.332 mg/mL (MIC_{90}) for inhibition. *S. enterica* showed intermediate susceptibility, with MIC_{50} and MIC_{90} values of 1.042 and 1.235 mg/mL, respectively (Table 3). For Gram-positive bacteria, *L. monocytogenes* was the most susceptible strain ($MIC_{50} = 0.381$ mg/mL; $MIC_{90} = 0.420$ mg/mL). *S. aureus* and *E. faecalis* exhibited similar responses, with MIC_{50} values of 0.762 and 0.783 mg/mL, respectively. Among the yeasts, *C. albicans* showed the lowest MIC values ($MIC_{50} = 0.077$ mg/mL; $MIC_{90} = 0.102$ mg/mL), followed by *C. glabrata* ($MIC_{50} = 0.136$ mg/mL; $MIC_{90} = 0.156$ mg/mL). Conversely, *C. krusei* displayed reduced sensitivity, with MIC_{50} and MIC_{90} values of 0.718 and 0.836 mg/mL, respectively. Overall, the MIC data demonstrate that LCEO exerts inhibitory effects across a broad spectrum of microorganisms, with yeasts and *Y. enterocolitica* being the most susceptible, and *E. coli* the least sensitive.

3.4 | In Situ Antimicrobial Effect of LCEO

The vapour-phase activity of LCEO on apple slices is presented in Figure 1. Among Gram-negative bacteria, the strongest inhibition was achieved at 500 μg/L, reaching 74.08% for *E. coli*, 74.26% for *S. enterica* and 74.04% for *Y. enterocolitica*. In the group of Gram-positive bacteria, the maximal effect was also observed at 500 μg/L, with inhibition levels of 55.15% for *E. faecalis*, 55.86% for *L. monocytogenes* and 54.48% for *S. aureus*. A different response was recorded for yeasts. Their highest susceptibility appeared at 125 μg/L, with inhibition rates of 75.30% for *Candida albicans*, 74.52% for *C. glabrata*, 74.86% for *C. krusei*, 72.30% for *C. parapsilosis* and 73.30% for *C. tropicalis*. At higher concentrations, the inhibitory effect declined. For the biofilm-forming *S. enterica* strain, the maximum inhibition was also detected at 125 μg/L, reaching 74.49%. In summary, LCEO exhibited the most pronounced antimicrobial activity on apple slices against Gram-negative bacteria at 500 μg/L, whereas yeasts and biofilm-forming *S. enterica* were most sensitive at 125 μg/L. Gram-positive bacteria were less affected, with maximum inhibition not exceeding 56% (Figure 1).

The antimicrobial activity of LCEO on pear slices increased with concentration, but the extent of inhibition differed across microbial groups (Figure 2). Among Gram-negative bacteria, the strongest response was recorded at 500 μg/L. *E. coli* showed 93.82%, *S. enterica* 91.67% and *Y. enterocolitica* 91.93%. At 62.5 μg/L, inhibition remained moderate (55.78%–56.78%), while intermediate concentrations (125–250 μg/L) produced values above 66%, reaching up to 91.34% in *Y. enterocolitica*. For Gram-positive bacteria, the inhibitory effect also increased with concentration. At 500 μg/L, *E. faecalis* reached 93.15%, *L-monocytogenes* 92.67% and *S. aureus* 93.49%. In contrast, the lowest concentration (62.5 μg/L) resulted in weaker activity, with values around 24.34%–24.93%. The yeasts were less sensitive overall. *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. tropicalis* exhibited maximum inhibition between 76.23% and 80.45% at 500 μg/L (Figure 2). At 62.5 μg/L, their inhibition remained low, ranging from 15.12% (*C. tropicalis*) to 16.56%

TABLE 3 | Minimal inhibition concentration (MIC_{50} and MIC_{90}) and minimal biofilm inhibition concentration ($MBIC_{50}$ and $MBIC_{90}$) of LCEO in mg/mL.

Microorganism	MIC_{50}	MIC_{90}
G-negative bacteria		
Escherichia coli CCM 3953	2.218 ± 0.177^{a}	2.332 ± 0.218^{a}
Salmonella enterica CCM 3807	1.042 ± 0.046^{b}	1.235 ± 0.056^{b}
Yersinia enterocolitica CCM 7204T	0.156 ± 0.011^{fg}	0.191 ± 0.016^{de}
G-positive bacteria		
Enterococcus faecalis CCM 4224	0.783 ± 0.107^{c}	0.940 ± 0.122^{c}
Listeria monocytogenes CCM 4699	0.381 ± 0.015^{e}	0.420 ± 0.040^{d}
Staphylococcus aureus CCM 4423	0.762 ± 0.065^{cd}	0.887 ± 0.085^{c}
Yeast		
Candida albicans CCM 8136	0.077 ± 0.018^{g}	0.102 ± 0.019^{e}
Candida glabrata CCM 8270	0.136 ± 0.009^{fg}	0.156 ± 0.010^{e}
Candida krusei CCM 8271	0.718 ± 0.025^{cd}	0.836 ± 0.062^{c}
Candida parapsilosis CCM 8260	0.580 ± 0.025^{d}	0.719 ± 0.063^{c}
Candida tropicalis CCM 8264	0.159 ± 0.009^{fg}	0.170 ± 0.010^{e}
BFB	$MBIC_{50}$	$MBIC_{90}$
Biofilm forming *S. enterica*	0.328 ± 0.013^{ef}	0.347 ± 0.019^{de}

Note: Data represent the mean (±standard deviation) of three samples. Different letters in each column indicate significant differences ($p \leq 0.05$).

(*C. krusei*). The most pronounced increase occurred between 125 μg/L (33.45%–35.12%) and 250 μg/L (59.16%–61.23%). The biofilm-forming *S. enterica* showed an intermediate response compared with the planktonic bacteria. Inhibition was 54.89% at 62.5 μg/L, increased to 64.53% at 125 μg/L and reached 84.41% at 500 μg/L. In summary, LCEO exhibited the strongest antimicrobial activity on pear slices against Gram-positive and Gram-negative bacteria, with inhibition consistently exceeding 90% at 500 μg/L. Yeasts were less affected, with maximum inhibition around 76%–80%. The biofilm-forming *S. enterica* showed intermediate sensitivity, peaking at 84.41%.

The antimicrobial response of *Litsea cubeba* essential oil (LCEO) on potato slices showed marked differences among microbial groups and concentrations (Figure 3). Among Gram-negative bacteria, inhibition was initially high at 62.5 μg/L, reaching 83.27% for *E. coli*, 87.64% for *S. enterica* and 87.30% for *Y. enterocolitica*. However, further increase in concentration led to a progressive decline in activity. At 500 μg/L, inhibition dropped to 22.63%–23.56%, indicating a strong biphasic effect with maximum suppression occurring at the lowest dose. A similar trend was observed in Gram-positive bacteria. At 62.5 μg/L, inhibition ranged from 85.86% (*E. faecalis*) to 87.08% (*S. aureus*). Intermediate concentrations (125 μg/L) yielded slightly reduced effects (74.82%–75.75%), while at 500 μg/L, inhibition decreased sharply to 22.35%–23.52%. In contrast, the yeasts demonstrated the opposite response pattern. At 62.5 μg/L, inhibition remained low (16.05%–18.92%). A strong concentration-dependent increase was evident, culminating in maximum inhibition between 82.04% and 85.23% at 500 μg/L across all five species (*C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*). The biofilm-forming *S. enterica* showed the most extreme biphasic effect. At 62.5 μg/L, inhibition peaked at 88.60%, whereas at 500 μg/L, it decreased to 22.63%, paralleling the response of planktonic Gram-negative and Gram-positive bacteria (Figure 3). In summary, LCEO displayed contrasting behaviours on potato slices: Gram-negative and Gram-positive bacteria were most sensitive at the lowest concentration (62.5 μg/L) with inhibition above 85%, while yeasts responded in a dose-dependent manner with maximum inhibition (82%–85%) at 500 μg/L. Biofilm-forming *S. enterica* mirrored the bacterial response, with strong inhibition at 62.5 μg/L followed by a steep decline at higher concentrations.

The inhibitory activity of *Litsea cubeba* essential oil (LCEO) on parsley slices is presented in Figure 4. Among Gram-negative bacteria, inhibition increased progressively with concentration. At 62.5 μg/L, suppression was modest (36.93% for *E. coli*, 35.71% for *S. enterica* and 36.27% for *Y. enterocolitica*). Strong concentration-dependent responses were recorded, reaching 86.68% for *E. coli*, 86.82% for *S. enterica* and 86.38% for *Y. enterocolitica* at 500 μg/L. For Gram-positive bacteria, a similar pattern was observed. At 62.5 μg/L, inhibition ranged between 36.15% (*L. monocytogenes*) and 36.52% (*E. faecalis*). With increasing concentrations, activity intensified, culminating at 85.86% (*L. monocytogenes*), 86.08% (*E. faecalis*) and 86.04% (*S. aureus*) at 500 μg/L. The yeasts showed generally weaker inhibition compared to bacteria. At 62.5 μg/L, values were low (34.12%–36.45%). Even at the highest concentration, inhibition remained moderate, ranging from 62.78% (*C. krusei*) to 66.82% (*C. tropicalis*). None of the yeast species exceeded 67% inhibition at 500 μg/L, indicating a reduced sensitivity on parsley slices. The biofilm-forming *S. enterica* exhibited a distinct response. At 62.5 μg/L, inhibition was 35.45%, increasing steadily with concentration and peaking at 85.82% at 500 μg/L, the highest value among all tested microorganisms on parsley (Figure 3). In summary, the strongest inhibitory effect of LCEO on parsley was observed against *S. enterica* (86.82% at 500 μg/L), followed by both Gram-negative and Gram-positive bacteria (85%–87%). Yeasts demonstrated the lowest susceptibility, with inhibition not exceeding 67% even at the maximum concentration tested.

3.5 | Antibiofilm Activity of *Litsea cubeba* EO

The antibiofilm activity of *Litsea cubeba* EO against *Salmonella enterica* was assessed using the crystal violet method (Table 3). At the tested concentrations, the EO significantly reduced biofilm

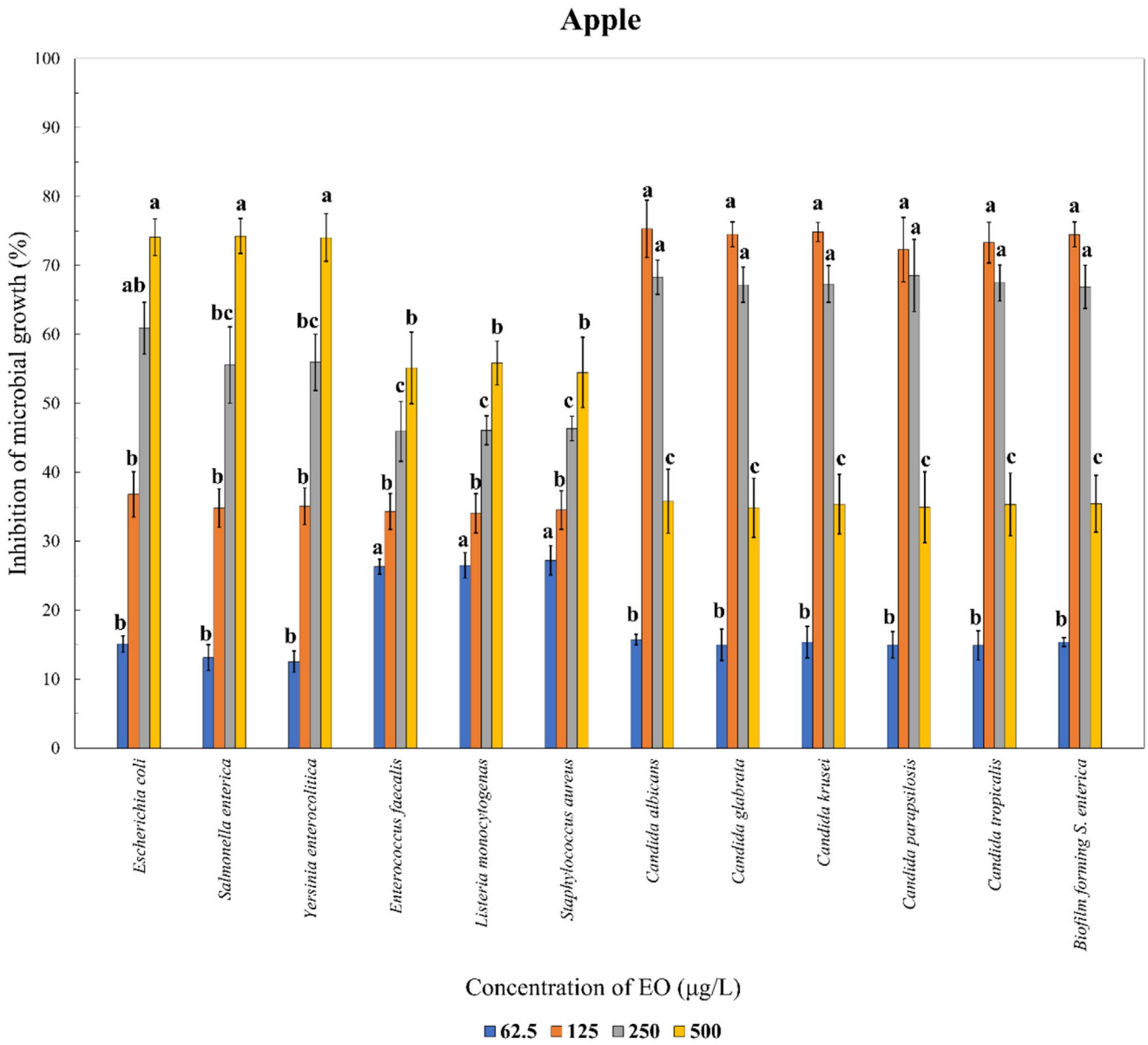


FIGURE 1 | In situ analysis of the inhibition of microbial growth (%) of the vapour phase of LCEO in apple. Data are the mean (bars indicate ± standard deviation) of three samples. Different letters for each EO concentration refer to significant differences ($p \leq 0.05$).

biomass, with $MBIC_{50} = 0.33$ mg/mL and $MBIC_{90} = 0.35$ mg/mL. These values indicate a strong antibiofilm effect at relatively low concentrations (Table 3).

MALDI-TOF MS spectra confirmed structural and compositional differences between planktonic cells and biofilm-associated forms on glass and stainless-steel surfaces (Figure 5A–C). Planktonic cells (SEPC 3) displayed dominant peaks at 3403.0, 4763.6, 5144.9, 7264.1, 9524.6 and 10,190.8 *m/z*, together with high-mass signals above 12,000 *m/z* (12,229.2, 15,417.2 and 17,751.5 *m/z*). The biofilm formed on glass (SEP 3) exhibited a more complex spectral profile, with additional peaks in the 4000–9000 *m/z* range (4356.4, 6257.2, 7305.4, 8901.2 *m/z*), while the intensity of high-mass signals was reduced, indicating altered protein expression upon surface attachment. The biofilm on stainless steel (SESS 3) retained some characteristic planktonic peaks (4760.6, 5256.3, 7304.3, 9523.1 *m/z*), but new signals appeared in the low and mid-*m/z* range (2624.7, 4363.2, 5381.4 *m/z*), suggesting compositional adjustments specific to stainless-steel adherence (Figure 5A).

SEPC 7 spectra showed higher overall complexity compared with Day 3, with numerous dominant peaks in the range of 3000–12,000 *m/z* (3402.7, 4762.1, 6483.9, 8739.9, 9236.8 *m/z*), and extended high-mass peaks (11,890.3, 12,228.7, 15,495.3, 17,844.7 and 18,697.3 *m/z*). The glass biofilm (SEP 7) preserved the dominant planktonic peak at 4763.3 *m/z*, while also presenting new signals (4186.3, 7941.5, 10069.3 *m/z*). Compared to planktonic cells, the intensity of peaks was generally lower, but the spectrum contained distinct new features, especially in the higher mass range (13,520.1 and 15,883.7 *m/z*). Stainless-steel biofilms (SESS 7) retained several of the planktonic signals (4762.6, 6485.8, 9522.9 *m/z*), yet additional peaks (8090.5, 11,827.5, 15,414.2, 17,747.4 *m/z*) revealed structural

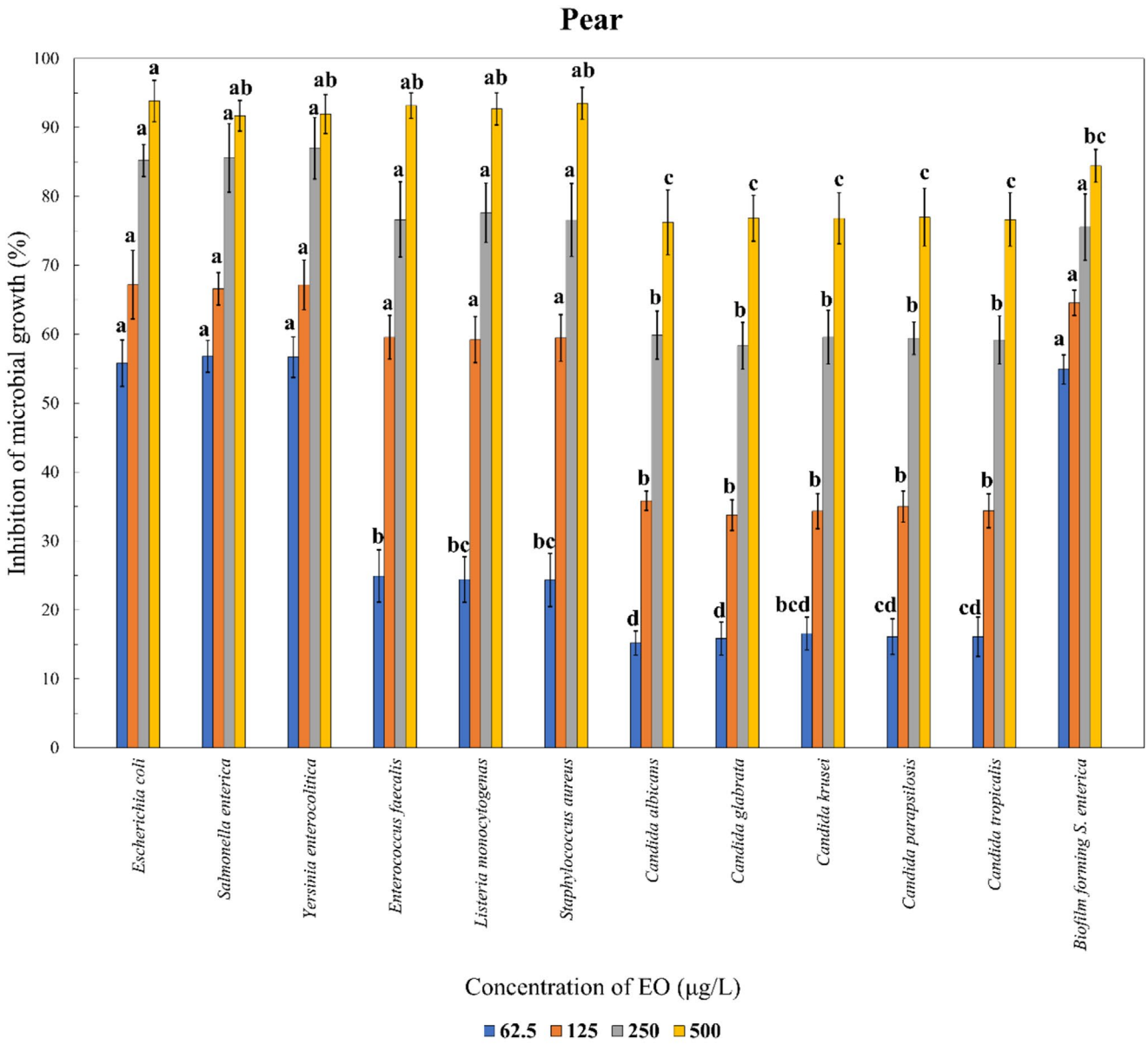


FIGURE 2 | In situ analysis of the inhibition of microbial growth (%) of the vapour phase of LCEO in pear. Data are the mean (bars indicate ± standard deviation) of 3 samples. Different letters for each EO concentration refer to significant differences ($p \leq 0.05$).

changes associated with biofilm maturation on stainless steel (Figure 5B).

Planktonic cells (SEPC 14) demonstrated highly complex spectra with dominant peaks at 7169.1 and 9637.5 *m/z*, accompanied by strong signals at 2381.2, 4817.2, 6568.4, 7711.6, 9248.9 and 10,200.5 *m/z*. High-mass signals were also abundant (12,216.6, 15,424.8, 16,623.9 and 17,760.9 *m/z*). Biofilm formed on glass (SEP 14) maintained the dominant 7167.4 *m/z* peak, while exhibiting new mid- and high-mass signals (4018.8, 8043.9, 10,278.3, 15,536.1 *m/z*), indicating increased spectral heterogeneity. Biofilms on stainless steel (SESS 14) showed a less intense but stable profile, retaining peaks at 7176.4, 8047.8, 9233.4 and 10,211.9 *m/z*, with additional signals at 12,352.4 and 15,559.8 *m/z*. Compared with glass biofilms, stainless steel adherence resulted in lower overall intensity but greater stability of dominant features (Figure 5C).

Across all time points, planktonic cells consistently exhibited complex and intense spectral profiles with multiple dominant peaks, particularly in the mid- to high-*m/z* range. Biofilms formed on glass demonstrated progressive spectral diversification, with the appearance of new peaks and increased heterogeneity over time, most pronounced at Day 14. In contrast, biofilms formed on stainless steel showed distinct compositional changes already at Day 3, retained several planktonic-like features, but overall peak intensity was lower, suggesting stable yet less heterogeneous biofilm development. These observations indicate that *S. enterica* biofilm formation is strongly influenced by the type of surface, with glass favouring higher variability and stainless steel supporting more stable but compositionally distinct biofilm structures during maturation.

The MSP dendrogram derived from MALDI-TOF MS spectra illustrates the clustering relationships between planktonic and

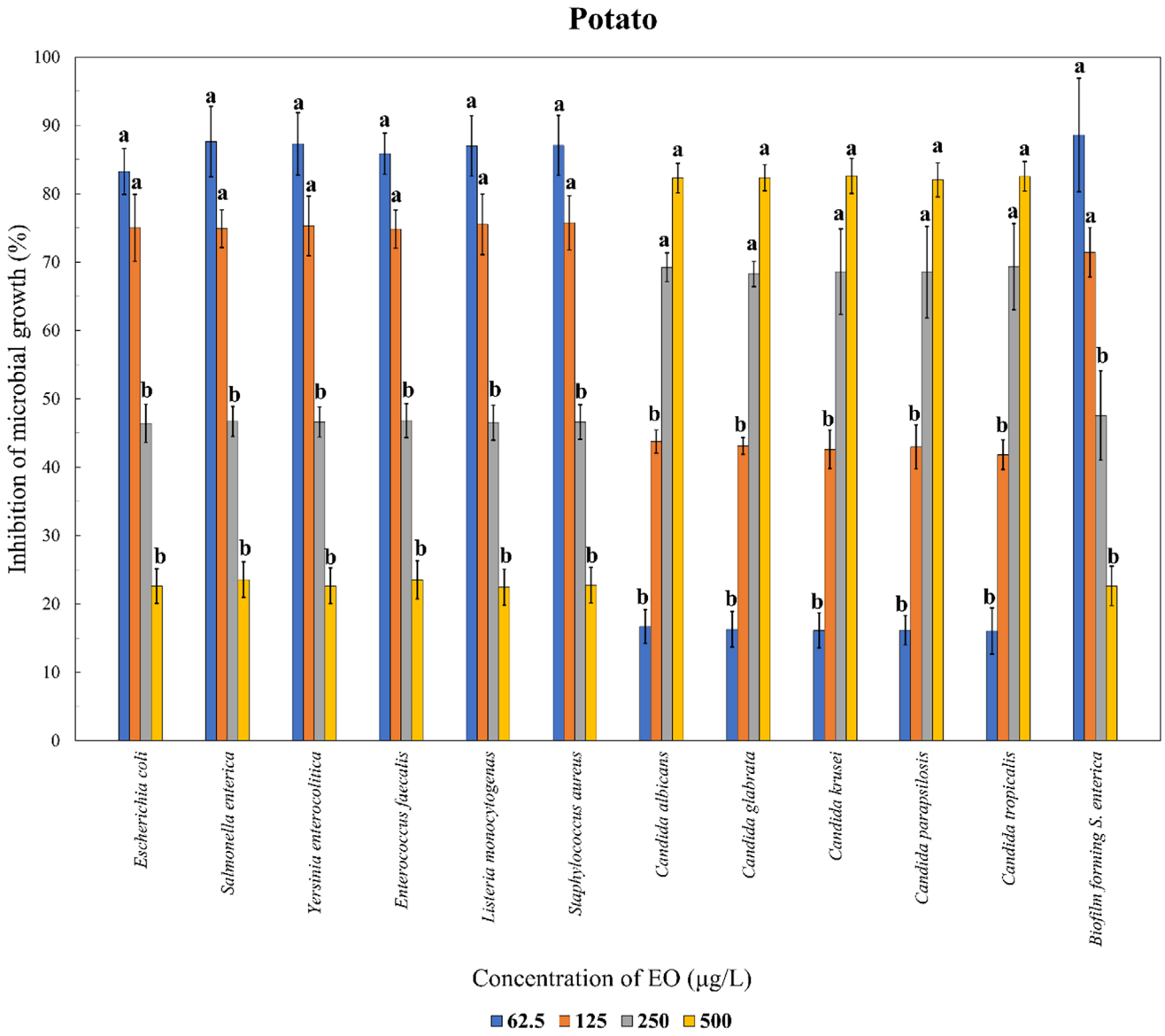


FIGURE 3 | In situ analysis of the inhibition of microbial growth (%) of the vapour phase of LCEO in potato. Data are the mean bars indicate ± standard deviation of three samples. Different letters for each EO concentration refer to significant differences ($p \leq 0.05$).

biofilm-associated cells of *Salmonella enterica* after 3, 7 and 14 days of incubation in the presence of *Litsea cubeba* essential oil (LCEO, LC) (Figure 6).

Planktonic controls (PC) from different days grouped closely together, indicating a high degree of spectral similarity and stability in their protein profiles across the incubation period (Figure 6). In contrast, biofilm-associated cells showed a clear separation from planktonic forms. At Day 3, biofilms on glass (SELCP) and stainless steel (SELCSS) clustered together and remained distant from the corresponding planktonic cells, reflecting early biofilm-specific spectral changes. By Day 7, the spectra of surface-attached cells again grouped separately from the planktonic controls, but glass- and steel-associated biofilms displayed closer relationships with each other than with PCs, suggesting convergence of biofilm-associated profiles regardless of the surface material.

At Day 14, biofilms formed on both surfaces exhibited distinct clustering, separated not only from planktonic controls but also from early biofilm stages (Day 3). This indicates progressive divergence in the protein composition of mature biofilms compared with both planktonic cells and younger biofilm forms. Overall, the dendrogram demonstrates that biofilm development under the influence of LCEO is accompanied by characteristic and time-dependent spectral changes, with stronger differences emerging at later stages of incubation.

3.6 | Assessment of the Insecticidal Potential of LCEO

The insecticidal activity of *Litsea cubeba* essential oil (LCEO) against adults of *C. maculatus* is summarised in Table 4. Complete mortality (100.00% ± 0.00%) was observed at the

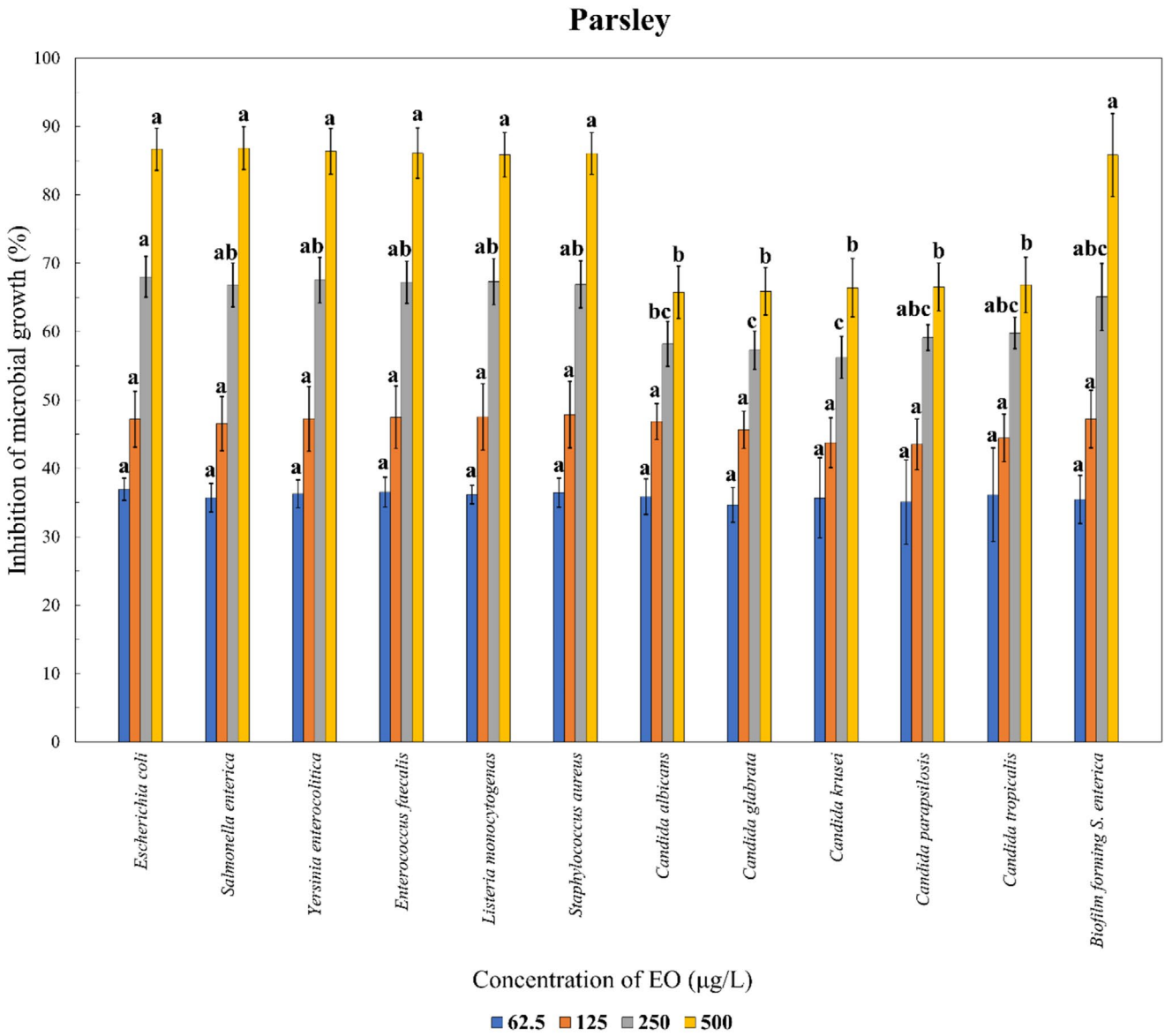


FIGURE 4 | In situ analysis of the inhibition of microbial growth (%) of the vapour phase of LCEO in parsley. Data are the mean (bars indicate ± standard deviation) of three samples. Different letters for each EO concentration refer to significant differences ($p \leq 0.05$).

two highest concentrations tested (100% and 50%), indicating a strong fumigant effect of LCEO. At 25%, mortality reached 90.00% ± 0.00%, while at 12.5% the effect decreased to 50.00% ± 0.00%. Sublethal concentrations of 6.25% and 3.125% resulted in only 10.00% ± 0.00% and 0.00% ± 0.00% mortality, respectively, values comparable to the untreated control (0.00% ± 0.00%). These results demonstrate a clear dose-dependent insecticidal activity of LCEO, with full effectiveness achieved at ≥ 50% concentration.

4 | Discussion

Litsea cubeba essential oil (LCEO) is increasingly highlighted as a multifunctional natural agent with antibacterial, antifungal and antibiofilm potential, attributable largely to its monoterpene-rich, citral-dominated profile and associated membrane disruptive mechanisms. Prior work documents substantial chemical diversity and composition–activity links across plant parts and geographies, as well as formulation-enabled enhancements of efficacy (e.g., nano–/micro-structures). Our findings extend this evidence base by evaluating LCEO across in vitro assays, vapour-phase in situ models on fresh produce, biofilm systems profiled by MALDI-TOF MS, and an insecticidal test [2, 17–21].

The chemical profile of our LCEO, dominated by neral (31.5%), α-citral (30.1%) and limonene (19.6%), is consistent with the citral-rich chemotype frequently reported for fruits of *L. cubeba* from subtropical Asia. Si et al. [22] analysed oils from eight Chinese regions and similarly identified citral isomers as the main constituents, though with variable limonene levels depending on geographic origin. Liu et al. [1] confirmed citral dominance but noted notable contributions of citronellal and

linalool in Vietnamese samples, highlighting the influence of regional and ecological factors. Likewise, Chen et al. [23] reported β-citral, citronellal and limonene as leading volatiles, underlining the chemotypic diversity of this species. Studies focusing on non-fruit plant parts further illustrate variability: leaf and twig oils often contain higher proportions of 1,8-cineole, sabinene, or α-terpineol, with relatively lower citral content [24]. Such variation across geography, plant part and extraction conditions underscores the need for detailed chemical characterisation when linking composition to biological activity. In our case, the comparatively high limonene fraction may help explain the pronounced vapour-phase efficacy observed on potato and parsley, where volatility and sorption behaviour are critical.

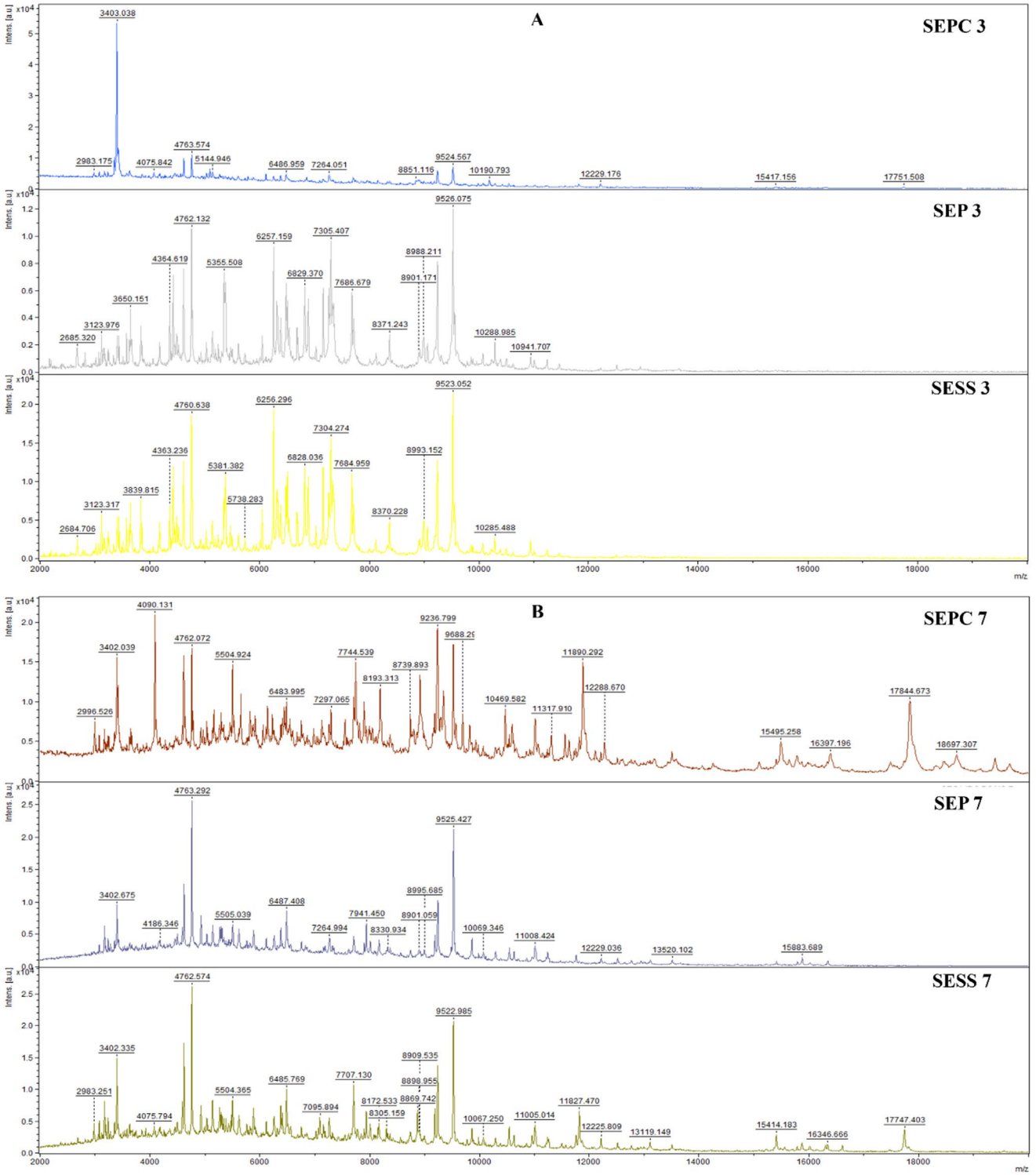


FIGURE 5 | MALDI-TOF MS spectral profiles of *Salmonella enterica* planktonic culture and biofilms developed on plastic and stainless-steel surfaces after 3 (A), 7 (B) and 14 (C) days of incubation. PC—planktonic culture; P—biofilm on plastic; SS—biofilm on stainless steel.

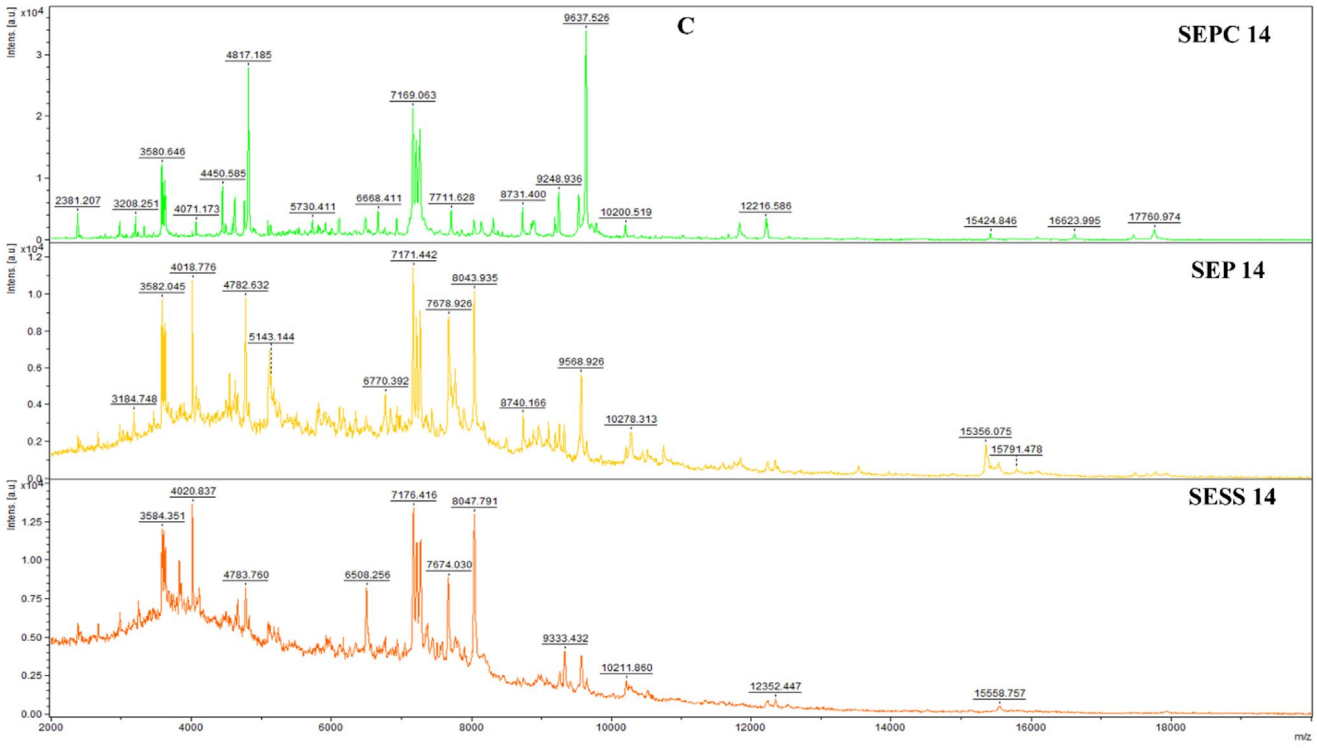


FIGURE 5 | (Continued)

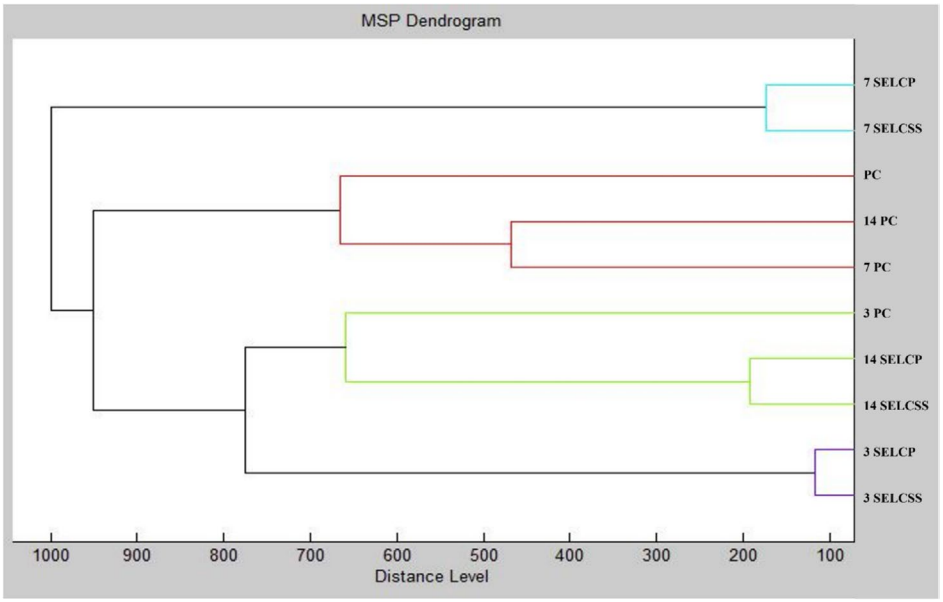


FIGURE 6 | MALDI-TOF MS dendrogram illustrating the clustering of *Salmonella enterica* planktonic cells and biofilms formed on glass and stainless-steel surfaces at different incubation times. PC, planktonic control; SEG, cells adhered to glass; SESS, cells adhered to stainless steel.

In the agar diffusion assay, LCEO produced measurable but comparatively smaller zones than reference antibiotics, with the familiar pattern of stronger effects on Gram-positive bacteria than on Gram-negative taxa consistent with literature reporting higher EO susceptibility among Gram-positives and reduced Gram-negative susceptibility linked to outer-membrane barriers and stress-response mechanisms [1, 17, 25]. Fungal isolates typically show lower and more variable responses to EOs, which aligns with our observations [1, 26, 27]. Composition and chemotype (notably citral isomers) likely underpin these trends [23, 27, 28], and prior studies with oral, food-borne and clinically relevant bacteria including *Staphylococcus aureus*, *Streptococcus mutans*, *Acinetobacter baumannii* and *Salmonella* spp. report convergent activity patterns and mechanisms for LCEO [19, 25, 29].

In the MIC assays, susceptibility patterns followed established structure–activity expectations for essential oils: Gram-negative

TABLE 4 | Insecticidal activity of LCEO against *C. maculatus* adults ($n = 50$).

Concentration (%)	Number of living individuals	Number of dead individuals	Insecticidal activity (%)
100	0	100	100.00 ± 0.00
50	0	100	100.00 ± 0.00
25	10	90	90.00 ± 0.00
12.5	50	50	50.00 ± 0.00
6.25	90	10	10.00 ± 0.00
3.125	100	0	0.00 ± 0.00
Control group	100	0	0.00 ± 0.00

bacteria tended to be less responsive than Gram-positive counterparts, whereas several yeasts showed comparatively favourable responses to LCEO. Such differences are plausibly explained by chemotype and distillation effects that tune citral-isomer ratios and minor terpenoids, alongside geographic/organ variability [1, 23, 27, 28]. Mechanistic reports across pathogens including *Staphylococcus aureus*, *Acinetobacter baumannii*, *Salmonella* spp., and filamentous fungi consistently implicate membrane damage, permeability changes and energetic stress, providing a rationale for the observed MIC ordering [17, 19, 20, 25]. Moreover, formulation strategies that improve dispersion and surface contact (e.g., polysaccharide nanofibers or peptide micro/nanotubes) can enhance apparent potency, which aligns with the relative performance shifts seen across taxa [30, 31]. Studies on oral and food-borne bacteria further corroborate these trends and mechanisms for LCEO [29].

On apple slices, vapour-phase LCEO suppressed all tested groups but with matrix-dependent patterns: bacteria showed progressively stronger inhibition with increasing vapour-phase dose, whereas yeasts and the biofilm-forming *Salmonella* displayed peak susceptibility at intermediate levels consistent with volatile partitioning/condensation behaviour on sugar- and moisture-rich fruit surfaces and the need for controlled release to stabilise activity. Prior work shows that formulation (e.g., chitosan LCEO systems) can enhance dispersion and sustain antimicrobial effects, which is directly applicable to vapour-phase delivery on produce [32]. Mechanistic studies further support a membrane-centric action with attenuation of virulence functions (e.g., listeriolysin O) and rapid damage in marine food-borne pathogens, consistent with the responses observed here [30, 33]. Extraction/fractionation choices also tune volatile profiles and antifungal potency relevant for apples where surface chemistry modulates EO retention while regional/oil-origin variability can shift activity baselines [34, 35].

On pear slices, vapour-phase LCEO produced a monotonic concentration–response in both Gram-negative and Gram-positive bacteria, consistent with headspace partitioning and favourable retention of citral-rich volatiles on sugar- and pectin-rich surfaces that can sustain contact over time. Yeasts were comparatively less responsive, in line with reports that fungal cell-wall architecture and stress defences temper EO efficacy, while the biofilm-forming *Salmonella* showed intermediate susceptibility consistent with partial biofilm tolerance. These matrix-linked outcomes agree with evidence that LCEO activity is strongly conditioned by chemotype and distillation (which tune citral isomers and minor terpenoids) and can be further optimised by formulation strategies that stabilise release and improve surface coverage (e.g., polysaccharide or peptide carriers) [1, 25, 27, 28, 30, 31, 35].

On potato slices, LCEO showed a biphasic response: bacteria were most responsive at the lowest vapour-phase dose, whereas yeasts required higher doses for maximal suppression. This divergence is consistent with differences in cell-envelope architecture and stress responses between bacteria and fungi, together with matrix-driven headspace dynamics on a starchy, low-acid surface that can favour rapid volatilisation at low doses yet increase condensational/contact effects at higher doses for fungi. The bacterial pattern aligns with reports that citral-rich LCEO disrupts membranes and energy metabolism in diverse pathogens, while fungal outcomes reflect known variability of EO sensitivity across taxa [17, 26, 29]. Composition/chemotype considerations (citral isomers and accompanying monoterpenes) also provide a plausible basis for these matrix-conditioned effects [36].

On parsley, vapour-phase LCEO produced a clear concentration-dependent enhancement in bacterial inhibition across both Gram-negative and Gram-positive groups, whereas yeasts remained comparatively less responsive an outcome consistent with fungal cell-wall constraints and stress defences against essential oils. The pronounced increase at higher vapour-phase doses is compatible with surface–headspace partitioning on herbaceous, cuticle-bearing tissues and suggests that maintaining volatile availability (rather than only initial load) is critical on leafy matrices. Literature indicates that chemotype and fractionation can tune citral-isomer balance and minor terpenoids that shape volatility and antimicrobial impact, while formulation strategies (e.g., polysaccharide fibres or peptide micro/nanotubes) improve dispersion, surface contact and persistence, directly applicable to fresh herbs [1, 25, 28, 30, 31, 35]. Across the four matrices, vapour-phase LCEO showed clear matrix-dependent behaviour governed by volatility–sorption and chemotype (citral-isomer balance and minor terpenoids) [27, 35]. On apple, bacterial inhibition increased with dose while yeasts and biofilm Salmonella peaked at intermediate headspace levels, consistent with transient headspace dynamics on moist, sugar-rich tissues [19, 33]. Pear displayed a more monotonic concentration–response, compatible with steadier headspace retention on pectin-rich surfaces ([34]). Potato showed a biphasic split strong early bacterial effects at low dose vs. fungal gains

at higher dose reflecting envelope differences and starchy, low-acid surface dynamics [26, 29]. Parsley (cuticle-bearing tissue) required sustained availability for bacterial/biofilm forming *Salmonella*, with yeasts remaining comparatively less responsive [17]. Practically, formulation/controlled release can stabilise headspace and improve contact across these matrices [20, 32].

Crystal-violet assays showed that LCEO suppressed *S. enterica* biofilm biomass, and MALDI-TOF patterns revealed time- and surface-dependent divergence of attached cells from planktonic controls consistent with biofilm-specific remodelling. Mechanistically, this fits with reports that LCEO (and its citral-rich profile) disrupts *Salmonella* membranes, impairs ATPase activity, and inhibits biofilm formation traits (motility/hydrophobicity), thereby undermining early attachment and matrix accrual [19]. Beyond direct action, formulation work indicates that nanoemulsified LCEO and ultrasound + LCEO nanoemulsion can intensify antimicrobial performance against *Salmonella*, a delivery angle that can translate to better surface contact on glass/stainless steel without raising dose [37]. Complementary overviews specific to *Salmonella* biofilms show that terpenes (citral among them) consistently reduce biomass and alter 3-D structure via EPS depletion and membrane stress aligning with the phenotypic shifts captured by your MALDI dendrograms [38]. Finally, LCEO's antibiofilm efficacy in other foodborne bacteria (e.g., *Cronobacter sakazakii* on stainless steel) supports a generalisable mode of action on food-contact materials, strengthening the rationale for LCEO in sanitation scenarios [10].

Our dose–response data for *C. maculatus* (complete adult mortality at ≥ 50% LCEO under closed conditions) align with independent evidence that *Litsea cubeba* oil is a potent arthropod toxicant in vapour/contact assays and that citral-type unsaturated aldehydes drive the effect. In ticks, *H. longicornis*, LCEO and its principal constituent 3,7-dimethyl-2,6-octadienal showed strong acaricidal and repellent activity, with structure–activity pointing to the importance of C=C unsaturation and methyl substitution mechanistic features consistent with fumigant lethality we observed in beetles [39]. Broader stored-product literature confirms essential oils as effective biofumigants/biopesticides against bruchids and other coleopterans, supporting the feasibility of LCEO for cowpea weevil control when delivery and headspace are managed [40, 41]. Recent EO-based insecticide studies further emphasise formulation (e.g., encapsulation) to enhance stability and persistence in stored-legume systems, which is directly applicable to translating our lab mortality into practice [42].

Overall, the present results support LCEO as a practical candidate for vapour-phase sanitation of fresh produce (apple, pear, potato, parsley) and for closed-space fumigation against adult *C. maculatus*. Moving from laboratory to use will require batch standardisation toward a citral-rich chemotype (once composition is reported), selection of delivery formats that maintain a stable vapour phase over product surfaces and inside semi-closed containers (e.g., sachets, thin coatings, nanoemulsions), and verification on food-contact materials (stainless steel, glass) in semi-closed test boxes that mirror routine handling and storage. Follow-up work should repeat the same endpoints used here agar diffusion and MIC, in situ inhibition on the four matrices, crystal-violet biofilm assays with MALDI-TOF profiling, and adult mortality of *C. maculatus* to define practical dose ranges.

5 | Conclusion

This study demonstrated that *Litsea cubeba* essential oil (LCEO) possesses broad-spectrum antimicrobial and antibiofilm activities. The strongest in vitro effects were observed against Gram-positive bacteria, while yeast susceptibility varied by assay and matrix; Gram-negative strains were least susceptible in vitro but showed substantial inhibition in situ. In situ assays highlighted matrix-dependent responses, with pears and parsley showing the highest inhibition levels, while potatoes revealed a biphasic effect. Biofilm formation of *Salmonella enterica* was effectively suppressed, as confirmed by both crystal violet assays and MALDI-TOF MS spectral analyses. In addition, LCEO exhibited strong insecticidal activity, achieving complete mortality of *Callosobruchus maculatus* adults at concentrations ≥ 50%. These findings emphasise the potential of LCEO as a natural preservative and biocontrol agent applicable to food safety and storage-related protection.

Author Contributions

Miroslava Kačániová: methodology, investigation, writing – original draft, formal analyses, supervision, project administration, funding acquisition, visualisation. **Minhang Qiao:** formal analyses, writing – review and editing. **Alessandro Bianchi:** statistics, formal analyses, methodology, investigation, writing – original draft, writing – review and editing. **Joel Horacio Elizondo-Luevano:** formal analyses, writing – review and editing. **Anis Ben Hsouna:** formal analyses, writing – review and editing. **Rania Ben Saad:** formal analyses, writing – review and editing. **Zhaojun Ban:** formal analyses, writing – review and editing. **Li Li:** formal analyses, writing – review and editing. **Jian Lou:** formal analyses, writing – review and editing. **Wissem Mnif:** formal analyses, writing – review and editing. **Stefania Garzoli:** formal analyses, data curation, methodology, investigation, writing – review and editing, supervision.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data will be available upon request to the authors.

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