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The potential of essential oils as a botanical control for bacterial leaf blight pathogen in paddy

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Abstract

Xanthomonas oryzae pv. *oryzae* (Xoo) is the causal agent of bacterial leaf blight (BLB), one of the most serious diseases affecting the rice industry. The Xoo bacteria enter either through wounds or hydathodes, multiply in the epitheme, and move to the xylem vessels, where active multiplication results in blight disease symptoms on rice leaves. The prolonged use of chemicals or antibiotics to prevent the disease outbreak may cause undesirable effects such as residual toxicity, development of pathogen resistance, environmental pollution, health hazards to humans and animals, and increased expenditure for plant protection. Meanwhile, essential oils provide a natural, sustainable, and eco-friendly approach to pest management, promoting healthier ecosystems and reducing reliance on synthetic pesticides. Seven plant essential oils were selected for the study. The antibacterial activity was assessed regarding their ability to inhibit Xoo growth on peptone sucrose agar (PSA). Essential oils from lemongrass (LgEO) showed a wide length of inhibition zone diameter (25.67 ± 2.52 mm). The result of the minimum LgEO concentration that kills *Xanthomonas oryzae* pv *oryzae* bacteria was identified at about 0.1% (v/v). This study indicated that LgEO can be developed individually as an environmentally safe bactericidal formulation against BLB in paddy.

Keywords: *bacterial leaf blight, lemongrass, botanical control, essential oil*

Introduction

Rice (*Oryza sativa* L.) is the third important cereal crop worldwide after wheat and maize and still the number one Malaysian staple food. According to the Department of Statistics Malaysia, the country produced 2.639 million mt of paddy in 2018. This number went down to 2.356 million mt in 2020. The import value of rice was recorded at RM 2.38 million in 2021 compared to just about RM 1.63 million in 2018 due to the COVID-19 pandemic (Booklet of Crop Statistics 2022). The emergence of the COVID-19 pandemic in 2020 around the world may increase Malaysian rice importation value. Like other crops, paddy suffers many constraints and threats, including diseases and pests, which cause substantial losses to global food security. The most common and widely distributed seed-borne bacterial diseases of paddy are: bacterial leaf streak (*Xanthomonas oryzae* pv. *oryzicola*), bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*), bacterial sheath brown rot (*Pseudomonas fuscovaginae*), bacterial brown stripe (*Acidovorax avenae* subsp *avenae*), bacterial grain rot (*Burkholderia glumae*) and bacterial sheath rot (*Sarocladium oryzae*). Bacterial leaf blight (BLB) was first observed in Japan in 1884 and has since become one of the most serious rice diseases worldwide (Chukwu et al. 2019 and Zhong et al. 2024). Yield loss due to BLB disease can be as much as 70% when susceptible varieties are grown, in environments favourable to the disease (Ooi et al. 2022 and Din et al. 2023). The yield was not much affected when plants were infected with BLB at the booting stage, however, it may result in poor quality of produced grains and a high proportion of broken rice kernels. Higher yield losses were recorded if the disease occurred at earlier stage of the rice plant. Therefore, it is important to discover a solution to this problem.

According to Ali et al. (2016), an ideal agent for chemical control should function at minimal concentration by either killing or inhibiting the multiplication of the pathogen by blocking its important metabolic pathway.

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Recently, growing public concern over the health and environmental hazards associated with the increased levels of chemical pesticides and the lack of approval for the renewal of some of the most effective active molecules has led to the development of safe, alternative, and natural methods of post-harvest disease control (Elshafie and Camele, 2023).

In the face of this threat, finding effective and environmentally sustainable control measures is paramount to ensure food security and protect the environment. To overcome the risks associated with chemical pesticides, research focusing on plant-derived natural bactericides and their potential applications in agriculture to control plant bacterial diseases has intensified, as this approach holds enormous potential to inspire and influence modern agrochemical research. Naturally occurring and biologically active plant products such as essential oils and organic extracts could be a source of alternative classes of natural biopesticides to serve as templates for new and more effective compounds in controlling plant pathogenic microorganisms. Essential oils offer a more sustainable and environmentally friendly approach to pest and disease management compared to conventional agrochemicals. They degrade easily, leaving fewer residues, and are less harmful to non-target organisms (Alonso-Gato et al. 2021). There is a need to use environmentally safe approaches to overcome the loss of grain yield in rice due to BLB disease. Active compounds from plant especially essential oils, have been demonstrated to possess potent antibacterial, antifungal, insecticidal and nematocidal activity. Therefore, this study on the effect of seven essential oils against *Xanthomonas oryzae* pv. *oryzae* (Xoo) was initiated.

Essential oils (EOs) are concentrated hydrophobic liquids containing volatile aromatic compounds obtained from medicinal and herbal plants. They are recognised for their antioxidant and antimicrobial properties (Elshafie and Camele, 2023). From a farming standpoint (Bunawan et al. 2023), EOs demonstrate effective antimicrobial activity against a wide variety of plant pathogens, including bacteria and fungi. Their efficacy makes them promising alternatives to synthetic pesticides for crop protection. The antimicrobial action of EOs typically involves disrupting microbial cell membranes, leading to leakage of cellular contents and eventual cell death. Components like thymol, eugenol, and carvacrol are especially potent (Alonso-Gato et al. 2021). There is no doubt that essential oils have important antimicrobial properties, which contribute significantly to medicinal and therapeutic practices. The antibacterial mechanisms of EOs are due to their ability to penetrate bacterial membranes into the cell, causing inhibition of vital functions (Bajpai et al. 2012). Therefore, research has been conducted to study the antimicrobial activities of selected essential oils with the potential as botanical control agents for BLB pathogen in paddy.

Materials and method

Essential oils

Six essential oils which are lemongrass (*Cymbopogon citratus*), citronella (*Cymbopogon nardus*), cinnamon (*Cinnamomum verum*), kaffir lime (*Citrus hystrix*), cajuputi (*Melaleuca cajuputi*) and tea tree (*Melaleuca alternifolia*) were supplied from MARDI Kuala Linggi, Malacca, Malaysia. The local essential oils are produced using an industrial scale steam distiller system (Essential oil incubator) located at MARDI Kuala Linggi, using a Standard Operation Procedures (SOP) for essential oil productions established by MARDI. Meanwhile, garlic (*Allium sativum*) was the only essential oil (Blackmores®) bought from Big Pharmacy, Serdang, Selangor, Malaysia. The seven EOs were chosen to be explored based on numerous works of literature reporting their antibacterial features (Wulansari et al. 2017 (tea tree), Sreepian et al. 2019 (kaffir lime), Hong et al. 2021 (cinnamon), Kaur et al. 2021 (citronella), Sarangi et al. 2021 (garlic), Schweitzer et al. 2022 (lemongrass) and Wahab et al. 2022 (cajuputi).

Bacteria

The strain of *Xanthomonas oryzae* pv. *oryzae* (Xoo) was obtained from MARDI Seberang Prai, Penang, Malaysia. The strain was cultured on potato sucrose agar (PSA) at 30°C for 48 hrs. The mass-produced bacteria on PSA agar were collected by washing the colony surface on the agar plate with 1 mL of sterile ultra-pure water. Cells were harvested by centrifugation at 5,000 × g for 15 min at 4°C on a benchtop centrifuge. The pelleted cells were then washed with 0.01 M phosphate buffer saline (PBS) at pH 7.4 and the procedures were repeated thrice. The bacterial cells were then re-suspended in PBS and the bacterial suspensions were adjusted to optical densities (OD) at 600 nm of between 1.0 ± 0.1 to obtain bacterial concentrations at 1×10^9 colony forming unit (CFU)/mL (Srinivas et al. 2024) on a UV/VIS spectrophotometer. The number of organisms in one visible colony is about one billion cells (1×10^9 or log 9 cells). Since a round visible colony could have been developed from one or more individual cells, it is referred to as a Colony Forming Unit or CFU. The bacterial concentrations were confirmed by a spread plate method on PSA agar.

Antimicrobial activity

The study on antimicrobial properties of essential oil used a completely randomised design (CRD) involving Kirby-Bauer disc diffusion protocol with some modification (Hudzicki 2009). The bacterial lawn was prepared by spreading 100 µL of a suspension containing 10^9 CFU/mL of Xoo on PSA using a sterile cotton bud. Then, a 6 mm diameter filter paper disc was individually impregnated with 3 µL of essential oil and placed in the middle of the inoculated agar. Negative control samples

were prepared by replacing essential oils with mineral oil while positive control was prepared using streptomycin sulphate at the concentration of 0.5 mg/mL. Petri dishes were then incubated at 30°C for 7 days. After incubation, the streptomycin and essential oils diffuse into the agar and inhibit the germination and growth of Xoo. The antimicrobial activities of the essential oils were evaluated by measuring the zone of inhibition in diameter (cm) around the discs against Xoo. The length of inhibition or halo zone diameter were recorded at day 1, 2 and 3 post inoculation. Each test assays were done in five replications (n=5).

Minimal bactericidal concentration (MBC) determination

The best essential oil that demonstrated the widest zone of inhibition in diameter (cm) around the discs against Xoo in the antimicrobial activity test was chosen for minimal bactericidal concentration (MBC). The MBC value for LgEO against Xoo was studied through the broth dilution method according to Gormez et al. (2013) with slight modification. The bacterium inoculum was prepared in lysogeny broth cultures and adjusted to an optical density (OD) at 600 nm of 0.1 equal to 0.5 McFarland Standard turbidity. The LgEO was prepared by diluting 10% Dimethyl sulfoxide (DMSO) in lysogeny broth. Then, 2-fold dilutions were performed to prepare a 500 µL solution of the desired concentration percentage of EOs, ranging from 0.5% (v/v) to 0.032% (v/v), in a sterile 1.5 mL Eppendorf tube (Bouaichi et al. 2015 and Proto et al. 2022). Each tube was inoculated with previously prepared Xoo inoculum and then incubated at 30 °C for 24 hours. Then 100 µL Xoo cultured in lysogeny broth were transferred and spread on PSA using a sterile cotton swab. The petri dish was sealed with parafilm and incubated in the oven at 30 °C for 3 days. The growth of Xoo on the PSA petri dish was observed and recorded. These tests were done in three replicates. This is the qualitative assay. The PSA plate with the lowest concentration of LgEO showing no visible growth on all replicates was regarded as the MBC value.

Determination of bioactive compounds in lemongrass essential oil using Gas Chromatography-Mass Spectrometry (GC-MS)

The lemongrass essential oil obtained by steam distillation was analysed by Gas Chromatography-Mass Spectrometer (GC-MS) for the identification of their bioactive compounds. The GCMS (Perkin Elmer) equipped with HP-5MS (5% Phenyl Methyl Silox) column (30 m × 250 µm × 0.25 µm) was used. The oven temperature was programmed as isothermal at an initial 60 °C for 10 minutes, then increased to 3 °C/min and 180 °C for 15 minutes. Helium gas was used as carrier gas at the rate of 3 mL/min. The effluent of GC column was directly introduced into the source of the MS via a transfer line with a temperature program of 280 °C. The sample injection flow rate was at 1 mL/min, and the total run time was 65 minutes. Identification of the essential oil components was performed by computerised matching of the acquired mass spectra with those stored in the mass spectral library of the GC-MS data system (Padrilah et al. 2020).

Data analysis

The study was conducted using a completely randomised design (CRD) with five replicates for each treatment. All data were subjected to analysis using an ANOVA (analysis of variance) test, followed by Tukey's HSD (honestly significant difference) test to ascertain significant differences between treatments.

Results and discussion

Antimicrobial activity

The completely randomised design (CRD) of antimicrobial activities of EOs against Xoo are summarised in *Table 1*. The results show the diameter of the inhibition zone including the diameter of the paper disk (6 mm) at day 3 post-inoculation as visualised in *Figure 1*.

A broad variation in antimicrobial properties of the analysed EOs was observed in this study. The effect of essential oil against Xoo growth in PSA was observed. The widest inhibition zone diameter goes to lemongrass essential oil at 25.67 ± 2.52 mm as well as positive control (streptomycin sulphate) value at 26 ± 1.73 mm. Then followed by citronella and cinnamon with a smaller length of inhibition zone diameter (< 25mm but > 15 mm). Meanwhile, kaffir lime essential oil shows a very small inhibition zone diameter (less than 15 mm). No inhibition zone was recorded on Xoo growth with cajuputi, tea tree, garlic essential oil, and mineral oil. Due to this finding, lemongrass essential oil (LgEO) was chosen to perform the MBC and further analysed with GC-MS.

Table 1. Antibacterial activity of EOs determine by filter disk diffusion assay

Essential oil	Inhibition zone diameter (mm)
Negative control	No inhibition
Positive control	26 ± 1.73
Lemongrass (*Cymbopogon citratus*)	25.67 ± 2.52
Tea tree (*Melaleuca alternifolia*)	No inhibition
Cinnamon (*Cinnamomum zeylanicum*)	21.67 ± 3.05
Citronella (*Cymbopogon nardus*)	15 ± 3.61
Kaffir lime (*Citrus hystrix*)	12.33 ±1.15
Paper bark (*Melaleuca cajuputi*)	No inhibition
Garlic (*Allium sativum*)	No inhibition

** Significantly different (p <0.05)*
** Values are the mean diameter of the inhibitory zone (mm), ±SD of five replicates*
** The diameter includes the size of the paper disk (6mm)*

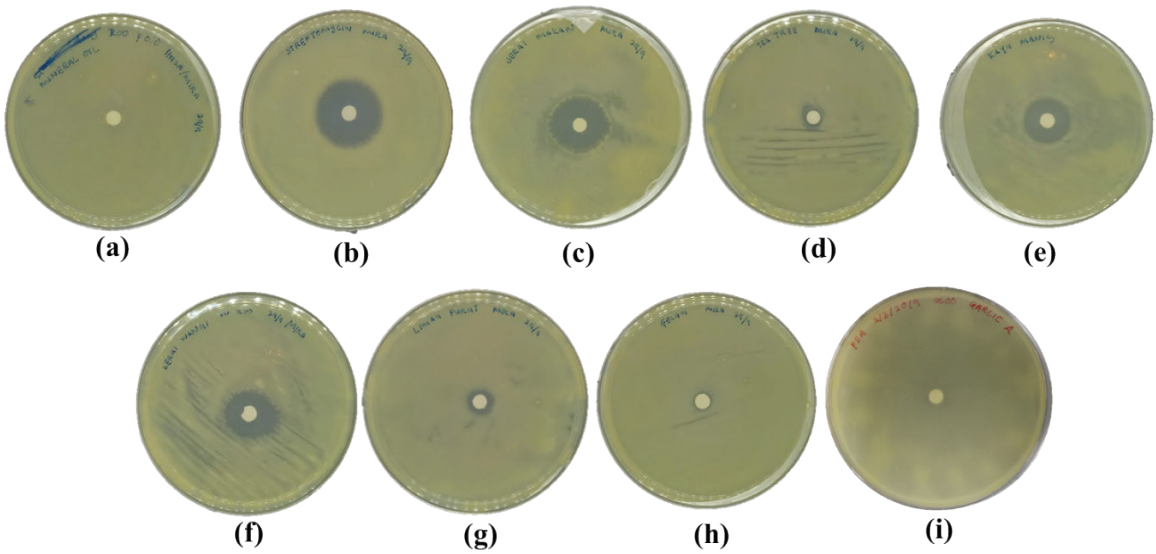

Figure 1. Diameter measurement of inhibition zones obtained from filter paper disc diffusion assay for; (a) negative control (mineral oil), (b) positive control (5mg/ml streptomycin sulphate), (c) lemongrass (Cymbopogon citratus), (d) tea tree (Melaleuca alternifolia), (e) cinnamon (Cinnamomum zeylanicum), (f) citronella (Cymbopogon nardus), (g) kaffir lime (Citrus hystrix), (h) paper bark (Melaleuca cajuputi), and (i) garlic (Allium sativum)

Minimal bactericidal concentration (MBC) determination

The effect of an antibacterial agent against microorganisms is often measured as the minimum bactericidal concentration (MBC). The lemongrass essential oil (LgEO) was chosen to perform the MBC due to its antibacterial ability by showing the largest inhibition zone on Xoo growth on PSA petri dishes. The MBC value is the lowest concentration percentage of LgEO resulting in killing 99.9% of the *Xanthomonas oryzae* pv *oryzae* bacteria tested with about 0.1% (v/v) against *Xanthomonas oryzae* pv. *oryzae* bacteria as highlighted in *Table 2*.

Table 2. The minimum bactericidal concentration percentage value of lemongrass (*Cymbopogon citratus*) essential oil

Concentration (%)	Day 1	Day 2	Day 3
0.5	-	-	-
0.4	-	-	-
0.3	-	-	-
0.25	-	-	-
0.2	-	-	-
0.15	-	-	-
0.125	-	-	-
0.1	-	-	-
0.075	-	+	+
0.063	-	+	+
0.05	-	+	+
0.038	-	+	+

+: indicates the presence of Xoo bacterial growth on PSA
-: indicates no Xoo bacterial growth on PSA was observed
* These tests were done in three replicates. The growth of Xoo on the PSA petri dish was observed and recorded qualitatively.

Starliper et al. (2015) studied the bactericidal properties of selected plant-derived essential oils against *Aeromonas* spp., the causal agent of fish diseases found that the mean MBCs for lemongrass oils from three sources, Stony Mountain Botanicals (0.10 ± 0.04%), Now Foods (0.36 ± 0.22%) and Puritan's Pride was 0.65 ± 0.39%. Schweitzer et al. 2022 studied the MBC of LgEO on the three bacterium species associated with pitted keratolysis, *B. thuringiensis* (0.2 ± 0 mg/mL), *D. congolensis* (0.15 ± 0.02 mg/mL), and *K. sedentarius* (0.15 ± 0 mg/mL).

Determination of active compounds in lemongrass essential oil using GC-MS

Cymbopogon spp. from the grass family Poaceae, are fast-growing and are primarily cultivated for their essential oils. The great commercial interest of these aromatic grass due to their wide applications in different areas such as the food, pharmaceutical, and cosmetic industries (Mukaram et al. 2021). Essential oils are complex mixtures of numerous active compounds. It has been debated if their biological effects are the result of synergism of all the compounds or reflect only those of the main compound present at the highest levels according to gas chromatographical analysis (Bakkali et al. 2008 and Tilaoui et al. 2015). The GC-MS chromatography analysis of LgEO showed the two dominant compounds at a retention time of 26.618 (neral) and 28.301 minutes (geranial) as demonstrated in *Figure 2*.

Meanwhile, *Figure 3* shows the percentage of major active compounds greater than 1.0% (≥1.0%) in lemongrass essential oil. The highest active compound in LgEO is citral (consisting of isomers, geranial and neral) at 83.12%. Overall active compounds in lemongrass (*Cymbopogon citratus*) essential oil identified using GC-MS were listed in *Table 3*.

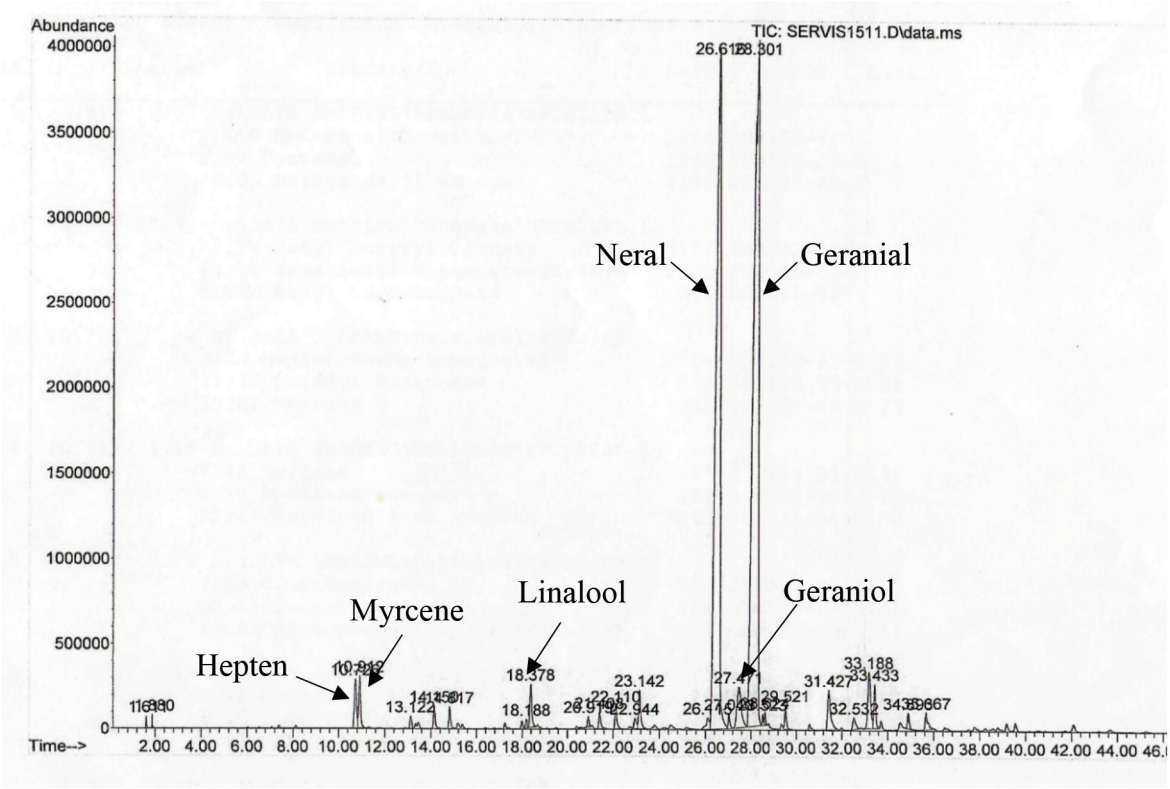

Figure 2. GC-MS chromatograph of lemongrass (Cymbopogon citratus) essential oil

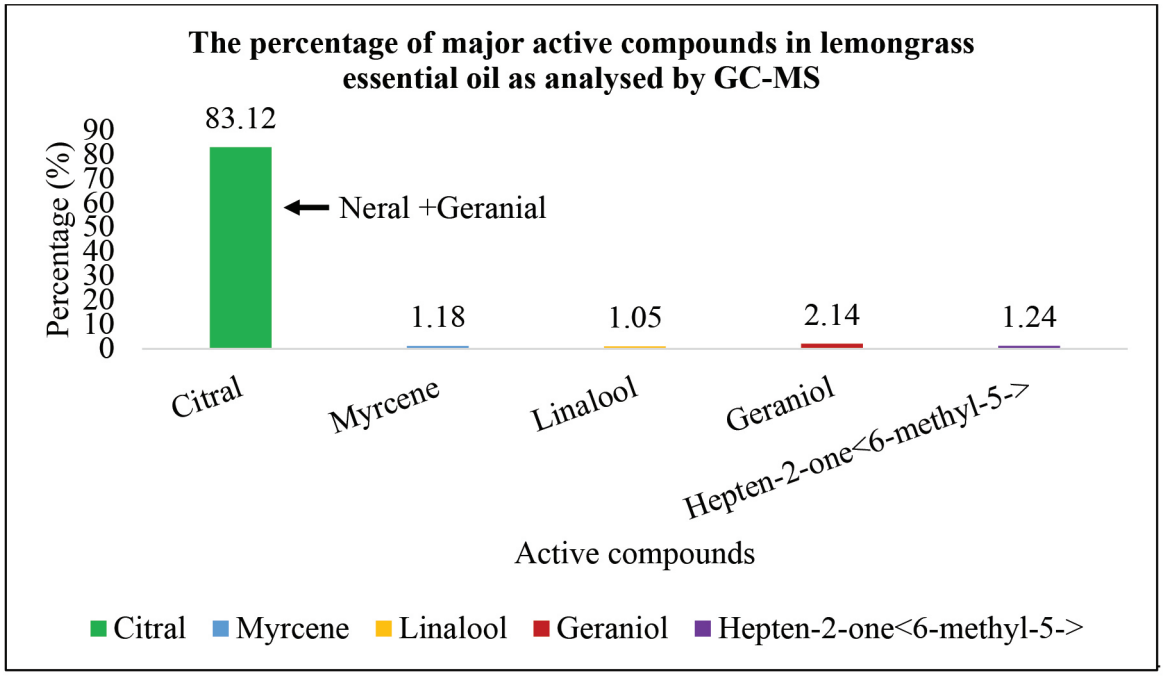

Figure 3. The percentage of major active compounds (≥1.0 %) in lemongrass (Cymbopogon citratus) essential oil

Table 3. Percentage of active compounds in lemongrass (*Cymbopogon citratus*) essential oil identified using GC-MS

No	Compounds	Retention time (minutes)	Percentage (%)
1	Hepten-2-one<6-methyl-5->	10.728	1.24
2	Myrcene	10.911	1.18
3	Cymene<para->	13.120	0.26
4	Ocimene<(Z)-beta->	14.150	0.47
5	Perillene	18.190	0.18
6	Linalool	18.379	1.05
7	Citronellal	21.411	0.38
8	Isocitral-E	23.139	0.96
9	Citronellol	26.115	0.46
10	Neral	26.618	37.95
11	Piperitone	27.042	0.31
12	Geraniol	27.471	2.14
13	Geranial	28.301	45.17
14	Geranyl formate	29.519	0.52
15	geranyl acetate	33.433	0.98
16	E-caryophyllene	34.892	0.37
17	Bergamotene <alpha-cis->	35.665	0.56

Citral is a collective term covering two geometric isomers with their own separate names; the E-isomer is named geranial or citral A, and the Z-isomer is named neral. The stereochemical structures of citral, geraniol, and linalool were illustrated in *Figure 4*. Citral ($C_{10}H_{16}O$) is also called 3,7-dimethyl-2,6-octadienal which is a pale-yellow liquid like, with a strong lemon odour that also occurs in some other plants. This essential oil is insoluble in water but soluble in ethanol (ethyl alcohol), diethyl ether, and other mineral oils. The effectiveness of lemongrass essential oil in inhibiting *Xoo* growth in this study could be due to the presence of citral (neral and geranial) as an active component as it displays spasmolytic, antimicrobial, antiinflammatory, analgesic, and chemo-preventive properties (Aprotosoaie et al. 2018). The antibacterial properties of citral (neral + geranial) as botanical control were proven by Schweitzer et al. 2022, where citral from lemongrass extract produced large clearing zones by diameter against three different bacterium species associated with pitted keratolysis [*D. congolensis* (23 ± 2 mm), *B. thuringiensis* (29 ± 2 mm), and *K. sedentarius* (14 ± 2 mm)].

In addition, the presence of geraniol, an acyclic monoterpene alcohol with the chemical formula $C_{10}H_{18}O$ also played a role as an antimicrobe. Geraniol is chemically related to neral and geranial because it can be produced by reducing these aldehydes. Geraniol may disrupt the lipid structure of the microorganism's cell membrane, and interact with its components, making it more permeable also for other compounds (Lira et al. 2020). Meanwhile, linalool which is a colourless liquid, relatively soluble in water and can be an alcoholic with a floral scent when present at high concentrations. It is found in many plants such as *Lavandula* sp., coriander, basil, cinnamon, and rosewood (Quintans et al. 2013). Numerous studies have been reported on linalool which include the contributions of linalool antimicrobials, anti-leishmanial, anti-inflammatory, and antioxidant biological activities (Beier et al. 2014). In term of the relationship between the most active compound found in GCMS, neral and geranial are aldehyde isomers and together makeup citral. Geraniol is an alcohol that is chemically related to neral and geranial because it can be produced by reducing these aldehydes. Meanwhile, Myrcene is a hydrocarbon monoterpene that is often found in the same oils as citral but does not directly interact with it. Linalool is a monoterpene alcohol that can coexist with citral and myrcene, contributing a floral fragrance. Lastly, Heptene is an alkene, which is chemically distinct from the other compounds, and although it may appear in small amounts in essential oils, it does not directly interact with them.

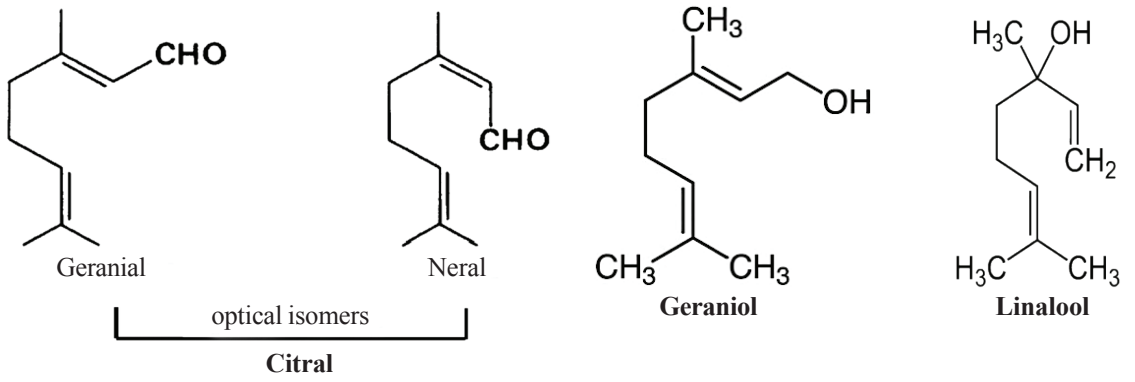

Figure 4. Stereochemical structures of citral, geraniol and linalool

Conclusion

Using botanical or plant-based pesticides can be a good, environmentally-friendly approach for controlling and managing the BLB disease in paddy fields. These in-vitro studies demonstrate that essential oils from lemongrass, citronella, and cinnamon have great potential as antibacterial agents against Xoo growth. Meanwhile, kaffir lime essential oil shows less ability as antibacterial agent against Xoo. Unfortunately, cajuputi, tea tree and garlic essential oils do not have any antibacterial ability to inhibit the Xoo growth. Based on the largest inhibition zone produced in Xoo bacterial lawn on PSA by LgEO, this EO was further studied for their MBC and active compound determination using GCMS. It is discovered that the effectiveness of lemongrass essential oil in inhibiting Xoo growth could be due to the presence of various active compounds such as citral, geraniol and linalool which are previously known to have antimicrobe properties. However, this potential antibacterial property of lemongrass for pest and disease management in the agriculture sector needs to be further explored and validated.

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References

Ali, H., Abbasi, F.M. & Ahmad, H. (2016). Bacterial blight, a serious threat to productivity of rice (*Oryza sativa* L.), an overview. *International Journal of Bioscience*, 9(6), 154-169.

Adamu, A., Ahmad, K., Siddiqui, Y., Ismail, I. S., Asib, N., Kutawa, A .B., Adzmi, F., Ismail, M. R. & Berahim, Z. (2021). Ginger Essential Oils-Loaded Nanoemulsions: Potential Strategy to Manage Bacterial Leaf Blight Disease and Enhanced Rice Yield. *Molecules*, 26, 3902. https://doi.org/10.3390/molecules26133902

Alonso-Gato, M. Astray, G. Mejuto, J. C. & Simal-Gandara, J. (2021). Essential oils as antimicrobials in crop protection. Antibiotics. Volume: 10 Issue:1. 1–12.

Aprotosoaie A. C., Luca V.S., Trifan A. & Miron, A. (2018). Chapter 7: Antigenotoxic Potential of Some Dietary Non-phenolic Phytochemicals. Studies in Natural Products Chemistry 60: 223-297

Bajpai, V. K., Baek, K. H. & Kang, S. C. (2012). Control of Salmonella in foods by using essential oils: A review. Food Research International 45: 722-734

Bakkali, F., Averbeck, S., Averbeck, D. & Idaomar, M. (2008). Biological effects of essential oils – A review. Food and Chemical Toxicology 46: 446-475

Beier, R. C., Byrd, J. A., Kubena, L. F., Hume, M. E., McReynolds, J. L., Anderson, R. C. & Nisbet, D. J. (2014). Evaluation of linalool, a natural antimicrobial and insecticidal essential oil from basil: Effects on poultry. *Journal of Poultry Sciences* 93: 267-272.

Bouaichi, A., Benkirane, R., Habbadi, K., Benbouazza, A. & Achbani, E. H. (2015). Antibacterial activities of the essential oils from medicinal plants against the growth of *Pseudomonas savastanoi* pv. *savastanoi* causal agent of olive knot. *Journal of Agriculture and Veterinary Science*, *8* (12), 41-5.

Bunawan, S. N., Masdor, N. A., Yasid, N. A., Halmi, M. I. E. & Abd Shukor, M. Y. (2023). Effect of different hydrophilic-lipophilic balance (HLB) on droplet size, polydispersity index and stability of Lemon Myrtle nanoemulsion. International Journal of Nanoelectronics and Materials, 16, 431-440.

Chukwu, S. C., Rafii, M. Y., Ramlee, S. I., Ismail, S. I, Hasan M. M, Oladosu, Y. A., Magaji, U. G., Akos, I. & Olalekan, K.K. (2019). Bacterial leaf blight resistance in rice: a review of conventional breeding to molecular approach. *Molecular Biology Reports* 46: 1519-1532.

Din, B. N. M., Ali, N. S., Hun, T. G. & Yusop, M. R. (2023). Current Status of Bacterial Leaf Blight in Malaysian Rice Plants. *Journal of Current Opinion in Crop Science, 4*(1), 1-12.

Elshafie, H. S. & Camele, I. (2023). Plant Essential Oil with Biological Activity (II). *Plants* 12, 3616. https://doi.org/10.3390/plants12203616

Gormez, A., Bozari, S., Yanmis, D., Gulluce, M., Agar, G. & Sahin, F. (2013). Antibacterial activity and chemical composition of essential oil obtained from *Nepeta nuda* against phytopathogenic bacteria. *The Journal of Essential Oil Research* 25 (2):149-153.

Hong, Z., Talib, K. M., Mujtaba, K. G., Dabin, H., Yahya, F., Congying, Z. & Fukai, W. (2021). Antifungal potential of cinnamon essential oils against *Phytophthora colocasiae* causing taro leaf blight. *Chemical and Biological Technologies in Agriculture*, 8, 1-9.

Hudzicki J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. *American Society for Microbiology*. 15:55–63.

Kaur, H., Bhardwaj, U. & Kaur, R. (2021). *Cymbopogon nardus* essential oil: A comprehensive review on its chemistry and bioactivity. *Journal of Essential Oil Research*, *33*(3), 205-220.

Lira, M. H. P. D., Andrade Júnior, F. P. D., Moraes, G. F. Q., Macena, G. D. S., Pereira, F. D. O. & Lima, I. O. (2020). Antimicrobial activity of geraniol: An integrative review. *Journal of Essential Oil Research*, *32*(3), 187-197.

Mukarram, M., Choudhary, S., Khan, M. A., Poltronieri, P., Khan, M. M. A., Ali, J., Kurjak, D. & Shahid, M. (2021). Lemongrass essential oil components with antimicrobial and anticancer activities. *Antioxidants*, *11*(1), 20.

Ooi, Y. S., Mohamed Nor, N. M. I., Furusawa, G., Tharek, M. & Ghazali, A. H. (2022) Application of Bacterial Endophytes to Control Bacterial Leaf Blight Disease and Promote Rice Growth. *The Plant Pathology Journal,* 38(5):490-502.

Padrilah, S. N., Masdor, N. A., Ghazali, M. S., Khalid, K. & Samsudin, N. I. P. (2020). Antifungal effect of cinnamon (*Cinnamomum verum* J. Presl) essential oil against aflatoxigenic *Aspergillus flavus* Link. *Transactions of the Malaysian Society of Plant Physiology,* Vol. 27:241-246.

Proto, M., Biondi, E., Baldo, D., Ferranti, C., Filippini, G., Mattarelli, P., Ratti, C. & Minardi, P. (2022). Biological control of *Erwinia amylovora* using essential oils-based products in vitro and in vivo. In *Book of Abstracts: 4th International Erwinia Workshop (4th IEW)* (pp. 10-10). Progetto grafico-editoriale esecutivo, Diletta Pini.

Quintans-Júnior, L. J., Barreto, R. S., Menezes, P. P., Almeida, J. R., Viana, A. F. S., Oliveira, R., Oliveira, A. P., Gelain, D .P., Lucca Júnior, W. & Araújo, A.A. (2013). β-Cyclodextrin-complexed (-)-linalool produces antinociceptive effect superior to that of (-)-linalool in experimental pain protocols. *Basic Clinical Pharmacology and Toxicology* 113: 167-172.

Sarangi, A., Das, B. S., Sahoo, A., Jena, B., Patnaik, G., Giri, S., Chattopadhyay, D. & Bhattacharya, D. (2024). Deciphering the Antibiofilm, Antibacterial, and Antioxidant Potential of Essential Oil from Indian Garlic and Its Phytocompounds Against Foodborne Pathogens. *Current Microbiology*, *81*(8), 245.

Schweitzer, B., Balázs, V. L., Molnár, S., Szögi-Tatár, B., Böszörményi, A., Palkovics, T., Horváth, G. & Schneider, G. (2022). Antibacterial Effect of Lemongrass (*Cymbopogon citratus*) against the Aetiological Agents of Pitted Keratolyis. *Molecules*, *27*(4), 1423.

Raveau, R. Fontaine, J. & Lounès-Hadj S. A. (2020) Essential oils as potential alternative biocontrol products against plant pathogens and weeds: A review. Foods. Volume: 9 Issue 3.

Sreepian, A., Sreepian, P. M., Chanthong, C., Mingkhwancheep, T. & Prathit, P. (2019). Antibacterial activity of essential oil extracted from *Citrus hystrix* (kaffir lime) peels: an in vitro study. *Tropical Biomedicine*, *36*(2), 531-541.

Starliper, C. E., Ketola, H. G., Noyes, A. D., Schill, W. B., Henson, F. G., Chalupnicki, M. A. & Dittman, D. E. (2015). An investigation of the bactericidal activity of selected essential oils to *Aeromonas* spp. *Journal of advanced research*, *6*(1), 89-97.

Srinivas, B., Patil, V. A., Venu, E., Hariprasath, M., Purushotham, P., Rajeswari, E., ... & Rajesh, K. (2024). Isolation, Purification and Identification of *Xanthomonas oryzae* pv. *oryzae*. *International Journal of Economic Plants*, *11*(1), 32-37.

Tilaoui, M., Ait, M. H., Jaafari, A. & Zyad, A. (2015). Comparative phytochemical analysis of essential oils from different biological parts of Artemisia herba alba and their cytotoxic effect on cancer cells. *PloS one*. 10(7): e0131799.

Wahab, N. Z. A, Ja'afar, N. S. A. & Ismail, S. B. (2022). Evaluation of Antibacterial Activity of Essential Oils of *Melaleuca cajuputi* Powell. *Journal of Pure and Applied Microbiology*, *16*(1), 549-557.

Wulansari, A., Jufri, M. & Budianti, A. (2017). Studies on the formulation, physical stability, and in vitro antibacterial activity of tea tree oil (*Melaleuca alternifolia*) nanoemulsion gel. *International Journal of Applied Pharmaceutics*, *9*, 135-139.

Yasmin, S., Hafeez, F. Y., Mirza, M. S., Rasul, M., Arshad, H. M. I, Zubair, M. & Iqbal, M. (2017). Biocontrol of Bacterial Leaf Blight of Rice and Profiling of Secondary Metabolites Produced by Rhizospheric *Pseudomonas aeruginosa* BRp3. *Frontier Microbiology* 8:1895.

Zhong, Q., Xu, Y. & Rao, Y. (2024) Mechanism of Rice Resistance to Bacterial Leaf Blight via Phytohormones. *Plants* 13, 2541. https://doi.org/10.3390/plants13182541.