HIGH SCHOOL EDITION
Journal of Student Research

Volume 12 Issue 3 (2023)

The Effect of Lavender Essential Oil on *Escherichia coli* Growth and its Potential Applications

Allyson Muller[1], Shreya Dhara[2], Paige Herrin[3#] and Paul Muller[#]

[1]Gilmer High School
[2]South Forsyth High School
[3]Kihei Charter High School
[#]Advisor

ABSTRACT

Escherichia coli (*E. coli*) infects millions worldwide annually, leading to thousands of deaths, particularly impacting those low to middle-income countries where *E. coli* can be found in contaminated soil, water, and surfaces (Winstead et al. 2019). In this investigation, lavender essential oil and ampicillin disks were tested against *E. coli* grown on an agar plate to see if they could be used as a potential antimicrobial agent in regions where *E. coli* infections are largely prevalent. Based on the Kirby-Bauer tests conducted in this investigation, lavender essential oil displayed a clear zone of inhibition, though it was relatively smaller than the zone of inhibition for ampicillin. Future applications to such findings could be to utilize lavender in both plant and essential oil form as a treatment for *E. coli* soil contaminations and a natural pest repellent, both in the agricultural industry and in regions where cost-effective, environmentally sustainable treatments may not currently exist.

Introduction

Escherichia coli (*E. coli*) infections typically occur due to fecal contamination of vegetables, raw milk products, and meats, often by livestock populations, contributing to 111 million infections and 63,000 deaths per year (Winstead et al., 2019). In prime soil conditions, *E. coli* can naturalize into the soil microbiome surviving for an extended amount of time without being reintroduced by feces or water contamination (Petersen et al., 2020). Soil aggregate remnants on produce pose a significant health threat, particularly in regions where the resources to decontaminate produce may be unavailable.

Because few commercial treatments for *E. coli* in soil exist, farmers often expose soil to extreme temperatures and drought to remove the bacteria (Ray, 2011). Implementing a plant-derived environmentally sustainable *E. coli* treatment for soil is one of the main goals of this research, with lavender essential oil as a primary ingredient. Lavender essential oils (LEOs) are typically derived from lavender species such as *Lavandula angustifolia* and *Lavandula latifolia*, and are composed of the organic compounds a) linalool and b) linalyl acetate as shown below (Kwiatkowski et al., 2019).

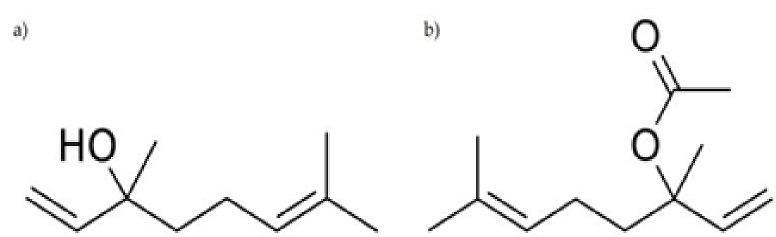

Figure 1: (Kwiatkowski et al., 2019)

LEOs have been shown to have synergistic effects when used in combination therapy alongside antibiotics due to their ability to neutralize the lipopolysaccharide (LPS) on the outer membrane of gram-negative bacterial species such as Methicillin-resistant Staphylococcus aureus bacteria, also known as MRSA (Leong et al., 2021). While antibiotics like ampicillin successfully inhibit the building of the bacterial cell wall, inevitably causing the cell to burst, the lipophilic and hydrophobic properties of LEO compounds allow the compounds to infiltrate the phospholipid bilayer of the cellular membrane of gram-negative bacteria, serving as a potent natural antimicrobial substance (Leong et al., 2021).

Due to the synergistic antimicrobial properties of lavender essential oil, it was of interest to investigate how LEOs alone impact *E. coli* growth conditions in comparison to traditional antibiotics such as ampicillin, commonly used to treat *E. coli* infections in humans. In terms of applications, a long-term goal is to implement cost-effective, natural methods of preventing soil and water contamination, specifically in agricultural areas where *E. coli* poisoning may pose a health threat due to a lack of proper treatments, or in cases where artificial treatments may not be viable. Lavender essential oil will decrease the growth rate of *E. coli* because of its hydrophobic and antimicrobial properties against gram-negative bacteria.

Methods

Mayjam Therapeutic Grade 100% lavender essential oil was obtained (07/05/2022) and one 7mm paper disk was soaked in the lavender essential oil for 20 minutes within a centrifuge tube. Next, the *E. coli* broth (K12 Broth #15-5068 Nutrient Broth 37, Carolina Biological Supply, Item #155068, 07/05/2022) was inoculated using an inoculating loop onto two plates of agar growth medium, with each plate divided into two segments. Standard sterilization techniques were used, as laid out in the Carolina Biological Supply manual, including flaming the inoculating loop before and after collecting *E. coli* broth alongside flaming the entrance of the tube where the *E. coli* broth was stored (Carolina BioKits® Antibiotic Sensitivity Teacher's Manual). The LEO disk was placed on one half of the agar medium with the ampicillin disk (10 mcg Biogram from Carolina Biological Supply as a part of Item #154740P, 07/05/2022) on the other half. Then, the plates were inspected following a 24-hour incubation period at 35.0 C.

Results

Table 1: Zone of Inhibition for Ampicillin and Lavender in *E. coli*

	Disk	Zone of Inhibition after 24 hours (mm)
1	Ampicillin	20.3
	Lavender	13.1
2	Ampicillin	20.8
	Lavender	11.0

Figure 2: Picture of Plate 1 (left) and Plate 2 (right)

The ampicillin portion (Figure 2, top half of plates) in plate 1 had a clear zone of inhibition with large colonies growing approximately 3.0 mm directly at the edge of the zone of inhibition. Smaller, more lawn-like colonies formed in between the larger colonies and the zone of inhibition The lavender (Figure 2, bottom half of plates), in comparison, had a smaller and less clear zone of inhibition, with a section of small colonies growing around the edge of the LEO's zone of inhibition, the same as around the ampicillin disk. From the clear zone of inhibition to the start of the larger colonies is approximately 8.2 mm. Interestingly, the ampicillin in comparison to the lavender did not have a smaller colony growth outside the zone of inhibition.

The ampicillin in Plate 2 again had a clear zone of inhibition expanding outwards from the ampicillin disk with larger colonies growing 2.7 mm away from the end of the zone of inhibition with smaller colonies in the middle. The lavender's "secondary" zone was oblong and circular, a clear circle was not shown as was in disk 1. Larger colonies start growing 9.0 mm away from the "secondary" zone with the largest point over 25 mm away, due to the irregular shape.

Discussion

Considering that the standard zone of inhibition for ampicillin on *E. coli* is 26 mm, there appears to have been some inconsistencies with the *E. coli* growth in this particular medium. However, the lavender, although not as effective as the ampicillin at preventing *E. coli* growth, maintained a zone of inhibition at a maximum of 13.1 mm. The data suggests that lavender did have an antibacterial effect to some extent on the *E. coli*; since statistical standards specifically for lavender essential oils on *E. coli* are scarce, further testing would be recommended to standardize the effects of LEO on *E. coli* and determine whether our findings are statistically significant based on minimum inhibitory concentrations. This investigation primarily offers a qualitative and visual representation of the impact of LEO versus ampicillin on E. coli. Furthermore, various species of lavender and their growth conditions, along with the specific impacts of the oils should be further investigated since this experiment did not include the extraction or harvesting process of the lavender buds and essential oil.

Additionally, based on the image of the results, there is a possibility that some fungal contamination occurred, potentially from the age of the agar growth medium or the air, which could be a potential limitation on the results. Despite the potential contamination, both the lavender and ampicillin disks maintained clear zones of resistance, suggesting that both substances potentially had an overall antimicrobial effect. Further studies would identify a minimum inhibitory concentration for lavender essential oil as well as identifying its interactions with *E. coli* when in a natural environment rather than in a lab.

In recent studies conducted on the soil microbiome, it was found that such microbiomes are typically sensitive, and *E. coli* invasions can cause several harmful consequences, including the death of natural soil microbes and a decrease in the functional microbial community, therefore affecting the soil's ability to cycle certain nutrients and increasing stress on the environment (Yao et al., 2014). In these studies, experiments were done under greenhouse conditions in soil with little to no large organic matter, which are different from the conditions one would see in the natural world. Although there are microbes in the soil that may naturally fight against *E. Coli* with compounds like bacteriocins, it is not enough to fully stop contamination (Yao et al., 2014).

E. coli contamination and illness continue to threaten public health, particularly in developing or underdeveloped regions, where access to appropriate sanitation or other resources may not be available. In a recent study on *E. coli* contamination levels in soil in peri-urban Bagamoyo, Tanzania, sources of soil contamination were analyzed, including pit latrines and wastewater disposal (Navab-Daneshmand et al., 2018). Both *E. coli* and ENT contamination was found in soil and water nearby households (Navab-Daneshmand et al., 2018). This is only one example of a region in which dangerously high *E. coli* levels exist.

Identifying a natural and cost-effective substance to fight *E. coli*, such as lavender, might present a viable method to prevent and treat further soil contamination and provide relief to contaminated regions. Current research would support the use of lavender essential oil as a useful agricultural and antimicrobial agent. According to a study on the effects of *Lavandula Angustifolia* as a companion plant against aphid populations, lavender, which emits volatile organic compounds, was also shown to have a significant effect on diminishing adult aphid and nymph numbers (Issa et. al). This property allows the *Lavandula Angustifolia* grown in a farmer's garden to serve two purposes: soil treatment and an alternative to pesticides. If these properties can be easily replicated, *Lavandula Angustifolia* may be a valuable tool to farmers.

Conclusion & Limitations

The antimicrobial properties of lavender essential oils against *E. coli* were assessed by measuring the zone of inhibitions of lavender and ampicillin. While lavender did not appear to be as effective against *E. coli* as the ampicillin was, it still exhibited antimicrobial properties. This property may have a beneficial application when it comes to agriculture in dry, arid regions with little water (lavender's preferred climate) where *E. coli* contamination serves as a significant health threat. Theoretically, farmers in such regions could plant lavender as a companion plant to their crops to ward off harmful insect pests, then harvest some of the buds every year to make essential oil, which is then applied back to the plots to help the soil microbes fight against *E. coli* contamination. This process may be cost-effective since certain species of lavender are native to regions in, and the process of making lavender essential oil is simple, only requiring dried buds and a carrier oil. However, most aspects of this scenario will need further experimental testing to quantitatively determine if lavender essential oil is safe to add to soil on a commercial scale and if so, how much and at what concentration? Will it negatively affect the beneficial microbes in the soil? Is the process sustainable? Despite these concerns, our findings demonstrate that lavender essential oil may have extensive agricultural applications, and further experimentation should be done to pursue its potential.

The primary limitations of this investigation were the time and resources available. Due to time constraints, this investigation included a growth or observation period of 24 hours for the E. *coli* colonies and did not utilize lab-extracted lavender essential oil in order to best simulate the real-world application conditions There was also only access to two agar plates, limiting the experiment to only two trials. Once our incubation time on the plates was finished, signs of contamination were observed and noted, likely occurring while the plates were in storage. This conclusion came from the fact that it could not have happened during inoculation due to the use of proper, sterile plating techniques. However, the lavender and ampicillin still had zones of resistance to the *E. coli* and the contaminating bacteria, therefore not impacting the results too significantly. However, this could give a hint as to how lavender could affect natural soil microorganisms and bacteria, but more testing would be needed to confirm that. A more apparent limitation was the lack of established baselines for the zone of resistance and minimum inhibitory concentrations of LEOs and *E. coli*. This investigation served as a baseline for the qualitative relationship between LEOs and *E. coli* and would require further studies to evaluate the minimum inhibitory concentrations for treatment with LEOs.

Acknowledgments

This endeavor would not have been possible without the Georgia Office of Student Achievement, the Georgia Governor's Honors program, and Berry College for providing the space, materials, and time needed to complete this research. We would also like to extend our sincere thanks to Mrs. Paige Herrin for advising and aiding us in our experimentation and Mrs. Erika Erickson for advising us on the publication process. Lastly, we would like to thank our parents, peers, and teachers for their unwavering support through the ups and downs of this project. Once again, thank you.

References

Carolina BioKits® Antibiotic Sensitivity Teacher's Manual. (n.d.). [PDFX]. Carolina Biological Supply. Retrieved July 9, 2022, from https://www.carolina.com/pdf/manuals/154740_Antibiotic_Sensitivity_SAM.pdf

Issa, R. B., Gautier, H., & Gomez, L. (2016). Influence of neighboring companion plants on ... - Wiley Online Library. Royal Entomological Society. Retrieved July 9, 2022, from https://onlinelibrary.wiley.com/doi/abs/10.1111/afe.12199

Kwiatkowski, P., Łopusiewicz, Ł., Kostek, M., Drozłowska, E., Pruss, A., Wojciuk, B., Sienkiewicz, M., Zielińska-Bliźniewska, H., & Dołęgowska, B. (2019). The Antibacterial Activity of Lavender Essential Oil Alone and In Combination with Octenidine Dihydrochloride against MRSA Strains. Molecules (Basel, Switzerland), 25(1), 95. Retrieved July 9, 2022, from https://doi.org/10.3390/molecules25010095

Leong, W.-H., Lai, K.-S., & Lim, S.-H. E. (2021). Combination Therapy Involving Lavandula angustifolia and Its Derivatives in Exhibiting Antimicrobial Properties and Combating Antimicrobial Resistance: Current Challenges and Future Prospects. Processes, 9(4), 609. Retrieved July 9, 2022, from https://doi.org/10.3390/pr9040609

Navab-Daneshmand, T., Friedrich, M. N. D., Gächter, M., Montealegre, M. C., Mlambo, L. S., Nhiwatiwa, T., Mosler, H. J., & Julian, T. R. (2018). *Escherichia coli* Contamination across Multiple Environmental Compartments (Soil, Hands, Drinking Water, and Handwashing Water) in Urban Harare: Correlations and Risk Factors. *The American journal of tropical medicine and hygiene*, *98*(3), 803–813. Retrieved July 9, 2022, from https://doi.org/10.4269/ajtmh.17-0521

Petersen, F., & Hubbart, J. A. (2020). Physical Factors Impacting the Survival and Occurrence of Escherichia coli in Secondary Habitats. Water, 12(6), 1796. MDPI AG. Retrieved July 9, 2022, from http://dx.doi.org/10.3390/w12061796

Ray, C. C. (2011, September 5). Cleaning the soil. The New York Times. Retrieved July 8, 2022, from https://www.nytimes.com/2011/09/06/science/06qna.html

Winstead, A., Hunter, J. C., & Griffin, P. M. (2019). Escherichia coli, diarrheagenic - chapter 4 - 2020 yellow book. Centers for Disease Control and Prevention. Retrieved July 8, 2022, from https://wwwnc.cdc.gov/travel/yellowbook/2020/travel-related-infectious-diseases/escherichia-coli-diarrheagenic

Yao, Z., Wang, H., Wu, L., Wu, J., Brookes, P. C., & Xu, J. (2014). Interaction between the microbial community and invading Escherichia coli O157:H7 in soils from vegetable fields. Applied and environmental microbiology, 80(1), 70–76.. Retrieved July 9, 2022, from https://doi.org/10.1128/AEM.03046-13

Yao, Z., Wei, G., Wang, H., Wu, L., Wu, J., & Xu, J. (2013). Survival of Escherichia coli O157:H7 in Soils from Vegetable Fields with Different Cultivation Patterns. American Society For Microbiology. Retrieved July 9, 2022, from https://journals.asm.org/doi/full/10.1128/AEM.03605-12#F