POTENTIAL OF PLANT ESSENTIAL OILS IN THE SUPPRESSION OF *E. COLI* AND *SALMONELLA* spp. – *IN VITRO* STUDY

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Abstract
One of the major public health concerns is food contamination by pathogenic bacteria. Fresh food products can be contaminated during each phase of food production. Many factors are involved in the survival and growth of pathogenic bacteria, such as environmental and agricultural practices. Several methods are proposed for pathogen removal from fresh food products; one of them is the application of essential oils. Thus, the aim of this research was to determine the effects of caraway, coriander, and thyme essential oils on *E. coli* and *Salmonella* spp. growth. Mueller-Hinton agar, previously inoculated by overnight bacterial cultures, was used for the estimation of particular effects. Sterile filter paper discs impregnated with essential oil were placed on the surface of the agar. After incubation, the inhibition zone was measured. The olive oil macerate was used as a control. The results showed that the highest impact was noticed in treatment with caraway essential oil, while thyme essential oil showed the lowest inhibition zone. The obtained results confirm the potential of natural products in the suppression of pathogen growth.

Key words: caraway essential oil, suppression, *Escherichia coli*, *Salmonella* spp.

Introduction
Food contamination due to the presence of pathogens has been reported as one of the major public health concerns globally (Asiegbu et al., 2016). There are more than 5 million cases of illnesses in Europe that originated from food poisoning (Jevšnik et al., 2008). World Health Organisation (2015) estimated that 28 microbial pathogenic species were included in 600 million cases of foodborne illness worldwide in 2010. Fresh plant products can be contaminated at different points during production, harvesting, and processing, and such contamination can be transmitted to consumers (Nuesch-Inderbinen and Stephan, 2016). Although Murray et al. (2017) showed that food contamination can be reduced by post-harvest washing, Gombas et al. (2017) revealed that washing has several limitations and may lead to cross-contamination. In addition, incomplete food processing, inadequate storage objects, and basic infrastructure play an important role in increasing health risks (Campos et al., 2015) and food borne illness accidents. Food quality depends on various factors, such as pH, temperature, handling, processing, clean water supply, etc. (Birgen et al., 2020), and its shelf life is determined by physical, chemical, and microbiological factors (Tesfaye et al., 2016).
Although food can be contaminated with various biological agents, bacterial infections are one of the most dangerous sources of food contamination in developed and developing countries (Ehuwa et al., 2021). Among pathogens, bacteria associated with food contamination belong to the following genera: *Clostridium*, *Escherichia*, *Salmonella*,

Shigella, *Campylobacter*, *Staphylococcus* and *Bacillus* (Nobili et al., 2017). The most detected source of food contamination in EU countries during 2018 was *Salmonella* – about one third of all outbreaks (Ehuwa et al., 2021). On the other hand, among gram-negative bacteria, E. coli is one of the most important and widespread pathogens and an indicator of insufficient hygiene and fecal contamination (Balpetek, 2010; Uçar et al., 2015). Thus, different approaches have been proposed to control the bacterial pathogens and reduce the risks caused by bacterial infections (FAO/WHO, 2006). One of them is the use of plant essential oil (Akhtar et al., 2014); many medicinal and aromatic plants, due to the chemical compounds of essential oils, can be used for the suppression of pathogen growth. Essential oils have huge market potential, fragrance properties, and a plethora of biological activities (Swamy and Sinniah, 2016) and can be used in the therapy of several diseases (Ali et al., 2015). Nazzaro et al. (2013) indicated the synergistic impact of essential oil ingredients against various pathogens.

The objective of this paper was to estimate the potential of coriander, caraway, and thyme essential oils in the suppression of *E. coli* and *Salmonella* spp. growth in laboratory conditions.

Material and methods

In this research, three essential oils were used in order to inhibit the growth of two bacteria from the family Enterobacteriaceae (*E. coli* and *Salmonella* spp.). Plant material (coriander shoots, caraway seeds, and thyme shoots) was obtained from the local distributor. Extraction of the essential oil from plant material was performed in the Laboratory of Microbiology (University of Sarajevo, Faculty of Agriculture and Food Sciences, Sarajevo, Bosnia and Herzegovina) using Clevenger apparatus.

The antimicrobial properties of the obtained essential oils were detected using the disc diffusion method. As an experimental medium, Mueller-Hinton agar was used. This nutrient medium was inoculated with the overnight cultures of selected bacteria. Three sterile filter paper discs (6 mm in diameter), previously impregnated with 10 µl of essential oils, were placed onto the agar surface. The treatments were as follow: Treatment 1- filter paper discs impregnated with coriander essential oils; Treatment 2- filter paper discs impregnated with caraway essential oils; Treatment 3 - filter paper discs impregnated with thyme essential oils and Treatment 4 (control) - filter paper discs impregnated with olive oil macerate. The experiment was done in triplicate.

Incubation of Petri dishes was performed at 37°C for 24 h in the dark (Binder, Latvia). The measurement of the inhibition zone was performed after 24 h of incubation, and the determination of essential oils impact on bacteria was carried out.

Results and discussion

The results showed that essential oils had an impact on the growth of pathogenic bacteria. The inhibition zone depends on the type of essential oil used (Tables 1 and 2).

Table 1. Impact of coriander, caraway ,and thyme essential oils against *E. coli*

Repetition	Average inhibition zone (mm)			
	Treatment 1	Treatment 2	Treatment 3	Treatment 4 (control)
I	27.0	28.0	14.0	7.0

II	30.0	33.0	15.0	8.0
III	26.0	31.0	16.0	7.0
average	27.6	30.6	15.0	7.3

As can be seen from Table 1, the highest inhibition zone was obtained using treatment 2 - caraway essential oil (30.6 mm), while thyme essential oil (treatment 3) showed the lowest impact against *E. coli* (15.0 mm). In control, a negligible impact of olive oil macerate was detected.

Although numerous studies confirm the significant impact of thyme essential oil against *E. coli* (Benameur et al., 2019; Sateriale et al., 2022), Duckova et al. (2014) found the growth of several gram-negative strains after the addition of thyme essential oil. Fabian et al. (2006) revealed that thymol, a component of thyme essential oil, showed no cytotoxic effect on *E. coli*. On the other hand, the high potential of caraway and coriander essential oils was confirmed by Strasakova et al. (2021) and Thompson et al. (2013).

Table 2. Impact of coriander, caraway, and thyme essential oils against *Salmonella* spp.

Repetition	Average inhibition zone (mm)			
	Treatment 1	Treatment 2	Treatment 3	Treatment 4 (control)
I	26.0	29.0	17.0	6.0
II	31.0	34.0	18.0	7.0
III	27.0	28.0	16.0	7.0
average	28.0	30.3	17.0	6.6

Table 2 shows the impact of the three essential oils on *Salmonella* spp. growth. The lowest impact was detected using thyme essential oil (treatment 3), while caraway (treatment 2) and coriander oils (treatment 1) showed similar suppression effects. Posgay et al. (2022) suggest that the practical application of thyme essential oil is limited due to its unstable nature, low solubility in water, and volatility. In contrast, Simić et al. (2008) confirmed the inhibition of microbial growth using caraway essential oil, probably due to the presence of carvone, which acts as an antimicrobial agent. In addition, Rezaei et al. (2016) found a significant inhibitory effect of coriander essential oil against various gram-negative bacteria, including *Salmonella*. Olive oil macerate showed low efficiency in suppressing selected bacteria. Zullo et al. (2018) showed that bacteria may survive in olive oil products containing low phenolic content.

Conclusion

Our research showed that the growth of pathogenic bacteria (*E. coli* and *Salmonella* spp.) can be suppressed using caraway, coriander, and thyme essential oils. The highest inhibition zone was noticed using caraway essential oil, while thyme essential oil showed the lowest impact on the growth of *E. coli* and *Salmonella* spp. Further research will be focused on the estimation of essential oils impact on other gram-negative bacteria and their mechanism(s) of action.

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