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Investigation of *Lavandula angustifolia* Mill. extracts as anti-*Escherichia coli* agents and microbial additives: are they an alternative to enrichment, decontaminating and deodorizing agents for organic soil improvers?

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Abstract

Microflora composition and antibacterial effect against *Escherichia coli* of plant extracts from different parts of *Lavandula angustifolia* and with different solvents were analyzed to determine variants to be used as microbial additives for enrichment with beneficial microflora, decontamination and deodorization of composts contaminated with *E. coli*. The highest total quantity of microorganisms was found in the decoction variant (whole plant and roots), and the lowest in the tincture variant (whole plant). The quantity of non-sporulating bacteria predominates in most variants. A significant increase of actinomycetes was found in the tincture and medical vinegar variants. The acidification of the environment leads to an increase in the quantity of mold fungi in the medicinal vinegar. Antibacterial effect against *Esherichia coli* for three tested strains (NMIMCC 3397 (WDCM 00012; ATCC 8739), NMIMCC 3398 T (WDCM 00090; ATCC 11775), NMIMCC 8905 (ATCC 35218)) was similar for the individual plant parts and the same for variants with whole plants. Lavender extracts, medicinal vinegar variant (individual plant parts and whole plant), as well as a decoction variant with a whole plant, showed stronger antibacterial activity against *Escherichia coli*. The concentration of all parts of the plant in "whole plant" extracts increased antibacterial activity compared to extracts with a concentration from a single specific part of the plant (roots, leaves, stems). Antibacterial activity against *Escherichia coli* remained lower even when whole plant was used for medicinal wine and medicinal oil variants. The choice of solvent probably has some effect on the diameter of the growth inhibition zone. The addition of Lavender extract (decoction variant, whole plant) to compost variants increased the quantity of non-pathogenic microflora in them. At the same time, the composts were decontaminated from the presence of *Escherichia coli*. The composts smelled of soil and lavender. The antibacterial effect of the lavender extract (decoction variant) against *Escherichia coli* strongly positively depends on the quantity of non-pathogenic microflora in it and in the composts after its application. The studied lavender plant extracts (best variant decoction, whole plant) can be applied as microbial additives, disinfectants against *Escherichia coli* and deodorizers of composts.

1. Introduction

Essential extracts and oils are concentrated natural extracts from plants that have been shown to be good sources of bioactive compounds with antibacterial, antifungal and antioxidant properties. They contain a whole range of pharmacologically active compounds with pronounced antimicrobial action. Analysis of medicinal essential oil cultures as biological agents possessing antimicrobial activity is essential to determine their qualities as biocides against pathogenic microorganisms. Chemical substances extracted from them attack and inactivate microbes usually by some of the following mechanisms: disruption of the homeostasis of the bacterial cell, lysis followed by leakage of the internal contents of the cell, inhibition of the catalytic function of bacterial enzymes, disruption of electron transport and oxidation processes, negative interaction with macromolecules and biosynthetic processes of bacteria (McDonnell and Russell, 1999).

Lavender flowers (*Lavandula angustifolia*) are commonly used for decoration and fresh fragrance. Lavender essential oil is used cosmetically and therapeutically, but whether it has significant clinical potential on its own or as additive to other products is still being debated (Cavanagh and Wilkinson, 2002). According to these authors, there is considerable variation in the antimicrobial properties of different lavender cultivars and species, ranging from excellent antibacterial/antifungal activity to no antibacterial/antifungal activity at all. The antimicrobial activity of lavender oil is affected not only by the types of lavender used, but also by the methods of preparing the extracts and the methods of reporting the antimicrobial activity – the use of different methods makes direct comparison between published results practically impossible according to Cavanagh and Wilkinson (2002). Future research should investigate the mechanism of individual components of essential oils, along with initiating a systematic investigation of synergistic mechanisms between different components (Chouhan et al., 2017).

Lavender has been found to have antimicrobial activity against pathogenic species: *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, with aqueous extracts showing generally lower bacteriostatic and bactericidal activity than micellar suspension (Man et al., 2019). The minimum inhibitory concentration of lavender essential oil against *Escherichia coli* is 2000 ppm and 1000–1200 ppm against *Staphylococcus aureus*, and mixing with oregano essential oil (50:50) is recommended, which has lower MIC values (1600–1800 ppm against *E. coli*; 800–900 ppm against *S. aureus*), due to its higher content of phenolic compounds (Martucci et al., 2015).

Antimicrobial (Gende et al., 2010; Martucci et al., 2015; Kačániová et al., 2017; Man et al., 2019), antibacterial (Canillac and Mourey, 2001; Burt, 2004; Teixeira et al., 2013; Martucci et al., 2015; Man et al., 2019), antifungal (Blazhekovikj–Dimovska et al., 2012; Bona et al., 2016; Blazhekovikj–Dimovska et al., 2019; Othman et al., 2020; Tian et al., 2022) and antioxidant properties (Kulevanova and Panovska, 2001; Danh et al., 2012; Teixeira et al., 2013; Hamad et al., 2013; Martucci et al., 2015; Kačániová et al., 2017; Kozics et al., 2017) of the essential extracts and oils can be attributed to the high content of terpene compounds (α-pinene, β-pinene, 1,8-cineole, menthol, linalool) or phenolic compounds such as carvacrol, eugenol and thymol (Burt, 2004). Lavender oil (mainly *L. angustifolia*) has been found to have antibacterial and antifungal effects against many types of bacteria and fungi (Nelson, 1997; Lis-Balchin et al., 1998; Hammer et al., 1999; Martucci et al., 2015; Man et al., 2019), as well as antioxidant activity (Hamad et al., 2013; Kozics et al., 2017). The main constituents of lavender oil are linalool and linalyl-acetate, which are more than 50% of the total composition, followed by smaller quantities of sesquiterpenes and terpenes (Umezu et al., 2006). The high content of linalyl-butyrate (26.5%) explains the good antibacterial activity of the micellar form of lavender, while linalool (25%) and its solubility in water can explain the good inhibitory effect of its aqueous extract (Man et al., 2019). Ciorcarlan et al. (2021) found 41 constituents in lavender oil, the main constituents being monoterpenes (84.08–92.55%), followed by sesquiterpenes (3.30–13.45%) and some aliphatic compounds (1.42–3.90%). Other authors found no correlation between linalool or linalyl acetate content in lavender oil and antibacterial or antifungal activity (Lis-Balchin et al., 1998).

The cited published results regarding the antimicrobial activity of lavender oil are somewhat contradictory and its antimicrobial effect is not sufficiently well studied. The aim of the study was to analyze the the composition of microflora and antibacterial activity against *Escherichia coli* of plant extracts from different parts of *Lavandula angustifolia* and with different solvents to determine variants to be used as microbial additives enriching with beneficial microflora, disinfectants and deodorizers of compost-like mixtures contaminated with *Escherichia coli* as a possible method of obtaining safe organic soil improvers to be used for fertilization and increasing soil fertility. The main method found in literature about decontaminating substrates with *Escherichia coli* is liming. The positive side of the content of beneficial microorganisms in the extracts is not considered. We describe groups of microorganisms contained in the extracts, which, falling into the soil or compost, have a positive influence on increasing soil fertility and compost quality. In addition, the strong aroma of *Lavandula angustifolia* Mill. allows the lavender extracts to be used to deodorize compost-like mixtures that have an unpleasant odor in the composting phases.

2. Materials and methods

The experiment was carried out under controlled conditions in the greenhouse of the educational and experimental field of the Department of Plant Breeding at the Technical University – Varna. The experiment was carried out with *Lavandula angustifolia* in two replicates.

The total extracts and oils from roots, stems, leaves and whole plant were prepared by 5 methods (Table 1).

Variant decoction was used for *Escherichia coli* decontamination of two compost variants (prepared in composters), in which the presence of *Escherichia coli* was detected (species identity confirmed with Erba Scan – 99%). Composts (compost 1 and compost 2) contain residues of lemons, potatoes, cucum-

Table 1
Variants of extracts and oils

Variants	Method of preparation	Solvent
Decoct	boiling the crumbled plant material; hot filtration	water
Tincture	soaking the crumbled plant material for 8–10 days; periodic shaking; filtering	30% ethyl alcohol
Medicinal vinegar	soaking the crumbled plant material for 7–10 days; periodic shaking; filtering	good quality wine vinegar
Medicinal wine	soaking the crumbled plant material for 7–10 days; periodic shaking; filtering	good quality and well stabilized wine
Medicinal oil	soaking the crumbled plant material for 4–6 weeks; storage in closed glass containers in the dark and cool place; filtering	good quality olive oil

bers, tomatoes, zucchini, wheat, leaves of fruit trees; starter – soil. A microbial additive (solution concentration 1:100, 25 l per 1 m^3) containing lactic acid and photosynthetic bacteria, nitrogen-fixing bacteria and and yeast from genus *Saccharomyces*, was added to compost 2. In pots with part of the finished composts, lavender extract was added as an improver and to decontaminate the composts from *Escherichia coli* (100% concentrate, 25 mL per 500 g of compost), every 15 days, for a period of two months. The organoleptic properties of the composts were determined. The assessment is visual, by smell (before and after adding lavender extract) and touch.

For the microbiological analysis of plant extracts and compost variants (after dilution), we applied the triplicate inoculation of solid nutrient media method with subsequent counting and calculation of colony-forming units (CFU) in 1 mL (g) substrate (Mishustin and Emtsev, 1989; Malcheva and Naskova, 2018; Nustorova and Malcheva, 2020). Systematic and physiological groups of aerobic microbes were determined – bacilli and non-spore-forming bacteria (on Nutrient (meat peptone) agar), micromycetes (mold fungi) – on Chapek-Dox agar, actinomycetes (on Actinomycetes isolation agar). The total microflora was calculated as the sum of the studied groups of microorganisms. The following solid food media were used to isolate the pathogenic microflora: Desoxycholate Citrate Agar (*Salmonella* sp.), ChromoBio Listeria Agar (*Listeria* sp.), Endo Agar (*Escherichia coli* and coliforms), ChromoBio Enterococcus Agar (*Enterococcus*).

The agar diffusion method was applied to determine the antibacterial effect against *Escherichia coli* of plant extracts (Nustorova and Malcheva, 2020). Three strains of *Escherichia coli* were used (Certified Reference Materials: NMIMCC 3397 (WDCM 00012; ATCC 8739), NMIMCC 3398 T (WDCM 00090; ATCC 11775), NMIMCC 8905 (ATCC 35218)). After dense surface inoculations for *Escherichia coli* (0.5 MFU – McFarland Unit) the wells were inoculated with plant extracts in a volume of 60 µl. Inoculations were incubated in a thermostat at 37 °C for 24h. After cultivation, the growth inhibition zone is measured with a ruler, in mm or cm.

The statistical processing of the results of the studied indicators includes the calculation of the average value of three repetitions and the determination of the coefficient of variation (CV). A correlation and regression analyses was applied to establish the relationships between some of the studied indicators. The software product Microsoft Excel 2010 was used for the statistical analysis.

3. Results and discussion

The composition of the microflora in lavender plant extracts is presented by examining non-spore-forming bacteria, spore-forming bacteria (bacilli), actinomycetes and micromycetes (mold fungi) (Table 2).

The variants are intended to be used as additives to improve the quality of compost-like products. In all variants of the study, the presence of pathogenic microflora was not detected: *Escherichia coli* and coliforms, *Salmonella* sp., *Listeria* sp., *Enterococcus*. In the Ordinance on separate collection of biowaste and biodegradable waste treatment (2017) are specified norms for *Escherichia coli* and *Salmonella,* but there are no norms for other pathogenic species and for the indicator "total quantity of microorganisms" in compost and organic soil improver. These results show that the addition of the extracts in compost-like mixtures will not lead to violation of the quality requirements of the final product by microbiological criteria. The highest quantity of total microflora was established in the water solvent variant, decreasing in the order: whole plant > roots > stems > leaves. Obviously, this solvent creates the best conditions suitable for the reproduction of aerobic groups of microorganisms, regardless of the fact that only in this variant there is boiling and some of the microorganisms die. Getting into compost mixtures and the soil, these microorganisms play a major role in the destruction of organic substances. Therefore, in terms of microflora useful for soil fertility and vegetation development, the decoction variant is most suitable for use as a microbial additive to organic fertilizers and soils. The total quantity of microorganisms is at its highest when using roots for the tincture, medicinal wine, medicinal oil and medicinal vinegar variants, and second highest in the decoction. Epiphytic microorganisms from the rhizoplane of the roots best continue to grow in the solvents used in the preparation of the extracts. The lowest total microflora is

Table 2
Quantity and composition of the microflora of lavender plant extracts and oils (cfu/mL)

Variants	Plant parts	Non-spore-forming bacteria	Bacilli	Actinomycetes	Micromycetes	Total microflora
Decoct	Roots	6980	700	100	20	7800
	Stems	6760	680	20	20	7480
	Leaves	6420	380	0	0	6800
	Whole plant	10060	1160	200	240	11660
Tincture	Roots	1350	660	660	40	2710
	Stems	300	260	300	20	880
	Leaves	320	280	320	40	960
	Whole plant	20	20	300	20	360
Medicinal vinegar	Roots	50	40	800	80	970
	Stems	20	40	780	60	900
	Leaves	0	0	180	60	240
	Whole plant	40	280	540	80	940
Medicinal wine	Roots	940	340	280	40	1600
	Stems	90	280	200	20	590
	Leaves	180	340	20	0	540
	Whole plant	400	280	240	20	940
Medicinal oil	Roots	660	260	200	40	1160
	Stems	120	220	140	20	500
	Leaves	160	240	160	0	560
	Whole plant	240	200	200	40	680

* CV up to 10% for all variants (low dispersion)

found in the tincture variant with a whole plant, which is influenced by the suppression of the development of microorganisms by the ethyl alcohol solvent. However, the concentrated extracts from the individual parts of the plants showed high total quantity of microorganisms also with the solvent ethanol. Of course, the lower concentration of ethyl alcohol used – 30% – has an influence on this.

The percentage distribution of microorganisms in the composition of the total microflora is different for the individual variants (Fig. 1).

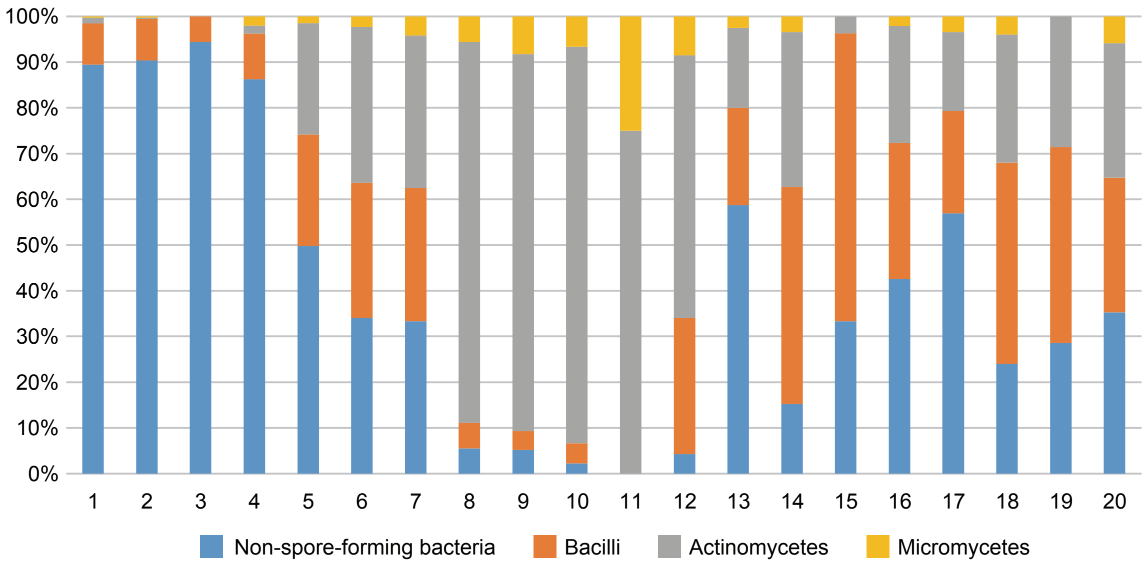

Fig. 1. Percentage distribution of microorganisms in the composition of the total microflora

In the decoction variant, for all parts of the plant, as well as the whole plant, the highest percentage is of the non-spore-forming bacteria (between 86% and 94%), followed by bacilli (between 6% and 10%), and less represented are actinomycetes and micromycetes – up to 2%. Non-spore forming bacteria remained the highest quantity also when using roots for the tincture, medicinal wine and medicinal oil variants. In general, a significant increase in the quantity of actinomycetes (up to 87% in stems, medicinal vinegar) was found in the tincture, medicinal vinegar, medicinal wine and medicinal oil variants, and in the tincture and medicinal vinegar variants, their quantity reached higher values than that of bacilli and non-spore-forming bacteria (except for roots, tincture variant). Except for the decoction, whole plant, the quantity of mold fungi was the highest in the medicinal vinegar variant for all plant parts and whole plant used. This is probably due to the vinegar solvent used, which lowers the pH and creates acidic environmental conditions that are most favorable for mold and acetic acid bacteria growth. The strongest reduction in the quantity of non-spore-forming bacteria and bacilli was found when using medicinal vinegar. According to a study by Ciorcarlan et al. (2021) lavender essential oil shows good antibacterial activity against *Bacillus subtilis*, *Pseudomonas fluorescens*, *Xanthomonas campestris*, *Erwinia carotovora* at a concentration of 300 μg/mL and *Erwinia amylovora*, *Candida utilis* at a concentration of 150 μg/mL, respectively. According to these authors, the lavender plant material, as well as residual water and ethanol extracts from the solid waste residue, showed high antimicrobial activity against strains of *Aspergillus niger*, *Alternaria alternata*, *Penicillium chrysogenum*, *Bacillus* sp. and *Pseudomonas aeroginosa*, at 0.75–6.0 μg/mL, 0.08–0.125 μg/mL, and 0.05–4.0 μg/mL, respectively. These tendencies indicate that lavender plant extracts may have antimicrobial activity against bacilli and molds and therefore reduce their quantity, noted when determining their total microflora.

The antibacterial activity of lavender plant extracts against *Escherichia coli* is presented in the following table 3.

Antibacterial effect against *Esherichia coli* for the three tested strains was similar for the variants with individual plant parts and the same for variants with whole plants. The data show that with medicinal vinegar, the retention zone is the largest, decreasing in the following descending order: whole plant > leaves > stems = roots. The following are the decoction and tincture options with the whole plant. It becomes obvious that the solvent affects the growth inhibition zone for different plants. Medicinal vinegar reduces the pH of the medium, which also influences the higher effect of this variant against *Escherichia coli*. To colonize the human gastrointestinal tract, the intestinal bacterium *Escherichia coli* must be able to grow between pH 4.5 and pH 9. In this wide pH range, *E. coli* retains enzyme activity as well as protein and nucleic acid stability. by maintaining the cytoplasmic pH in the range of pH 7.2 to 7.8. E. coli responds rapidly to a change in intracellular pH; after acidification of the external medium, *E. coli* intracellular pH begins to recover within 1 min, and complete recovery occurs within 5 min (Wilks and Slonczewski, 2007).

Decoction with lavender stems has a larger growth inhibition zone than the same variant with roots and leaves. Medicinal vinegar with stems has a weaker antibacterial effect compared to the leaves and the same compared to the roots. Medicinal oil

Table 3
Antibacterial activity against *Escherichia coli* of lavender plant extracts and oils

Variants	Decoct	Tincture	Medicinal vinegar	Medicinal wine	Medicinal oil
Plant part	Growth inhibition zone, cm				
Escherichia coli NMIMCC 3397					
Roots	0,5	0,6	1,0	0,5	0,6
Stems	0,6	0,4	1,0	0,4	0,6
Leaves	0,5	0,3	1,3	0,5	0,4
Whole plant	1,3	0,8	1,4	0,6	0,6
Escherichia coli NMIMCC 3398 T					
Roots	0,5	0,6	0,9	0,5	0,6
Stems	0,6	0,4	0,9	0,3	0,5
Leaves	0,4	0,3	1,3	0,5	0,4
Whole plant	1,3	0,8	1,4	0,6	0,6
Escherichia coli NMIMCC 8905					
Roots	0,4	0,6	1,0	0,5	0,6
Stems	0,6	0,3	1,0	0,4	0,5
Leaves	0,5	0,3	1,2	0,5	0,4
Whole plant	1,3	0,8	1,4	0,6	0,6

* CV up to 10% for all variants (low dispersion)

with lavender stems has a stronger antibacterial effect compared to the leaves and the same compared to the roots. Medicinal wine with stems has a lower growth inhibition zone value than the same variant with roots and leaves. The tincture with lavender stems has a stronger antibacterial effect compared to the leaves and a weaker one compared to the roots. In terms of variants, the growth inhibition zone decreases in the following order: medicinal vinegar > medicinal oil = decoction > tincture = medicinal wine.

Data about leaf extracts show better results than the medicinal vinegar variant. Regarding stemmed variants, the growth inhibition zone decreases in the following order: medicinal vinegar > decoction = medicinal wine > medicinal oil > tincture.

The tincture and medicinal oil of lavender root extracts are more effective against *Escherichia coli* compared to the leaves and stems of the plant. While lavender leaf medicinal vinegar has a higher antibacterial effect than lavender roots and stems medicinal vinegar.

In general, all whole-plant variants have a stronger antibacterial effect against *Escherichia coli* than the same variants with roots, stems and leaves (except for medicinal lavender oil – stems, roots and whole plant have the same effect). Regarding the whole plant variants, the growth inhibition zone decreases in the following order: medicinal vinegar > decoction > tincture > medicinal oil = medicinal wine.

The antimicrobial activity of lavender and oregano extracts against *Escherichia coli* has been also found by other authors, indicating a lower minimum inhibitory concentration for oregano (Man et al., 2019; Martucci et al., 2015). According to our research, extracts with lavender reached a higher antibacterial activity against *Escherichia coli* compared to those with coriander (Naskova et al., 2023), caraway (Malcheva et al., 2023a), thyme and basil (Malcheva et al., 2023b), and lower than oregano (Malcheva et al., 2023c). Similar to our results against *Escherichia coli* were obtained in the study of antibacterial activity of Satureja hortensis L. essential oil – 7 mm growth inhibition zone at an oil concentration of 600 µg/mL (Blažeković et al., 2010). Lavender extracts have been found to have antimicrobial and antifungal activity against many species of bacteria and fungi (Nelson, 1997; Lis-Balchin et al., 1998; Hammer et al., 1999; Martucci et al., 2015; Man et al., 2019).

It is likely that additional conditions such as reducing the pH of the medium when using medicinal vinegar, the inclusion of additional plants – grapes (wine), vinegar (apples, grapes), olives (solvent olive oil for the medicinal oil), the time of action of the extracts also affect the the detention area. In the medicinal vinegar variants, a common growth inhibition zone is formed around the wells.

Lavandula angustifolia extracts can be used to decontaminate and deodorize compost-like products containing *Escherichia coli*. The most favorable variants for *Escherichia coli* decontamination and enrichment with beneficial microflora are medicinal vinegar and a decoction with a whole plant. Their solvents – respectively water for decoction and vinegar for medicinal vinegar – also make these variants the most suitable to use in the preparation of organic soil improvers.

The antimicrobial activity of plant extracts and oils has been shown to be due to their chemical composition (Burt, 2004; Umezu et al., 2006; Ciorcarlan et al., 2021). Essential oils are characterized by changes in their chemical composition depending on the development and condition of the plant, the part used for extraction, the geographical location and the physical and chemical characteristics of the soil and climate (Gende et al., 2010).

We chose to apply a decoction variant (whole plant) as a microbial additive and decontaminating agent of *E. coli*, because of the relatively neutral solvent (water), the highest quantity of beneficial microflora for fertility and a high antibacterial effect against *E. coli* when using a whole lavender plant. We investigated the use of lavender extract (decoction variant) to increase the quantity of non-pathogenic microflora and reduce the quantity of *Escherichia coli* in two compost variants. The quantity of microorganisms was higher in the compost with microbial addition (Compost 2) compared to that without addition (Compost 1). Actinomycetes make up the majority of the total microflora in both composts, followed by non-spore-forming bacteria, bacilli, and micromycetes (mold fungi) are the least represented. The addition of Lavender extract (decoction variant, whole plant) to both compost variants increased the quantity of the tested microorganisms over a period of two months. At the same time, the composts were decontaminated from the presence of *Escherichia coli*, which confirmed the antibacterial effect of the Lavender extract against *Escherichia coli* during its application. Lavender extract was added to the finished composts every 15 days and the enrichment of the composts with beneficial microflora and their decontamination were being monitored over for a period of two months (Table 4).

Both composts are dark brown, loose, soft, with a uniform structure without lumps, and with an earthy smell, and after adding lavender extract and with a lavender scent. There is no smell of ammonia. The lavender aroma is due to linalool molecules. Lavender extracts have aromatic properties with strong herbal, clove and floral odors (Guo and Wang, 2020).

The change in the antibacterial activity against *E. coli* and the total microflora of the extract decoction (whole plant) was also monitored while the extract was being cultivated for the same period of time (two months, determination of the effect every 15 days, before adding the composts). Over a period of two months, the growth inhibition zone slightly varied from 1.3 to 1.5 cm, the quantity of non-pathogenic microflora from 11660 cfu/mL to 12380 cfu/mL. Correlation coefficients are presented to determine the relationship between the total quantity of microorganisms in an extract decoction and the antibacterial activity of the extract against *Escherichia coli*, between the total quantity of microorganisms in the composts and the presence of *Escherichia coli* in them, as well as between the antibacterial activity of the extract inserted in the composts and the presence of *Escherichia coli* in composts. A correlation matrix was constructed to test for factor independence. The presence of correlation coefficients greater than 0.7 (even if it is only one in the matrix) indicates that there is multicollinearity between the factor variables and regression analysis can be applied (Table 5).

Table 4
Quantity and composition of the microflora in the compost variants (cfu/g)

Variants	Non-spore-forming bacteria	Bacilli	Actino-mycetes	Micro-mycetes	*Escherichia coli*	Total microflora
Before adding Lavender extract						
Compost 1	1360000	304000	1600000	272000	595	3536595
Compost 2	1508800	393600	1738400	328000	574	3969374
15th day after adding Lavender extract (extract reapplication)						
Compost 1	1440000	334000	1728000	320000	500	3822500
Compost 2	1590800	403000	1869600	393600	470	4257470
30th day after adding of Lavender extract (extract reapplication)						
Compost 1	1536000	428000	1888000	416000	330	4268330
Compost 2	1656400	493200	2017200	475600	300	4642700
45th day after adding Lavender extract (extract reapplication)						
Compost 1	1632000	575400	2096000	560000	158	4863558
Compost 2	2017200	645000	2296000	623200	136	5581536
60th day after adding Lavender extract (extract reapplication)						
Compost 1	1760000	620200	2240000	608000	0	5228200
Compost 2	2132000	693200	2443600	672400	0	5941200

* CV up to 10% for all variants (low dispersion)

Table 5
Correlation and regression analyses

Correlation coefficients	Antibacterial activity-extract	Total microflora-extract	Total microflora-compost	*E. coli*-compost
Compost 1				
Antibacterial activity-extract	1			
Total microflora-extract	0,943420833	1		
Total microflora-compost	0,939531441	0,99632628	1	
E. coli-compost	–0,956124415	–0,99732292	–0,9984499	1
Compost 2				
Antibacterial activity-extract	1			
Total microflora-extract	0,943420833	1		
Total microflora-compost	0,911253816	0,98499432	1	
E. coli-compost	–0,951329393	–0,99815515	–0,9872201	1

Regression Statistic

	Compost 1	Compost 2
Multiple R	0,999984821	0,99916905
R Square	0,999969643	0,99833879
Adjusted R Square	0,999878571	0,99335517
Standard Error	2,680650937	19,1515291
Observations	5	5
Significance F	0,007015179	0,00518802

The antibacterial effect of the extract against *Escherichia coli* strongly positively depends on the quantity of non-pathogenic microflora in it (R=0.94) and in the composts (R=0,94 (compost 1); R=0,91 (compost 2)) after its application, i.e. as the quantity of non-pathogenic microflora increases, the antibacterial effect against *Escherichia coli* increases. There is a strong negative correlation between the antibacterial effect and the presence of *Escherichia coli* in the composts (R=–0.96 (compost 1); R=–0,95 (compost 2)) – as the strength of the antibacterial effect of the added extract increases, the content of *Escherichia coli* in the composts decreases. The correlation between the quantity of non-pathogenic microflora in the extract and in the composts and the quantity of *Escherichia coli* in the composts is also strongly negative. The regression model is adequate. It is proved by the level of significance of the F test, denoted as Sig. F. It has a value less than the error $\alpha = 0.05$.

5. Conclusions

The quantity and composition of the microflora in the studied lavender extracts indicates that they can be used as a microbial additive for the enrichment of compost mixtures and organic soil improvers and accordingly be used as a possible method to improve soil fertility in fertilization. The highest total quantity of microorganisms was found in the decoction variant with whole plant and roots, and the lowest in the tincture variant with whole plant. The type of solvent has an influence on these tendencies – water for decoction and ethyl alcohol for tincture. The quantity of non-sporulating bacteria predominates in most variants. A significant increase of actinomycetes was found in the tincture and medicinal vinegar variants, where their quantity reached higher values than that of bacilli and non-sporulating bacteria. The acidification of the environment leads to an increase in the quantity of mold fungi in the medicinal vinegar variant compared to the variants: tincture, medicinal wine and medicinal oil.

Antibacterial effect against *Esherichia coli* for the three tested strains (NMIMCC 3397 (WDCM 00012; ATCC 8739), NMIMCC 3398 T (WDCM 00090; ATCC 11775), NMIMCC 8905 (ATCC 35218)) was similar for the individual plant parts and the same for variants with whole plants. *Lavandula angustifolia* extracts, medicinal vinegar variant, showed stronger antibacterial activity against *Escherichia coli*, characteristic of all plant parts and whole plant. In general, all whole-plant variants had a stronger antibacterial effect against *Escherichia coli* than the same variants individually with roots, stems and leaves. Therefore, the concentration of all parts of the plant in "whole plant" extracts increases the antibacterial activity against *Escherichia coli* compared to extracts with concentration from a specific part of the plant (roots, leaves, stems).

Antibacterial activity against *Escherichia coli* remains persistently lower in all variants with medicinal wine. While in the decoction (solvent water) and tincture (solvent ethanol) variants, the effect is lower in the extracts with separate plant parts and higher in the variants with the whole plant. Medicinal oil with lavender stems and roots ranks second in antibacterial effect against *Escherichia coli* compared to the use of the same plant parts in the other variants. Therefore, the choice of solvent probably has some influence on the diameter of the growth inhibition zone.

Lavender extracts (best decoction, whole plant) can be used as anti-*E. coli* agents and microbial additives, enriching, disinfecting and deodorizing agents for organic soil improvers. The addition of Lavender extract (decoction variant, whole plant) to compost variants increased the quantity of non-pathogenic microflora in them. At the same time, the composts were decontaminated of *Escherichia coli*. The composts smelled of soil and lavender. The use of natural sources with microbiological and antibacterial activity could simultaneously solve two essential agricultural and environmental problems: to increase soil fertility and remove pathogenic microflora from organic soil improvers and soils.

The antibacterial effect of the lavender extract (decoction variant) against *Escherichia coli* strongly positively depends on the quantity of non-pathogenic microflora in it and in the composts after its application. There is a strong negative correlation between the antibacterial effect and the presence of *Escherichia coli*, as well as between the quantity of non-pathogenic microflora in the extract applied to the composts and the quantity of *Escherichia coli* in the composts.

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