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Antibacterial Activity of Ocimum Basilicum L. and Thymus Vulgaris L. Extracts against Escherichia coli as a future method for decontamination and deodorization of compost-like mixtures

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Abstract: Ocimum basilicum L. and Thymus vulgaris L. are natural plant sources of antimicrobial activity, which is essential to determine their qualities as biological agents against pathogenic microorganisms. The aim of the study is the analysis of the antibacterial activity of plant extracts of basil and thyme against Escherichia coli for their subsequent application in agricultural practice as additives for decontamination and deodorization of compost-like mixtures. Plant extracts from roots, stems, leaves and whole plants of basil and thyme were obtained by various methods: decoction, tincture, medicinal vinegar, medicinal wine and medicinal oil. Agar diffusion method was used to determine the antimicrobial activity of the extracts against Escherichia coli. The analyzed essential oil cultures increase the microbiological and enzymatic activity of soils. Basil extracts showed stronger antimicrobial activity against Escherichia coli than thyme extracts, differing for individual plant parts - roots variants showed higher antimicrobial activity compared to variants with leaves, stems and whole plant. In general, a lower antimicrobial activity of the plant extracts was found in the medicated wine and tincture variants (except for roots tincture), compared to the decoction, medicated vinegar and medicated oil variants. The antibacterial activity of the stem extracts depended significantly, and that of the root, leaf and whole plant extracts moderately, on the biogenicity of the soils. The analyzed essential oil cultures can be applied for decontamination (against Escherichia coli) and deodorization of compost-like mixtures. The use of basil and thyme extracts decontaminated composts from E. coli content in a repeated 4-month application.

Keywords: *Antibacterial activity, Basil, Soil biogenicity, Thyme.*

1. Introduction

The study of essential oil crops as biocides possessing antimicrobial activity is essential to determine their qualities as biological agents against pathogenic microorganisms. Their cultivation depends directly on the fertility of the soil, which in turn is determined by its biogenicity. Soil biogenicity depends on a number of factors – temperature, humidity, mechanical composition, soil pH, soil nutrient content, type of vegetation, soil tillage and fertilization methods, and other factors [1]. Soil microorganisms and the enzymes produced by them are sensitive indicators of ongoing changes, including when growing essential oil crops [2].

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The term "biocide" is an umbrella term for the antibacterial action of various substances [3]. By their functional essence, these chemical substances attack and inactivate bacteria, exerting a toxic effect on their cells. Most often, the mechanisms include: 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 [4].

Medicinal essential oil plants are a natural source of antimicrobial activity, as they contain a whole range of pharmacologically active compounds with pronounced antimicrobial action. Essential oils are used as a source of carbon and energy by quite ubiquitous soil microorganisms and provide evidence that they will not accumulate in soil if environmental conditions favor the growth of these microorganisms [5].

Basil (*Ocimum basilicum* L.) and thyme (*Thymus vulgaris* L.) are herbaceous plants of the Lamiaceae family that are used as spices in cooking and as medicinal plants in medicine. According to a study by Stanojevic et al. (2017) [6] essential oils from *Ocimum basilicum* L. exhibited significant antimicrobial activity against multiple pathogenic microorganisms, with the best effect against coagulase-positive *Staphylococcus*. Basil has proven its antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* [7]. According to the same study, basil extracts have antimicrobial properties against strains of *Pseudomonas aeruginosa*, *Shigella* sp., *Listeria monocytogenes*, *Staphylococcus aureus* and two different strains of *Escherichia coli*. Basil also exhibits antimicrobial activity against *Helicobacter pylori*, making it potentially effective in the fight against *Helicobacter* infections [8]. According to a scientific study by Karagözlü et al. (2011) [9] basil essential oil has an antimicrobial effect on the survival of *Escherichia coli* and *Salmonella typhimurium*.

Thyme essential oil exhibits potent antimicrobial activity against a number of bacterial strains, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* [10]. Another study [11] reported that thyme extract was effective against *Escherichia coli* and *Listeria monocytogenes*. According to a study by He et al. (2022) [12] thyme essential oil is effective against a variety of fungi, including *Candida albicans*, *Aspergillus niger*, and *Penicillium expansum*. Overall, the research suggests that thyme has significant antimicrobial activity and may have potential as a natural alternative to conventional antimicrobial agents. Thyme has demonstrated significant antimicrobial activity against a variety of bacterial and fungal pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger* [13]. Thyme essential oil exhibits antimicrobial activity against *Helicobacter pylori*, which is the causative agent of chronic gastritis and gastric ulcer [14]. Thyme extract has demonstrated strong antimicrobial activity against *Escherichia coli* and other bacteria [15, 16, 17, 18, 19].

The antimicrobial activity of plant extracts is determined by their chemical composition. Eleven components were identified in fresh basil leaves, the main constituents being methyl cinnamate (70.1%), linalool (17.5%), β-elemene (2.6%) and camphor (1.52%) [20]. The chemical compounds found in *T. vulgaris* are terpenes and aromatic compounds, the main compounds being thymol, camphene, caryophyllene, humulene, α-terpeniol and para-cymene [21].

Plant essential oils are being explored as a promising replacement for currently used antimicrobials, and to date many plants essential oils have been reported to exhibit significant antimicrobial activity [22]. The study of extracts from the essential oil crops of basil and thyme aims to determine their antibacterial activity against *Escherichia coli* for their subsequent application in agricultural practice as additives for decontamination and deodorization of compost-like mixtures.

2. Material and Methods

For the preparation of plant extracts, roots, stem, leaves and whole plant of two essential oil crops were used: basil (*Ocimum basilicum* L.) and thyme (*Thymus vulgaris* L.).

The experiment on the cultivation of the crops was carried out under controlled conditions in the greenhouse of the educational and experimental field of the "Crop Cultivation" department at the Technical University - Varna. Before planting the experiment and at the end of the growing season, soil samples were taken for each of the variants for agrochemical and microbiological analysis.
Decontamination of Escherichia coli has been applied to composts from agricultural crop wastes (vegetables, fruits, starter: soil).

2.1. Agrochemical Analyses

- Ammonium and nitrate nitrogen content was determined photometrically with Nitrospectral.
- The content of phosphorus and potassium was determined by the Egner-Riem double-lactate method.
- Soil reaction values were measured potentiometrically with a pH meter (ISO 10390:2005).
- The moisture content of the samples was determined on a moisture balance, model DBS.

2.2. Microbiological Analyses

For the microbiological analysis of the soils and composts, the method of dilution and three-fold inoculation of solid nutrient media was used with subsequent counting of colony-forming units (CFU) in 1 g abs. dry soil [23, 24, 25]. Systematic and physiological groups of aerobic microbes - bacilli and non-spore-forming bacteria (on meat-peptone agar), micromycetes (mold fungi) - on Chapek-Dox agar, actinomycetes and bacteria assimilating mineral nitrogen (on Actinomycetes isolation agar) were determined. The general microflora was determined. The mineralization coefficient was calculated [26, 24].

Before testing the extracts for antimicrobial activity, they were subjected to sanitary-microbiological control. For the isolation of pathogenic microflora, the following solid nutrient media were used: Desoxycholate Citrate agar (*Salmonella* sp.), ChromoBio Listeria agar (*Listeria* sp.), Endo agar (*Escherichia coli* and coliforms), ChromoBio Enterococcus agar (*Enterococcus*), Baird-Parker agar (*Staphylococcus aureus*). A sanitary-microbiological control was carried out on the water for preparing the decoction variant (solvent water), the irrigation water and the composts for the content of intestinal bacteria (*Enterococcus*), *Escherichia coli* and *Salmonella* sp.

The catalase activity of soil microorganisms was determined by the titration manganese-metric method [27].

The cellulase activity of soil microorganisms was determined dynamically by tracking the percentage of degraded area of cellulosic material (filter sheets) placed on loose soil in a petri dish [27].

2.3. Antimicrobial Activity

An agar-diffusion method is used, pouring 25 ml of liquefied and cooled solid Endo agar nutrient medium, suitable for cultivating the pathogenic species *Escherichia coli*, into 90 mm sterile petri dishes [25]. A solid culture is made for *Escherichia coli* (Certified reference material: *Escherichia coli* NBIMCC 3397). Using a sterile instrument, wells with a diameter of 7 mm are prepared, equidistant from each other and at a distance of 2.5 cm from the center of the petri dish. The wells are inoculated with plant extracts, volume 60 µL. Incubate in a thermostat at 37 °C for 24h. After cultivation, it is measured with a line, in mm, the so-called sterile zone (retention, suppression), resp. sensitivity of *Escherichia coli* to a certain plant extract. The diameter of the zone is proportional to the sensitivity of the tested microorganism.

2.4. Methods for Obtaining the Extracts

Total extracts were prepared by 5 methods (Table 1):

Table 1.
Extract variants.

Variant	Method of preparation	Solvent
Decoct	Boiling the chopped plant product with the necessary solvent; the extractive solution is filtered while hot	Water
Tincture	Soaking for 8-10 days in solvent, shaking periodically during the extraction period; filtering	Ethyl alcohol (30%)
Medicinal wine	Previously crushed herbs are soaked for 7-10 days in a solvent; filtering	Good quality and well stabilized wine
Medicinal vinegar	Previously crushed herbs are soaked for 7-10 days in a solvent; filtering	Good quality wine vinegar
Medicinal oil	Soaking the herbs in a solvent for 4-6 weeks; storage in closed glass containers in the dark and cool; filtering	Good quality olive oil

2.5. Statistical Analysis

Statistical data processing from the microbiological indicators included calculating the average value of three repetitions and determination of coefficient of variation (CV). Correlation analysis was applied to establish relationships between soil biogenicity and antibacterial activity of plant extracts. Microsoft Excel software product was used for the statistical analysis.

3. Results and Discussion

The content of nutrients in the soil before sowing and at the end of the growing season of crops is presented in the following table 2.

Table 2.
Content of nutrients in the soil.

Variant	pH	Nutrients			
Control (Before starting the experiment)		**NH_4 mg/kg**	**NO_3 mg/kg**	**P_2O_5 mg/100g**	**K_2O mg/100g**
	7.25	4.08	8.2	16.9	17.5
At the end of the growing season:					
Ocimum basilicum	7.23	3.95	5.98	16	15.2
Thymus vulgaris	7.2	4	5.03	14.1	15

Comparing with the limit values for soil stocking with available nitrogen compounds, mobile phosphates and absorbable potassium, it can be concluded that the soil is poorly stocked with nitrogen, but has a good stocking degree in terms of phosphorus and potassium, and the soil reaction is relatively favorable for plant development. The agrochemical analyzes before planting the experiment and at the end of the growing season of the two plant species in terms of pH showed a neutral reaction (in the range 7.20 - 7.23). The values of nitrogen, phosphorus and potassium are close for the two research

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periods, slightly decreasing at the end of the growing season, to a higher degree for thyme compared to basil.

The biogenicity of the soils before sowing and at the end of the vegetation of the crops is presented in the following table 3.

Table 3.
Quantity and qualitative composition of soil microorganisms (CFU x 10^3 / g abs. dry soil); ±CV.

Variant	Total micro-flora	Non-spore-forming bacteria	Bacilli	Actino-mycetes	Micro-mycetes	Bacteria assimilating mineral nitrogen	Mineralization coefficient
Control (Before starting the experiment)	3256	2678±0.013	380±0.053	60±0.333	138±0.072	4900±0.020	1.6
At the end of the growing season:							
Ocimum basilicum	4082	2940±0.014	500±0.040	290±0.034	352±0.028	5920±0.014	1.72
Thymus vulgaris	3890	2820±0.012	450±0.059	280±0.071	340±0.059	5400±0.019	1.65

The results show that soil biogenicity is higher in the vegetated variants compared to the control (no vegetated). This trend applies to the individual studied groups of microorganisms and, accordingly, to the general microflora. Basil increases the amount of microorganisms to a higher degree than thyme. The rate of decomposition of organic substances in soils correlates with the amount of microorganisms - the values of the mineralization coefficient decrease in the following order: basil > thyme > control.

In all three variants, the main share in the composition of the total microflora is occupied by non-spore-forming bacteria (82% in the control, 72% in basil, 73% in thyme), followed by bacilli (about 17% in all variants), and the least represented are micromycetes (moulds) (4% in the control, 9% in the vegetated variants) and actinomycetes (2% in the control, 7% in the vegetated variants).

In the variants with vegetation, an increase in the percentage participation of actinomycetes and micromycetes was found at the expense of non-sporulating bacteria (compared to the control), but their share remained the lowest in the composition of the total microflora. Actinomycetes and micromycetes are involved in the more extreme stages of decomposition of organic matter.

Catalase is a respiratory enzyme that breaks down the toxic hydrogen peroxide that is released when proteins are broken down. The catalase activity of soil microorganisms is presented in the following Figure 1.

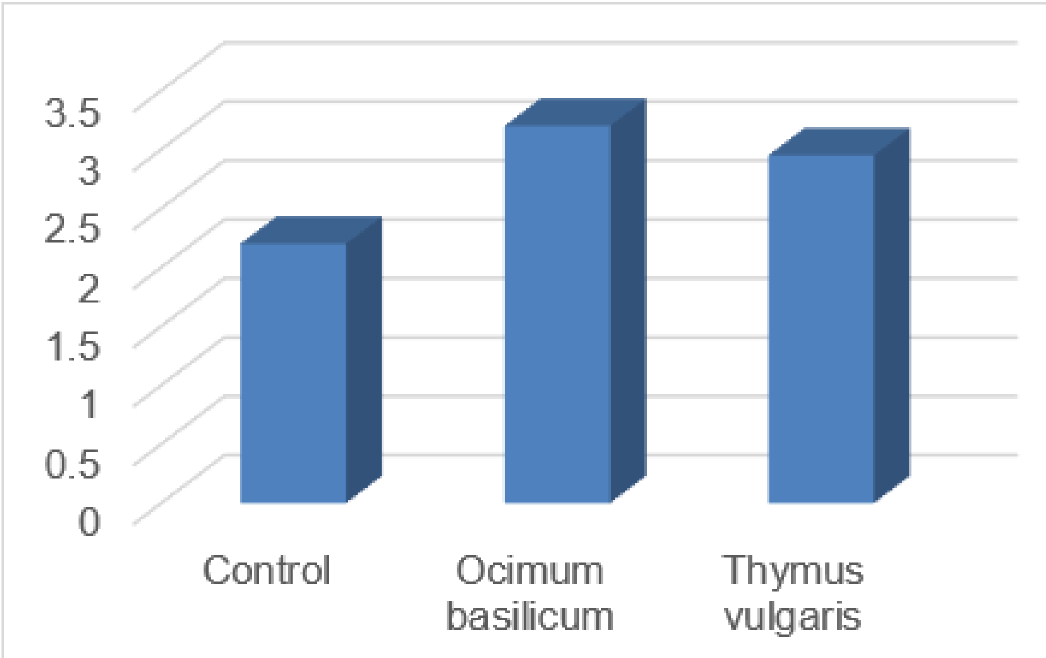

Figure 1.
Catalase activity of soil microorganisms (ml O_2/30 min).
Note: * CV up to 10% for all variants (low dispersion).

The results showed that catalase activity increased in vegetated variants, to a higher extent in basil. In addition to microbial origin, there is also catalase of plant origin. Catalase values correlate with the amount of soil microorganisms. A number of factors are important for enzyme activity: soil type, soil humidity and temperature, nutrient content, amount and composition of microflora, type of vegetation and others.

Cellulase catalyzes the hydrolysis of cellulose, in which cellulose is initially broken down to cellobiose, which under the action of ß-glucosidase is broken down to glucose. The cellulase activity of the investigated variants is presented in the following Figure 2.

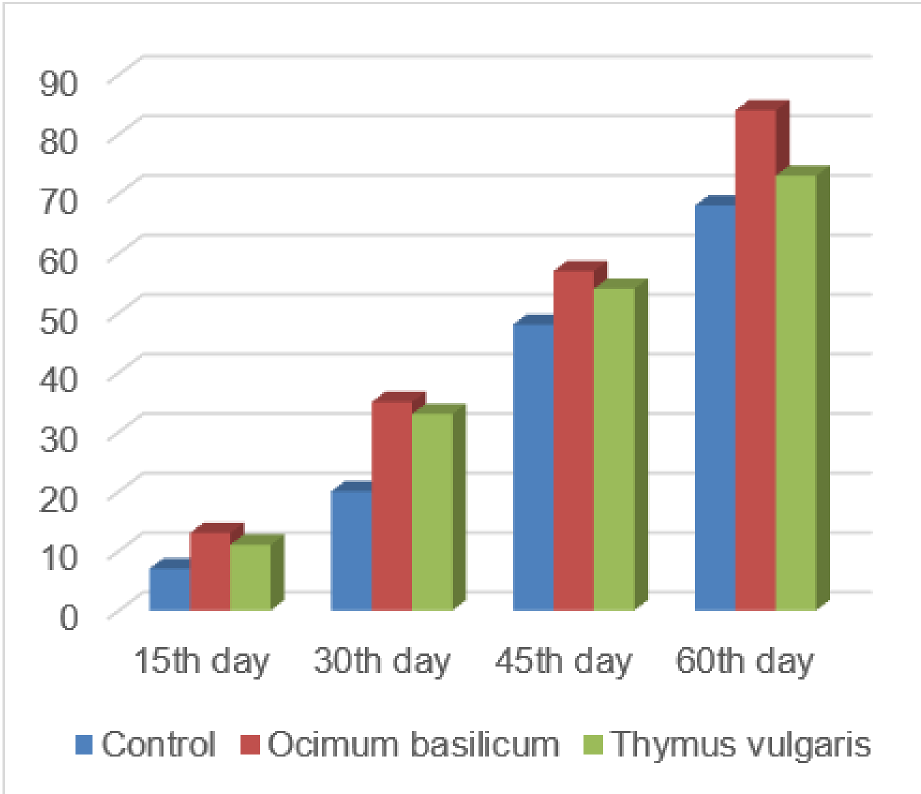

Figure 2.
Cellulase activity of soil microorganisms (% decomposed area).
Note: * CV up to 10% for all variants (low dispersion).

Microbiological and enzymatic indicators are sensitive indicators of soil fertility, as well as in the presence of contamination, including pathogenic microorganisms [28, 29, 30, 31, 32, 33; 34, 35, 36, 37].

To determine the antimicrobial activity of the extracts in each Endo agar petri dish after inoculation with an inoculum containing *Escherichia coli*, four wells were prepared for root, leaf, stem and whole plant, respectively. The results are presented in the following tables 4, 5, 6 and 7.

Table 4.
Antimicrobial activity of the studied extracts (Roots).

Variant: Roots	Sterile zone, cm	
	Ocimum basilicum	Thymus vulgaris
Decoct	1.4	1.0
Tincture	1.0	0.9
Medicinal vinegar	0.8	0.7
Medicinal wine	0.6	0.5
Medicinal oil	1.0	0.9

Data on root extracts show better results with basil than thyme. In terms of variants, the sterile zone decreases in the following order: decoction > medicinal oil = tincture > medicinal vinegar > medicinal wine. The results of the microbiological control of the extracts showed the absence of the tested pathogenic microorganisms: *Escherichia coli*, *Salmonella* sp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Enterococcus*. All figures and tables should be placed in the text, where most suitable.

Table 5.
Antimicrobial activity of the studied extracts (Leaves).

Variant: Leaves	Sterile zone, cm	
	Ocimum basilicum	**Thymus vulgaris**
Decoct	0.8	0.7
Tincture	0.4	0.3
Medicinal vinegar	0.9	0.8
Medicinal wine	0.5	0.4
Medicinal oil	0.9	0.8

Data on leaf extracts show better results with basil compared to thyme. In terms of variants, the sterile zone decreases in the following order: medicinal oil = medicinal vinegar > decoction > medicinal wine > tincture. Compared to root extracts, the sterile zone of leaf extracts had lower values, indicating that basil and thyme roots were more effective against *Escherichia coli* than their leaves.

Table 6.
Antimicrobial activity of the studied extracts (Stems).

Variant: Stems	Sterile zone, cm	
	Ocimum basilicum	**Thymus vulgaris**
Decoct	0.8	0.6
Tincture	0.6	0.5
Medicinal vinegar	1.1	0.8
Medicinal wine	0.7	0.6
Medicinal oil	0.9	0.7

Data on stem extracts show better results with basil than thyme. In terms of variants, the sterile zone decreases in the following order: medicinal vinegar > medicinal oil > decoction > medicinal wine > tincture. In general, compared to root and leaf extracts, the sterile zone of stem extracts was intermediate between the results for root and leaf, indicating that basil and thyme roots were more effective and leaves less effective against *Escherichia coli* relative to their stems. For the medicinal oil, an equal effect against the pathogen was found for leaf and stem extracts in basil and higher for leaves versus stems in thyme.

Table 7.
Antimicrobial activity of the studied extracts (Whole plant).

Variant: Whole plant	Sterile zone, cm	
	Ocimum basilicum	**Thymus vulgaris**
Decoct	0.8	0.7
Tincture	0.6	0.5
Medicinal vinegar	1.0	0.9
Medicinal wine	0.7	0.6
Medicinal oil	0.7	0.6

Data on whole plant extracts show better results with basil than thyme. In terms of variants, the sterile zone decreases in the following order: medicinal vinegar > decoction > medicinal wine =

medicinal oil > tincture. In whole plant extracts, the sterile zones are more comparable to those of leaves and stems. The concentration of all parts of the plant ("whole plant" extracts) did not increase the antimicrobial activity of the tested plants compared to extracts with a concentration of a certain part of the plant (roots, leaves, stems).

Other authors [7, 9] also found antimicrobial activity of basil extracts against *Escherichia coli*. Thyme extract shows strong antimicrobial activity against *Escherichia coli* and other pathogenic microorganisms [10, 11, 13, 15, 16, 17, 18, 19].

The chemical composition of the extracts is of primary importance for their antimicrobial activity. The main constituents in basil are methyl cinnamate, linalool, β-elemene and camphor [20], and in thyme: thymol, camphene, caryophyllene, humulene, α-terpeniol and para-cymene [21]. Additional conditions - lowering the pH of the medium when using medicinal vinegar and medicinal wine, the inclusion of additional plants - grapes (wine, vinegar), olives (solvent olive oil for the medicinal oil), the time of action of the extracts probably also affect the zone of detention. Similar trends are described by Naskova et al. (2023) [38] and Malcheva et al. (2023) [39] in a study of the antimicrobial activity against *Escherichia coli* of *Coriandrum sativum* L. and *Mentha spicata* L. extracts. Blažeković et al. (2010) [40] found that the concentration of *Satureja hortensis* L. essential oil as well as the type of bacteria determined different antibacterial activity against *Escherichia coli*, *Salmonella enteritidis*, and *Bacillus subtilis*.

We established positive relationships between the content of beneficial microorganisms in the extracts and their antimicrobial activity [41]. However, the content of microbes in the extracts depends on the biogenicity of the soils, the development of the vegetation (the presence of microbes in the extracts from the individual parts of the plants), and their reproduction in the extracts from the environmental conditions – the type of solvent, the temperature at which the extracts are stored, pH of the extracts, the period of analysis after preparation of the extracts. A correlation matrix was created to determine correlation coefficients between the total microflora and the antimicrobial activity of individual plant parts and the whole plant. We found a significant and moderate correlation between the total soil microflora and the antimicrobial activity of the stem (significant) and whole plant, root and leaf (moderate) extracts, respectively. A high positive correlation was found between the antibacterial activities of leaf, stem and whole plant extracts (Table 8).

Table 8.
Correlation analysis.

Indicators	**Total microflora**	**Anticacterial activity (Roots)**	**Anticacterial activity (Leaves)**	**Anticacterial activity (Stems)**	**Anticacterial activity (Whole plant)**
Total microflora	1				
Anticacterial activity (Roots)	0.333333	1			
Anticacterial activity (Leaves)	0.231869	0.328482	1		
Anticacterial activity (Stems)	0.536895	0.089482	0.816098	1	
Anticacterial activity (Whole plant)	0.345857	0.034586	0.721744	0.854169	1

A regression analysis was applied to determine to what extent the average antimicrobial activity from all parts of the plant and whole plant depends on the total amount of microorganisms (Table 9).

Table 9.
Regression analysis.

Summary output					
Regression statistics					
Multiple R	0.983018				
R square	0.966325				
Adjusted R square	0.855214				
Standard error	0.146384				
Observations	10				
ANOVA					
	df	**SS**	**MS**	**F**	**Significance F**
Regression	1	5.53402	5.53402	258.2577	2.25578E-07
Residual	9	0.192855	0.021428		
Total	10	5.726875			

Source: *P<0,001.

A photo material of the preparation of the extracts, the biogenicity and antimicrobial activity of some of the variants is presented in the following Figure 3.

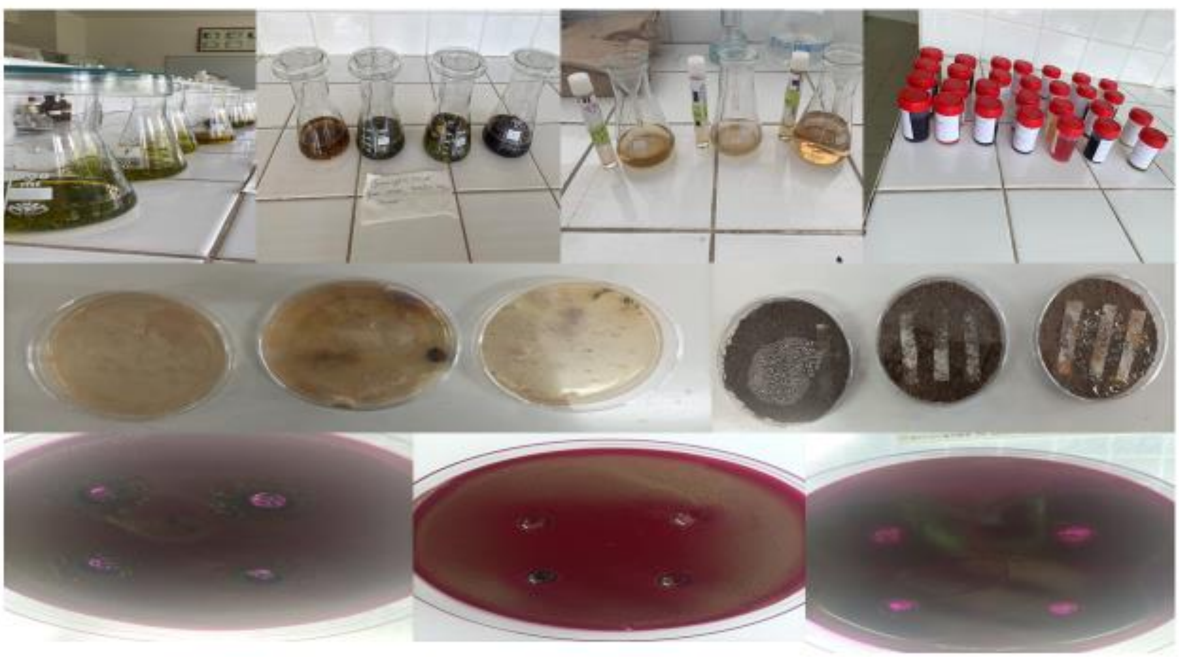

Figure 3.
Photo material of some variants and results.

The sanitary-microbiological control of the water for preparing the variant decoction, as well as the water for irrigation, shows the absence of pathogenic microflora (*Enterococcus*, *Escherichia coli*, *Salmonella* sp.) in the tested waters. Application of extracts of basil and thyme (individually and in combination), whole plant, decoction variant resulted in gradual complete decontamination of composts containing *Escherichia coli* over a period of four months, with monthly re-application of the extracts. Lavender and oregano decontaminated composts in a shorter period of two months [41]. In addition, the composts were enriched with microflora useful for soil fertility after the application of the extracts. Composts are dark brown, loose, soft, with a uniform structure without lumps and lumps, and with an earthy smell (no ammonia smell), and after adding the extracts of basil and thyme and with the aroma of these plants.

5. Conclusions

The obtained results of the agrochemical analyzes show that basil and thyme do not have a great influence on the dynamics of macroelements in the soil. Macronutrient values were close at the beginning and end of the experiment.

The variants with basil and thyme slightly increase the biogenicity of soil microorganisms (to a higher extent with basil), the percentage participation of non-spore-forming bacteria and bacilli is preserved, but the share of mold fungi and actinomycetes increases compared to the control. In all variants, the main share in the composition of the general microflora is occupied by non-spore-forming bacteria, followed by bacilli, and the least represented are actinomycetes and micromycetes.

The values of the enzymes catalase and cellulase correlate with the amount of microorganisms and also their activity increases in the variants with vegetation – to a higher degree in basil. A number of factors are important for enzyme activity: soil type, soil humidity and temperature, content of nutritional elements, amount and composition of microflora, type of vegetation and others.

Basil extracts showed stronger antimicrobial activity against the pathogenic microorganism *Escherichia coli* compared to thyme extracts, differing for individual plant parts. Root extracts showed higher antimicrobial activity compared to leaf, stem and whole plant extracts. The leaves are less effective against *Escherichia coli* than their stems. In whole plant extracts, the sterile zones are more comparable to those of leaves and stems. The concentration of all parts of the plant ("whole plant" extracts) did not increase the antimicrobial activity of the tested plants compared to extracts with a concentration of a certain part of the plant (roots, leaves, stems). The indicated trends are indicative for both plants – basil and thyme.

A persistent trend for weaker antimicrobial activity of the plant extracts was found in the medicinal wine and tincture variants (except for the root tincture). In the other variants, the antimicrobial effect has similar values. The choice of solvent and the exposure time of the extracts probably also influence the diameter of the retention zone.

The antibacterial activity of stem extracts depends significantly on the total amount of microorganisms in the soils. A moderate correlation was found between soil biogenicity and antimicrobial activity of whole plant, root and leaf extracts.

The analyzed essential oil cultures can be used for disinfection (against *Escherichia coli*) and deodorization of compost-like mixtures.

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