Technical University of Varna
Annual Journal
https://doi.org/10.29114/ajtuv.vol8.iss1.304
Vol. 8 Issue 1 (2024)
Published: 2024-06-28
ISSN 2603-316X (Online)

Combination of factors influencing the antibacterial activity of oregan extracts as future additives for compost decontamination

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Abstract. *Raw materials of plant origin can serve as an inexhaustible source of biologically active sub-stances, which in turn are used in various fields of medicine, especially in treatment of diseases that require an integrated approach. One of the contemporary trends is the search for herbal medicines that have a certain antimicrobial activity. The objects of our study were extracts and oils obtained from different plant fractions of oregano (roots, leaves, stems, whole plant) and using different solvents (water, ethanol, vinegar, wine, olive oil), grown in a vegetation cage and under greenhouse conditions. It was also determined that the antibacterial activity against Escherichia coli to the same plant fractions. The studied oregano extracts can be used for compost decontamination and other substrates in which Escherichia coli is found. For defining dependencies and trends among the experimental data were used mathematical statistics tools.*

Keywords: antimicrobial activity, mathematical modeling, oregano, extract

1 Introduction

Plants play an important role in the development of contemporary research, as a starting point for new developments in medicine, as they are a source of many antimicrobial compounds (Ibrahim & Kebede, 2020; Cowan, 1999). Medicinal plants are known to cure many infectious diseases and this justifies their use as a source of antimicrobials to expand the range of available antibiotics (Zaidan et al., 2005). Most of them contain substances that are precursors for the synthesis of drugs or substances that can be used for therapeutic purposes. Such a plant is the oregano (*Origanum vulgare*). Its therapeutic properties are determined by its chemical composition. It contains essential oil (up to 1. 2%), flavonoids, tannins, antimicrobial phenols (thymol and carvacrol), sesquiterpenes, free alcohols, geranyl acetate, organic acids, macro- and trace elements, vitamins C, B1, B2, D, A, K and other biologically active compounds (Terenina et al., 2011; Burlakova et al., 2012). Oregano essential oil predominantly contains carvacrol (80. 5%) (Man et al., 2019).

The Oregano essential oil has been used in folk medicine since ancient times and has antioxidant and antibacterial effects (Asensio et al., 2017; Rodriguez-Garcia et al., 2016). It inhibits the growth of *Escherichia coli* and *Staphylococcus aureus*, being more sensitive to gram-positive bacteria (Martucci et al., 2015).

The objective of the study was to determine the best method for solution preparation in terms of solvent, preparation time and used plant parts. In terms of experimental data processing, we put set ourselves the task of finding an optimal combination of factors influencing the antibacterial activity of oregano essential oil solution obtained from different plant parts under different conditions. The study also aimed to characterize the antibacterial activity of oregano extracts against Escherichia coli for developing a future method for composts decontamination.

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2 Material and methods

Oregano was grown in two experimental setups: a greenhouse with uncontrolled conditions at the experimental training field of Technical University-Varna and a vegetation cell at Dobrudja Agricultural Institute - General Toshevo. Extracts of different plant fractions were prepared and used for Escherichia coli decontamination of various compost variants.

Extract and oils from various parts of the oregano (roots, leaves, stems, whole plants) were analyzed using different solvents (decoction - solvent: water; tincture - solvent: 30% ethanol; medical vinegar - solvent: vinegar; medical wine - solvent: wine; medical oil - solvent: olive oil) and preparation methods.

In decoction, the chopped plant product is boiled with solvent water and the resulting extractive solution is hot filtered hot. Tincture is obtained by soaking the herb with 30% ethyl alcohol, usually for a time ranging between 8-10 days. The operation is carried out in well-closed glass containers. Shaking, for good extraction, is necessary throughout the whole extraction period. For medicinal wine preparation, the previously crumbled plants are soaked in wine (good quality and well stabilized) for 7-10 days, after which the preparation is filtered. The extraction was carried out in a weak hydroalcoholic medium, at slightly acid pH. For medicinal vinegar preparation, the previously crumbled plants are soaked in vinegar (benign) for 7-10 days, after which the preparation is filtered. The extraction takes place in Store in well-closed glass bottles, in a dark and cool place.an acidic medium. To obtain the medicinal oil, the herb is soaked in olive oil for 4 to 6 weeks.

The antibacterial activity of the extracts and oils against *Escherichia coli* was recorded on the 2nd, 10th and 20th day after preparation, with readings at the 24th and 48th hour after plating (in Endo agar) for Escherichia coli and the extracts and oils in the wells. Agar diffusion method was used to determine the antimicrobial activity (Нусторова и Малчева, 2020).

Prior to testing the extracts and oils for antibacterial activity, a sanitary microbiological control was conducted to verify the microbiological purity of the variants. The following solid culture media were used to isolate pathogenic microflora: Desoxycholate Citrate Agar (*Salmonella* sp.), ChromoBio Listeria Agar (*Listeria* sp.), Endo Agar (*Escherichia coli* and coliforms), ChromoBio Enterococcus Agar (*Enterococcus*).

Mathematical methods for processing multivariate experimental data

Working with experimental data often leads up to working with a large amount of information. The resulting data are rarely used in its pure form. They undergo various pre-treatments. Data preprocessing serves as a means of assessing the quality of the collected information, providing insight into the informational value of individual groups of observables. The types of the examined quantities may be called recognition features or factors depending on the processing method.

The question arises to what extent the individual factors are able to provide independently in-formation about the state of the object of analysis. Typically, control data are not available in advance for a posteriori assessment of the methods information value or attributes associated with it. Often the data set is required to be arranged or classified into separate self-contained groups, or if using the terminology from pattern recognition, state classes. For most tasks, including diagnostic (Недев и др., 2011) specialists have to define boundaries between classes. On this basis, can be sought criteria based on the distribution (location) of the measurement data in the feature space or some other space. Then are sough methods for estimating the information value of features, in the sense of space separability. In the light of the above, it can be argued that one feature or a set of features (S2 in Fig. 1 b) is better than another feature or another set (S1 in Fig. 1 a), if it provides a better opportunity to recognize the condition (Недев и Тенекеджиев, 1994).

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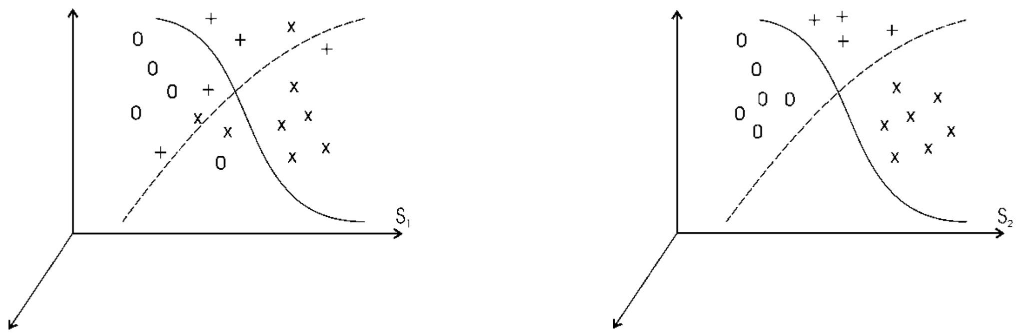

a) b)

Fig. 1. Data layout in the feature space.

In applied research, is posed the task the task of finding the best coordinate system in which to visualize data grouped by class.

The classical procedures for solving both problems can be sought in factor analysis, or principal component analysis. The purpose of the factor analysis is to represent the data in a space of decreasing dimensionality, taking into account the correlation between features in the prior space.

One possible method for finding the best clustering of multivariate data is the factor analysis. It is used when, the study of objects are influenced by factors of a different nature that cannot be measured directly - the so-called. indirect factors. Available for observation and measurement are multiple (larger number of factors) variables and their functions (usually linear). If the initial variables are denoted by x1,x2,. . . ,xp and X is the data matrix, it is sought a data a representation of the following type:

$$X = FL + E \quad (1)$$

where F - is a matrix record of factor values,

L is a matrix of factor weights,

E - is a matrix of errors.

The main tasks of factor analysis are (https://store.fmi.uni-sofia.bg/fmi/statist/personal/vandev/lectures/stat1/Stat.htm -Записки по Приложна статистика, Димитър Въндев, 1999):

1) Determine the sufficient number of factors to describe the phenomenon (the second dimension of the F matrix);

2) To find the factors. This means to calculate the coefficients of the linear functions L representing the variables (by means of the factors) and the values for the factors determined on the individual objects F.

3) Interpret the results.

Object of the classical factor analysis is the correlation matrix R with dimension dxd and elements rij. It reflects on the sampling variance and does not depend on the coordinate systems in which the measurements were taken. Given that for uncorrelated traits ρ_ij=0 and for fully correlated (linearly dependent) traits ρ_ij=1 , we can assume that the correlation coefficient acts as a function of the similarity of the traits. Two features for which it is close to one can be merged into one, thereby reducing the dimensionality by one. Consistent repetition of this process leads to the known in theory hierarchical grouping procedure for dimensionality reduction.

1. Let $\hat{d} = d$ – dimension and $F_i=\{X_i\}$; i=(1÷d) - signs

2. If $\hat{d} = d'$ – stop (d'– set minimum dimension)

3. The correlation matrix is calculated and the most correlated pair of the group of traits is found signs: F_i and F_j

4. Fi and Fj are merged; is erased and the dimension of the space d ̂is reduced by one ;

4. Fi and Fj are merged; the dimension of the space d ̂is erased one unit reduced;

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5. Execute the loop until the specified minimum dimension is reached.

Another method for representing multivariate data is the principal components method. It allows to determine to what extent a certain number of factors describe the phenomenon. It is based on transformations of a larger number mostly correlated variables into a smaller number of uncorrelated ones. The new variables are called principal components and they form the axes of the new space. Each axis, or basis vector, is qualitatively independent of all the others. The first axis is chosen to represent the direction with the largest variance, the second axis shows the second largest variance, and so on. In other words, the ordering of the principal components is by the degree to which they describe the behavior of the original variables - the first principal components are of greatest importance, each subsequent explains a smaller part of the "non-described" dependencies (https://store.fmi.uni-sofia.bg/fmi/statist/personal/vandev/lectures/stat1/Stat.htm - Записки по Приложна статистика, Димитър Въндев, 1999). For this transformation to be meaningful, it must be proven that it preserves the entire amount of useful information, and the remaining principal components after dimensionality reduction are carriers of the bulk of this information.

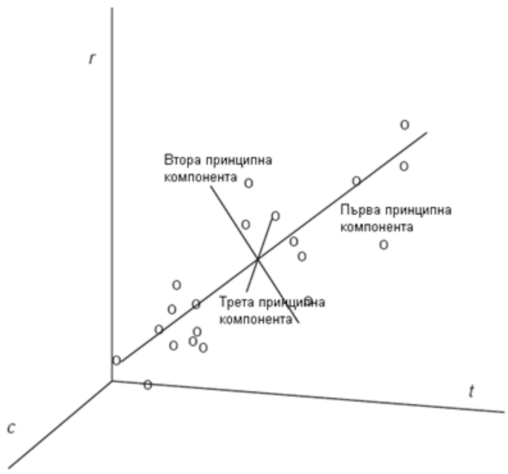

Fig.2. Character space and principle components (Консулова-Бакалова, 2021)
фиг.2. Пространство на признаците и принципни компоненти [Консулова-Бакалова, Мария Иванова, Автоматизация на инженерния труд. Автоматизирано проектиране и анализ, Геа принт – Варна, 2021, ISBN 978-619-184-047-2]

The procedure allows data to be represented in two or three dimensional space. In most cases, it is possible to present more than 80% of the information using only two strands. Researchers very often gain insight into the nature of processes by considering these first couple of new variables.

The full set of principal components is as large as the number of original variables. If there are cross-sensitivities between the output values, respectively, the variables will be at least partially correlated. This means that the actual dimensionality of the data set is smaller than the maximum dimensionality.

In the variables space the observed quantities, is formed the so-called ellipsoid of scatter points representing the information data. The principal components are the axes of this ellipsoid and are completely independent. In doing so, some of the factors that have little variance can be removed and to be worked with a reduced number of variables.

The task that requires the use of this method is the improved interpretation of the regression model similar to that in factor analysis - the response is represented as a function of new artificial variables - factors.

The solution by the method of principal directions gives the minimum of the functional trE'E. A representation of the matrix X of the form X = UDV is usually called svd or singular value decomposition. Here U and V are orthogonal and D is "diagonal" with non-negative elements. In a more detailed

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record it looks like this (https://store.fmi.uni-sofia.bg/fmi/statist/personal/vandev/lectures/stat1/Stat.htm - Записки по Приложна статистика, Димитър Въндев, 1999):

$$\mathbf{X}_{M\times N} = \mathbf{U}_{M\times N} D_{N\times N} \mathbf{V}^{T}_{N\times N} =$$

$$= (u_1 u_2 \ldots u_N) \begin{bmatrix} \sigma_{11} & 0 & \cdots & 0 \\ 0 & \sigma_{22} & \cdots & 0 \\ \vdots & \vdots & \ddots & \\ 0 & 0 & \ldots & \sigma_{NN} \end{bmatrix} (v_1, v_2, \ldots v_N) \ , \qquad (2)$$

Dual representation of the covariance matrix:

$$X'X = V'D'U'UDV = V'D'DV = \sum d^2_{i,i} v_i v'_i \qquad (3)$$

The representation of the matrix X'X is analogous, but the eigenvectors are in a different space (https://store.fmi.uni-sofia.bg/fmi/statist/personal/vandev/lectures/stat1/Stat.htm - Записки по Приложна статистика, Димитър Въндев, 1999).

We also have $d^2_{i,i} = \lambda_i$.

Thus, fixing k and denoting by ()k the "truncation" operation on the first k columns of a matrix, we obtain F = n-1/2(UD)k, L'=(V')k. The numbers explain how much of the variance of the factors is explained by the selected factors.

The matrix L is sometimes called "raw" and is very often represented in normalized form - its poles are normalized by the square root of the communities. This eases interpretation.

In specialized software products for performing mathematical calculations, simulations and visualization, there are often specialized functions that perform the transformation by the principal components method. The data is thus transformed into a new coordinate system called the principal directions domain. Usually they are represented in the new orthogonal coordinate system by the so-called. "scatter plot" as a set of points. The distances between the points represent the known Mahalonobis distance (https://encyclopediaofmath. org/index. php?title=Mahalanobis_distance).

3 Results and discussions

The analyses showed that the plant extracts and oils did not contain pathogenic microflora - *Salmonella* sp., *Listeria* sp., *Escherichia coli* and coliforms, *Enterococcus* were found to be absent. These results warranted assays to report antimicrobial activity. Tables 1 and 2 present the numerical data for antibacterial activity when oregano was grown in a greenhouse and in a growing cage, respectively. The graphical representation of the data can be seen in Table 3.

Table 1. Antibacterial activity against *Escherichia coli* (oregano, greenhouse)

Variants	Plant parts	2nd day		10th day		20th day	
		Antibacterial activity (sterile area, cm)					
		24 h	48 h	24 h	48 h	24 h	48 h
Decoct	Roots	0,4	0,5	0,5	0,6	0,6	0,6
	Stems	0,2	0,3	0,3	0,4	0,4	0,5
	Leaves	0,3	0,3	0,3	0,5	0,4	0,4
	Whole plant	0,6	0,7	0,7	0,8	0,8	0,9
Tinc	Roots	0,3	0,3	0,3	0,4	0,3	0,4

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	Stems	0,4	0,5	0,4	0,5	0,4	0,4
	Leaves	0,4	0,5	0,4	0,5	0,4	0,4
	Whole plant	0,4	0,5	0,5	0,6	0,5	0,6
Medicinal vinegar	Roots	1	1,1	1,1	1,2	1,2	1,3
	Stems	1,2	1,3	1,3	1,4	1,4	1,4
	Leaves	1,2	1,3	1,3	1,3	1,4	1,4
	Whole plant	1,4	1,5	1,5	1,6	1,5	1,6
Medicinal wine	Roots	0,3	0,4	0,3	0,4	0,3	0,4
	Stems	0,3	0,4	0,3	0,4	0,3	0,4
	Leaves	0,3	0,4	0,4	0,4	0,4	0,4
	Whole plant	0,5	0,6	0,5	0,6	0,6	0,7
Medicinal oil	Roots	0,6	0,7	0,6	0,7	0,6	0,7
	Stems	0,6	0,6	0,6	0,7	0,8	0,8
	Leaves	0,8	0,8	0,8	0,9	0,9	0,9
	Whole plant	0,8	0,9	0,8	0,9	0,9	1

Table 2. Antibacterial activity against *Escherichia coli* (oregano, vegetative cell)

Variants	**Plant parts**	2nd day		10th day		20th day	
		Antibacterial activity (sterile area, cm)					
		24 h	48 h	24 h	48 h	24 h	48 h
Decoct	Roots	0,5	0,6	0,6	0,6	0,7	0,7
	Stems	0,4	0,5	0,5	0,5	0,6	0,7
	Leaves	0,4	0,5	0,5	0,6	0,6	0,6
	Whole plant	0,6	0,7	0,7	0,8	0,9	1
Tincture	Roots	0,4	0,5	0,4	0,5	0,4	0,5
	Stems	0,5	0,6	0,5	0,5	0,5	0,5
	Leaves	0,5	0,6	0,6	0,6	0,6	0,6
	Whole plant	0,5	0,6	0,6	0,7	0,7	0,9
Medicinal vinegar	Roots	1,2	1,3	1,3	1,4	1,4	1,5
	Stems	1,3	1,4	1,4	1,5	1,5	1,6
	Leaves	1,3	1,4	1,4	1,5	1,5	1,7
	Whole plant	1,4	1,5	1,5	1,6	1,6	1,8
Medicinal wine	Roots	0,4	0,5	0,4	0,5	0,4	0,5
	Stems	0,4	0,5	0,4	0,4	0,4	0,5
	Leaves	0,5	0,6	0,5	0,5	0,5	0,5
	Whole plant	0,6	0,7	0,7	0,8	0,7	0,8

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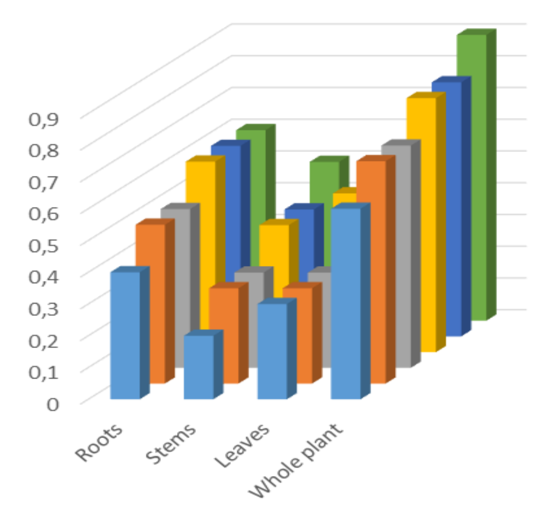
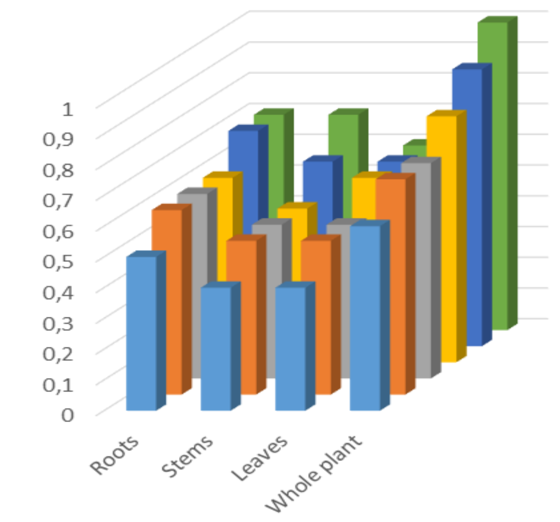
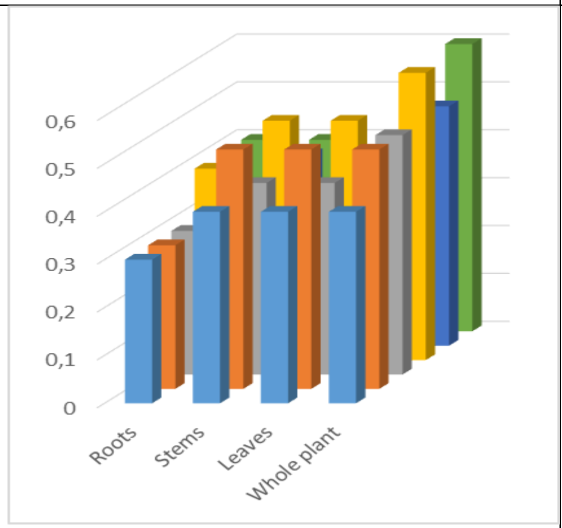
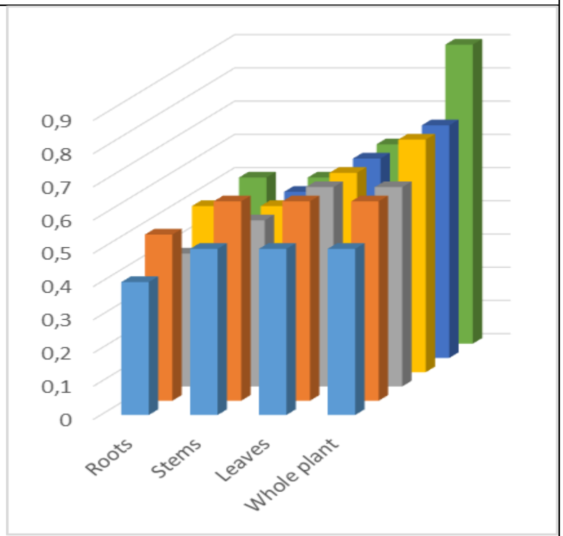
Medicinal oil	Roots	0,6	0,7	0,7	0,8	0,7	0,8
	Stems	0,7	0,8	0,8	0,8	0,8	0,8
	Leaves	0,8	0,9	0,9	0,9	0,9	0,9
	Whole plant	0,8	0,9	0,9	1	0,9	1

Table 3. Antibacterial activity (sterile area, cm)

	Greenhouse	Vegetative cell
Decoct		
Tincture		

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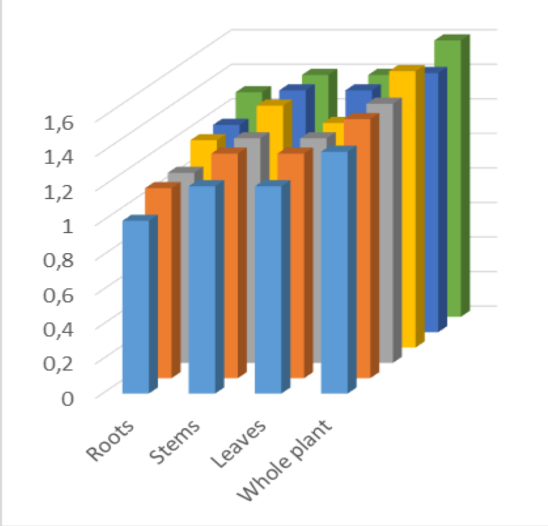
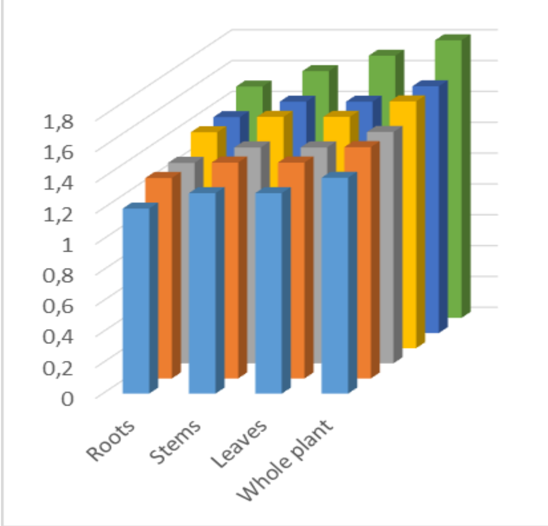
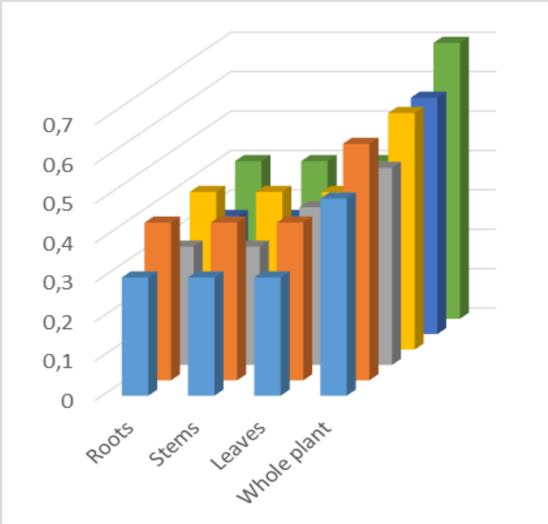
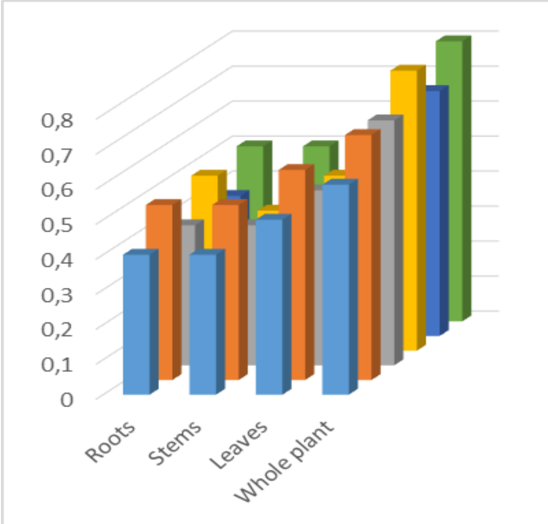
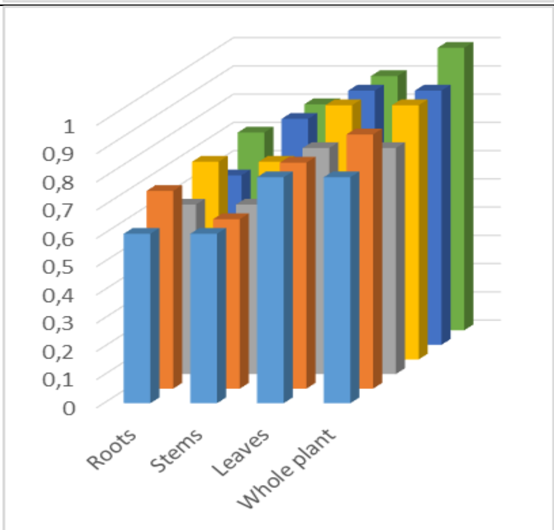
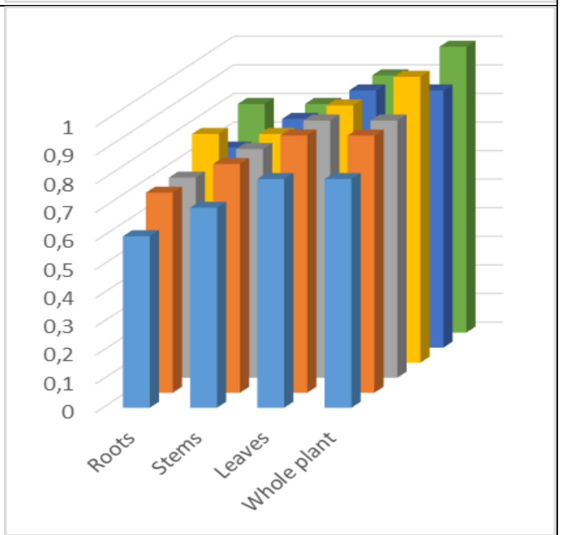
Medicinal vinegar		
Medicinal wine		
Medicinal oil		
	■ 24 часа след посяване (2-ри ден) ■ 48 часа след посяване (2-ри ден) ■ 24 часа след посяване (10-ти ден)	■ 48 часа след посяване (10-ти ден) ■ 24 часа след посяване (20-ти ден) ■ 48 часа след посяване (20-ти ден)

To detect dependencies and trends in the data obtained from the experiments, it is appropriate to use the tools of mathematical statistics (table 4). The main objective here is to determine the best method

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for obtaining an antibacterial solution, including the choice of solvent type, time to obtain the solution, and the choice of plant part from which to obtain the solution. In this setting of the problem, several factors are formed, which represent independent quantities and one quantity (dependent) on which the factors show influence. Despite the relatively small amount of data, the task is multidimensional due to the significant number of factors whose influence must be assessed. In the table below can be seen the factors whose influence on antibacterial activity we would like to evaluate:

Table 4. Factors affecting antibacterial activity

Factor	Abbreviation	Borrowed values	*Coded factor levels*
Time to obtain a solution	T, days	{2 days, 10 days, 20 days}	{2, 10, 20}
Sowing time	σ, hours	{24 hours, 48 hours}	{24, 48}
Type of solution	V	{Decoct, Tincture, Medicinal vinegar, Medicinal wine, Medicinal oil}	{1, 2, 3, 4, 5}
Part of the plant	P	{Roots, Stems, Leaves, Whole plat}	{1, 2, 3, 4}
Cultivation method	M	{Greenhouse, Vegetative cell}	{1, 2}

Most statistical analyses involve the use of purely numerical data when analyzing the influ-ence of various factors on a dependent variable. For this reason, it is appropriate to code the level factors so that they can be used in standard statistical procedures.

One possible procedure for examining the significance of individual factors is the regression analysis. It was conducted on the entire data set. The independent variables were the factors shown in Table 3 and the dependent variable was antibacterial activity. The results of the proce-dure conducted in Excel are summarized in Table 5.

Table 5. Regression analysis results

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95,0%*	*Upper 95,0%*
Intercept	0,0877	0,1270	0,6905	0,4906	-0,1625	0,3378	-0,1625	0,3378
M	0,1042	0,0459	2,2691	**0,0242**	0,0137	0,1946	0,0137	0,1946
V	0,0446	0,0162	2,7470	**0,0065**	0,0126	0,0766	0,0126	0,0766
P	0,0728	0,0205	3,5477	**0,0005**	0,0324	0,1133	0,0324	0,1133
T	0,0058	0,0031	1,8621	0,0638	-0,0003	0,0119	-0,0003	0,0119
σ	0,0033	0,0019	1,7245	0,0859	-0,0005	0,0071	-0,0005	0,0071

From a statistical point of view, it can be said that the influence of the method of cultivation (green-house or vegetation cell), the type of solution and the parts of the plant from which it is prepared are the most significant. Solution preparation time and seeding time in this problem setting did not show statis-tical significance. The conclusion is based on the P-value.

The conclusion obtained so far, however, does not answer the problem posed at the beginning. The regression analysis procedure provided an answer for the significance of the factors, but not a combina-tion between them that produced optimal results. The simplest would be to find the maximum value of antibacterial activity and state under what conditions it is obtained. With modern computational tools, this represents a very simple procedure, but in terms of the accuracy of scientific experiments it cannot be considered completely sufficient. Often the maximum value may be a momentary condition and if not enough experiments have been conducted, considering only a single value to bet on results that cannot be obtained in a subsequent similar experiment. The task we set ourselves here is not only to answer the questions posed at the outset, but also to demonstrate an approach to solving similar problems in future work.

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Published: 2024-06-28
ISSN 2603-316X (Online)

Figure 3a shows the frequency and distribution of observations across all antibacterial activity values obtained. Most of the observations cluster in the range 0. 5 to 1. However, another smaller peak of values around 1. 5 is also visible. The picture formed may be due to the fact that it is possible from a statistical point of view to have a separation of the data into two groups, or class of condition based on the influence of the departmental factors and the values of the dependent variable. This leads to the idea of using procedures for clustering and recognizing multivariate data.

There are many methods for processing and analyzing experimental data, but most of them, as well as the regression analysis already performed, use only numerical data. Procedures for the representation and analysis of multivariate data that can work including non-digital input information are PCA (Principal component Analysis), cluster analysis and discriminant analysis. For these, despite the multidimensionality of the data, a representation can be found that groups them into distinct classes of state. XLSTAT application to Excel was used to conduct discriminant analysis. Table 6 shows some of the numerical results.

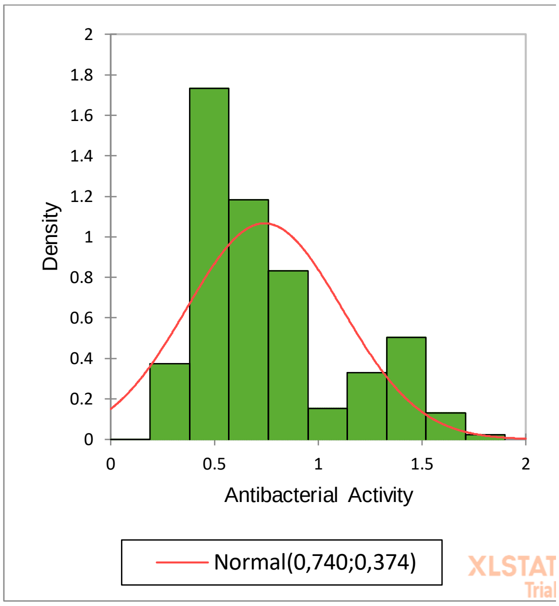
Table 6. Discriminant analysis results

	Lambda	F	DF1	DF2	p-value
method-cell	0,857	2,335	16	223	0,003
method-greenhouse			16	223	
variant-Medical oil	0,467	15,883	16	223	<0,0001
variant-Medicinal vinegar	**0,021**	655,063	16	223	<0,0001
variant-Medicinal wine	0,781	3,906	16	223	<0,0001
variant-decoct	0,884	1,823	16	223	0,030
variant-tincture			16	223	
plant-Leaves	0,897	1,598	16	223	0,071
plant-Roots	0,884	1,836	16	223	0,028
plant-Stems			16	223	
plant-Whole plant	0,737	4,974	16	223	<0,0001
tau-2	0,935	0,969	16	223	0,492
tau-10			16	223	
tau-20	0,921	1,199	16	223	0,270
t-24	0,915	1,296	16	223	0,201
t-48			16	223	

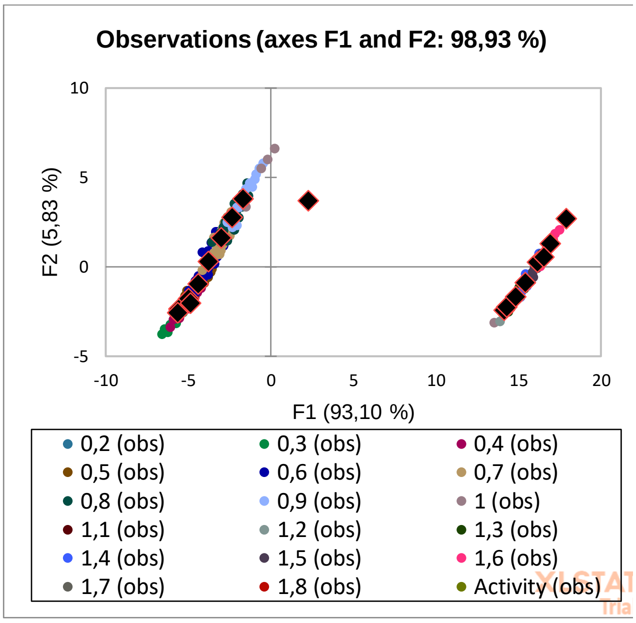
The differences in the Lambda values for the individual factors are clearly visible. The lowest value is for medical vinegar. It can be confidently asserted that the solution obtained with it gives results for antibacterial activity that differ markedly from those with the other substances.

The graphical representation of the data in the area of the first two strands after data transformation and presentation with eigenvalues gives an even better insight into their grouping into two distinct groups (Fig. 3b).

Technical University of Varna
Annual Journal
https://doi.org/10.29114/ajtuv.vol8.iss1.304
Vol. 8 Issue 1 (2024)
Published: 2024-06-28
ISSN 2603-316X (Online)



a) Antibacterial activity data distribution value



b) Presentation in the field of the first two main directions

Fig.3. Experimental data results

One group of data is the one obtained with medical vinegar solution, the other is all the others. In Fig. 3b very clearly distinguish the two sets of data. To refine and select the best condition of the remaining factors, dimensionality of the problem can be reduced by performing numerical experiments on the sample of data obtained with medical vinegar. The idea is to check if there is a split of the data within the group and this is due to some of the other factors. Unfortunately, however, the numerical experiments performed do not provide a basis for claiming that any of the other factors yield such a resolution. To some extent, it can be said that solutions obtained from the whole plant give higher levels of antibacterial activity, which is also evident from the raw data. Regarding the other factors, it can be said that their influence on the studied dependent variable is insignificant.

4 Conclusions

The statistical methods used for data processing and presentation allowed to determine the factors influencing the antibacterial activity. Combining several methods and representing the data in the feature space is an example of processing multivariate data without having a prede-fined grouping into several known states. When working with experimentally obtained results and looking for dependencies between them, the proposed approach would be a good oppor-tunity to split the data into several groups and determine the optimal set of factors influencing the output variables.

The capabilities of modern software products to perform complex computational procedures and the availability of specialized statistical processing programs make it possible to implement in practice and in a short time a large number of numerical experiments. Thanks to this, the best method or, as in this case, the method providing the best separation of the output data can be quickly and easily selected.

Plant extracts and oils of oregano exhibit antibacterial activity against Escherichia coli, with the most pronounced effect in the medicinal vinegar variant. The use of all parts of the plant (whole plant variants) enhances the antimicrobial activity of oregano relative to variants with a concentration of a specific part of the plant (roots, leaves, stems). The plant extracts and oils of oregano studied can be used to decontaminate compost variants and other substrates in which Escherichia coli is found to be present.

https://doi.org/10.29114/ajtuv.vol8.iss1.304
Vol. 8 Issue 1 (2024)
Published: 2024-06-28
ISSN 2603-316X (Online)

Acknowledgments

The research, the results of which are presented in this publication, were carried out under project NP 9/2023 "Investigation of the main indicators in the different phases of the production of quality compost from agricultural crop residues and monitoring of its impact on soil fertility" in the frames the inherent research activity of TU-Varna , financed targeted from the state budget.

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