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CONTENT

INTORDUCTION

In the past sixteen years the educational, research and practical activities of the Faculty of Agriculture – Stip, Goce Delcev University – Stip, contributed to the development of agriculture sector in the country and broader region.

The Faculty of Agriculture organized the 3rd International Meeting Agriscience & Practice (ASP 2023), giving an opportunity to the participants for presentation and discussion of original scientific and practical results in different fields of agriculture.

The 3rd International Meeting Agriscience & Practice (ASP 2023), heled on 19th and 20th of April 2023 at the Faculty of Agriculture - Stip, was organized with intention to bring together all agricultural stakeholders for sharing their knowledge, experience and obstacles. One of the main aims was to link research and field work in agricultural sector in the country and broader, giving it an international dimension. All oral presentations as well as poster presentations at ASP 2023 were organized in several scientific sessions:

- Agricultural economics,
- Plant biotechnology,
- Plant production,
- Plant protection,
- Quality control and food safety,
- Soil science and hydrology,
- Viticulture, enology and fruit production.

The main goal of the Meeting was linking and promoting scientific achievements and practical knowledge, presented in different thematic areas, which were achieved in the Republic of North Macedonia and wider in the region.

Journal of Agriculture and Plant Sciences Vol. 21, No. 1 mostly contains the presented papers from the 3rd International Meeting Agriscience & Practice (ASP 2023).

Editorial Board, **Editor in chief,**

June, 2023 **Prof. Liljana Koleva Gudeva, PhD**

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Original scientific paper

HETEROTHIC EFFECT OF SOME QUANTITATIVE TRAITS IN F_1 DIALLEL HYBRIDS OF VARIOUS TOBACCO TYPES

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Abstract

The mode of inheritance and heterotic effect were studied in ten F_1 crosses obtained by one-way diallel crossing between five parental genotypes: MV-1, P 76/86, Adiyaman, Basma-Djebel and P 66 9 7. The following quantitative traits were studied: the number of leaves per stalk, length of leaves from the middle belt of the stalk and yield of green leaf mass per stalk and per hectare. The trial was set up in the experimental field of Scientific Tobacco Institute-Prilep, using a randomized block design with four replications in the period 2018-2019. Traditional cultural practices were applied during the growing season of tobacco in the field.

The aim of this research was to study the mode of inheritance of the quantitative traits, to detect heterosis in the F_1 generation and to assess its economic viability.

Analysis of variance determined statistically significant differences in traits between parents and hybrids in the two-year investigation. The most common way of trait inheritance is partial-dominant, then intermediate. The negative heterotic effect on the number of leaves per stalk has P 76/86 x P 66 9 7. The hybrids MV-1 x Adiyaman, P 76/86 x Basma-Djebel, P 76/86 x P 66 9 7 and Basma-Djebel x P 66 9 7 have a positive heterotic effect on the length of the leaves. Oriental hybrids, where one of the parents is the variety P 66 9 7, have positive heterosis in the yield of green leaf mass per stalk. The investigation provides very useful guidance for future successive selection activities.

Key words: *Nicotiana tabacum L., F_1 crosses, inheritance, dominance, heterosis*

INTRODUCTION

The mode of inheriting traits is an important factor for determining the directions and duration in different selection programmes. Heterotic effect or F_1 vigour is a phenomenon that occurs in the first generation, where the offspring shows higher (or lower) values of particular trait from both parents. It is widely used in many crops where high-yielding hybrids are developed. In tobacco, F_1 hybrids with heterotic effect are rarely used due to their economic unviability. However, there are possibilities for their use, such as resistance to economically significant diseases.

The topic of this paper is focused on the mode of inheriting of the most important quantitative traits in F_1 offspring of hybrids obtained by diallel crossing between varieties of different tobacco types. In the last ten years, a number of authors – breeders have worked on this subject: Aleksoski (2010), in a two-year study of four parental genotypes and their six diallel F_1 hybrids, obtained a different way of inheriting of leaf length and a weak heterotic effect without economic justification. Gixhari & Sulovari (2010), conducted three-year research at two locations in a genetically diverse population of eight oriental tobaccos and their one-way diallel hybrids and obtained a dominant and partial-dominant mode of inheritance and heterosis in leaf size and yield. Aleksoski & Korubin – Aleksoska (2011), conducted three-year studies for green and dry mass yields in a one-way diallel on three oriental and one large-leaf variety and their six F_1 crosses and obtained positive and negative heterosis. Dimanov & Dyulgerski (2012), found heterosis with varying strength in number of leaves in ten F_1 crosses obtained from parent pairs of local and introduced Burley

tobacco varieties. Dyulgerski & Dimanov (2012), performed examinations of the dimensions of 7-8 leaves and 13-14 leaves in the P_1, P_2 and F_1 populations at ten crosses originating from local and introduced varieties of Burley tobacco and obtained a high heterotic effect of significant economic importance. In an experiment in Khan Gari, Mardan, Pakistan, with seven Virginia flue-cured genotypes and their 42 two-way diallel crosses, Imtiaz et al. (2014), found heterosis with high heterotic effect, with the possibility of using it on leaf number per stalk, leaf length and weight of green leaf mass per plant. Dyulgerski & Radoukova (2015), studied the mode of inheritance, the coefficient of inheritance and the expression of heterosis and transgression in seven Burley-type crosses and seven Virginia-type crosses of local and introduced origin in F_1 and F_2 offspring and found a dominant and partial - dominant inheritance in the length of the leaves, always in the direction of the parent with longer leaves, and the resulting heterosis had no economic importance. Ramachandra et al. (2015), obtained hybrids superior to the control variety in number and length of leaves, in genetic studies on the yield and quality of 62 genotypes (six lines of different types of tobacco, eight testers and their crosses), at the Agricultural Research Station, Nipaniat Belgaum, Karnataka - India. Shah et al. (2017), found highly significant differences in the number of leaves per stalk in a two-year study of ten Virginia flue-cured hybrids. The field experiment was set up in agro-ecological conditions at Khan Gari in Mardan, Pakistan.

The aim of these investigations was to study the mode of inheritance and to detect possible heterosis in the number of leaves per stalk, length of the leaves from the middle belt and yield of green leaf mass per stalk and per hectare. The F_1 generation obtained by diallel crosses of tobacco varieties from different types will give us an important guidance for future selection programs in tobacco breeding.

MATERIAL AND METHODS

In order to investigate the quantitative traits of tobacco, in 2016, five varieties of different tobacco types were selected with previous studies of the available assortment at the Scientific Tobacco Institute - Prilep: MV-1 is a large-leaf variety of the Virginia type, P 76/86 is an oriental variety of the Prilep type, Adiyaman is an oriental variety of the Kabukalak type, Basma-Djebel (Basma-Dzebel or Basma-Cebel) is an oriental variety of the type Basma, P 66 9 7 is an oriental variety of the Prilep type.

In 2017, ten one-way diallel crosses were made in field conditions, by applying the diallel method of crossing, using hand castration and pollination method: MV-1 x P 76/86, MV-1 x Adiyaman, MV-1 x Basma-Djebel, MV-1 x P 66 9 7, P 76/86 x Adiyaman, P 76/86 x Basma- Djebel, P 76/86 x P 66 9 7, Adiyaman x Basma-Djebel, Adiyaman x P 66 9 7 and Basma-Djebelx P 66 9 7.

In 2018, the trial with 15 genotypes (5 parents and 10 F_1 hybrids) was set up in the experimental field of STI-Prilep, using randomized block system in four replications. The same year, seeds for the second generation were collected and again diallel crosses between the listed parents for the first generation were made.

In 2019, a trial was set up using the same method, in which the same set of 15 genotypes was planted. The total area of the trial (working area-405m^2 + area of paths-477 m^2) was 882 m^2. 826 plants (770 plants + 56 plants as protection) were planted to fill the surface of one repetition. A total of 3304 plants (stalks) were planted in the experiment.

This paper places the analysis based on: number of leaves per stalk, length of leaves from the middle belt and yield of green mass per stalk and hectare. Measurements of leaf number and length were taken during flowering, in late July and August, when the population is in lush growth. Due to the uniformity of the homozygous parent genotypes and the heterozygous F_1 offspring, 20 stalks of each variant were measured in one replication, i.e., 80 plants in the four replications (1200 plants in total). The yield of green leaf mass was measured after each harvest, the weight of all harvests from each plot was added and then it was divided by the number of plants from which the tobacco was harvested, which gave us the weight of a green leaf per stalk. The yield of green leaf mass per hectare was obtained by multiplying the weight of the green leaf per stalk by the number of plants planted on the surface of one hectare.

Statistical data processing was performed using the measurements of the subject traits of each variant. The mode of inheritance of traits is determined on the basis of test-significance from

the mean value of the F_1 generation in relation to the average of both parents. Intermediate mode of inheritance (i) occurs when the mean value of a trait in the offspring is equal to the parent average. There is a partial-dominant mode (pd) when the mean value of the hybrid offspring approaches one of the parent varieties. Dominance in inheritance (d) - positive or negative, occurs when the mean value of the cross coincides with the mean value of one of the parents (+d - when the parent with a higher mean value dominates, -d - when the parent with a lower mean value dominates). Positive heterosis (+h) occurs in the hybrid with a significantly higher value than that of the parent with a higher mean value, while negative heterosis (-h) occurs in the hybrid with a significantly lower value than the one from the parent with a lower mean value.

Climatic and soil conditions in the area of investigations

A more realistic vision for the inheritance of quantitative traits is obtained by displaying data on climatic conditions during the tobacco vegetation in 2018 and 2019 (Table 1).

Table 1. Weather conditions in Prilep presented by monthly averages for 2018, 2019.

Year	Climatic parameters	May	June	July	August	September	$\bar{x}$
2018	Avg. temp. (°C)	19.80	22.40	24.40	23.90	20.10	22.12
	Min. temp. (°C)	11.00	12.90	15.80	15.30	12.00	13.40
	Max. temp (°C)	22.30	25.90	27.50	29.80	23.60	25.82
	Humidity (%)	81.00	80.00	78.00	75.00	80.00	78.80
	Precipitation /Rainfall (mm)	18.00	20.00	21.00	20.00	90.00	∑ = 169.00
2019	Avg. temp. (°C)	15.77	22.77	24.26	27.39	21.97	22.43
	Min. temp. (°C)	8.00	16.00	14.00	22.00	14.00	14.80
	Max. temp (°C)	20.00	28.00	29.00	32.00	27.00	27.20
	Humidity (%)	71.30	67.17	59.42	42.61	53.00	58.70
	Precipitation / Rainfall (mm)	124.10	139.90	91.80	9.50	39.50	∑ = 404.80

Source: https://en.climate-data.org/europe/macedonia/prilep/prilep-37313/

The results of the temperature and relative humidity are within the optimal limits for normal development of tobacco and obtaining quality tobacco raw material. In July and August, one irrigation was performed with a watering norm of 300 m^3/ha of water.

The soil with its mechanical composition and nutrient content is the substrate on which tobacco grows and develops. Our investigations were performed in the experimental field of Scientific Tobacco Institute - Prilep on deluvial soil type. This soil is characterized by low humus and total nitrogen content, moderately acidic to neutral reaction, low to extremely low supply with readily available phosphorus and medium to good potassium supply. Throughout its depth, the soil is carbonate-free. Taking into account the stratigraphy of the profile and the agrochemical traits of the soil for the performance of the profile, it was properly prepared. One autumn and spring plowing were carried out along with basic tillage. The basic fertilization was performed with the spring plowing, using 250 kg / ha NPK fertilizer 8:22:20.

RESULTS AND DISCUSSION

The number of leaves is a trait that is directly related to yield. Because of that, it is the most common subject of research in all selection programs for tobacco breeding.

In 2018 the most common way of inheriting of the number of leaves per stalk in the F_1 generation is the partial-dominant. Intermediate inheritance occurs only in Adiyaman x P 66 9 7. The hybrid P 76/86 x P 66 9 7 has heterosis with a negative heterotic effect, which means that the offspring in the F_1 generation has less leaves than both parents. In 2019 we found an identical scheme for this trait. This indicates the fact that these are stable homozygous parents whose offspring forms the first investigated generation. The number of leaves is a highly inherited trait on which environmental factors have limited influence.

Table 2 shows the mean values of the number of leaves per stalk in the parental genotypes and their diallel F_1 offspring, as well as the mode of inheritance of this trait in 2018 and 2019.

Table 2. Mode of inheritance of the number of leaves per stalk in diallel F_1 hybrids in 2018 and 2019.

Parents	Number of leaves per stalk				
	MV-1	P 76/86	Adiyaman	Basma-Djebel	P 66 9 7
2018					
MV-1	27.65	37.90 pd	28.09 pd	25.02 pd	32.75 pd
P 76/86		60.09	35.09 pd	28.60 pd	48.55 -h
Adiyaman			29.89	25.86 pd	44.62 i
Basma-Djebel				18.07	29.84 pd
P 66 9 7					54.81
2019					
MV-1	28.52	39.32 pd	28.88 pd	24.62 pd	33.93 pd
P 76/86		59.56	36.68 pd	27.54 pd	49.72 -h
Adiyaman			30.82	26.46 pd	46.08 i
Basma-Djebel				17.26	27.51 pd
P 66 9 7					56.44

In the inheritance of the leaves' length from the middle belt of the F_1 offspring in 2018, all the modalities are present.

There is intermediate inheritance in Adiyaman x P 66 9 7. There is a partial-dominant way of inheritance in MV-1 x Basma-Djebel, MV-1 x P 66 9 7, P 76/86 x Adiyaman and Adiyaman x Basma-Djebel. Positive dominance occurs in MV-1 x P 76/86. Positive heterosis in inheritance of this trait occurs in MV-1 x Adiyaman, P 76/86 x Basma-Djebel, P 76/86 x P 66 9 7 and Basma-Djebel x P 66 9 7, which means that the F_1 generation has longer leaves than the leaves of the both parents.

The same mode of inheriting in crosses was obtained in 2019, which means that the trait is highly inherited and it is a varietal characteristic.

Table 3 shows the mean values of the leaf length of the middle belt of the stalk in the parental genotypes and their diallel F_1 offspring, as well as the mode of trait inheritance in 2018 and 2019.

Table 3. Mode of inheritance of the length of the leaves from the middle belt of the stalk in diallel F1 hybrids in 2018 and 2019.

Parents	Length of the leaves from the middle belt of the stalk (cm)				
	MV-1	P 76/86	Adiyaman	Basma-Djebel	P 66 9 7
2018					
MV-1	50	48.51 $^{+d}$	55.22 $^{+h}$	45.12 ^{pd}	46.23 ^{pd}
P 76/86		23.62	31.55 ^{pd}	25.04 $^{+h}$	24.47 $^{+h}$
Adiyaman			35.75	30.53 ^{pd}	30.26 ^{i}
Basma-Djebel				20.57	24.39 $^{+h}$
P 66 9 7					23.01
2019					
MV-1	52.57	51.86^{+d}	56.57 $^{+h}$	43.78 ^{pd}	47.05 ^{pd}
P 76/86		23.44	35.22 ^{pd}	24.36 $^{+h}$	24.16 $^{+h}$
Adiyaman			37.29	32.05 ^{pd}	29.28 ^{i}
Basma-Djebel				20.74	23.73 $^{+h}$
P 66 9 7					22.49

Table 4. Mode of inheritance of green mass yield per stalk in diallel F_1 hybrids in 2018 and 2019.

Parents	Green mass yield per stalk (g)				
	MV-1	P 76/86	Adiyaman	Basma-Djebel	P 66 9 7
2018					
MV-1	970.94	716.14 ^{pd}	742.64 ^{pd}	567.41 ^{i}	667.67 ^{pd}
P 76/86		168.95	165.25 ^{pd}	163.45 $^{+d}$	177.58 $^{+h}$
Adiyaman			153.81	144.79 $^{+d}$	168.07 $^{+h}$
Basma-Djebel				63.59	148.85 $^{+h}$
P 66 9 7					137.12
2019					
MV-1	990.42	751.47 ^{pd}	757.34 ^{pd}	581.72 ^{l}	683.86 ^{pd}
P 76/86		172.45	174.54 $^{+d}$	177.28 $^{+d}$	182.49 $^{+h}$
Adiyaman			150.38	135.15 ^{pd}	176.80 $^{+h}$
Basma-Djebel				69.84	153.35 $^{+h}$
P 66 9 7					129.39

In 2018, the most common way of inheritance of the yield of green leaf mass per stalk in F_1 offspring is the partial-dominant in the direction of the parent with higher yield, followed by the positive-dominant. Intermediate mode is found only in MV-1 x Basma-Djebel. Crosses where one parent is P 66 9 7 have positive heterosis, which means that they are more productive than the parent with higher yield (with the exception of MV-1 x P 66 9 7 in which there is partial dominance).

In 2019, the vision for complete identity is changed by the cross P 76/86 x Adiyaman (with positive dominance) and Adiyaman x Basma-Djebel (with partial dominance). If we take into account that the yield is a variable quantity that is greatly influenced by environmental factors, then the obtained values are very reliable and reflect the professional setting of the experiment and timely activities for its cultivation and analysis.

Table 4 shows the mean values of the yield of green leaf mass per stalk in the parent genotypes and their diallel F_1 offspring, as well as the mode of inheritance of this trait in 2018 and 2019. In 2018, the mode of inheritance of the yield of green leaf mass per hectare in F_1 offspring is partially-dominant and positively dominant. Negative heterosis is present in crosses where one parent is MV-1 (with the exception of MV-1 x Basma-Djebel in which there is partial dominance in the direction of the weaker parent). The occurrence of positive heterosis has the Oriental crosses where one parent is P 66 9 7.

Table 5. Mode of inheritance of green mass yield per hectare in diallel F_1 hybrids in 2018 and 2019.

Parents	Green mass yield per hectare (t)				
	MV-1	P 76/86	Adiyaman	Basma-Djebel	P 66 9 7
2018					
MV-1	21.576	15.914 $^{-h}$	16.503 $^{-h}$	12.609 ^{pd}	14.837 $^{-h}$
P 76/86		25.030	24.482 ^{pd}	24.215 $^{+d}$	26.307 $^{+h}$
Adiyaman			22.786	21.450 $^{+d}$	24.899 $^{+h}$
Basma-Djebel				9.420	22.051 $^{+h}$
P 66 9 7					20.314
2019					
MV-1	22.009	16.699 $^{-h}$	16.830 $^{-h}$	12.927 ^{pd}	15.197 $^{-h}$
P 76/86		25.548	25.858 $^{+d}$	26.264 $^{+d}$	27.035 $^{+h}$
Adiyaman			22.278	20.022 ^{pd}	26.193 $^{+h}$
Basma-Djebel				10.347	22.718 +h
P 66 9 7					19.170

In 2019, there are changes in the way of inheritance of the yield of green leaf mass per hectare and the yield of green leaf mass per stalk (P 76/86 x Adiyaman - positive dominance and Adiyaman x Basma-Djebel - partial dominance).

The analysis of the heredity of the yield of green leaf mass per stalk and the yield of green leaf mass per hectare indicates drastic differences and therefore it is necessary to indicate the reasons. From the shown heredity in the crosses where the parent pairs are of oriental type, the reliability of the results is confirmed as a reflection of the professional approach to the overall work. Namely, all oriental hybrids showed the same inheritance pattern in yield per stalk and yield per hectare. But this is not the case with hybrids where one parent is the large-leaf variety MV-1.

Here, instead of partial dominance, negative heterosis occurs (with the exception of MV-1 x Basma-Djebel, where instead of intermediate, partial dominance occurs).

The reason for this outcome is in the different planting distance of the plants in the experiment. Oriental parents and oriental hybrids are planted with a row spacing of 45 cm and plant spacing of 15 cm, while the large-leaved MV-1 and its crosses are planted with a row spacing of 90 cm and plant spacing of 50 cm, which means that the calculation of yield per hectare is different, depending on the plant composition of the genotype.

Table 5 shows the mean values of the yield of green leaf mass per hectare in the parent genotypes and their diallel F_1 offspring, as well as the mode of inheritance of the trait in 2018 and 2019.

CONCLUDING REMARKS

Inheritance of the number of leaves per stalk in F_1 generation in 2018 and 2019 is partial- dominant (only Adiyaman x P 66 9 7 has intermediation). The hybrid P 76/86 x P 66 9 7 showed a negative heterotic effect.

All the modalities in the two years of investigations can be found in the length of the leaves from the middle belt of the stalk. Positive heterosis in this trait occurs in MV-1 x Adiyaman, P 76/86 x Basma-Djebel, P 76/86 x P 66 9 7 and Basma-Djebel x P 66 9 7.

Inheritance of the number of leaves per stalk and the length of the leaves from the middle belt of the stalk in F_1 hybrids shows identical values for both years of research, which indicates the fact that the parents in the diallel are stable and homozygous and the environmental factors have a limited impact on these traits.

The mode of inheritance of the yield of green mass per stalk is partially-dominant in the direction of the parent with higher yield and positively-dominant (only in MV-1 x Basma-Djebel there is an intermediate mode). Positive heterosis occurs in Oriental crosses where one parent is P 66 9 7.

The mode of inheritance of the yield of green leaf mass per hectare is partially-dominant and positively dominant. Negative heterosis is present in hybrids where one of the parents is MV-1 (with the exception of MV-1 x Basma-Djebel where there is partial dominance in the direction of the weaker parent). The occurrence of positive heterosis has the Oriental hybrids where one of the parents is P 66 9 7.

The picture of the heritability of the yield detects small differences in relation to the two years of research, which indicates the fact that it is a trait that is greatly influenced by the environmental factors, but also that there is a professional approach to the overall work.

From the analysis of the inheritance of the yield of green leaf mass per stalk and the yield of green leaf mass per hectare, it is concluded that there are no differences with the oriental hybrids, but there are differences with the hybrids where one of the parents is MV-1, and that is a consequence of the different planting distance of plants in the experiment.

The results of this paper are good original source material for further successive selection activity.

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ХЕТЕРОТИЧКИ ЕФЕКТ НА НЕКОИ КВАНТИТАТИВНИ КАРАКТЕРИСТИКИ КАЈ ДИЈАЛЕЛНИТЕ F1 ХИБРИДИ ОД РАЗЛИЧНИ ВИДОВИ ТУТУН

Јане Алексоски

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Резиме

Трудот опфаќа проучувања за начинот на наследување и хетеротичниот ефект кај десет F_1 крстоски добиени со еднонасочни дијалелни вкрстувања помеѓу пет родителски генотипови: MV-1, P 76/86, Adiyaman, Basma-Djebel и P 66 9 7. Анализирани се следниве квантитативни карактеристики: број на листови по страк, должина на листовите од средниот појас на стракот и принос на зелена лисна маса по страк и по хектар. Опитот беше поставен на експериментално поле при Научен институт за тутун – Прилеп, по рандомизиран блок-систем со четири повторувања во периодот 2018-2019 година. За време на вегетациониот период во полето беа применети традиционални културни практики.

Целта на овие истражувања беше да се проучи начинот на наследување на квантитативните особини, да се открие хетерозис кај F_1 генерацијата и да се процени неговата економска исплатливост.

Со анализата на варијансата се утврдија статистички значајни разлики во особините помеѓу родителите и нивните хибриди во двегодишното истражување. Најчестиот начин на наследување на особините е парцијално доминантниот, потоа интермедијарниот. Негативен хетеротичен ефект за бројот на листови по страк има кај P 76/86 x P 66 9 7. Хибридите MV-1 x Adiyaman, P 76/86 x Basma-Djebel, P 76/86 x P 66 9 7 и Basma – Djebel x P 66 9 7 имаат позитивен хетеротичен ефект за должината на листовите. Ориенталните хибриди, каде еден од родителите е сортата P 66 9 7, имаат позитивен хетерозис за приносот на зелената лисна маса по страк. Истражувањата нудат многу корисни насоки за идните сукцесивни селекциони активности.

Клучни зборови: *Nicotiana tabacum L., F_1 крстоски, наследност, доминантност, хетерозис.*

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Professional paper

REMOTE MANAGEMENT IN SYSTEMS FOR IRRIGATION OF SOLAR ENERGY BUNARIES IN THE VITICULTURE

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Abstract

The cultivation of the vine by the people has been dated for centuries or thousands of years, yet the progress of knowledge in the cultivation of the vine has grown enormously over the last fifty years.

Mass production with high-grade grape varieties, wine and table, thick planting, the use of fertilizers, the application of irrigation, and the acquisition of high yields, contributed to the intensification of conditions for disease development, harmful insects and weed development. For that purpose, mobile solar aggregates are very suitable today for agriculture (viticulture, fruit growing, crop production, gardening, animal husbandry, etc.), especially in those locations where there is no possibility of availability of power supply, but also in cases where we have organic food production, because they satisfy even the most stringent environmental standards.

More recently today, the world is working on the intensive development and application of mobile solar generators that include solar tracking systems and they find application in agriculture.

Key words: *Grape production, Remote management, Irrigation systems*

INTRODUCTION

In this paper we show how the solar energy is used by the farmers in the Republic of Macedonia for irrigation and management of the distance using the GSM module.

Over the last three decades, a number of advanced process controls have been installed in production plants. The need for upgrading these systems over an Internet network provides the ability to design and analyze remote-controlled systems with immense stability and reliability. In the last two decades, web based systems in real time play an important role in the production of a quality product in our case raw grapes. This paper is a solution that allows us to obtain quality and remote-controlled equipment for low-cost equipment, which is specialized for activating and disconnection of pumps powered by solar energy and for irrigation of vineyards. Use existing available technologies that are free to use and in combination with the new solutions from this paper, a managed system is run that will work seamlessly with mechanisms through which all errors will be annulled.

Several systems are offered that offer cheap solutions that will meet the requirements of the growers in the Republic of North Macedonia.

The solutions are characterized by a short reaction time, at any time and under any conditions, to respond in a reverse manner without the need for additional intervention by users and system supervisors. The application of communication technology services to GPRS, EDGE and UMTS with the regional management and management of water systems ensures a quality system operated and monitored remotely.

Measuring the current value of water supply facilities, such as water, water pressure in the pipeline, top, bottom and current tank levels in reservoirs, pump status and exclusion devices have become a necessity today.

The transfer of these data from the peripheral stations to the central unit, linking them to a single system has been made through the UKV radio link.

The infrastructure security costs needed to handle the UKV radio connection and the inconsistency of data transmission have caused UKV's radio link to become a thing of the past.

The new way of connecting is through GPRS connectivity that does not need a separate infrastructure because it uses existing communication, and the transmission itself is safe, fast and accurate. GPRS connectivity enabled us to react quickly in case of any disruption, while at the same time significantly reducing the costs associated with the round and control of water supply facilities.

The transmission of measured values from the peripheral stations to the distribution center, as well as the collection of information as indicated by using GPRS communication technology is something new in our wine-growing region. The status display and data processing is provided via mobile phones and the Internet, in order to ensure mobility with the control center and individual peripheral cells from anywhere regardless of distance and without the need for additional infrastructure investment.

At the same time, the use of GPRS technology significantly reduces the cost of communication.

REMOTE MONITORING AND WATER SUPPLY CONTROL USING GPRS TECHNOLOGY

Modern water distribution systems require continuous monitoring of water flow, water pressure in the pipeline, water level in the reservoirs, pump status and closing bodies, as well as other necessary parameters through their transfer to the remote control center. In addition to remote monitoring, the system must also provide a secure remote control with water pumps, regulating the locking of valves and the like.

At the same time, the system must also provide feedback, ie confirmation of the executed order.

In addition to the above, the system must also provide reliable reception and transmission of signal and information for future measurement control equipment.

The present mode of communication and connection of peripheral objects with the control center is achieved through the UKV radio link, which as such is subject to:

- Uncertainty and sluggishness in data transfer
- Meteorological impacts
- The congestion of communication channels
- Dependent on the configuration of the terrain
- Reflection of the signal, etc…

In addition to the above-mentioned shortcomings in the operation, this way of communication already at the very beginning required a large investment in the radio-communications infrastructure UKV needed for radio link operation.

It was necessary to prepare project documentation for a radio connection and to obtain the necessary annual licenses with the competent institutions.

The use of a radio link was paid as a fee for radio frequency utilization.

Recognizing the above-mentioned shortcomings of the UKV radio connection, the data transmission was changed using a GSM communication link.

This was a good time, but communication using the GSM connection showed some drawbacks:

- The high cost of data transfer, because the calculation is done according to the time of the opening
- Relatively slow transfer of data about 9.6 KB / sec
- The connection is made by reference to a given number
- During a connection of about 45 seconds
- Relatively long data transmission time
- Only one link is possible at the same time.

To avoid the disadvantages of GSM and FM radio and to access remote data transfer from one another, as well as to connect to peripheral facilities for the supervision centre in a single system requires the implementation of GPRS communication technoogy.

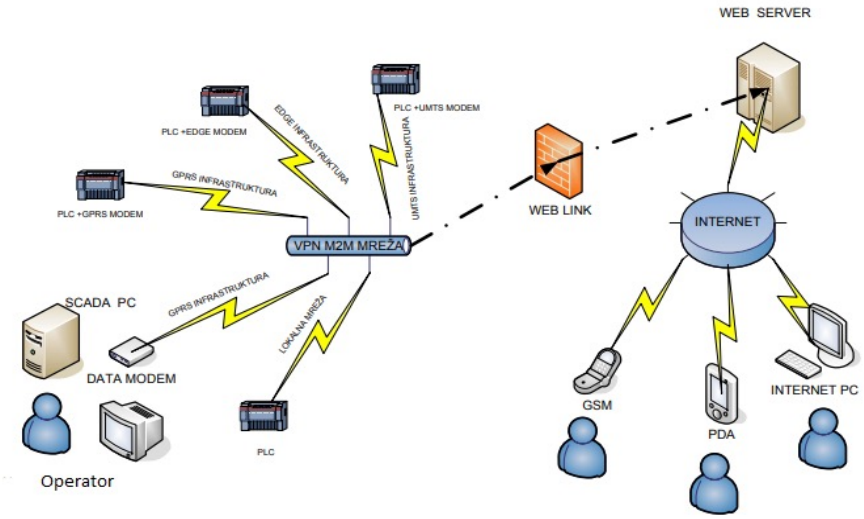

Figure 1. GPRS technology

BASIC POINTS AND THEIR CHARACTERISTICS OF GPRS

General packet radio service or shorter GPRS is defined as an additional service on the GSM network which, when added to the packet transmission protocol provides shorter connectivity time and faster and safer data transfer.

GPRS supports transmission speeds of 20 to 30 kbps (theoretical maximum is 171.2 kbps) and provides a permanent connection that does not charge the connection time but the amount of data transmitted.

In today's GSM 2.5G network, GPRS is the most important step towards next generation 3G networks, for which GPRS is the basis for communication.

Telemetry is defined as the transmission of the measured values of the observed physical quantities in the central supervisory point, where these values can be processed and based on them, information can be obtained which can be used for the remote-control processes.

GPRS TELEPHONE SYSTEM

GPRS telemetry system is the name of the new generation telemetry system, and includes the use of all public infrastructure networks and the protocol for network communication.

By using the GPRS connection for data transmission to the supervisory control system (shorter NUS), in addition to the aforementioned advantages of GPRS, they realize that there are other options that the said system offers for its communication concept to the users.

By using GPRS services in the "leased" infrastructure, it also provides the user with notifications via SMS, fax and e-mail.

The way of functioning sense is described in a system that collects all measurements of water supply facilities and a GPRS connection, so the collected data, at regular intervals, sends control to a computer. All communication, respectively. Control, communication and management of water supply facilities takes place in a closed VPN network. In order to use the VPN service, this request must be for the authorized person in the company›s mobile network.

Upon request and approval by the mobile operator, each member receives a VPN card with your PIN, which he defines access levels and privileges. At the request of an authorized person, at any time, change the level of users or revoke the card. So only a member of the VPN network can be monitored at the factory and allow the user to manage and with the installed equipment, a water supply system, other than what must be a member of the VPN network must know the password and password for the management level of installation suitable water tanks or pumping stations. At the time of the change of each reference value, all authorized persons in the form of SMS messages receive information about the change in the status or value of the user. Control of the water supply system is possible, except within the VPN network, as well as on the Internet.

In fact, if you want, the user is enabled on the web server, periodically, sending data on the state of the measured values of the water supply system. Anyone who knows the correct password and password can have an insight into the status of the water supply system being watched over the Internet from anywhere in the world. On the web server, in addition to the status display, statistical processing of the measured values was made. We must emphasize that the management of the water supply system can be done within the VPN network, while over the Internet can be monitored only on the system, because there is no possibility of entering the Internet in the VPN network.

FUNCTIONAL LOOK AND SECURITY WITHIN THE COMMUNICATION WITH USING GPRS TECHNOLOGY

GPRS technology was adopted and approved by the European Institute of Technology. Telecommunication standards ETSI and consists of a set of standards to be noticed by equipment manufacturers, network elements and mobile terminals, as well as the mobile telecommunications operators themselves. Several standards are embedded in the standard security itself to protect the "privacy" of data and of the senders, including the most important:

- Data encryption is done on GPRS / UMTS / EDGE connection between your mobile phone terminal and operator system
- allows the authorization of mobile stations by the Vip or T-mobile operator at any time, the system recognizes the sender and checks the identity through appropriate tests.

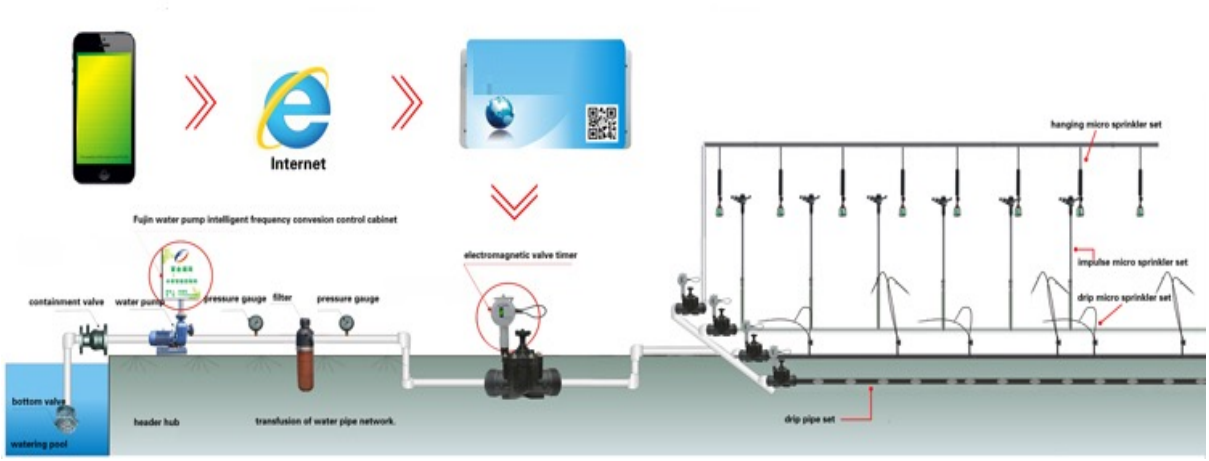

Figure 2. Indication of irrigation by means of pumps operated via a mobile phone

REMOTE MANAGER

All equipment with electronic output (analogue or digital) can be controlled from a remote monitor and those that have the possibility of electronic input can be managed.

Parameters that can be read on a PC or PDA, for example, or a GSM / GPRS module (with an active GPRS service and a web out) for example:

- Water flow (electricity and total)
- Water level in water - current, minimum and maximum
- Water pressure in pipelines
- Water turbidity
- The residual concentration of chlorine in water
- Concentration of chlorine in the air
- The pump status
- Valve status
- Status of the hydro-blocks
- The status of other equipment such as compressors, hydraulic shock compensators, etc.
- Notification of entry to a remote object
- A power failure report
- Alarm

SMS messages:

- ALARM (location) - power failure
- ALARM (location) - the level of water in the water is too low - the danger of operation of

the pump is dry
- ALARM (location) - The water level in the water is too high - the risk of spillage
- ALARM (location) - the door of the building is open
- ALARM (location) - network service is established
- ALARM (location) - the pressure of the water in the pipeline is too low
- ALARM (location) - flow of water above the set value
- ALARM (location) - concentration of residual chlorine in large / small water
- ALARM (location) - the appearance of chlorine in the air
- ALARM (location) - the valve is not fully closed / open

Management:
- Change in the level of water in which the pumps are switched on / off
- Change in the pressure of the water in the pipeline in which the pumps are switched on / off
- Selecting the pumping order
- Change in the concentration of the rest of the chlorine
- Setting the operation of the plant - manually / automatically
- Reset the default values

Figure 3. Solar collector through which the irrigation pump is powered

ADVANTAGES OF THE WORK OF THE SYSTEM FOR SUPERVISION AND MANAGEMENT

The described system of remote monitoring and management of water supply systems allows the user to have a number of advantages and benefits, among which the most important in relation to FM radio connection are:
- Continuity of connection independent of external influences, field configuration, meteorological conditions.
- Significantly lower installation costs because existing HT-mobile or VIP networks are used by an operator
- Significantly lower maintenance costs
- Low power consumption related to GSM radio connection:
- Great security in communication
- High speed data transfer - 115 kb / sec
- The price of the transfer is significantly lower, because the calculation is done according to the amount of the transferred data
- The connection is made by calling a static IP address
- The current connection is established as a permanent connection
- The duration of the transmission is short
- The number of concurrent connections is unlimited
- Fast and reliable insight into the state of the system over the Internet
- Manage your computer via a GPRS connection
- PDA control via GPRS connection
- Management via GSM via GPRS connection
- Security against attempts for unauthorized control of the system using multi-level user protection

- Continuous monitoring of 24 hours and recording of all parameters of the event followed in the process of measuring and controlling the equipment

However, the main advantage of the GPRS telescope is the mobility and security of the surveillance and management of water supply systems. Supervision and management are designed to provide a breakdown or if the requested value is reached through today's known SMS messaging channel, the event will be reported to the responsible person. For example, a power failure in a plumbing facility will consequently be notified to electricians, managers and technical director.

In the example above, in the event of a power failure, the system remains in the control function for 24 hours from the moment of power failure, since it is powered by its own source, which is an integral part of the device. System overview of the system is available at anytime and anywhere by connecting to the Internet server, allowing the current status of all elements to be managed, as well as a review of the processed statistics of all analogue and digital sizes such as pressure on the gas pipeline, the water level in the water tank and the like.

The GPRS telemetry system connects to the web server and periodically, at predefined times when the time interval is switched on, sends data on the measured physical quantities and status of the measurement and control equipment. The data is stored and processed on the server side. The authorized user can see the status of all water supply system facilities, while at the same time providing a quick insight into the status of all significant parameters are monitored and analysed. Viewing all the parameters that are followed during the time when some changes are made are made possible by a subsequent analysis of all events, and increased security of predicting possible future events. In the graphic display it is possible to select a view for a certain period, or a view for a particular day, where there is a visible change in a specific time unit. The ultimate advantage of this monitoring and management system is its mobility and a variety of management interfaces. One of them is SCUBA Duplika which represents a novelty in SCADA for managing water supply systems. The advantage is, except for a friendly graphical user interface, and Jscada itself, which is written in Java.

Java scada is compatible with all existing operating systems and is thus flexible in terms of installing existing or new computers. In cases where our computers are not available, remote management may be via a mobile device that supports GPRS data transmission and has an integrated web browser, connecting to a telemetry server for GPRS. It is important to note that regardless of the way the information is viewed or how the stations are operated, all measured and managed variables are programmed at any time flexible to users.

DESCRIPTION OF THE SYSTEM AND THEORY

The system features are simplicity, practicality and relatively low cost.

It is basically based on the ATMEL ATmega328P microcontroller, which is tested in practice and features stability, quality and low cost, which allows application in irrigation systems. The system monitors the humidity of the soil and depending on how much the soil is damp, returns to the server side the humidity level. Depending on this, the operator can activate the pump or the pump to be put into automatic mode itself to activate if there is water in the tank. If there is no water, the system will generate a signal and the operator will know that there are not enough water in the wells to fill the tank. The system has an LCD display to display the status locally. The real time is also displayed on the display.

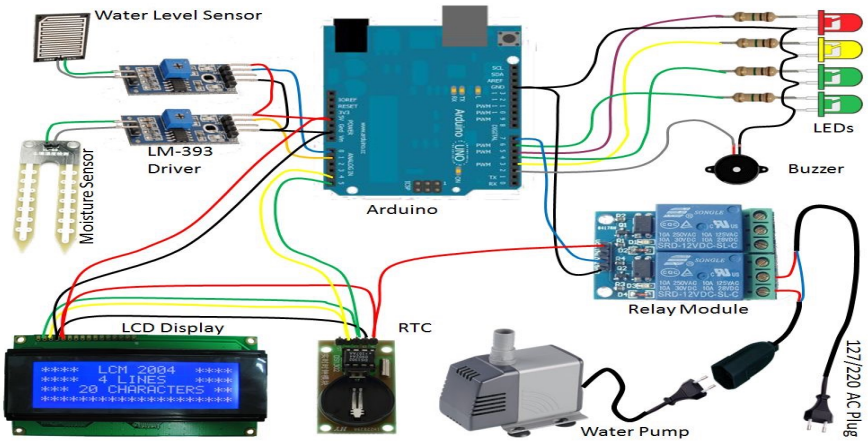

Figure 4. Connection diagram for humidity sensors, controller, display, diode and pump

This report serves as an overview of the use of solar energy by farmers and farmers in the United States that identifies trends and future potential.

Agriculture increasingly includes quality remote applications through which all time parameters are monitored. These applications are low-cost and efficient in their work, but in the last decade, agriculture has noted the number of systems connected to the network and the average size of solar systems is increasing. Some solar thermal installations are also used in agriculture, but are currently overshadowed by solar electric power. Although solar energy can reduce energy costs volatility and greenhouse gases, its high capital costs and lower average cost of competitive fuel remain obstacles to growth. For this reason, the development of solar energy is aimed at politics. The report examines the regulations and incentives available to farmers and farmers, and recently increased installations and examined major financial impacts. The development of solar energy in agriculture varies considerably by the state, incentives and energy prices.

In this project, we'll see how we can manage a variety of devices using a mobile phone under the Android platform. The project is based only on switching on / off the device, i.e., when switching on / off the relay with any device can be powered, in our case a water pump. The project uses the Arduino Pro Mini development system, as well as the Bluegiga WT11 Bluetooth module.

The device consists of two parts:

Hardware, receiving commands and turning on / off the output relay and Software running under the Android platform and sending hardware commands through which the current state of the device is displayed.

These two elements communicate via Bluetooth connection. The block diagram of a part of the device on the device is shown in the picture.

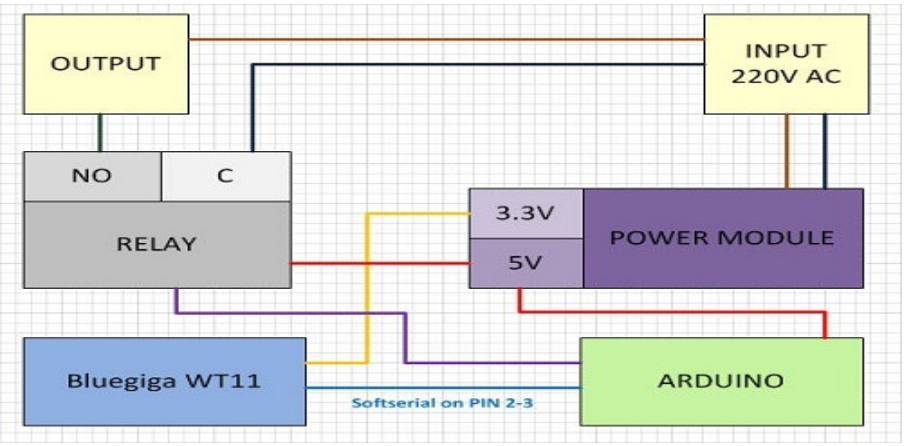

Figure 5. Bluetooth module connected to the arduino programmable logic controller

The basic element of the device is the Arduino IDE Pro Mini development system. It manages the output relay (which is powered by a 5V DC voltage) based on the command it receives from the Bluegig WT11 Bluetooth module.

To operate the device, a power module is required, which supplies all components of the system. The power supply module should provide 3.3V and 5V DC.

An automatic irrigation system for farmers or any user who needs to fill the reservoirs, as well as to use the water supply.

The system consists of a pump motor, together with an LCD and GSM modem controlled by a microcontroller.

The system allows users to remotely switch on / off the engine. The system consists of an automatic water measurement system that automatically switches off the pump motor as soon as it senses that the water supply is stopped and the engine is dry.

Our system uses a GSM modem to send and receive user commands and then manages the pump engine based on user SMS. The system also consists of infrared sensors to detect the flow of water through the tube until the flow of water is detected through the pump's pump tube. As soon as the sensor detects that the water supply is stopped, the microcontroller is notified of the dry start state. The system now informs the user that it has turned off the engine when it noticed a dry condition.

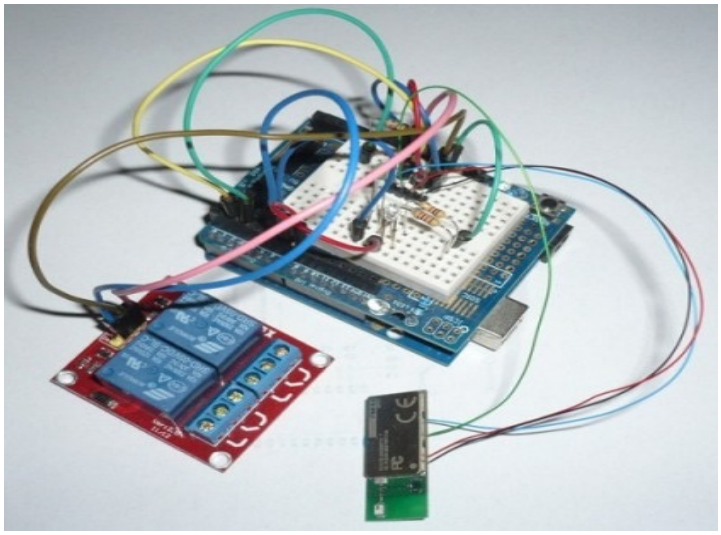

Figure 6. Communication system with mobile phone via Bluetooth module

Arduino and Bluetooth module are connected to serial communication. A part of the microcontroller software, which is in charge of their communication, was developed with the help of Software Serial Library.

Figure 7. Complete system placed in the box

The electrical pattern of the device, as well as the PCB designed in the Eagle software, can be found at the end of the text. In the picture you can see what the prototype of the device looks like and the picture shows the finished device.

The RX pin of GSM is connected to the TX pin from the arduin while the TX pin from GSM is connected to the RX pin from the arduin. The line view is shown below.

ARDUINO
TX ---------- RX GSM modem
RX ---------- TX

The zeros of the connection links are connected at the same point.

The whole system works with SMS messages. Below is the program code that is with special comments explained each line separately.

PROGRAM CODE:

```
int LED = 8; // an ice-connected diode 8
int motor = 9; the water pump is connected to pin 9
int temp = 0; // auxiliary variable
int i = 0; // auxiliary variable
char str [15]; // initialization of a string with a length of 15
void setup () // start of the main initialization
{
Serial.begin (9600); // serial communication at a speed of 9600 characters per second
pinMode (motor, OUTPUT); // initialization of the engine outlet from the water pump
pinMode (LED, OUTPUT);
digitalWrite (motor, LOW);
digitalWrite (LED, LOW);
delay (20000); // time delay of 2 seconds
delay (20000); // time delay of 2 seconds
delay (20000); // time delay of 2 seconds
Serial.println ("AT + CNMI = 2,2,0,0,0"); // serial data transfer for SMS commands
delay (1000);
Serial.println ("AT + CMGF = 1"); // serial data transfer for SMS commands
delay (500);
Serial.println ("AT + CMGS = \" + 38971826952 \ "\ r"); // serial data transfer for SMS commands
delay (1000);
Serial.println ("System is ready to receive commands."); // Text sent via SMS containing a message that the system is ready to receive commands
delay (100); // time delay of 0.1 second
Serial.println ((char) 26); // ASCII code for CTRL + Z
delay (1000);
}
void loop () // main loop
{
if (temp == 1)
{
check (); // activate the check function
temp = 0;
i = 0;
delay (1000);
}}
void serialEvent () // if there is a serial transmission then check the sign of the slash is activated
{
while (Serial.available ())
{
```

```
if (Serial.find ("/"))
{
delay (1000);
while (Serial.available ())
{
char inChar = Serial.read ();
str [i ++] = inChar;
if (inChar == '/')
{
temp = 1;
return;
}}}}}
void check ()
{
if (! (strncmp (str, "motor on", 8))) // check if the engine is activated
{
digitalWrite (motor, HIGH);
digitalWrite (LED, HIGH);
delay (1000);
Serial.println ("AT + CMGS = \" + 38971826952 \ "\ r"); // Send a message to a mobile phone
delay (1000);
Serial.println ("Motor Activated"); // SMS text you want to send
delay (100);
Serial.println ((char) 26); // ASCII code for CTRL + Z
delay (1000); // time delays
}
else if (! (strncmp (str, "motor off", 9)))
{
digitalWrite (motor, LOW); // turn off the engine
digitalWrite (LED, LOW); // turn off the light diode
delay (1000); // time delays
Serial.println ("AT + CMGS = \" + 38971826952 \ "\ r");
delay (1000); // time delays
Serial.println ("Motor deactivated"); // SMS Message that the engine has been deactivated
delay (100);
Serial.println ((char) 26); // ASCII code for CTRL + Z
delay (1000);
}
else if (! (strncmp (str, "test", 4)))
{
Serial.println ("AT + CMGS = \" + 38971826952 \ "\ r");
delay (1000);
Serial.println ("The System is Working Fine.");
// SMS message that sends the mobile phone to our phone
delay (100);
Serial.println ((char) 26); // ASCII code for CTRL + Z
delay (1000);
}
}
// ---------- end of the program ---------- //
```

The GSM / GPRS shield works primarily with Arduino sending a communication sketch through the terminal, then connecting to the GSM / GPRS shield.

It's important to have a shield especially the power that connects to the Arduino microcontroller, because without an extra external powerful shield it cannot be connected to the cellular network. You need to connect to the network to protect your computer and start the terminal after you press the "PWR" button GSM / GPRS display. When connecting to a network, the blue guidance with the name "NET" starts flashing and the shield sends a response to the terminal.

The terminal must be configured as Arduino speed 9600. After that the shield is ready for use. Shield is used with the previous described commands, allowing all basic functions like a normal mobile phone. When sending SMS messages, you must enter the number of the SIM operator whose SIM card is used, because otherwise the messages will be late or not at all. The module can also be used with the Arduino scheme, so it is necessary to define in the scheme all orders are fine and send them through a sketch of a shield instead of a terminal.

The advantage of using a sketch over the terminal is what makes it easy to use the screen, we may be able to set an automatic action or reduce the requirements for entering a command terminal. The screen connects to the planned location on the screen and is defined in the sketch, and you must download a command library for that screen on the Internet. The screen should be pushed into the screen or wire so it can be connected and comes in white and blue.

Initially, the device should have a keyboard that was supposed to enable management, but this was not achieved. No device and no keyboard work great and performs all the functions that are made they can request a mobile device.

It can receive and receive calls, send and receive SMS messages, and display information on the screen. The problem that arises when making the device is a failure of the rectifier that comes with the GSM / GPRS shield. The device's adapter works great despite sending SMS messages.

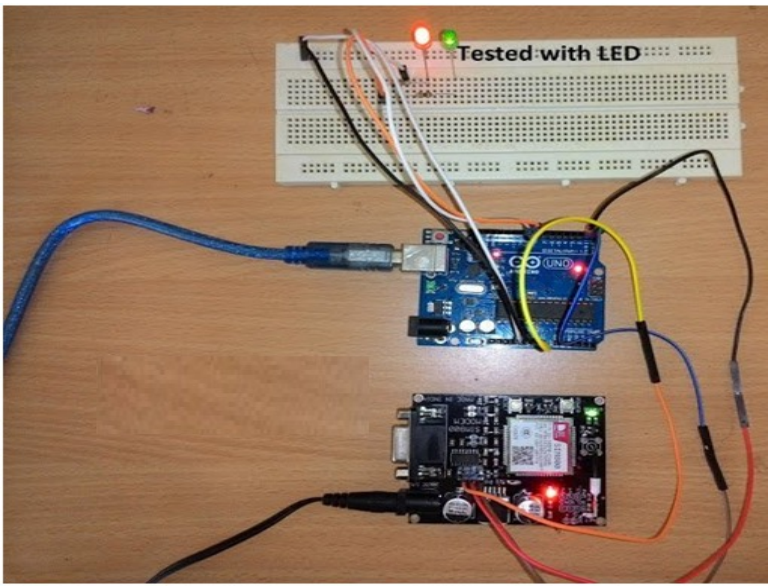

Figure 8. Test version of GSM module in combination with Arduino controller

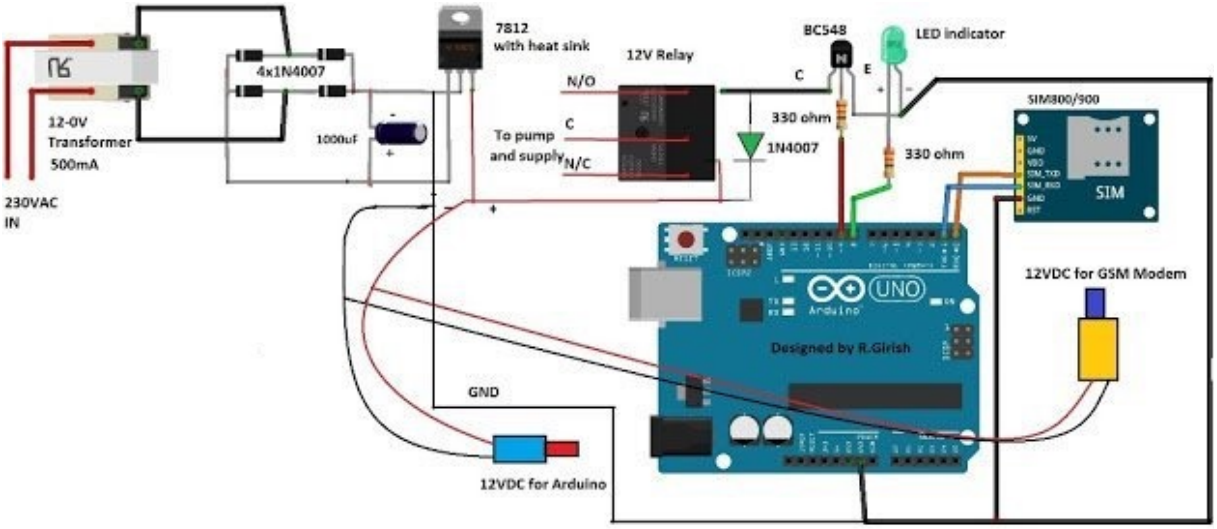

Figure 9. Test version of GSM module in combination with Arduino controller and relay for pump

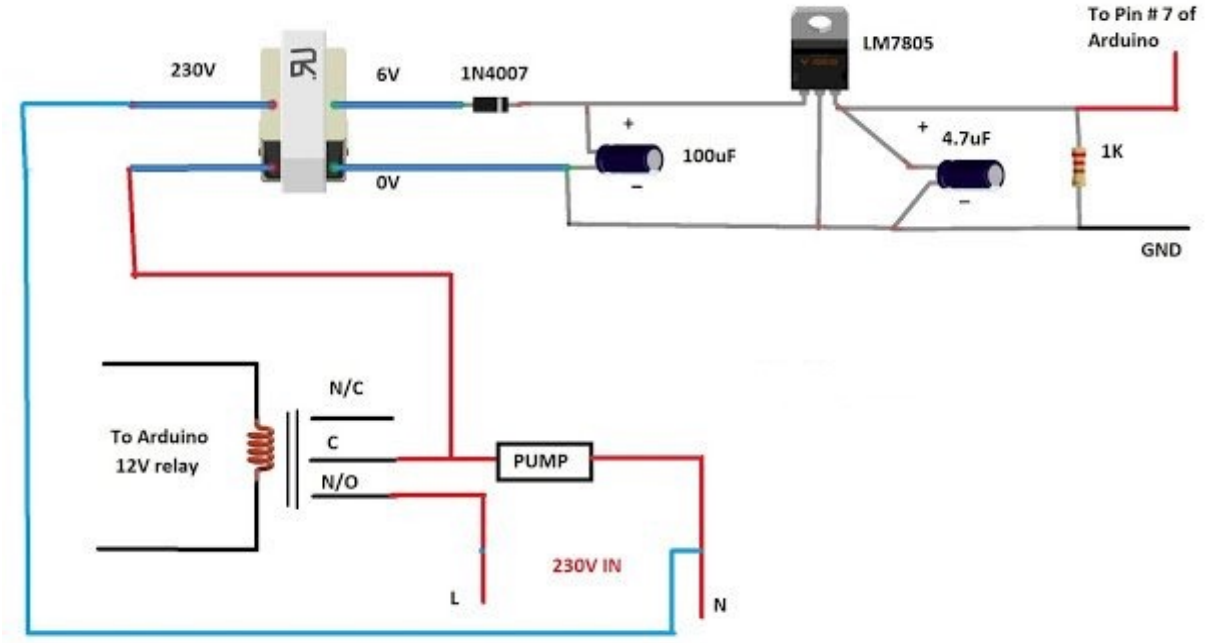

Figure 10. Solution for the return connection of the pump

LIST OF PARTS

1) Transformer 12-0V
2) Diode IN4007 x5
3) LM7812 x1 stabilizer
4) Relay 12V x1
5) BC548 Transistor x1
6) Electrolytic capacitor 1000uF x1
7) GSM Module: SIM 800 or SIM 900 model
8) 330 Ohm Resistor x2
9) LEDs Red / Green x1
10) Arduino Uno or Arduino nano or Arduino Mega

Figure 11. Functional system

CONCLUSION

Remote monitoring and management systems based on GPRS, UMTS and EDGE technologies have largely shifted to the boundaries of the meaning of the concept of the remote control with highspeed transmission of large amounts of data, the system also features stability connectivity and the use of existing infrastructure resources. It is very characteristic that reduces financial start-up, as well as the cost of maintaining the system, as well as for use GSM networks are minimal. In addition to all above, the security of the system is an excellent data transfer as well as management. Multiple log encryption and encryption are used, so access is allowed exclusively for authorized users. On the end, we have to say that the solution described is new in our market and it is completely the result of the work of local experts. Advantages of such a system were observed by many utility companies, and this was implemented in several water supply systems in the Republic of North Macedonia.

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ДАЛЕЧИНСКО УПРАВУВАЊЕ СО СИСТЕМИ ЗА НАВОДНУВАЊЕ НА БУНАРИ ЗА СОЛАРНА ЕНЕРГИЈА ВО ЛОЗАРСТВОТО

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Резиме

Одгледувањето на виновата лоза од страна на човекот датира со векови или илјадници години, но, сепак, прогресот на знаењето во одгледувањето на виновата лоза порасна енормно во последните педесетина години.

Масовното производство со високородни сорти од грозје,ински и трпезни, густото садење, употребата на ѓубривата, примената на наводнувањето, добивањето на високи приноси, придонесоа за интензивирање на условите за развој на болестите, штетните инсекти и развојот на плевелите.

За таа цел мобилните соларни агрегати денес се многу погодни за примена во земјоделството (лозарството, овоштарството, полјоделството, градинарството, сточарството и др.), поготово на оние локации на коишто не е можна достапност од мрежно напојување, но и во случаи каде што имаме органско производство на храна бидејќи ги задоволуваат и најстрогите еколошки стандарди.

Во поново време денес во светот се работи на интензивен развој и примена на мобилни соларни генератори коишто во себе содржат системи за следење на сонцето и истите наоѓаат примена во земјоделството.

Клучни зборови: *лозарство, далечинско управување, системи за наводнување.*

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Original scientific paper

THE INFLUENCE OF DIFFERENT CLIMATIC TYPES ON THE NUMBER OF *Bacillus* spp. ISOLATED FROM SOIL IN NORTH MACEDONIA

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Abstract

Soil microorganisms play an important role in the biogeochemical processes of various elements vital to plant growth and animal life. Understanding and predicting the effects of climate change on soil microorganisms and their role in the ecosystem is a major challenge and provides an opportunity to focus research efforts on one of the most pressing problems facing our planet. *Bacillus* is a widely distributed genus with 347 species and 7 subspecies known to date. Members of this genus are capable of forming spores that are resistant to extreme heat, bactericidal agents and chemical disinfectants. Many *Bacillus* species are used in medicine and agriculture to produce antibiotics and also serve as probiotics in foods. Climate, as an abiotic factor, influences soil microorganisms by controlling the rate of soil formation and the chemical composition of the soil. Most soil microbiology studies focus on the diversity and abundance of soil microorganisms and on documenting the effects of environmental changes on these microorganisms. This new research trend can be applied to *Bacillus* spp. from soils in North Macedonia in the three climate types represented, mainly due to the climatic differences between them. This research focuses on the determination of soil geochemical parameters and microbiological analyses. A total of 36 strains of *Bacillus* spp. were isolated, 6 of which showed antimicrobial activity against certain test microorganisms. According to the results, it was also found that the diversity of *Bacillus* species changes depending on the soil microenvironment under the influence of different climatic conditions.

Key words: *soil bacteria, climate change, agriculture, antibiotics, probiotics*

INTRODUCTION

Climate change has major direct and indirect impacts on the composition and functions of terrestrial microbial communities. There has been a list of climate change impacts on terrestrial microbial communities. Among these are mortality and disturbance, direct and indirect effects on metabolic activity, biomass reduction (stimulation), diversity and composition leading to extinction/displacement, with negative or positive effects on physiology and greenhouse gas emissions (Tyagi et al., 2014). Microbial community structures tend to change with temperature increases, and respiration, fermentation, and methanogenesis processes are accelerated (Weiman, 2015). The risk of damage, disease, and death from heat waves, fires, severe storms, floods, natural disasters, extreme heat, poor air quality, drought, and the spread and development of disease are all part of the impact of climate change on biotic and abiotic components (Castro et al., 2010). Climate change is affected by bacteria, fungi, algae, and archaea (Fierer & Schimel, 2003). They contribute to global warming by increasing the rate of decomposition of organic matter, thereby increasing the amount of CO_2 in the atmosphere (Bardgett et al. 2008).

Microbial biodegradation of carbon in soil provides positive feedback for increasing the global temperatures. Microbial biomass and enzymes are efficient tools to enhance warming by breaking down carbon-containing organic matter and releasing hazardous substances into the environment while preventing climate change (Allison et al., 2010). The impact of temperature on enzyme activity and microbial physiological properties is direct (Friedlingstein et al., 2006). The rate at which soil microorganisms use carbon directly influences the soil response to climate change. When microorganisms are exposed to new extreme environmental conditions, their community composition, occurrence, and ecosystem function change, i.e., environmental changes or global warming/ climate disturbances affect microbial ecology, ecosystem structure, and function (Steinweg et al., 2008). In addition, significant changes in their functional genes and properties occur over time. Every biogeochemical cycle is subjected to these types of impacts/influences (Bradford et al., 2008). Generalizing the effects of climate change on soil microbial populations in different soil ecosystems is challenging because soils vary widely in terms of their biotic and abiotic properties. There are variations in biogeochemistry that control the types of microorganisms found in a given soil type, including pH (Fierer & Jackson, 2006) and salinity (Thompson et al., 2017). In addition, soil structure and moisture content influence the establishment of microbial niches (Schimel & Schaeffer, 2012) which in turn has a cascading effect on carbon and nutrient transformation. In order to understand better how climate change affects community species relations and metabolism (Cordero & Datta, 2016) the distribution and interconnectivity of soil microbial communities must be first examined. Considerable part of modern research in the field of soil microbial ecology concentrates on categorization of soil bacterial diversity and documentation of how soil bacterial communities change in response to certain environmental changes or disturbances (Fierer & Jackson, 2006; Thompson et al., 2017) and other research of similar nature could focus on the distribution of *Bacillus* spp. population in the soil of the Republic of North Macedonia, to which climatic variability is applied.

In this study, a strategy was used to determine the number of *Bacillus* spp. in different soil types from three different climatic regions in the Republic of North Macedonia: continental, submediterranean and alpine, in order to have a better understanding of the important species of *Bacillus* spp. in the soil in different climatic zones, and also about how the number of species of *Bacillus* spp. differs in various geographic and climatic areas.

MATERIAL AND METHODS

Sample collection

Samples were collected from different locations in the Republic of North Macedonia in three types of climatic zones. Soil samples were collected in May 2021 from three different locations in each climatic zone, ranging from the southwest to the southeast of the Republic of North Macedonia. The soil from the continental zone was collected from the village of Bistrica (Bitola), the soil from the alpine area from Mount Baba (Bitola) and the soil from the submediterranean area was taken from Vardarski Rid (Gevgelija). The selection took into account conservation, availability of the sample site and anthropogenic disturbances (proximity to a forest road, etc.). During sampling, the temperature in the respective area and the corresponding geographical information (coordinates, altitude) were recorded. The collection of the material in the field was carried out on experimental plots (wooden frames) of 1 m x 1 m. Samples were collected 15-20 cm below the surface. Soil was collected with a sterile spatula and transported in sterile bags in a refrigerator box with ice to the laboratory of the Department of Microbiology and Microbial Biotechnology at the Faculty of Natural Sciences and Mathematics, where a laboratory was accessed within 24 hours after the material was collected (Figure 1).

Figure 1. Soil sample collection.

Soil geochemical parameters

A total of three soil geochemical parameters were determined, including moisture content, soil pH, and organic carbon. After collecting the samples of soil, the moisture content was determined for each soil type. The samples were dried at 105 °C to a constant mass, after which the percent moisture loss in the samples was determined. The pH of the soil was determined using a pH meter. 20 g sample of each soil type was placed in a 100 ml glass beaker and 40 ml of distilled water was added. After stirring the suspension vigorously, it was left for 1 hour to enable the clay particles to precipitate, and the pH of the soil was measured. Organic carbon was determined by the Kotzman method, which is based on oxidation of organic carbon with $KMnO_4$ solution and retitration with oxalic acid (Figure 2).

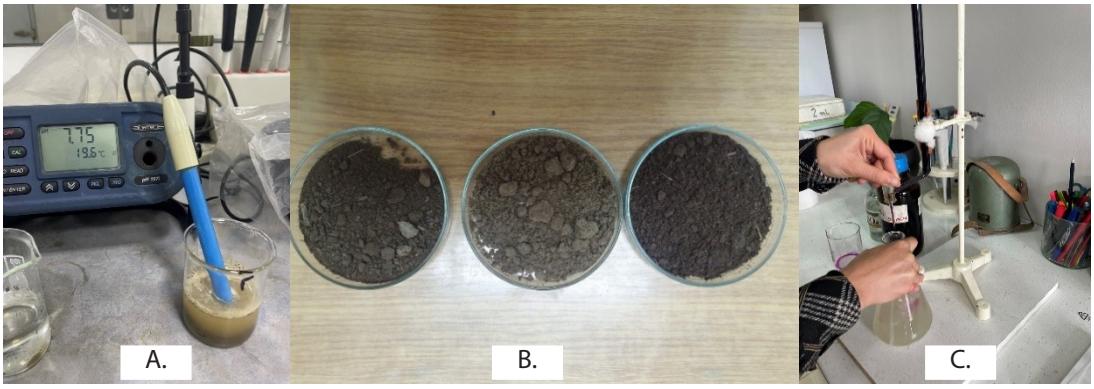

Figure 2. A. Determination of the pH value of the soil. B. Determination of moisture content. C. Determination of the organic carbon.

Determination of the number of *Bacillus* spp.

To determine the number of *Bacillus* spp. a dilution series of each soil type was prepared. For this purpose, 10 g of each soil sample was transferred to a sterile bottle containing 90 ml of sterile distilled water. After mixing the bottle vigorously, 5 ml of each was transferred to sterile empty test tubes, which were placed in a water bath at 70°C for 30 minutes. After expiration of this time, the sample was serially diluted. Each of the dilutions (10^{-1}-10^{-8}) was inoculated onto a nutrient agar plate (NA) using the pour plate method. The inoculated plates were incubated at 37 °C, and bacterial growth was monitored after 24 and 48 hours. Quantitative analyses of bacterial growth in the samples were carried out by determining the number of bacterial colonies expressed as CFU/g of the original sample. Qualitative analyses were determined by the morphological characteristics of the microorganisms of the genus *Bacillus*.

For this purpose, the macroscopic characteristics were then observed to describe the colony's morphological shape and colour. Before beginning the microscopic analysis, pure cultures were isolated from the previously inoculated plates and then microscopic slides were prepared using the Gram stain method. Microscopic observation was performed using a microscope with a x100 objective.

Determination of antimicrobial activity

The determination of the antimicrobial activity of the isolates against the test microorganisms was conducted using the agar well diffusion method. The test bacteria (*Salmonella enterica* ATCC 10708, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Listeria monocytogenes* ATCC 13393, *Bacillus subtillis* ATCC 6633, *Staphylococcus aureus* ATCC 25922) and test fungi (*Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 10231) used in this analysis are included in the microorganism collection of the Microbiology Laboratory at the Faculty of Natural Sciences and Mathematics, Skopje, North Macedonia. All test bacteria were inoculated in nutrient broth (NB) and incubated at 37°C for 24 hours, while the test fungi were inoculated in Sabourad-dextrose broth (SDB) and incubated at 25°C for 72 hours. Test microorganisms were inoculated onto each sterile nutrient agar (NA) Petri dish using a sterile swab, and then wells with a diameter of 6 mm were placed on the nutrient medium. Into these wells, cultures of *Bacillus* spp. were added which were allowed to pre-incubate at 37°C for 24 hours. The plates thus inoculated were incubated at 37°C for 24 hours, and then the diameter of the zone of inhibition was measured for each isolate.

RESULTS AND DISCUSSION

Sample collection

Geographic information, altitude, and temperature during soil collection are shown in Table 1.

Table 1. Geographic information, altitude, temperature for each soil type.

Location	Village Bistrica (Bitola)	Mount Baba (Bitola)	Vardarski Rid (Gevgelija)
Coordinates	N40°59'3"E21°22'40"	N41°02.3974'E21°13.0749'	N41°08'44"E22°21'30"
Altitude	587 m	1380 m	55 m
Temperature	20°C	11°C	24°C

Several factors influence the microbial community in the soil. One of them is climate, which is considered an important abiotic factor affecting other sub-factors that form the community of Bacillus spp. in soil regardless of climatic conditions and artificial changes. Climate change and global warming are causing a paradigm shift in soil microbial communities, making the study of microbial communities in different terrestrial ecosystems increasingly important. Several studies analysing seasonal variations in bacterial community profiles in alpine forest soils confirm that this approach is gaining importance in the study of soil microbial community composition at different climatic and geographic scales (Cordero & Datta, 2016). The Balkan Peninsula has very different climatic conditions that have a considerable impact on terrestrial ecosystems, changing the physiochemical properties of the soil and thus altering the profile of soil bacterial communities. This opens many opportunities to study the diversity of microbial communities in the soil of the Republic of North Macedonia in climatic and geographic dimensions that currently receive little attention.

Soil geochemical parameters

After determining the moisture content of the different soil types, the highest pH value was observed for the soil from the alpine area (Mount Baba, Bitola) (Table 2). When determining the pH value of the given soil types, the highest pH value (7.67) was found for the soil from the submediterranean area, followed by the soil from the continental area with a pH value of (7.53), while the lowest pH value (6.91) was determined for the soil from the alpine region.

Table 2. Moisture content for each soil type.

Location	Village Bistrica (Bitola)	Mount Baba (Bitola)	Vardarski Rid (Gevgelija)
% moisture	11,3%	19,08%	9,8%

According to the humus content, Gračanin divided the soils into: very low humus content (<1%), low humus content (1-3%), medium humus content (3-5%), high humus content (5-10%), very high humus content (>10%). From the results of the determination of organic carbon in the soils by the use of Kotzman method, the soil from the continental area (Bistrica village) is slightly rich in humus, the soil from the mountain area (Mount Baba) is strongly rich in humus, while the soil from the submediterranean area (Gevgelija) is extremely poor in humus (Table 3).

Table 3. Humus content in soil according to Kotzman's method.

Location	Village Bistrica (Bitola)	Mount Baba (Bitola)	Vardarski Rid (Gevgelija)
Humus (%)	2,097 2,057	5,017 5,022	0,77 0,52

Soil parameters pH, moisture content and organic carbon showed significant differences between climatic zones. Moisture content was lower in the submediterranean region, but *Bacillus* spp. were more diverse than in other climatic zones, indicating a minor influence of moisture content on the survival of different species of *Bacillus* spp. This study shows the difference in the number of Bacillus spp. isolated from different soil types in different climates, despite the same growth conditions in the laboratory, which has never been reported in the literature. According to the results obtained in the determination of antimicrobial activity, several of these isolates have the potential for enzyme production as well as probiotic properties. Further research is needed to determine the relationship between the microbial population and other ecological characteristics in the area of Republic of North Macedonia, which could face catastrophic consequences as a result of urbanization and climate change. The extreme flexibility of the microbial genome has impacted microbial adaptation over time, allowing bacteria to rearrange and exchange genomic sequences and acquire advantageous traits in response to changing environmental conditions. Because members of soil biological communities vary in their physiology, growth rate, temperature sensitivity, and other environmental parameters (Delgado-Baquerizo et al., 2014) climate change is likely to alter their relative abundance and function.

Determination of the number of *Bacillus* spp.

The results of the determination of the number of *Bacillus* spp. for each soil type are presented in Table 4. According to the results, the highest number of *Bacillus* spp. was found in the mountain region and the lowest value for the number of *Bacillus* spp. in the soil was found in the submediterranean region, expressed as CFU/g dry soil.

Table 4. Number of *Bacillus* spp. for each type of soil

Location	Village Bistrica (Bitola)	Mount Baba (Bitola)	Vardarski Rid (Gevgelija)
Log10 CFUs/g	4,68	6,69	4,97

*CFUs (Colony Forming Units)

The bacteria isolates from soil samples from different climatic regions of the Republic of North Macedonia were isolated and sampled on nutrient agar plates. Morphological analysis showed that the isolates were Gram-positive, rod-shaped, sporulating bacteria (Figure 3).

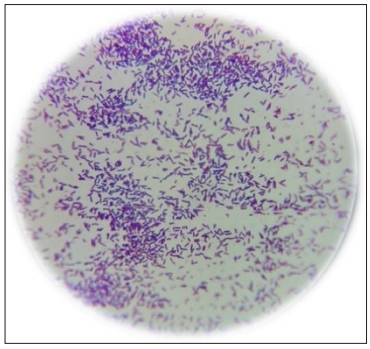

Figure 3. Microscopic characteristics of *Bacillus* spp. isolated from soil stained by the Gram method (x100).

Based on the data, although the genus *Bacillus* is ubiquitous in all major soil types of different climatic zones, the number of this genus's species varies considerably, indicating that climate has a significant influence on the microbial population of *Bacillus* spp. in different soils. The highest value for the number was found in the alpine region and the lowest value in the submediterranean region, which is associated with an increase in temperature and a decrease in altitude. It is probable that bacterial diversity and abundance decrease with increasing altitude and are affected by environmental and geological factors such as vegetation, temperature, and pH, which form a complex interaction. However, numerous studies have yet to uncover consistent changes in microbial populations along altitudinal gradients. Alterations in the relative abundance of organisms involved in regulating certain soil processes can directly affect the rate at which these are carried out, which in turn has implications for soil functioning and hence pollutant degradation rates. Because these processes can be carried out by a variety of species, phylogenetically broad process are generally more strongly linked to abiotic parameters (e.g., temperature, moisture) than to microbial community composition (Hooper et al., 2005).

Determination of antimicrobial activity

Screening of antimicrobial activity of the 6 isolates was performed against 9 test microorganisms. All isolates were found to have inhibitory activity against *Escherichia coli* ATCC 8739 and *Bacillus subtillis* ATCC 6633 (Table 5).

Table 5. Antimicrobial activity of *Bacillus* spp. isolated from soil against test bacteria

	Escherichia coli ATCC 8739	*Bacillus subtillis* ATCC 6633
Bacillus 1	/	8 mm
Bacillus 2	4 mm	4 mm
Bacillus 3	/	8 mm
Bacillus 4	2 mm	4 mm
Bacillus 5	4 mm	10 mm

Most of the isolates showed antimicrobial activity against the Gram-positive bacteria *Bacillus subtillis* ATCC 6633, 3 of the isolates showed antimicrobial activity against the Gram-negative bacteria *Escherichia coli* ATCC 8739. The first two isolates presented in Tables 4 and 5 were isolated from the sub-Mediterranean area, i.e., Gevgelija, while the others from the continental area, village Bistrica, Bitola. None of the isolates from the alpine area, Mount Baba, showed antimicrobial activity. The isolate *Bacillus* 5, isolated from the continental area, showed the greatest inhibitory effect on the test bacteria, i.e., the largest zone of inhibition for this isolate was observed against *Staphylococcus aureus* ATCC 25922 with a diameter of 10 mm (Figure 4).

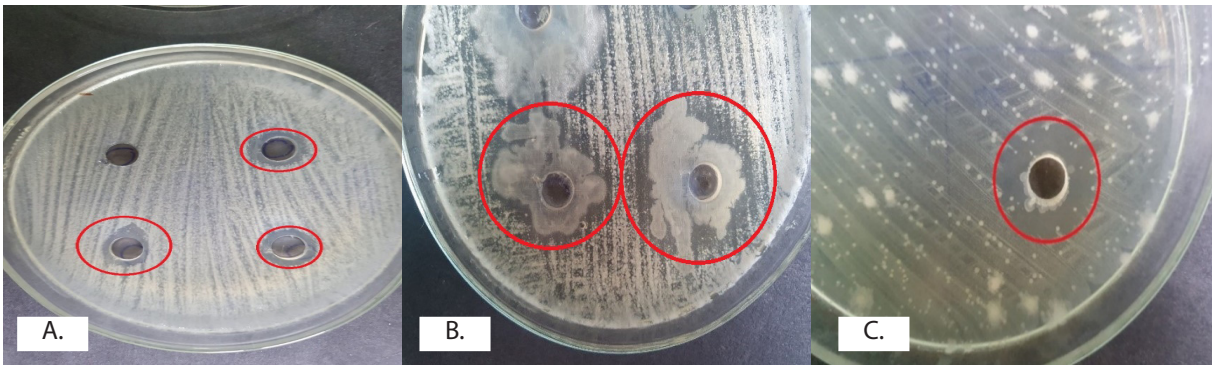

Figure 4. Antibacterial activity (zones of inhibition) of *Bacillus* spp. from soil against:
A. *Escherichia coli* ATCC 8739. B. *Bacillus subtillis* ATCC 6633. C. *Staphylococcus aureus* ATCC 25922

In terms of the antimicrobial activity against the test fungi, only two of the isolates showed a zone of inhibition against *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404 (Table 6). Both isolates from the continental and submediterranean areas showed a zone of inhibition with a diameter of 12 mm against *Aspergillus niger* ATCC 16404 (Figure 5).

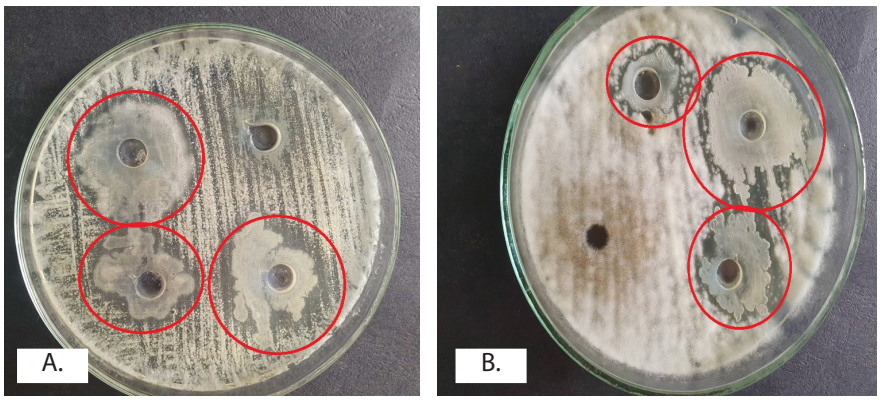

Figure 5. Antifungal activity (zones of inhibition) of *Bacillus* spp. from soil against:
A. *Candida albicans* ATCC 10231. B. *Aspergillus niger* ATCC 16404.

Table 6. Antimicrobial activity of *Bacillus* spp. isolated from soil against test fungi

	Test microorganisms	
	Candida albicans ATCC 10231	*Aspergillus niger* ATCC 16404
Bacillus spp.		
Bacillus 1	/	/
Bacillus 2	2 mm	12 mm
Bacillus 3	/	/
Bacillus 4	4 mm	12 mm
Bacillus 5	/	/

The ecosystem functions of soil microorganisms are crucial for maintaining soil carbon and providing nutrients to plants, and their value in preserving healthy soils for future generations cannot be overstated. There is an urgent need to better understand the effects of climate change on important biogeochemical processes carried out by soil microorganisms and to use this information to better predict climate and ultimately develop microbial strategies to counteract further climate warming and soil degradation (Classen et al., 2015). Although numerous approaches are being explored to use soil microorganisms to reduce the impacts of climate change, these would not be sufficient to compensate for the soil loss and greenhouse gas emissions that are occurring (Whitaker et al., 2014). To achieve this, details obtained from microbial population surveys need to be linked to ecosystem function resolution and various climate models, consistent with the recent "Warning to Humanity" reminder of the importance of the microbiota in maintaining ecosystem stability during climate change (Cavicchioli et al., 2019).

CONCLUDING REMARKS

Soil microbial populations are incredibly diverse and represent an important aspect of ecosystems that contribute to climate change through the breakdown of soil organic matter. Soil is the primary store of global terrestrial carbon, and fractional-level changes in the total soil carbon cycle can have significant consequences on atmospheric carbon dioxide density. As a result, the change in soil carbon is a key regulator of future climate in response to environmental change. In general, the consequences of global warming on the microbial community and subsequent decomposition processes are more pronounced in alpine, arctic and highland sites. However, compared to the diversity of macroorganisms, which has been studied for millennia, microbial population patterns have not been widely explored and remain poorly understood.

In the present study, some of the isolated *Bacillus* spp. showed antimicrobial activity against *Escherichia coli* ATCC 8739, *Bacillus subtillis* ATCC 6633 and *Staphylococcus aureus* ATCC 25922, these strains make excellent candidates for use as brand-new probiotic strains. Around the world, there has been a continuous search for innovative probiotics that are beneficial in industrial, agricultural, and medical settings. Potential probiotics must be able to pass the basic probiotics characteristics test, be able to withstand digestive factors especially those with low pH and high bile concentrations, produce antimicrobial substances, and adhere to the intestinal mucosa (Kesen & Olayinka, 2018). In addition, since these are preliminary results, to evaluate the long-term probiotic capability of these strains, it will be necessary to determine their antimicrobial activity against the most frequently used antibiotics as a control test and on a wider variety of pathogenic microorganisms, as well as on animals.

There are several ways that climate change affects our world, including changes in water temperature, pH, or oxygen content; a rise in desertification; wind patterns that are affected by land erosion; and prolonged dry spells in tropical forests. Each of these geographical shifts has an effect on the microbial communities in the area, which affects their metabolic capabilities and activities. The regional as well as the global climate can then be affected by these changes. According to the results, it was found that the diversity of *Bacillus* species changes depending on the soil microenvironment under the influence of different climatic conditions. There is still a lack of knowledge about how *Bacillus* assembles in the soil ecosystem, particularly throughout the year's several seasons. The primary driver of the Bacillus community was temperature and humus content. Thus, the sum of our data offers a thorough understanding of the *Bacillus* community assembly in the soil ecosystem under different climatic conditions.

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ВЛИЈАНИЕТО НА РАЗЛИЧНИТЕ КЛИМАТСКИ ТИПОВИ ВО РС МАКЕДОНИЈА ВРЗ БРОЈНОСТА НА *Bacillus* spp. ИЗОЛИРАНИ ОД ПОЧВА

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Резиме

Почвените микроорганизми имаат главна улога во биогеохемиските процеси на различни елементи кои се од витално значење за растот на растенијата и животот на животните. Разбирањето на влијанието на климатските промени врз почвените микроорганизми и нивната улога во екосистемот претставуваат голем предизвик и можност за насочување на истражувачките напори кон еден од повеќето итни проблеми со кои се соочува нашата планета. *Bacillus* е широко распространет род кој брои 347 видови и 7 подвидови познати до денес. Членовите на овој род имаат способност да формираат спори кои се отпорни на екстремна топлина, бактерицидни агенси и хемиски средства за дезинфекција. Многу видови на *Bacillus* имаат широка примена во медицината и земјоделството поради производството на антибиотици, а се користат и како пробиотици во исхраната. Климата како абиотички фактор влијае врз почвените микроорганизми преку контролирање на брзината на формирање на почвата и нејзиниот хемиски состав. Поголемиот дел од истражувањата во областа на микробна екологија се фокусираат на бројноста на почвените микроорганизми, како и на документирање на влијанието на промените во околината врз истите. Овој нов тренд на истражувања може да се примени на *Bacillus* spp. од почва во РС Македонија во трите климатски типови поради разликите помеѓу нив. Ова истражување се фокусира на определување на геохемиските параметри на почвата и микробиолошки анализи. Беа изолирани вкупно 36 соеви на *Bacillus* spp., а 5 од нив покажаа антимикробна активност врз одредени тест микроорганизми. Според добиените резултати исто така беше детерминирано дека разновидноста на видовите на *Bacillus* се менува според микросредината на почвата под влијание на различни климатски услови.

Клучни зборови: *почвени бактерии, климатски промени, земјоделство, антибиотици, пробиотици.*

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THE QUALITY OF RED WINE VRANEC, MERLOT AND FRANKOVKA FERMENTED BY COMMERCIAL AND BAKER'S YEASTS

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Abstract

The aim of this study is determination of the quality of red wines from grape varieties Vranec, Merlot and Frankovka fermented by wine yeasts from the French manufacturer SELECTYS® LA DÉLICIEUSE and baker's yeast seeded in a ratio of 0.25 g/L. The research was done during 2020 and 2021 in the Vinica vineyard, where the three grape varieties are grown at an altitude from 400 to 520 m. Regarding enological parameters, the highest amount of alcohol was measured for Merlot wines fermented by French yeast (13.07%), Frankovka wine fermented by baker's yeast had the highest amount of sugar (9.71 g/L), Vranec wine produced by baker's yeast had the highest total acidity (7.37 g/L) while the highest concentration of volatile acids was determined for Frankovka wine fermented by French yeast SELECTYS® LA DÉLICIEUSE (1.58 g/L). The impact of wine yeast to the quantity of particular elements was the most statistically significant for Fe57 in Merlot and Vranec wines, Cu65 in Frankovka wines and Pb208 in Merlot. Vranec and Merlot wines fermented by baker's yeast indicated higher amounts of phenolic compounds while Frankovka wines produced by SELECTYS® LA DÉLICIEUSE showed opposite tendency with significantly higher amounts of polyphenols.

Key words: *yeasts, wine, fermentation, Vranec, Merlot, Frankovka, winegrapes*

INTRODUCTION

Wines are classified as red, white and rosé on the basis of grape variety, sugar content, alcohol content, carbon dioxide content, color, fermentation and ageing process or geographical origin (Jackson RS., 2000).

Wines can be differentiated by the geographical location of the vineyards, the different methods of vinification, the use of yeasts of different origins (wine yeasts, indigenous yeasts or baker's yeasts) and etc.

Red wines are obtained by alcoholic fermentation of the must in the presence of the solid parts of the berry (skin and seeds), unlike white wines, which are produced exclusively by fermentation of the grape juice (Ribéreau-Gayon P., et al. 2006).

The use of yeasts in wine fermentation is of great importance as they are largely responsible for the complexity and sensory quality of wines. For this reason, current studies are mainly focused on the search for new yeast species to be used in winemaking technology. Isolation, morphological and physiological characterization of *Saccharomyces cerevisiae* yeast strains from 15 microregions of Tikveš wine-growing region of Macedonia as well as their impact of the organoleptic profile of wines produced from Vranec and Cabernet Sauvignon grape variety were studied by Ilieva et al., (2019).

Wine fermentation is a series of ordered and complex processes of biochemical transformation of yeasts and bacteria. To facilitate control of fermentation conditions and reduce the risk of spoilage and unpredictable changes in wine taste, commercial active dry yeast is commonly used in current wine production (Liu et al., 2021; Liu et al., 2016). The increasing usage of traded selected yeasts for winemaking leads

inevitably to a loss of the autochthonous yeast populations naturally present in regional grapes and, consequently, drives to the potential loss of genetic diversity and heritage. Largely, the specificities, authenticity, uniqueness and, most importantly, the quality characteristics of the wine are dependent on the natural microbiota found in the grapes of each viticulture region (Ilieva et al., 2021).

Domestication of wine yeast, while inadvertent until recent decades, has generated strains that differ considerably from "wild" *S. cerevisiae* strains. Inoculations of "wild" *S. cerevisiae* yeasts can influence the process of fermentation and greatly affect the quality of the wine. Isolating strains from successful fermentations for inoculation in subsequent vintages was being practiced during winemaking in order to avoid unwanted malolactic or acetic fermentation. Largely, the specifics and the most important quality characteristics of the wine are due to the natural microflora of the grape of the viticulture region (Ilieva et al., 2017). In the work of Petrov et al. the correlation between wine yeast SELECTYS® LA DÉLICIEUSE and spontaneous fermentation by wild yeast was examined (Petrov, Ilieva, Kostadinović Veličkovska & Dimovska, 2022).

The main object of this study is comparison of the quality of the wines from Vranec, Merlot and Frankovka fermented by wine yeast SELECTYS® LA DÉLICIEUSE and baker's yeast. For this purpose, after fermentation of wines by both yeasts, the oenological parameters (% of alcohol, sugar, total and volatile acids, pH and free SO_2 were compared as well as the amount of 8 elements (Li7, Be9, Mn55, Fe57, Ni60, Cu65, Ga71 and Pb208).

MATERIAL AND METHODS

The research was carried out during 2020 and 2021, during which 3 varieties of grapes were used: Vranec, Merlot and Frankovka, produced in the Vinica vineyard is found at the altitude of 400 to 520 m.

Fermentation technology

The tested grape varieties were harvested at technological maturity and processed with an electric grape mill. The grape mash was placed in 6 barrels of 75L each, for fermentation, then grapes were added in an amount of 10 mg/kg. After two hours, all samples were inoculated with the respective yeasts. Three of the vessels were seeded with yeasts for general use (baker's yeast), three of the vessels were seeded with pure cultured wine yeasts from the French manufacturer SELECTYS® LA DÉLICIEUSE. In the samples with inoculated yeasts this was done at a ratio of 0.25 g/L. The maceration period is 5 days, during which the mixture is stirred 3 times a day. The temperature in the rooms was constant from 22 °C to 25 °C.

Reagents and standards

The following chemicals were used in this study: Acetonitrile CHROMASOLV™ gradient grade ≥99.9% (Honeywell Riedel-de Haën™, Seelze, Germany), acetic and nitric acids Optima® LC/MS glacial (Fisher Chemical, Geel, Belgium), acetone Reag. Ph. Eur. 100% (VWR, Fontenay-sous-Bois Cedex, France), methanol ≥99.8% HPLC grade (Fischer Scientific, Loughborough, United Kingdom), water (deionized, nanopure®, Werner, Leverkusen, Germany).

Determination of oenological parameters, macro and micro elements and total phenolic compounds (TPC)

Determination of oenological parameters of wines fermented by two type of yeasts were performed with FOSS WINESCAN. Determination of the amount of alcohol was performed ebuliometrically by a Dujardin-Salleron ebulliometer (GW Kent, Ypsilanti, USA) method (Zoecklein et al., 1995), and for the determination of total reducing sugars, the Luff-Schoorl method (ISI 28-1e: Determination of Reducing Sugar, DE by Luff-Schoorl's method) was used and the results expressed as g/L. Quantification of titratable (TA) and volatile (VA) organic acidity in wines was performed according to the methodologies described by Ilieva et al. (2017), and both are expressed as g/L. total phenolic content was performed by the AOAC International Method (AOAC SMPR 2015.009: Estimation of Total Phenolic Content Using the Folin–Ciocalteu Assay, 2015) and expressed as mg/L. The pH values of the wines were determined by the International Standard Method according to OIV-MA-AS313-01.

Macro and micro elements in wine were performed with ICP/MS (mass spectrometry, which uses inductively coupled plasma that ionizes the sample). Inductively coupled

plasma with mass spectrometry (ICP-MS, model 7500cx Agilent Technologies, USA) with a glass concentric nebulizer was used for analyses of the elements content. In this study, five step set or combination of power, pressure, and time conditions for microwave-assisted digestion were applied. Microwave-assisted digestion conditions involved the digestion of 0.5 g of the sample with 5 mL HNO_3 and 2 mL of H_2O_2 in the microwave digestion system CEM model MARS 5 (CEM Corporation, Matthews, NC, USA). After digestion, the vessels were allowed to cool until the pressure of the vessel was reduced to below 50 psi and temperature was below 40 °C. The caps of each vessel were then carefully removed and the contents were filtered using 2µm filter paper diluted to 25 mL in a volumetric flask using deionized water, and stored in polyethylene vial prior to the final determination of the elements' concentration.

RESULTS AND DISCUSSION

The process of fermentation in wine-making turns grape juice into an alcoholic beverage (Amerine et al., 1980). In our research, the highest alcohol percentage of 13.07% was measured for Merlot wine obtained by fermentation using commercial wine yeast from the manufacturer SELECTYS® LA DÉLICIEUSE, while the lowest percentage of 11.36% is the Vranec variety obtained by fermentation in which baker's yeast is used. Generally speaking, the percentage of the alcohol in wine depends from the percentage of sugar and activity of the wine yeasts. The higher alcohol content is associated with the "vinous" aroma of fermented must, the intensity depending on the type of alcohols present and their concentrations (Lambrechts & Pretorius, 2000).

The residual sugar in red wine usually is related to the type of yeast and environmental conditions during the process of fermentation. The highest and the lowest values of residual sugars were obtained in samples which were fermented using baker's yeast, 9.71 g/L in Frankovka, and 0.59 g/L in Vranec, respectively. The significant difference in the residual sugars between those two wines depends on the amount of sugar in grapes and different ability of wine yeast SELECTYS® LA DÉLICIEUSE and baker's yeast to consume the sugar and convert in alcohol (Bisson, 1999; Branco et al., 2014; Maicas, 2020). The use of *S. cerevisiae* as starter culture is the most widespread practice in winemaking but not always inoculation of musts using selected *Saccharomyces* strains does ensure their dominance at the final stages of fermentation (Capece et al., 2010).

Total acids showed a strong negative correlation with alcohol and sugars in red wines. The data obtained in the research showed the lowest value of 4.80 g/L for Frankovka wine fermented with beer yeast, while the highest value of 7.37 g/L for Vranec fermented with baker's yeast. There is a strong positive correlation between total acids and volatile acids.

Volatile acids are strongly correlated with alcohol, sugars and total acids. Frankovka fermented with commercial wine yeasts has the highest value of volatile acids of 1.58 g/L, while Vranec fermented with baker's yeast has the lowest value of 0.23 g/L.

Wine pH is strictly intertwined with the microbiological and physiochemical stability of the product, as it affects the selection of microorganisms as well as some key chemical reactions, including sulphur dioxide balance (Comuzzo & Battistutta, 2019). Also, acidity and pH play an important role on the sensory characteristics and balance of wines (Comuzzo & Battistutta, 2019).

The pH data obtained in our research ranged from 2.97 for Vranec fermented with baker's yeast to 3.44 for Merlot fermented with commercial wine yeast. The correlation coefficient showed that there is a strong positive correlation between pH and the content of alcohol, sugar and free SO_2, while there is a strong negative correlation with total acids.

The amount of SO_2 in wines has a very important role in preventing oxidation and maintaining freshness in wine. The highest SO_2 value of 17.32 mg/L was obtained for Merlot fermented with baker's yeast, and the lowest 7.27 mg/L for Vranec fermented with baker's yeast.

Macro- and macroelements in wine are very important for its quality. The obtained data indicated that the highest level of Li7, Ni60, Cu65, Ga71 and Pb208 has the Merlot variety fermented with selected wine yeasts, while Fe57 has the Vranec fermented with baker's yeast, Mn

55 has the Merlot fermented with baker's yeast and the highest level of Be 9 has Frankovka fermented with commercial wine yeast. However, significant difference was obtained by presence of Li 7 in Vranec wines fermented by both yeasts. In addition, Vranec wine produced by bakery yeast had more than double amount of Fe 56 (7868.90 ppb) in comparison with the same wine fermented by wine yeast SELECTYS® LA DÉLICIEUSE (3947.09 ppb). Opposite, Merlot wine produced by wine yeast had almost double of the amount of Mn55 in comparison to the same wine produced by bakery yeast (Table 2). Frankovka wine produced by wine yeast SELECTYS® LA DÉLICIEUSE had 63,24 ppb Cu65 which is almost double in comparison to the same wine produced by bakery yeast (37.82 ppb). Finally, Merlot wine produced by wine yeast SELECTYS® LA DÉLICIEUSE had more than three times higher amount of Pb208 (21.96 ppb) in comparison to the same wine produced by bakery yeast (6.98 ppb) (Table 2). According to the Blackwell and Tobin, greater amounts of metal may be accumulated by yeasts due to their mechanisms for transport of metal ions into microbial cells include lipid peroxidation, complex permeation, carrier mediation, ion channels/pumps and endocytosis (Blackwell and Tobin, 1995). Most mechanisms of metal transport appear to rely on the electrochemical proton gradient across the cell membrane, which has a chemical and electrical potential, both of which are responsible for driving transport of ionized solutes across membranes (Gadd, 1993).

Tab. 1. Oenological parameters of Vranec, Frankovka and Merlot wines fermented by wine and baker's yeasts.

Wine	Yeast	Alcohol %	Sugar (g/l)	Total acids (g/L)	Volatile Acidity (g/L)	pH	Free SO_2 (mg/L)
Vranec	SELECTYS® LA DÉLICIEUSE	12.15	2.3	5.91	0.34	3.25	9.24
Vranec	baker's yeasts	11.36	0.59	7.37	0.23	2.97	7.27
Frankovka	SELECTYS® LA DÉLICIEUSE	12.35	4.42	6.58	1.58	3.38	8.20
Frankovka	baker's yeasts	12.54	9.71	4.80	0.40	3.40	9.02
Merlot	SELECTYS® LA DÉLICIEUSE	13.07	4.67	5.15	0.78	3.44	7.58
Merlot	baker's yeasts	12.93	3.13	5.18	0.37	3.32	17.32

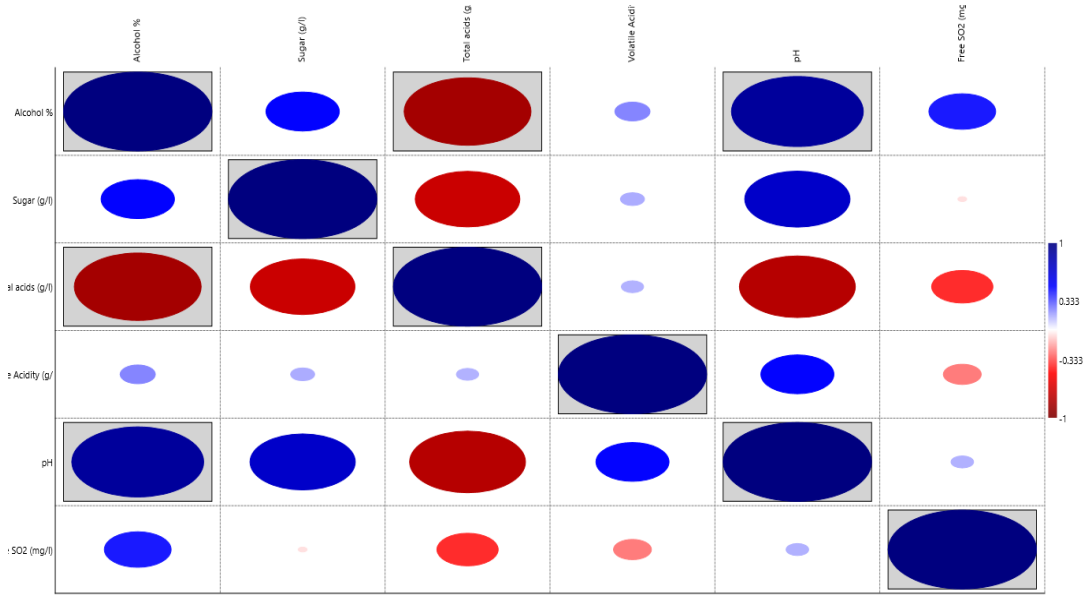

Figure 1. Correlation between investigated chemical characteristics of red wines

Table. 2. Multielement analysis of Vranec, Frankovka and Merlot wines fermented by wine and baker's yeasts.

Wine	Yeast	Li7 ppb ug/L	Be9 ppb ug/L	Mn55 ppb ug/L	Fe57 ppb ug/L	Ni60 ppb ug/L	Cu65 ppb ug/L	Ga71 ppb ug/L	Pb208 ppb ug/L
Vranec	SELECTYS® LA DÉLICIEUSE	16.64	0.29	1 311.18	3 947.09	30.93	50.31	3.03	5.86
Vranec	baker's yeasts	25.25	0.27	1 272.26	7 868.90	38.28	42.99	2.97	4.18
Frankovka	SELECTYS® LA DÉLICIEUSE	20.48	1 233.57	2 117.81	4 343.72	71.14	63.24	9.88	3.43
Frankovka	baker's yeasts	21.69	1 145.76	2 300.88	3 239.29	72.47	37.82	8.46	2.77
Merlot	SELECTYS® LA DÉLICIEUSE	53.44	0.39	3 000.61	5 037.64	97.82	163.08	15.47	21.96
Merlot	baker's yeasts	50.31	0.41	3 001.54	3 624.718	79.00	147.79	14.43	6.98

Polyphenols in grapes are phenolic acids, anthocyanins and flavonoids and their composition and content can vary depending on the location of grape cultivation (Leifert W.R. et. al. 2008).

Phenolic compounds can be useful markers for wine quality and its authenticity (Merkytė et al., 2020). Wine quality evaluation is based on taste, aroma and chemical characteristics. The sensory evaluation covers colour and taste, especially astringency which depends from the phenolic compounds presented in the wine. Thus, phenolic compounds are widely used for the wine quality and authenticity assessment (Chira et al., 2011; Heras-Roger et al., 2016; Lukic et al., 2016). In our research, the average polyphenolic profile obtained from three repetitions showed that the lowest value in the Vranec variety fermented with commercial wine yeasts, and the highest value of 43.281 mg/L in the Merlot fermented with baker's yeast. The highest significant differences were obtained for Frankovka wines fermented by wine and bakery yeasts. The total phenolic compounds obtained by wine yeast was 32. 132 mg/L while the same wine fermented by bakery yeast had 26.358 mg/L. This result can be explained by the fact that wine yeasts are more appropriate for fermentation of grape must and accumulation of phenolic compounds in wines (Ilieva et al., 2017).

Table 3. Determination of phenolic compounds in Vranec, Frankovka and Merlot wines fermented by wine and baker's yeasts (mg/L).

Wine	Yeast	First replication	Second replication	Third replication	Average
Vranec	SELECTYS® LA DÉLICIEUSE	24.042	22.763	22.993	23.266
Vranec	baker's yeasts	26.324	26.139	25.333	25.932
Frankovka	SELECTYS® LA DÉLICIEUSE	32.85	32.105	32.105	32.132
Frankovka	baker's yeasts	26.91	26.439	25.726	26.358
Merlot	SELECTYS® LA DÉLICIEUSE	41.475	41.071	40.79	41.112
Merlot	baker's yeasts	43.607	42.91	43.327	43.281

CONCLUDING REMARKS

The results from our study showed that type of the yeast strongly influenced wine fermentation and quality of the final wines. Vranec wine fermented by yeast strain SELECTYS® LA DÉLICIEUSE had higher amount of sugar while Frankovka wine had double amount of sugar fermented by bakery yeast in comparison to the same wine produced by wine yeast. Total and volatile activity was affected to the yeast strain to a lesser extent while amount of SO_2 was almost double in Merlot wine produced by bakery yeast. Regarding phenolic compounds, Frankovka wine was affected the most by the influence of yeast strain with significantly higher content of phenolic compounds produced by wine yeast SELECTYS® LA DÉLICIEUSE. Finally, the results from the element analysis indicated different influence of type of yeast strains to different elements. The most affected was Fe57 in Merlot and Vranec wines, Cu65 in Frankovka wines and Pb208 in Merlot wines.

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КВАЛИТЕТ НА ЦРВЕНИТЕ ВИНА ОД СОРТИТЕ ВРАНЕЦ, МЕРЛО И ФРАНКОВКА, ФЕРМЕНТИРАНИ СО КОМЕРЦИЈАЛЕН ВИНСКИ И ПЕКАРСКИ КВАСЕЦ

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Резиме

Целта на ова истражување е да се утврди квалитетот на црвените вина од сортите грозје Вранец, Мерло и Франковка ферментирани со вински квасец од францускиот производител SELECTYS® LA DÉLICIEUSE и пекарски квасец засеан во сооднос од 0,25 g/L. Во однос на анализираните параметри, најголемо количество алкохол е измерено во виното Мерло ферментирано со француски квасец (13,07 %), виното Франковка ферментирано со пекарски квасец има најголемо количество шеќер (9,71 g/l). Виното Вранец произведено од пекарски квасец има највисока вкупна киселост (7,37 g/L), додека највисока концентрација на испарливи киселини има виното Франковка ферментирано со француски квасец SELECTYS® LA DÉLICIEUSE (1,58 g/L). Влијанието на винскиот квасец врз количината на одредени елементи беше статистички најзначајно за Fe 57 во вината Мерло и Вранец, Cu 65 во виното Франковка и Pb208 во Мерло. Вината од Вранец и Мерло ферментирани со пекарски квасец покажаа повисоки количини на фенолни соединенија, додека виното Франковка произведено со SELECTYS® LA DÉLICIEUSE покажа спротивна тенденција со значително повисоки количини на полифеноли.

Клучни зборови: *квасец, вино, ферментација, грозје, Вранец, Мерло, Франковка.*

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Professional paper

CORN PRODUCTION IN THE REPUBLIC OF NORTH MACEDONIA AND POSSIBILITIES FOR ITS CULTIVATION WITHOUT INTERVENTIONAL IRRIGATION

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Abstract

Corn is an annual plant from the sub-group of millet grains. In the Republic of North Macedonia, there are relatively good soil and climate conditions for its production. It is the third cereal crop in terms of representation on arable land, after wheat and barley. The production of corn grain in North Macedonia in 2021 amounted to 130 769 tons. These quantities of corn in grain obtained from corn production on the agricultural lands in the Republic of North Macedonia do not satisfy domestic demand. The total annual production of wheat, barley and corn grain in 2021 was 526 045 tons. Rye, rice and oats are produced in much smaller quantities than the other cereal plants.

Taking into account the agrotechnical measures applied in the production of corn and the soil-climatic conditions that prevail in Macedonia, this article gives a special review of the problems faced by this production, as well as the possibilities and measures for its production without interventional irrigation.

Key words: *Zea mays, yield, measures, irrigation, grain*

INTRODUCTION

Cereal crops are very significant group of field crops (Vasilevski, 2004). Corn has many uses for human nutrition, domestic animals and the processing industry. The realization of high and stable production of corn is largely influenced by the soil and climate conditions of the region, as well as the degree of agricultural technology used. The quantities of corn produced do not meet the needs in Republic of North Macedonia, although it is the third cereal crop in terms of representation per area on which it is produced.

In relation to the average yield of other cereal crops grown in R. Macedonia, corn and rice have a significantly higher yield that approaches the regional and European averages. These situations are the result of improved agrotechnical measures in corn production, such as:

- using certified seed material of a hybrid nature,
- fertilization with satisfactory amounts of fertilizers,
- timely feeding of the crops at an appropriate stage of the organogenetic development of the plant,
- use of more modern machinery in the production process, etc.

Natural conditions for corn production in the Republic of North Macedonia

The natural conditions in the Republic of North Macedonia provide the opportunity for the cultivation of all cereal plants. Millet grains are very sensitive to low temperatures. Corn in

the phenophase of sprouting tolerates negative temperatures from -2 to -3°C. Corn, despite being a millet grain plant, due to the large habitus it forms, still has a relatively high need for water and it is hard to bare it, especially in the phenophase of fertilization, pouring and ripening of the grain. One of the main problems in the production of cereal plants, including corn, is precisely this factor.

Cereals are mostly grown on all soil types. According to the reaction of the soil solution, cereal plants are divided into two groups: cereal plants that normally grow and vegetate at a neutral or slightly acidic reaction (pH 6-7), like wheat, barley and corn, and cereal plants that tolerate a wider pH interval value, like rye, oats, millet and buckwheat (http://makstat.stat.gov.mk/). Regarding the soil, it should be emphasized the great heterogeneity of the soil types found in the territory of Macedonia and the diversity in terms of fertility and other characteristics of such soils. In this regard, we need consistency in soil fertility tests, all with the aim of proper nutrition of this crop.

Corn production in the Republic of North Macedonia

Table 1 provides data on the representation, yield and realized production of corn in the production years 2019, 2020 and 2021 and the average for the three-year period.

Table 1. Corn production(http://makstat.stat.gov.mk/)

Year	Area (ha)	Yeald (kg/ha)	Production (t)
2019	34 123	4 277	145 528
2020	32 013	4 589	146 434
2021	30 425	4 327	130 769
AVERAGE 2019/21	32 187	4 398	140 910

Data in the table show that the areas under corn have a tendency to decrease from year to year, but the data for the average yield per unit area at the republic level are constant and amount to over 4000 kg/ha. The average corn grain production for the three-year period is 140,910 tons. This amount is not enough for the needs of Republic of North Macedonia, so about 20-25% is imported from the world market.

In these three production years, corn was produced on an average area of 32,187 ha, with an average three-year yield of 4.4 t/ha.

Recommendations and measures for the improvement of production with corn in Republic of North Macedonia

Corn production in the last three-year period does not satisfy domestic demand, although it is the third cereal crop in terms of representation in crop production in our country. Domestic production meets about 75 to 80% of needs. The remaining quantity is still provided by imports.

In order to overcome these conditions and become market independent for corn grain, it is necessary to take certain bolder steps in the organization of production. One of the possibilities is to increase the areas with this cereal plant. At present, the areas with corn are 32 187 ha, as producers grow economically more profitable crops. In such market conditions, realistically, that is also more difficult to do.

Another possibility is to increase the average yield per unit area, which now amounts to 4398 kg/ha. The realization of the second possibility requires a series of steps that must be followed and implemented.

Those steps are as follows:

1. Alleviation of drought periods in the critical phenophases of growth and development of maize with intervening irrigation, especially in the phenophases of stem growth, filling and grain ripening. These phenophases are critical for the final result obtained in the yield and wherever there is no proper distribution of precipitation during the vegetation and the possibility of intervening irrigation of the crops in these phases, irreversible damage occurs in terms of the height of the yield. At the moment, in the Republic of North Macedonia, intervention irrigation is implemented on about 60 - 70% of the surfaces;

2. State investments in the improvement of outdated and non-functional irrigation systems and the construction of new agro-ameliorative systems, use of the drip irrigation system. The

current situation with the irrigation systems is unenviable, and the use of the drip system in the production of this crop is still at an early stage. The areas cultivated with this system are through certain grants and projects, and are very small. From the results obtained in projects where this irrigation system is incorporated, the yields per unit area have increased by 60 - 100%;

3. Increased and improved application of means for plant protection. In standard corn production at the moment, herbicides are used in a sufficient extent for weed protection. But the use of fungicides and insecticides for crop protection during the vegetation is minimal and should be increased;

4. Increased support in the area of subsidies for the production of this crop. At the moment, the subsidy is 10 000 denars/1 ha corn, but in order to help the producers, these funds should be increased by another 80%;

5. Development of measures for corn production in conditions without interventional irrigation, etc.

According to Glemoćlija (2004) agrotechnical measures, with the application of which higher yields can be achieved, among others, are:

- the correct selection of the pre-crop,
- cultivation in crop rotation and avoidance of cultivation in monoculture,
- correct selection of hybrids with appropriate length of vegetation,
- quality tillage,
- use of optimal seed quantities,
- appropriate additional plant nutrition with natural soil fertility,
- breeding pest, disease and weed resistant genotypes.

Proposed agrotechnical measures and steps in corn production in conditions without interventional irrigation

The production of corn without irrigation carries a certain degree of risk in the cultivation of this crop in the amount of yield per unit area, but the measures that are proposed are an option and a possibility for areas where there are no real opportunities for irrigation.

Those measures are the following:

Conservation and preservation of moisture in the soil;

- Cultivation of corn as a first crop;
- Avoidance of corn production as a second crop;
- Deep basic ploughing (35-40 cm);
- Deep pre-sowing soil preparation;
- Application of 2/3 of the nitrogenous and whole amounts of the phosphorous and potassium nutrients provided for this crop with the pre-sowing preparation of the soil;
- Timely sowing if possible and the earliest sowing according to the production area;
- Performing precision sowing;
- Fertilizing with 1/3 of nitrogen fertilizers (UREA 46%) in phenophase 7-8 leaves (stem growth);
- Selection of hybrids with short vegetation (FAO ripening groups 100, 200, 300 and possibly 400);
- Selection of hybrids which are more tolerant to dry conditions and intended for arid areas.

The proposed agrotechnical measures should improve the current state of a large number of areas where soil moisture conservation is not practiced with measures such as:

- mandatory basic ploughing in autumn and another one before sowing;
- early sowing in optimal terms when the temperature conditions allow it, unlike to the current sowing which takes place from the end of April until the beginning of July;
- avoiding hybrids with long vegetation, such as FAO 500, 600, 700 and 800, which are used in combination with later sowing dates;
- to avoid using inappropriate and adapted corn planters, which are often used, especially among younger producers;
- avoiding the standard way of fertilizing, where 2/3 of the required quantity of nitrogenous fertilizers is added by feeding during the vegetation. In this case, the amount of nitrogen fertilizers should be increased before sowing and only 1/3 during the vegetation, in the corresponding phenophases of the crop development, etc.

CONCLUDING REMARKS

Based on the above, the following conclusions and findings can be drawn:

- Corn production in the Republic of North Macedonia (2019/21) amounts to 140,910 tons of grain.
- Domestic production meets about 75 to 80% of needs. The remaining quantity is still provided by imports.
- In order to achieve a higher and stable production, to a large extent, in addition to the soil and climate conditions of the region, it is necessary to improve the degree of applied agricultural technology.
- In order to overcome the dependence on the import of corn grain, all efforts and measures should be aimed at increasing the average yield per unit area.
- Corn production without intervening irrigation carries a certain degree of risk in cultivation.
- The proposed measures are an option and opportunity for areas that do not have real possibilities for intervention irrigation in critical phenophases of the growth and development of corn.

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ПРОИЗВОДСТВО НА ПЧЕНКА ВО РЕПУБЛИКА СЕВЕРНА МАКЕДОНИЈА И МОЖНОСТИ ЗА НЕЈЗИНО ОДГЛЕДУВАЊЕ БЕЗ ИНТЕРВЕНТНО НАВОДНУВАЊЕ

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Резиме

Пченката е едногодишно растение од подгрупата на просовидни жита. Во Република Северна Македонија постојат релативно добри почвено-климатски услови за нејзино производство. Таа е трета житна култура по застапеност на ораничните обработливи површини, по пченицата и јачменот. Во 2021 година се произведени 130 769 тони пченка. Овие количини на пченка добиени од производство на пченка на земјоделски површини во Република Северна Македонија не ги задоволуваат домашните потреби. Вкупното годишно производство на зрно од пченица, јачмен и пченка во 2021 година изнесувало 526 045 тони. Од останатите житни растенија 'ржта, оризот и овесот се произведуваат во многу помали количини.

Имајќи ги предвид агротехничките мерки кои се применуваат во производството на пченка и почвено-климатските услови кои преовладуваат во Македонија, во трудот е даден посебен осврт на проблемите со кои се соочува ова производство, како и можностите и мерките за нејзино производство без интервентно наводнување.

Клучни зборови: *Zea mays, принос, мерки, наводнување, зрно.*

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USING BBCH SCALE AND GROWING DEGREE DAYS TO IDENTIFY THE GROWTH STAGES OF WINTER OILSEED RAPE GENOTYPES IN THE SKOPJE REGION

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Abstract

Identifying the growth stages on oilseed rape accurately is essential for effective crop management. Two commonly used methods for identifying growth stages are growing degree days (GDD) and BBCH scale, by measuring the heat accumulation on daily temperatures and describes the growth stages of plants. The main goal of this research is using a combination of these methods, where can identify the growth stages in production period. The three-year field experiments 2015/16 - 2017/18 were located in the Skopje Region, with two genotypes in 30 variants and 4 replications. Sowing was on October 1, with 8 kg ha^{-1} seeding rate. BBCH scale for oilseed rape was used to register the stages of development. Growing degree days - GDD were determined by the formula with corrections for Tmax and Tmin values calculated. Germination (09 BBCH), was 7 days in the first and third year and 79 ^{0}C - 65 ^{0}C GDD and 8 days in the second year - 65 ^{0}C GDD. The flowering (63 BBCH), begins at 202 days in the first, - 809 ^{0}C GDD, 199 days in the second year – 649 ^{0}C GDD, and 198 days third year with 633 ^{0}C GDD. Senescence (BBCH 97), began on days 254, with accumulate 1530 ^{0}C GDD, days 258 – 1577 ^{0}C GDD, and days 265 with 1542 ^{0}C GDD in 3, 1 and 2 years. All data obtained from the research are aimed at meeting the needs of producers and researchers related to rapeseed production in order to ensure optimal production.

Key words: *oilseed rape, GDD, BBCH-scale, heat accumulation, temperatures*

INTRODUCTION

The development of oilseed rape from the emergence of cotyledons to the flowering stage is controlled by photo-thermal factors and temperature from flowering to full maturity (Nanda et al., 1995). In winter genotypes, the initial stages of development, including sprouting, leaf development, and stem elongation, last the longest in terms of time (in days). When conditions are optimal, temperature is the main factor on which the dynamics of germination and sprouting of plants depends. At temperatures below 10°C, germination is slow (Ehrensing, 2008), which is why seed yield is often limited as a result of initial poor plant growth (Yang et al., 2014). From the beginning of leafs development formation to the end of the stem elongation stage, the timing of individual stages is controlled by temperature, vernalization stage, and photoperiodism (Böttcher et al., 2016). The optimal temperatures for the growth and development of oilseed rape (photosynthesis, vegetative, and generative stages) have been determined to be between 21-25°C (Deligios et al., 2013). The Skopje Valley is located in the Vardar region, in which a modified Mediterranean type of climate prevails (Filipovski et al., 1996), or a dry, cold semi-arid climate (BSk) according to the Köppen-Geiger climate classification (Beck, et al. 2018).

MATERIAL AND METHODS

In a three-year research, the stages of development were represented by their occurrence and duration, presented in days. Due to the simultaneous entry of plants into the stages for the entire period of vegetation, results were shown that applied to both genotypes respectively. The BBCH oilseed rape scale was used to register them (Weber & Bleiholder, 1990). Meteorological data were provided by Macedonian Hydrometerological service, from the free database Reanalysis data NOAA/OAR/ESRL PSD, Boulder, Colorado, USA, and as well from the wunderground database. Sowing was done on October 1, with a sowing rate for both genotypes of 8 kg ha^{-1}. Two genotypes of oilseed beet were used (variety Zorica, hybrid Rohan). The sum of active temperatures was obtained as a product of the average monthly temperatures and the number of days in the month during the vegetation period of the genotypes. The sum of GDD - growing degree days was determined from the values of the daily maximum and minimum temperatures and the base temperature, which for oilseed rape is 5 °C, during the vegetation, from the beginning to the end of development, according to the formula (Gordon and Bootsam 1993): GDD = (Tmax + Tmin) / 2 - Tbase (Tmax: daily maximum temperature, Tmin: daily minimum temperature, Tbase: base temperature (5 °C) (Vigil et al. 1997). In the calculation of GDD, if the maximum daily temperature exceeds 30 °C, the value for Tmax is corrected to 30 °C, while if the daily minimum temperature falls below 5 °C, the value for Tmin is corrected to 0 °C (Djaman et al. 2018).

RESULTS AND DISCUSSION

Development Stages according to BBCH scale

The stage of germination and sprouting in the first and third year was seven days, in the second eight days, data which is also confirmed in the research of Ferguson (2015). According to this research, under favourable agroecological conditions, sprouting occurs in seven days.

From germination to the appearance of two true leaves, plants took 7 and 8 days (I II and III year). In both genotypes, the appearance of four true leaves was ascertained on the 28th day from sowing in the year I and II and on the 30th day in the year III. In substage the four to six leaf substage was recorded on the 48th day in the first vegetation year, the 49th in the day second, and the 46th day in the third. The plants entered the leaf development stage with nine or more leaves formed in 86 days in the first, 85 days in the second, and 79 days in the third vegetation year. If the plants do not have sufficiently formed leaves before the onset of the winter months, the low temperatures and low light intensity during the winter can cause serious losses of the above-ground mass, and thus of the accumulated nitrogen, as well as of the leaf area index (Colnenne et al. 1998, Sierts et al. 1987).

The plants entered the stem elongation stage and the substage of two visible internodes in 167 days in the first, 168 days in the second, and 165 days in the third year, with a time difference of 81, 83, and 86 days after the leaf development stage.

The formation of flower buds in an inflorescence (visible flower buds green bud), started after the 178th day in the first, 177th day in the second, and 175th day in the third year, so that in the second substage, when the buds turned yellowish, the plants entered 8 or 10 days later.

The beginning of the flowering substage, when 30% of the flowers were opened, began on the 202nd day in the first, 199th day in the second, and 198th day in the third year of research, while the mass flowering substage was registered on the 207th day in the first, 211th day in the second, and 204th day in the third year. This stage is the most critical in the development of oilseed rape due to the reduction of total leaf area and reduced photosynthesis (Gabrielle et al.,1998; Robelin and Triboli, 1983). Minus temperatures at the beginning of flowering affect the intensive differentiation of plants, flowering lasts longer and fewer weakly developed fruits are formed on the lower branches (Balodis and Gaile 2016). On the other hand, intense rains in the flowering stage of the plant can have a negative effect on seed yield and, despite the formation of a larger biomass during flowering (Takashima et al., 2013). If there is a lack of water in the period after flowering in oilseed rape, the seed yield will be lower as a result of intensive transpiration and

the inability of the plants to meet the required amounts of water (Weymann et al. 2015).

The substage when 80% of the fruits were ripe and when the seeds acquired a black color began on day 251 in the first, day 259 in the second, and day 248 in the third year. In the seed formation stage, temperature has a significant influence on the yield potential (Balodis and Gaile, 2016).

Table 1. Stages of development and length of vegetation period (in days).

Stages description	BBCH	stages and substages - days		
		2015/16	2016/17	2017/18
Germination		7	8	7
Emergence: cotyledons emerge through soil surface	09	7	8	7
Leaf developmen		7	7	8
2 leaves unfolded	12	14	15	15
		14	13	15
4 leaves unfolded	14	28	28	30
		20	21	16
4 to 6 leaves unfolded	18	48	49	46
		38	36	33
9 or more leaves unfolded	19	86	85	79
Stem elongation		81	83	86
2 visibly extended internodes	32	167	168	165
Inflorescence emergence		11	9	10
Flower buds visible from above (“green bud”)	51	178	177	175
		9	8	10
First petals visible, flower buds still closed (“yellow bud”)	59	187	184	185
Flowering		15	15	13
30% of flowers on main raceme open	63	202	199	198
		5	12	6
Full flowering: 50% flowers on main raceme open	65	207	211	204
		7	8	6
End of flowering	69	213	219	210
Development of fruit		7	7	7
50% of pods have reached final size	75	220	226	217
		6	6	7
Nearly all pods have reached final size	79	226	232	224
Ripening- seed		20	21	18
50% of pods ripe, seeds dark and hard	85	246	253	242
		5	6	6
80% of pods ripe, seeds dark and hard	88	251	259	248
		7	6	6
Senescence - Plant dead and dry	97	258	265	254
Harvest		6	6	7
Length of vegetation period		264	272	261

The potential yield can be finally determined at the end of the flowering stage, but whether it will be achieved or not to a large extent depends on the temperature and the availability of water in the successive stages of crop development (Weymann et al. 2015).

From the sub- stage 80% ripe fruits in the ripening stage, the plants entered for 7 or 6 days. The ripening stage began on day 258 in the first, day 265 in the second, and day 254 in the third year.

The length of the vegetation period for the winter genotypes of oilseed rape ranged from 260 to 310 days (Димов, 2014; Mustapić, 1982). Represented by years in the first year of the research, the length of the vegetation period was 264 days, in the second 272 days, and in the third year 261 days for both genotypes (Table 1).

Table 2. Days stages/vegetation, sum on GDD ^{0}C stages/substages and vegetation.

Year	2015/16			2016/17			2017/18		
Stages description (BBCH Code)	Days stages/ vege.	GDD (^{0}C) stages	GDD (^{0}C) vege.	Days stages/ vege.	GDD (^{0}C) stages	GDD (^{0}C) vege.	Days stages/ vege.	GDD (^{0}C) stages	GDD (^{0}C) vege.
Germination / Emergence cotyledons emerge through soil surface (09)	7/ 7	79	79	8/8	67	67	7/7	65	65
Leaf development									
2 leaves unfolded (12)	7/14	72	151	7/15	52	119	7/15	65	130
9 or more leaves unfolde (19)	72/ 86	231	382	70/85	190	309	64/79	199	329
Stem elongation 2 visibly extended internodes (32)	81/167	162	543	83/168	98	406	86/165	87	416
Inflorescence emergence (51-59)	11/178	32	575	9/177	76	482	10/175	35	451
Flowering									
30% of flowers on main raceme open (63)	24/202	234	809	22/199	168	649	23/198	183	633
Full flowering (65)	5/207	40	849	12/211	72	721	6/204	76	699
Development of fruit									
50% of pods have reached final size (75)	13/220	100	949	15/226	175	896	13/217	160	762
Nearly all pods have reached final size (79)	6/226	66	1015	6/232	72	968	7/224	267	966
Ripening – seed									
50% of pods ripe, seeds dark and hard (85)	20/246	235	1250	21/253	292	1260	18/242	255	1220
80% of pods ripe, seeds dark and hard (88)	5/251	75	1325	6/259	100	1360	6/248	97	1317
Senescence plant dead and dry (97)	7/258	104	1429	6/265	89	1449	6/254	106	1423
Harvest time	6/264	102	1530	7/272	128	1577	7/261	119	1542
Vegetation length / Total GDD	264	1 530		272	1 577		261	1 542	

Growing degree days GDD - Accumulated sum of effective temperatures

The phenological development of plants is linked to the level of temperature or thermal accumulation, or the base temperature above which active growth begins in most plants. Temperature is an important factor that provides for the growth and development of plants, although it is conditioned by other climatic factors (humidity, solar radiation, day length, etc.) (Iljovski, 2012). The temperature effects on plant development are observed through the measurement of the sum of effective temperatures (GDD) or accumulated heat and temperature of the culture over a certain period of time/stage. In the Skopje Valley, annual sums of temperatures are 4410 ^{0}C (Filipovski et al., 1996), of which, based on the results from this research and the length of the winter oilseed rape vegetative period, 35% account for the total accumulated effective temperatures. The need for satisfactory effective temperatures is especially

In the development stage of two true leaves and the beginning of leaves development formation, the plants entered for approximately 14 and 15 days, with accumulated 151 GDD ^{0}C, 119 GDD ^{0}C and 130 GDD ^{0}C. For 13-16 days from sowing, the required accumulated temperature in the research of Martinez-Feria (2015) ranges from 129-156 ^{0}C, which coincides with our research both in calendar days and accumulated temperature. Wittman (2005) in (Arizona, USA) determined 237-314 GDD ^{0}C, while Miller et al., (2018) from 282-324 GDD ^{0}C. Martinez-Feria (2015) reported that from emergence to development of the five-leaf stage, 323 and 374 GDD ^{0}C were accumulated, which in our research coincided with the formation of 9 leaves (19 BBCH) in the first and third vegetative years, in which GDD amounted to 382 ^{0}C and 329 ^{0}C, sums that had significance in the development of the plants for overwintered. According to Estonian conditions, the best wintering of oilseed rape was obtained when sowing was done on August 15, ant the plants had accumulated 416 GDD ^{0}C at the end of the five-leaf stage. At early (August 8) and late sowing (August 29), the plants accumulate 500 GDD ^{0}C and 300 GDD ^{0}C, and only 50% of the plants overwintered (Lääniste et al. 2007). These findings were confirmed in our research, in which in the second vegetative year, when the genotypes accumulated the lowest amount of only 309 GDD ^{0}C in the five-leaf stage and 406 GDD ^{0}C in the stem elongation stage, the percentage of plants that did not overwinter was the highest (17.8%).

expressed in the stages of development: germination, onset of flowering and full maturity (Balodis et al., 2011). The number of days from sowing to germination is relatively identical, with accumulated effective temperatures amounting to 79 GDD ^{0}C (year I), 67 GDD ^{0}C (year II) and 65 GDD ^{0}C (year III). The values obtained were lower than those of Martinez-Feria (2015), who, upon sowing on October 1 in 2012, for this stage established 129 GDD ^{0}C, while in 2013, 156 GDD ^{0}C. Somewhat higher values of 152-186 GDD ^{0}C were determined in Montana USA in the period 1995-1998 (Miller et al. 2018). On the other hand, in conditions in Lithuania, upon sowing of September 1, the GDD value for the final stage of flowering in the first year (2009) was 60 GDD ^{0}C, and 38 GDD ^{0}C in 2010, (Balodis & Gaile, 2011). Data on required GDD for early stages of autumn genotypes of Brassica napus indicate a requirement of 90 to 115 ^{0}C, obtained with a base temperature (Tbase) of 4 ^{0}C (Vigil et al. 1997).

Already in the formation stage of flower buds (inflorescences) for the year II, the GDD sum (482 GDD ^{0}C) was equal to that of the year III (451 GDD ^{0}C), but both years had registered lower sums compared to the year I (575 GDD ^{0}C), due to the high average monthly (8.4 ^{0}C) and average monthly maximum (14.8 ^{0}C) temperatures in February that year.

In the flowering stage and the substage of 30% fully open flowers, the plants accumulated effective temperatures of 809 GDD ^{0}C (year I), 649 GDD ^{0}C (year II), and 633 GDD ^{0}C (year II). This condition is maintained in the full flowering substage with 849 GDD ^{0}C - year I, 721 GDD ^{0}C - year II, 699 GDD ^{0}C - year III. The high values in the first year are the result of high average monthly and maximum temperatures in February (8.4 ^{0}C, 14.8 ^{0}C) and March (8.1 ^{0}C, 14.1 ^{0}C). According to other research, for this stage and substage 50 % flowering accumulated effective temperatures range from 759 to 852 ^{0}C (Miller et al. 2018), but also higher GDD sums, 1153-1218 GDD ^{0}C - year I and 1259-1322 GDD ^{0}C - year II (Begna and Angadi 2016), differences in GDD values which are obtained due to the location.

In the flowering stage, accumulated effective temperatures are stabilisate, and approximately the same amounts of 1250 GDD ^{0}C, 1260 GDD ^{0}C, and 1220 GDD ^{0}C (I, II and III year) were obtained. In the same stage, another research (Miller et al. 2018) shows slightly higher effective temperatures from 1326 to 1445 GDD 0C.

Finally, the sums of accumulated effective temperatures for the genotypes yielded 1530 ^{0}C, 1577 ^{0}C, and 1542 ^{0}C (I, II and III year), obtained values that are close to another research where GDD ranges from 1432 to 1557 ^{0}C (Miller et al. 2018), but also lower than the 2283 GDD ^{0}C obtained in research with a longer vegetation (sowing early September - harvesting mid/ late July) (Djaman et al. 2018). The impact of temperature conditions in the year as key factors that determine the values of accumulated effective temperatures has great importance for the development of the oilseed rape during the respective stages or for the entire period of vegetation.

CONCLUSION

In a modified Mediterranean-type climate, or dry, cool semi-arid climate (BSk) the accumulated sum of effective temperatures for a significant stages are: from 119 to 151 GDD ^{0}C for stages leaf development and substage 2 leaves (12 BBCH), 406 to 543 GDD ^{0}C for substage 2 visibly extended internodes (32 BBCH), 575 GDD ^{0}C emergence stages to flowering, 633 to 809 GDD ^{0}C for flowering or 30% of flowers on main whol open (63 BBCH), 762 to 949 GDD ^{0}C for 50% of pods to reach final size (75 BBCH), from 1220 to 1260 GDD ^{0}C in stage 50% of pods ripe, seeds dark and hard (85 BBCH) and 1530 to 1577 GDD ^{0}C for the vegetation period. Displaying and identifying growth stages using the BBCH scale in combination with growing degree days (GDD) we believe will be important and useful for producers of winter oilseed rape genotypes.

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УПОТРЕБА НА BBCH СКАЛАТА И GDD МЕТОДОТ ЗА ИДЕНТИФИКАЦИЈА НА УПОТРЕБА НА BBCH СКАЛАТА И GDD МЕТОДОТ ЗА ИДЕНТИФИКАЦИЈА НА ФАЗИТЕ НА РАСТ НА ЗИМСКИ ГЕНОТИПОВИ МАСЛОДАЈНА РЕПКА ВО СКОПСКИОТ РЕГИОН

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Резиме

Идентификувањето на фазите на раст на маслодајната репка е од суштинско значење за ефикасно управување. Два често користени методи за идентификација на фазите и степенот на раст, BBCH скалата и GDD, се корисни за мерење на акумулираната топлина од дневните температури и опишување на фазите на раст на растенијата. Главната цел на истражувањето е користење на комбинација од овие методи за предикција на фазите на раст за целиот период на вегетација. Тригодишните опити 2015/16 – 2017/18 беа лоцирани во Скопскиот Регион, со два генотипа поставени на 30 варијанти и 4 повторувања. Сеидбата е изведена на 1 октомври, со сеидбена норма од 8 $kgha^{-1}$. За регистрирање на фазите на развој се користеше BBCH скалата за маслодајна репка. GDD методот беше одреден преку формула и пресметани корекции за Tmax и Tmin вредности. Никнувањето/'Ртењето (09 BBCH) започна за 7 дена во првата и во третата година – 79 ^{0}C – 65 ^{0}C GDD, а за 8 дена во втората година – 65 ^{0}C GDD. Цветањето (63 BBCH) започна за 202 дена во првата година – 809 ^{0}C GDD, 199 дена во втората година – 649 ^{0}C GDD и 198 дена во третата година – 633 ^{0}C GDD. Полната зрелост (BBCH 97) започна за 254 дена во првата година со акумулирани 1530 ^{0}C GDD, 258 дена во третата година – 1577 ^{0}C GDD и 265 дена со 1542 ^{0}C GDD во втората година. Добиените резултати се во насока на задоволување на потребите на производителите и истражувачите поврзани со производството на маслодајна репка со цел обезбедување на оптимално производство.

Клучни зборови: *маслодајна репка, BBCH, GDD, акумулирана топлина, температури.*

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ISOLATION, SCREENING AND CHARACTERIZATION OF CELLULOLYTIC BACTERIA FROM DIFFERENT SOIL SAMPLES FROM PELAGONIA REGION

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Abstract

Cellulose is the most abundant renewable natural product in the biosphere, so cellulolytic microorganisms are fundamental for the transformation of cellulose into sugars that are essential nutrients for various organisms and for biofuels. Additionally, since the annual production of cellulose is estimated at 4.0 x 10^7 tons, large quantities of industrial and agricultural cellulosic waste have been accumulated due to inefficient use. Different kind of soils could potentially support several microbes with potent cellulolytic enzyme activities and therefore the exploration of those communities could be useful for biotechnology as well as for ecological conservation.

The goal of this study was to conduct a survey for bacteria with cellulolytic potential, isolated from soils originating from Pelagonia Region. To select microorganisms with cellulolytic potential, qualitative cellulolytic activity was determined by culturing microorganisms in media containing cellulose as the only carbon source. After screening, fifteen colonies were isolated capable of degrading cellulase. Determination revealed the isolates were identified as *Bacillus spp., Bacillus weihenstephanensis, Pseudomonas putida* and *Staphylococcus spp*.

This study gives an overview of the potential microorganism that could be used for cellulose degradation in various biotechnological applications and for sustainable agricultural waste treatment.

Key words: *bacteria, enzymes, agricultural waste, Bacillus*

INTRODUCTION

Cellulose, which is part of the lignocellulosic biomass, is a common and easily accessible polymer in natural environment. It is an organic compound, which is the main component of plant cell walls. It creates the most resistant and stable skeleton built from cellulose fibrils, so called microfibrils and macrofibrils. Hydrogen bonds occurring between the neighbouring hydroxyl groups and Van der Waals forces ensure stabilisation of cellulose fibres and the required conformation of glucose particles. Its content in plants depends on their age, plant type and parts. For example, it is 45-50 % in leafy stems, 40-55 % in woody stems and 15-20 % in leaves (Reddy et al., 2017; Poszytek et al., 2016; Juturu et al., 2014; Eveleigh et al., 2009).

As the main source of carbon generated by photosynthesis, cellulose is a nutrient for cellulolytic microorganisms using lignocellulosic biomass. Cellulose decomposition undergoes through hydrolysis of β-1,4 glycoside bonds. However, due to stabilisation of cellulose microfibres with these bonds, cooperation of several enzymes is required for effective decomposition. Cellulose is mineralised by cellulolytic microorganisms belonging to various groups: fungi, aerobic and anaerobic bacteria (Juturu et al., 2014; Singhania, 2009; Lynd et al., 2002; Bayer, 1998).

Cellulolytic enzymes can be divided into three groups, taking into consideration their structure, enzymatic activity, specificity and

active centres. Cellulolytic enzymes are made of the so-called domains - catalytic and binding carbohydrates (CBM, Cellulose Binding Module) and have a characteristic shape active point, which determines their activity. CMB domain influences binding with cellulose surface and is located on the C-terminus of enzyme, connected with the catalytic domain at N-terminus. These enzymes (endoglucanases and exoglycanases) act in a complex way, resulting in shortening of cellulose chain, single units of cellobiose and glucose. β-glucosidase is part of cellulolytic enzymes complex. The enzyme does not attack cellulose but it decomposes cellobiose and reduces its blocking activity towards cellobiohydrolase and endoglucanase (Lugani et al., 2015; El-Hadi et al., 2014; Berlin, 2013; Horn et al., 2012; Shuangqi et al., 2011; Eveleigh et al., 2009; Singhania, 2009; Lynd et al.; 2002).

Considering this huge significance of cellulase and keeping the above in sight, the present work aimed to isolate and characterize bacterial isolates that were collected from five different soils of different areas of Pelagonia region district with high cellulase-producing ability determining by the zone size diameter around the colony degrading cellulose.

MATERIAL AND METHODS

Collection of soil samples

Soil samples were collected from different locations in Pelagonia region. Samples from five different areas namely leafy forest, winter green forest, stubble, meadow and compost were collected (Figure 1). Samples were collected 15-20 cm below the surface. Soil was collected with a sterile spatula and transported in sterile bags in a refrigerator box with ice to the laboratory of the Department of Microbiology and Microbial Biotechnology at the Faculty of Natural Sciences and Mathematics, where a laboratory was accessed within 24 hours after the material was collected (Figure 2).

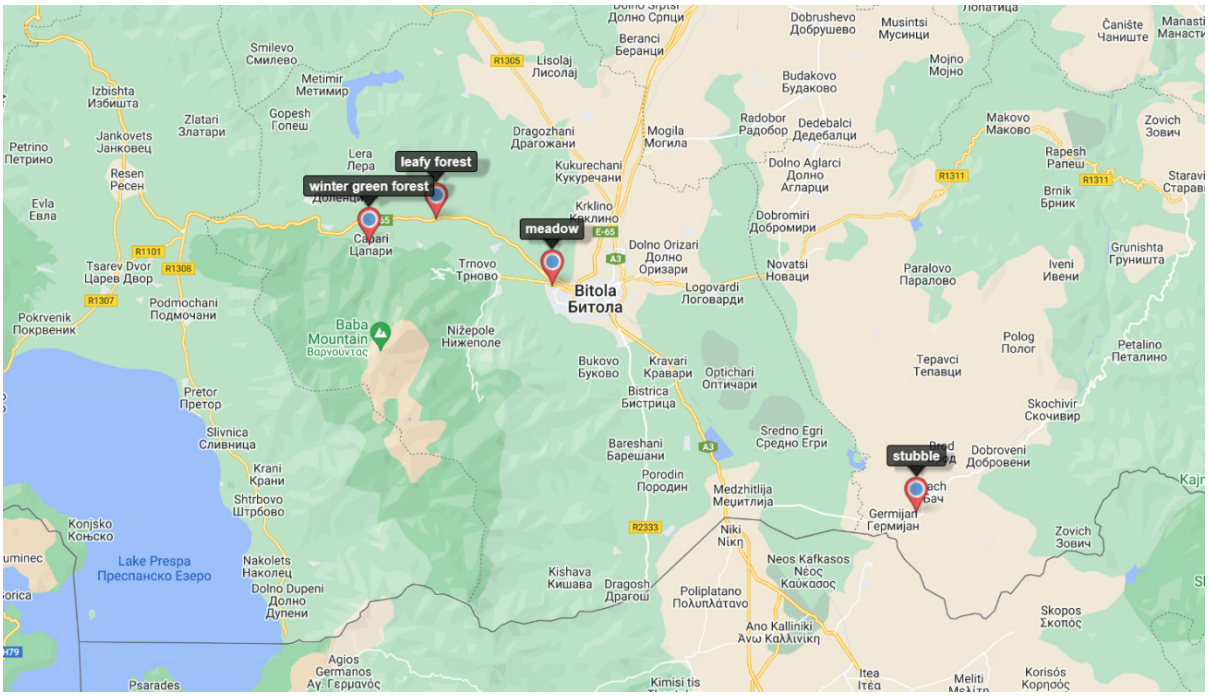

Figure 1. Locations of different areas namely leafy forest, winter green forest, stubble, meadow.

Figure 2. Soil sample collection

Screening of cellulolytic bacteria

In CMC agar plates, pure cultures of bacterial isolates were individually transferred. CMC agar plates were flooded with 1% Congo red and allowed to stand for 15 min at room temperature after 48 hours incubation. For counterstaining the plates, one molar NaCl was thoroughly applied. Around growing bacterial colonies, clear zones were appeared that was indicating cellulose hydrolysis taken place. For identification and cellulase production, the bacterial colonies were selected that having the largest clear zone (Gomashe et al., 2013) (Figure 3).

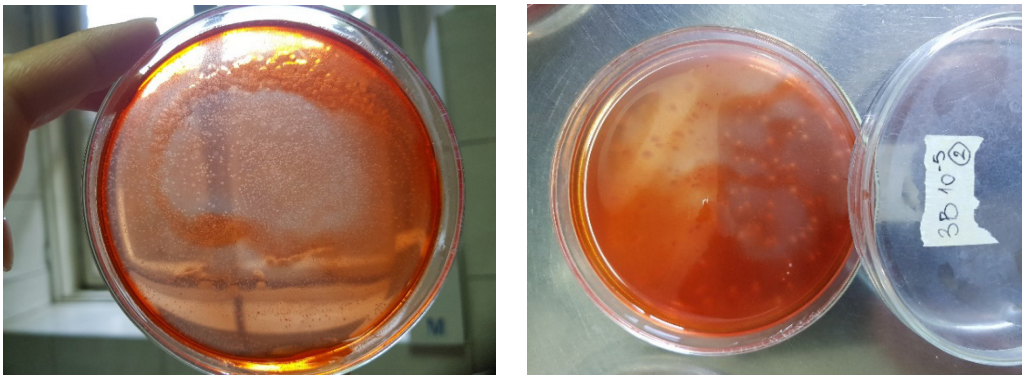

Figure 3. Clear zones around growing bacterial colonies

Secondary screening of cellulolytic bacteria

The selected cellulolytic bacteria isolates were cultured at 37°C at 150 rpm in a selective media composed of KH_2PO_4 0.5 g, $MgSO_4$ 0.25 g, and gelatine 2 g, distilled water 1 L and containing Whatman filter paper No.1 (1 × 6 cm strip, 0.05 g per 20 mL) and at pH 6.8–7.2. Broth culture after three days of incubation period was subjected to centrifugation at 5000 rpm for 15 min at 4°C. Supernatant was collected and stored as crude enzyme preparation at 4°C for further enzyme assays. Pellet recovered after centrifugation of broth culture was subjected to gravimetric analysis in order to determine the residual cellulose of filter paper (Tailliez et al., 1989).

Microscopic examination of bacterial morphology

Gram staining

Gram positive (+ve) and Gram negative (-ve) bacteria were distinguished through a differential staining method called Gram staining. Gram (+ve) cells appeared purple and Gram (-ve) cells were pink or red when the cells were examined under the light microscope. The examination of Cell morphology was also done and noted (Gomashe et al., 2013).

Biochemical Characterization

The colonies of the isolates were identified by performing various biochemical tests. Citrate utilization test, methyl red test, Voges-Proskauer (VP) test, Motility test, Catalase test, Triple sugar iron agar (TSI) test were performed, for Gram (+ve) bacteria and Gram (-ve) bacteria. All the tests were carried out according to the standard protocol as described in Bergey's Manual of systematic Bacteriology (Bergey *et al*., 1984).

Microbial identification by MALDI-TOF/ Saramis

Single cell colony from agar plate (incubated from 16-24 h) is transfer to the MALDI steel plate, Axima 384x2.8mm target plate (DE1580TA, Kratos Analytical Limited and Shimadzu Corporation). The cells are immobilized with addition of 1 µl matrix (40 mg/ml α-Cyano-4-hydroxycinnamic acid (CHCA) in water/acetonitrile/ethanol (1:1:1) with 0.03% trifluoroacetic acid) and air-dehydrated within 10–15 min at room temperature. The reference strain Escherichia *coli* DH5α is used as a standard for calibration and as reference for quality control.

The protein mass profiles (spectrum) is obtained using Linear acquisition mode of the MALDI-TOF-TOF mass spectrometer (Axima Performance, Shimadzu Corporation), with laser power of 56 V, frequency of 50 Hz and data acquisition range from 2 000 to 20 000 Da. Peak list obtained in form of ASCII file is transferred directly into the SARAMIS software, where the pattern is compared against the SARAMIS database and subsequently the microorganism is identified up to species level.

RESULTS AND DISCUSSION

Isolation and Screening of Cellulase Producing Bacteria

A total of 15 positive isolates of cellulase producing bacteria were obtained from five different soil samples. Out of these, 15 isolates showed maximum zone of clearance after staining with Congo red dye. A list of all the isolates along with area of clearance zone is given in Table 1. As shown in Table 1, isolates from the soil of winter green forest were coded as 1A 1, 1A 7, 1A 8 and isolates from the soil of leafy forest were coded as 2A 1A, 2A 4A. The bacteria isolated from the soil of meadow were coded as 4A 2, 4A 4, 4A 5, 4A 7, 4B 2. The isolates from the compost were named as 5A 4, 5A 8, 5B 1, 5B 2. The only isolate from the soil of stubble was named as 3B 1. This research indicates that the soil from meadow and compost will be effective in producing cellulase enzyme by different cellulolytic bacteria.

Table 1. Various 15 isolates from five different soil samples

S. no.	Isolates code	Type of soil
1	1A 1	Winter green forest
2	1A 7	Winter green forest
3	1A 8	Winter green forest
4	2A 1A	Leafy forest
5	2A 4A	Leafy forest
6	3B 1	Stubble
7	4A 2	Meadow
8	4A 4	Meadow
9	4A 5	Meadow
10	4A 7	Meadow
11	4B 2	Meadow
12	5A 4	Compost
13	5A 8	Compost
14	5B 1	Compost
15	5B 2	Compost

Secondary screening of cellulolytic bacteria

Secondary screening for cellulase activity on filter paper was found to be highest for 2A 1A, 4A 2, 4B 2 and 5B 1 (Figure 5). A list of all the isolates along with the weight of filter paper after incubation is given in Table 2. Gravimetric analysis shows that maximum and minimum rates of filter paper degradation were 37% and 20%, respectively, estimated at third day of incubation. Figure 4 shows that 5B 1 has highest filter paper degradation rate of 37%. In a result documented by Lu et al., 2004, the data for synergetic cellulose degradation detected in four groups of mixed cultures were only 23.5%, 26.3%, 19.4%, and 24.5%, respectively. Bichet-Hebe et al., 1999, reported the rates of paper degradation ranged from 31 to 60% after 10 days for mixed bacterial populations by gravimetric procedure.

Table 2. Four isolates that showed more cellulase production on secondary screening

Isolate code	Weight of filter paper
Control filter paper	0,0414 g
2A 1A	0,0402 g
4A 2	0,0400 g
4B 2	0,0402 g
5B 1	0,0392 g

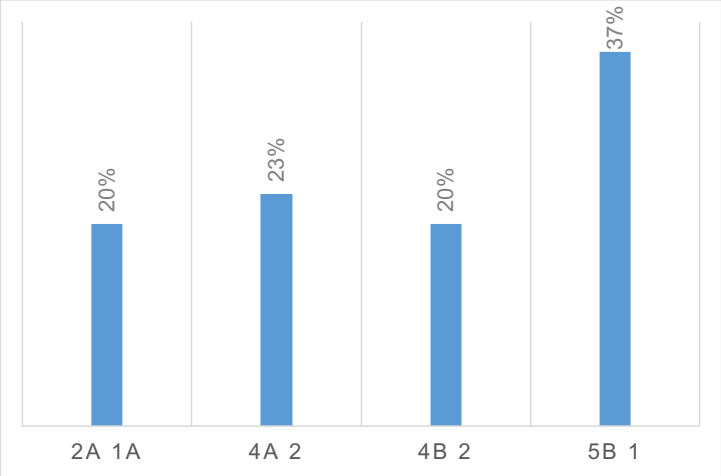

Figure 4. Percent filter paper degradation by various bacterial isolates by gravimetric method. Maximum percentage of filter paper degradation was found to be 37% by 5B 1.

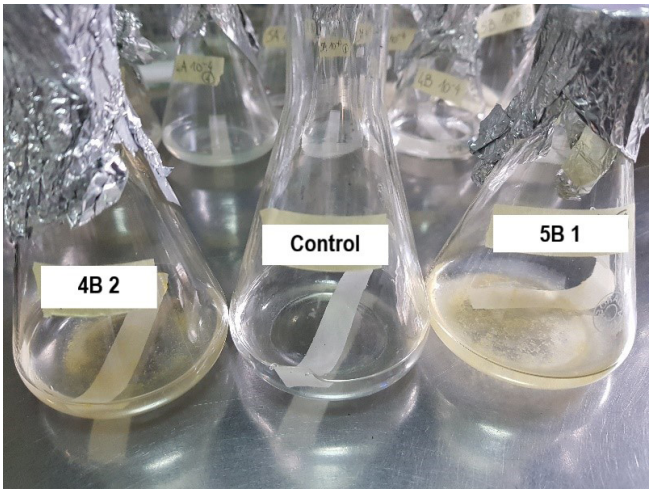

Figure 5. Filter paper degradation by isolates 4B 2 and 5B 1 cultured in selective medium supplemented with Whatman filter paper no.1 (1 × 6 cm strip × 2, 0.05 g per 20 mL) at the end of 96 hours of incubation. Flask control is the control for this experimental set up and does not show any filter paper degradation.

Microscopic Observation of the Isolates

All the isolates were examined microscopically after Gram staining to determine whether the isolates were Gram (+ve) or Gram (-ve) and to observe their arrangements. The results showed that sample 4B 2 was Gram (+ve) and samples 2A 1A, 4A 2 and 5B 1 were Gram (-ve) bacteria (Table 3). The colony characteristics of the isolates were found variable. Microscopic observation of the isolates revealed that most of them are rod shaped and motile. By Gram staining, morphological characteristics of different types of colonies of each sample were recorded. Isolates were identified by their microscopic examination and biochemical reaction.

Biochemical Identification

In this work, 4 isolates were found from 15 samples. All the isolates were subjected to different biochemical tests for their identification (Table 3).

Table 3. Morphological and biochemical test results of isolates

S. no.	Isolates code	Gram staining	Citrate utilization test	Methyl red test	Voges-Proskauer test	Motility test	Catalase test	Triple sugar iron test
1	2A 1A	- ve	- ve	- ve	- ve	+ ve	+ ve	- ve
2	4A 2	- ve	- ve	- ve	- ve	+ ve	+ ve	- ve
3	4B 2	+ ve	+ ve	- ve	- ve	+ ve	- ve	- ve
4	5B 1	- ve	- ve	+ ve	- ve	+ ve	+ ve	- ve

Microbial identification by MALDI-TOF/Saramis

Microbiological identification was performed only for 4 isolates that proved to be the most active isolates in the process of cellulose degradation in the in-vitro tests. On the basis of their morphological, biochemical characterization and microbial identification these isolates were confirmed as *Pseudomonas putida* (Isolate 2A 1A, 4A 2 and 5B 1) and *Bacillus weihenstephanensis* (Isolate 4B 2) (Table 4). According to Li et al. (2022), housefly larvae can considerably accelerate the breakdown of cellulose in silkworm faeces. In comparison to the groups (11.5%) without housefly larvae, the cellulose breakdown rate in the groups with housefly larvae reached 58.90% in 6 days. Three new cellulose-degrading bacteria, *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, and *Bacillus subtilis*, were isolated from silkworm feces substrates. Wang et al. (2022) However, Wang et al. (2022) demonstrated that exogenous cellulose-degrading bacteria (ECDB) boosted the potential activities and interactions of bacterial communities. Bacillus subtilis WF-8, B. licheniformis WF-11, B. cereus WS-1, and Streptomyces nogalater WF-10 were also identified as the ECDB. This degradation technique may be used to test the biodegradation of natural cellulosic fibres if suitable degrading bacteria are available (Lednicka et al., 2000). It has been discovered that many aerobic bacteria degrade cellulose, making them suitable candidates for use in investigations on the biodegradation of natural fibres.

Table 4. Microbial identification of isolates

Isolates code	Familiy	Genus	Species
2A 1A	Pseudomonadaceae	Pseudomonas	putida
4A 2	Pseudomonadaceae	Pseudomonas	putida
4B 2	Bacillaceae	Bacillus	weihenstephanensis
5B 1	Pseudomonadaceae	Pseudomonas	putida

CONCLUDING REMARKS

Treatment of cellulose by cellulase enzyme from different bacterial isolates has attracted the continuing interest of biotechnologists, taxonomists, enzymologists and even some industrialists in their own researches. This research indicates that the soil from meadow and compost will be effective in producing cellulase enzyme by different cellulolytic bacteria.

A total 15 isolates showed cellulase production on primary screening. Ultimately four isolates showing more cellulase production were identified as *Pseudomonas putida* and *Bacillus weihenstephanensis*.

The use of these cellulolytic bacteria as bio-inoculants can be incorporated to enhance organic matter decomposition in soil to increase soil fertility and to minimize the fertilizer application in the area of Pelagonia. These bacteria can also be applied to reduce the environmental pollution and promote sustainable agriculture.

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ИЗОЛАЦИЈА, СКРИНИНГ И КАРАКТЕРИЗАЦИЈА НА ЦЕЛУЛОЛИТИЧКИ БАКТЕРИИ ОД РАЗЛИЧНИ ПОЧВЕНИ ПРИМЕРОЦИ ОД ПЕЛАГОНИСКИОТ РЕГИОН

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Резиме

Целулозата е најзастапена, обновлива, природна материја во биосферата, а целулолитичките микроорганизми имаат фундаментално значење за трансформацијата на целулозата во шеќери кои се основни хранливи материи за различни организми, а исто така и за создавање на биогорива. Дополнително, бидејќи годишното производство на целулоза се проценува на 4,0 x 10^7 тони, се акумулира големо количество на индустриски и земјоделски целулозен отпад поради неговата неефикасна употреба. Различни видови почви можат да поддржат неколку видови микроорганизми со потенцијални целулолитички активности и затоа истражувањето на овие заедници би можело да биде корисно за биотехнологијата, како и за еколошката конзервација.

Целта на ова истражување е да се изолираат и детерминираат бактерии со целулолитички потенцијал, изолирани од почви кои потекнуваат од Пелагониискиот Регион. За да се селектираат микроорганизми со целулолитички потенцијал, квалитативната целулолитичка активност се одредува со култивирање на микроорганизмите во медиуми кои содржат целулоза како единствен извор на јаглерод. По извршениот скрининг се изолирани 15 колонии кои се способни да ја деградираат целулозата. Изолатите се детерминирани како: *Bacillus spp, Bacillus weihenstephanensis, Pseudomonas putida* и *Staphylococcus spp*.

Ова истражување дава преглед на потенцијалните микроорганизми кои би можеле да се користат за деградација на целулозата во различни биотехнолошки апликации и за одржлив третман на земјоделски отпад.

Клучни зборови: *бактерии, ензими, земјоделски отпад, Bacillus*.

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MICROBIOLOGICAL QUALITY OF MACEDONIAN WHITE BRINED CHEESE

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ABSTRACT

This scientific paper determined the microbiological quality of Macedonian white brined cheese. Four best variants of Macedonian white brined cheeses were analysed for their microbiological parameters. From pathogenic microflora the following parameters were examined: a total number of: *Coliforms*, *E. coli*, pathogenic *staphylococci*, *Molds*, *Yeast, Listeria* and *Salmonella*. From lactic acid bacteria the following parameters were examined: the total number of *Lactococcus* bacteria and the total number of *Lactobacillus* bacteria. In all examined cheese samples pathogenic bacteria were not detected. *Lactococcus* bacteria was dominant bacteria in all four cheese variants at the beginning of ripening period till day 20. After that and at the end of ripening period (60 day) the Lactobacillus bacteria were predominant.

Key words: *cheese variants, Lactic-acid bacteria, pathogenic bacteria, quality*.

INTRODUCTION

White brined cheese belongs to the group of cheeses that ferment in brine solution in anaerobic conditions. This product is characterized with acid-salty flavour, no rind, usually white colour, but sometimes with yellowish tint, anaerobic brine fermented in plastic cans and pieces which are usually in form of cubes with dimensions 10x10x10 cm, (Veleski, 2015).

The microbiological quality and safety of white brine cheese largely depends on the quality of raw milk, the type of heat treatment applied during milk processing, the degree of microbiological contamination during processing, the salt absorption and its final concentration in water content and also the pH dynamics during ripening period. These factors control the type and the number of microorganisms in the cheese, and play an important role in the safety of white brine cheese, and also affect the metabolic processes that lead to the development of the taste of the final product (Abd El-Salam & Alichanidis, 2004).

The dynamics of the ripening process of white brine cheese are greatly influenced by microorganisms, including bacteria, yeasts and moulds. Their effect can be direct (through their metabolic activity) or indirect (through the release of enzymes into the cheese matrix through autolysis). Pathogens that can be found in cheese during ripening have a negative effect on the quality of the final product, so the used technology should contain precautions to remove or prevent pathogen microorganisms to enter the cheese, (Beresford & Williams, 2004).

In white brined cheese except for non-dairy microflora (yeasts and moulds), micrococci and coliform bacteria can be found. Coliform bacteria usually disappear during the cheese ripening process and participate in the formation of CO_2 and H_2. Yeasts also form CO_2 from lactose, which is responsible for the early gas bloating that usually occurs in cheeses made from unpasteurized milk with poor hygiene, and also in cheeses exposed to high temperatures, (Alichanidis, 2007).

If we want to produce white brined cheese that fulfils all demanded microbiological parameters, we need to use high quality milk, but also hygiene maintenance in the premises where the production process takes place, the personal hygiene of the people involved in the production, the microbiological correctness of the ingredients used, the bins, the water, the salt, the sludge and all items that in any way come into direct contact with the cheese (Makarijoski, 2019).

MATERIAL AND METHODS

In order to make these examinations, cheese samples of four variants were provided from the producers, and were transported in the certified laboratory for testing milk and dairy product quality-LB Lact in Plovdiv, R. Bulgaria. Cheese samples for microbiological quality parameters were analysed at 8th and 60th day. Methods that were used for microbiological analysis of white brined cheese were as follow:

- Determination of total number of *Lactobacillus*, ISO 7889, IDF 117;
- Determination of total number of *Lactococcus*, ISO 7889, IDF 117;
- Determination of total number of *Escherichia colli* (BDS ISO 16649-2:2014);
- Determination of total number of *Staphylococcus* (BDS EN ISO 6888-1:2005/A1:2005);
- Determination of total number of *Listeria monocytogenes* (BDS EN ISO 11290-1:2000/A1:2005);
- Determination of total number of *Salmonella* spp. (BDS EN ISO 6579-1/2017);
- Determination of total number of Coliforms (ISO 4832:2006); и
- Determination of total number of yeasts and molds (BDS ISO 6611:2006).

RESULTS AND DISCUSSION

In order to determine the safety of the product that will be consumed by the final consumer, it was necessary to make a microbiological analysis according to the Rulebook on special requirements for food safety in terms of microbiological criteria (Official Gazette No. 100/2013). The microbiological analyses of the four varieties of white brine cheese were made in two time intervals, on the 8th day after production process (Tab. 1) and on the 60th day after the ripening process was already finalised (Tab. 2). It is of great importance to meet the microbiological criteria for a particular product, in order for it to be safe for people's consumption.

Table 1. Microbiological parameters of examined cheese variants (8 day).

Microbiological parameter / Variant	Coliforms Cfu/g	E. coli Cfu/g	Pathogenic staphylococci Cfu/g	Molds	Yeast	Listeria Cfu/g	Salmonella Cfu/g
Variant No. 1	$8.5x10^2$	$3.1x10^2$	< 10	< 10	$9.9x10^4$	Absence in 25 g	Absence in 25 g
Variant No. 2	$3.9x10^2$	$6.3x10^2$	< 10	< 10	$4.8x10^3$	Absence in 25 g	Absence in 25 g
Variant No. 3	< 10	< 10	< 10	< 10	$6.9x10^4$	Absence in 25 g	Absence in 25 g
Variant No. 4	< 10	< 10	< 10	< 10	$1.2x10^5$	Absence in 25 g	Absence in 25 g

Table 2. Microbiological parameters of examined cheese variants (60 day)

Microbiological parameter / Variant	Coliforms Cfu/g	*E. coli* Cfu/g	Pathogenic staphylococci Cfu/g	Molds	Yeast	*Listeria* Cfu/g	*Salmonella* Cfu/g
Variant No. 1	< 10	< 10	< 10	< 10	< 10	Absence in 25 g	Absence in 25 g
Variant No. 2	< 10	< 10	< 10	< 10	< 10	Absence in 25 g	Absence in 25 g
Variant No. 3	< 10	< 10	< 10	< 10	< 10	Absence in 25 g	Absence in 25 g
Variant No. 4	< 10	< 10	< 10	< 10	< 10	Absence in 25 g	Absence in 25 g

A total of nine parameters were examined to determine the microbiological quality of the four varieties of white brine cheese. Seven of the examined parameters referred to microorganisms that are undesirable in the cheese during and at the end of the ripening period (*Coliforms, E. coli, pathogenic staphylococci, moulds, yeasts, Listeria monocytogenes, Salmonella*). The other two parameters referred to the beneficial microflora, *Lactococcus* and *Lactobacillus*, which were found in the product.

From the point of view of obtaining a quality microbiological product, it is important to note that in both examined periods (day 8 and day 60) in none of the examined variants of white brined cheese was detected the presence of *Listeria monocytogenes* and *Salmonella* (absence in 25 g cheese).

Poor hygiene is the most common reason for the presence of coliform bacteria in white brine cheese. These bacteria have a wide range of distribution in soil, water, digestive tract of humans and animals. They usually cause premature bloating and cavities in cheeses because they produce gases such as hydrogen and carbon dioxide.

On the 8-th day of ripening process, coliform bacteria were observed in two variants Variant No. 1: $8.5x10^2$ cfu/g and Variant No. 2: $3.9x10^2$ cfu/g. In the other two variants Variant No. 3 and Variant No. 4 the number of coliform bacteria was insignificant <10. On the 60^{th} day when the ripening process was already finalised, the number of coliform bacteria in all variants was reduced to a minimum number <10, which is a good hygienic indicator.

Our results for the total number of coliform bacteria were in accordance with the data obtained by Stojiljkovic (2007), who found that in the industrial production of white brine cheese, the coliform bacterial species completely disappear or are present in minimal numbers.

The greatest impact on the number of coliform bacteria during the ripening period of white brine cheese had the decrease of the active acidity, the increase of the concentration of lactic acid, the concentration of salt in the brine and also the anaerobic way of ripening process of white brine cheese.

Escherichia coli and *Coagulase positive staphylococci* according to the Rulebook on special requirements for food safety in terms of microbiological criteria (Official Gazette No. 100/2013) should have values below 100 cfu/g cheese at the end of the ripening period. *Escherichia coli* in our examined variants were detected on the 8th day in Variant No.1: $3.1x10^2$ cfu/g and in Variant no.2: $6.3x10^2$ cfu/g, and in the other two variants No.3 and No.4 were detected in insignificant number <10 cfu/g cheese. After the completion of the ripening period in all variants the number of Escherichia coli bacteria was <10 cfu / g, which was a good hygienic indicator.

Pathogenic staphylococci in all variants were detected in insignificant numbers <10 cfu/g, (8th day and 60th day during ripening process).

The starter cultures that were used, play a significant role in preventing the development of harmful microorganisms. They successfully reduce the pH value to a level at which microorganisms cannot grow at all, so the created conditions do not suit them completely.

Bulajic & Mijacevic (2011) also confirm that the active acidity of the cheese is the main factor for the development of pathogenic microflora. The microorganisms listed in the Rulebook on microbiological criteria have optimal growth in the range of pH 6-7.5.

According to Cogan (2003), salt concentration also plays an important role in preventing the growth of harmful microorganisms, with the exception of *Staphylococcus aureus* which can grow in the presence of 6.5% salt and *Listeria monocytogenes* which grows in a concentration of 10% salt.

In all examined variants, the concentration of salt in the final product was between 3% and 4%, while the concentration of salt in the brine was about 10%, the presence of these bacteria was not detected.

Moulds were detected in the four examined variants in both study periods, but in insignificant numbers <10 cfu/g.

Yeasts were detected in all variants in high levels at 8th day, mostly in the Variant No.4: $1.2x10^5$ cfu/g, then in the Variant No.1: $9.9x10^4$ cfu/g, followed by the variants Variant No.3: $6.9x10^4$ cfu/g and Variant No.2 with the lowest number of $4.8x10^3$ cfu/g. At the end of the ripening period, the number of yeasts were insignificant in all examined cheese samples (<10 cfu/g).

Yeasts and moulds can enter the cheese

from a variety of sources. They can come from the starter culture, the air, the brine solution, the equipment used for production, as well as from workers (Mounier et al., 2006). Although yeast can have a positive effect on cheese (appearance, taste), some species can spoil it. If they are found on the surface of the cheese, they spoil it, create an unwanted aroma, taste, as well as contribute to the formation of some other metabolic products that reduce the quality of the cheese, (Bintsis & Papademas, 2002; Jakobsen & Narvhus, 1996).

Moulds as well as yeasts are added to some types of cheese in order to provide a characteristic appearance, consistency and taste, but also have the function of extending the shelf life of the product (Haasum & Nielsen, 1998). However, moulds that are found on the surface of the cheese under improper hygienic conditions can contaminate it with mycotoxins, and thus such a product is a potential risk to human health (Sengun et al., 2008).

From lactic acid bacteria, the total number of *Lactococcus* and *Lactobacillus* were observed. After 8 days, during ripening period, the number of *Lactococcus* bacteria was higher in all four variants compared to the number of *Lactobacillus* bacteria. The greatest number of *Lactococcus* bacteria were counted in Variant No.4: 10.49 log cfu/g, followed by the Variant No.3 with 10.38 cfu/g, then Variant No.2: 10.32 log cfu/g, and the lowest number was determined in Variant No.1: 10.27 log cfu/g (Fig. 1). In the same period, the total number of *Lactobacillus lactic* acid bacteria were from 9.28 to 9.74 log cfu/g. The highest number of *Lactobacillus* bacteria was counted in Variant No.3, and the lowest number was counted in Variant No.1, (Fig. 2). The highest number of *Lactococcus* bacteria was counted in Variant No.3, and the lowest number in Variant No.1, (Fig. 1).

Lactobacilli are normally present at the level of 10^9 bacteria/g, actively participating in the fermentation process during the ripening period. Their number decreases rapidly during the ripening period, and this depends on the sensitivity of the starter cultures to salt, active water, the power of autolysis of the species, (Coeuret *et al*., 2003). When the ripening process is finished, it is normal to expect a reduction in the number of lactic acid bacteria, and that was confirmed by our results. The total number of *Lactococcus* bacteria was ranged from 6.68 to 6.93 log cfu/g and *Lactobacillus* from 7.04 to 7.38 log cfu/g, which was in the same range as the results obtained by Mojsova *et al.*, (2013). In their research the number of *Lactobacillus* bacteria after the ripening period was between 5.35 to 7.43 log cfu/g and the number of *Lactococcus* bacteria was from 4.11 to 7.10 log cfu/g.

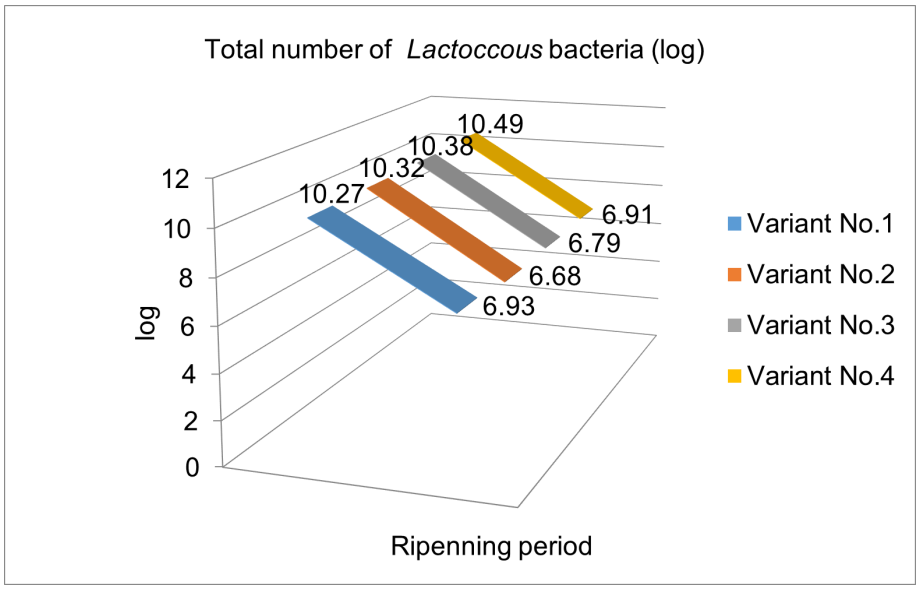

Figure 1. Dynamics of *Lactococcus* bacteria during ripening

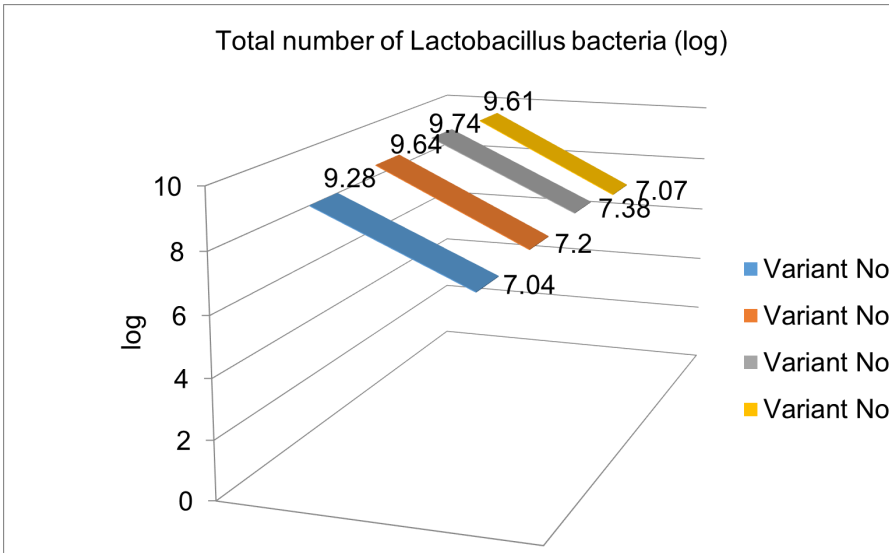

Figure 2. Dynamics of *Lactobacillus* bacteria during ripening

From the results obtained for lactic acid bacteria, it can be concluded that *Lactococcus* bacteria was dominant bacteria in all four cheese variants at the beginning of ripening period till day 20. After that and at the end of ripening period (60 day) the *Lactobacillus* bacteria were predominant.

The reason for this phenomenon is the greater resistance of *Lactobacillus* bacteria to increased salt concentration. The growth of *lactococci* is inhibited, the production of lactic acid is decreased due to the level of salt in aqueous phase (especially at the level of salt / water> 5.0 g/100g), and the inhibition of Lactobacillus bacteria growth that have a higher salt tolerance occurs when the aqueous salt level is greater than 6 g/100 g, (Fox et al., 2004).

Our results for the number of *Lactococcus* and *Lactobacillus* bacteria were approximately equal to the results obtained by Balabanova (2015), which determined the total number of bacteria of these species 7.4 ± 0.5 log cfu/g and 7.1 ± 0.5 log cfu/g, respectively.

According to Kayagil (2006) the number of *Lactobacillus* bacteria at the beginning of the ripening period (day 2) was between $1.8x10^7$ to $5.2x10^{10}$ cfu/g. This number was reduced to $9.0x10^6$cfu/g at day 30. The number of *Lactococcus* at the beginning of the ripening period (day 2) was between $2.1x10^7$ to $4.6x10^{10}$cfu/g. This number was reduced to $1.8x10^6$ cfu/g at day 30.

CONCLUSION

In all examined cheese samples pathogenic bacteria were not detected. Lactococcus bacteria was dominant bacteria in all four cheese variants at the beginning of ripening period till day 20. After that and at the end of ripening period (60 day) the Lactobacillus bacteria were predominant. In order to produce cheese that is microbiologically correct, we must use milk in production process with appropriate microbiological quality. Also, it is of great importance to maintain hygiene in the premises where the production process takes place, personal hygiene of people involved in production, microbiological correctness of ingredients that were used, bins, water, salt, strainers and all items that in any way come in direct contact with the cheese. It is also necessary to keep regular records and appropriate technology logs which are mandatory under HACCP standards.

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МИКРОБИОЛОШКИ КВАЛИТЕТ НА МАКЕДОНСКОТО БЕЛО САЛАМУРЕНО СИРЕЊЕ

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Резиме

Во овој научен труд е испитуван микробиолошкиот квалитет на македонското бело саламурено сирење. Беа анализирани микробиолошките параметри на четири најдобри варијанти македонско бело саламурено сирење. Од патогената микрофлора беа испитувани: вкупен број на колиформни бактерии, *E. Coli*, патогени стафилококи, квасци, мувли, *Listeria* и *Salmonella*. Од млечнокиселинските бактерии беа испитувани следните параметри: вкупен број на бактерии од видот *Lactococcus* и вкупен број на бактерии од видот *Lactobacillus*. Во сите испитувани примероци не беа детектирани патогени микроорганизми. Бројот на бактериите од видот *Lactococcus беа доминантни кај сите четири варијанти македонско бело саламурено сирење на почетокот од процесот на зреење до 20.* ден. Потоа и до крајот на процесот на зреење (60. ден) доминантни беа бактериите од видот *Lactobacillus*.

Клучни зборови: *варијанти сирење, млечнокиселински бактерии, патогени бактерии, квалитет*.

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OPPORTUNITIES – ALTERNATIVES FOR APPLICATION OF AGROECOLOGICAL MEASURES AND USE OF POST-HARVEST RESIDUES

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Abstract

In most of the cases, the standard cultivation of cereal crops by agricultural producers is carried out by applying agricultural practices, which are unsustainable in long term. Especially the treatment of post-harvest residues (mostly burning the stubble of rice, wheat and barley), contributes to the increase of the risk faster and mostly irreversible or very slow reversible degradation of natural resources.

The treatment of the post-harvest residues must be in accordance with the strategies and practices of good agricultural practice, which are compatible with the EU strategy in the field of agricultural policy. Only in Bregalnica region, on an annual level, around 100 000 tons (96,082 tons) of "post-harvest residues" remain on productive agricultural arable land. These amounts are obtained from around 31 100 hectares of arable land on which the most common cereal crops (wheat, corn, barley, rye and oats) are grown. On average, from all crops, on an annual level, about 3,2 t/ha of post-harvest aboveground plant biomass remained, which represents a significant resource, that is often used irrationally and sometimes harmful to the environment, by agricultural producers.

The practice of agricultural cereal crop producers has been analysed and alternative approaches for using post-harvest residues have been proposed. The additional financial implications for farmers, which can be achieved by proper treatments of the post-harvest residues are emphasized. Proposed measures and activities for sustainable management of plant residues after harvest are explained.

Key words: *cereals, agricultural practices, degradation, sustainable management*

INTRODUCTION

In most of the cases, the conventional cultivation of agricultural crops by farmers is carried out through the application of unsustainable long-term agricultural practices. The low level of adaptability of applied agricultural practices, together with inefficient risk management methods of their application, contributes to increasing the risk degree of faster and most often irreversible or very slow reversible degradation of natural resources.

This type of activities contributes to irreversible disruption of natural biocycles in natural but also in agrobiotopes and agrobiocenosis, associated with a strong reduction on soil fertility and reduction in resistance and functional integrity of ecosystems.

Agricultural producers, especially rice producers in Bregalnica region, in the past decades and currently, with very rare exceptions, have been implementing a multi-year practice of regular application of unpopular and legal prohibited agricultural measure of burning the post-harvest residues on the stubble. The correct and ecologically sustainable management of biomass obtained from harvest residues, carried out in accordance with good agricultural and hygienic practices (Ministry of Agriculture, Forestry and Water Management, 2010), which are the backbone of agroecological measures (Ministry of Agriculture, Forestry and Water Management, 2014), present a set of pre-planned, comprehensive, systemic and continuous activities and agrotechnical practices, which agricultural producers are recommended to implement in a coordinated manner.

The goal of good agricultural practice is

to determine the correct procedures in the process of agricultural production that would minimize the threats of degradation and the loss of this limited natural resource, and which refer to measures and procedures to preserve and improve soil fertility.

In the agricultural practice during the production of agricultural crops which leave a significant amounts of aboveground plant material, after the harvest in the Bregalnica region, as well as in the country, in an extremely small number of cases, it is acted in accordance with good agricultural practice. Most often, grain producers improperly deal with harvest residues, i.e. they burn them and on this way multiple damages and unwanted consequences are caused for people, animals and the environment.

In our country, in several official (published in the Official Gazette) laws, (for agricultural land, for hunting, for forests, for the environment, for nature protection), are foreseen penal provisions for lighting fire in the open air. A lot of articles of those laws, also contain and describe in detail the procedures that are prohibited to be carried out in the open field, which are related to burning the stubble and other plant residues, and high penalties are predicted for legal and natural persons.

In some parts of the law of environmental protection (Official Gazette of the Republic of Macedonia, 2005), is predicted the implementation of sustainable use of natural resources with a little disturbance of the natural balance and insignificant damages. This law also prohibits permanent disturbances of the natural balance by implementing agrotechnical measures recognized as harmful to the natural composition, development and maintenance of an ecological healthy environment. Common practices for burning post-harvest residues are contrary and violate the provisions of the environmental protection law, in which it is contained:

„In order to avoid destruction and degradation with long-term effects, a ban of on the use of nature is being approached in causes where it can cause: damage or destruction of biological and regional diversity; soil degradation and loss of its fertility; damage of surface or underground geomorphological values; pollution and change of water and air pollution."

The handling of post-harvest residues should necessarily be in accordance with the strategies and good agricultural practice, which are compatible with EU strategy in the field of agricultural policy, where stimulation of farmers for the use of agricultural land is predicted with adopted legal regulations which relate on a way that is consistent with the protection of environmental and biological diversity.

The principles of good agricultural practice usually predict: reduced or minimal soil tillage, application of an appropriate crop rotation with inclusion of cover crops, as well as use of organic fertilizers.

Plant biomass that remains after crop harvest, especially cereals, is an excellent raw material for its conversion into an energy or value-added product with use for many different purposes in agricultural production.

Beside being used for renewable energy source, plant post-harvest residues also can be used for: organic soil fertilizers; substrate for mushroom production; material for composting; fodder food; raw materials in furniture production; paper and building materials industries; directly for burning.

In this paper, the types and the volume of cultivated cereals in Bregalnica region my municipalities will be explained, as well as the yields obtained by them, the current situation with the management of harvest residues, farmers practice, alternative approaches for using post-harvest residues, additional financial farmers implications for straw collection, as well as proposing measures for sustainable management with harvest residues.

MATERIAL AND METHODS

The following basic sources of data are used:

- officially published data from SSO (State Statistical Office);
- data from original scientific research published in domestic and foreign scientific journals in this field;
- data from publications, project reports and monographs;
- data from the regional units of the Ministry of Agriculture, Forestry and Water Management (2014);
- data that results based on the practice of farmers.

Below are presented the methodologies used for this manuscript:

- recognition and collection of data, their

preparation, grouping, validation, tabular and text presentation and analysis;
- average annual hectares of cultivated cereals in the Bregalnica region by municipalities and obtained yields;
- the weight of the post-harvest biomass was theoretically calculated and analysed;
- methods and analyses (SWOT), oriented to the possibilities of the resource;
- description and analysis of the current situation with the management of harvest residues;
- field surveys with producers;
- alternative approaches for the use of harvest residues.

The biomass which remains on the agricultural land after harvest, could be estimated using various methodologies, assumptions and data. Statistical methods were used, oriented to the possibilities of the source. For more detailed analyses, it is necessary to assess: the theoretical and technical potential, as well as economic source potential.

The theoretical potential is the maximum amount of harvestable biomass, which is available according to the potential of its source. Technical potential is a part of the theoretical potential, which can be used for energy needs or for its use as a raw material for other purposes, taking into account the limitations related to the current technological possibilities, the possible use of live human labour, as well as environmental limitations. Economic potential is the potential that can be used under current economic conditions, i.e. it satisfies the criterion of being profitable.

The data for total post-harvest biomass, explained in this paper, are calculated based on the theoretical potential of harvest biomass.

RESULTS AND DISCUSSION

Grain production and post-harvest residues from cereals crop in Bregalnica region

In Table 1 data of wheat and corn grain production in 2017, in thirteen municipalities in Bregalnica region are presented.

Table 1. Production of wheat and corn grain, by municipalities in the Bregalnica region in 2017.

Bregalnica region-municipalities	Wheat				Corn			
	Area (ha)		Production		Area (ha)		Production	
	Sown area	Harvested area	Total t (tons)	Yield kg/ha	Sown area	Harvested area	Total t (tons)	Yield kg/ha
Berovo	366	366	1 024	2 798	135	135	225	1 667
Vinica	287	287	575	2 002	263	263	2 362	8 981
Delcevo	829	829	1 480	1 786	709	707	1 439	2 035
Zrnovci	229	224	572	2 555	294	258	1 878	7 278
Karbinci	1 081	1 041	1 068	1 026	382	318	1 265	3 978
Kocani	626	626	1 513	2 417	393	383	2 901	7 573
Lozovo	668	668	1 136	1 700	22	22	25	1 136
Makedonska kamenica	71	71	126	1770	87	87	146	1 672
Pehcevo	422	422	1 271	3 011	84	84	198	2 362
Probistip	1 507	1 507	3 261	2 164	248	248	330	1 332
Sveti Nikole	3 160	3 160	4 988	1 579	477	477	388	814
Cesinovo - Oblesevo	831	831	2 282	2 746	860	860	7 230	8 407
Stip	1 015	1 015	826	814	157	157	576	3 668
Total ha/ t (tons)/ Average (kg/ha)	11 092	11 047	17 918	1 622	4 111	3 999	18 963	4 742

Source: Statistical Review: Agriculture (Statistical Office of the Republic of Macedonia, 2018)

The values obtained for the weight of the harvest residues from the six most important crops (wheat, corn, barley, rice, rye and oats) which leave the largest amounts of harvest biomass, are calculated based on the harvest index. The harvest index represents the ratio between the yield (the mass of the rest of the aboveground part of the plant at the time of harvest) and the yield (the mass of grain). In Table 2 is given the harvest index for some crops.

Table 2. Harvest index (harvest residue index/grain yield) for some crops.

Plant species	Harvest index (harvest residue index / grain yield)	Range – Grain Yield Rank* (кg/ha)
corn	1.0	3 100 – 9 400
soybeans	1.5	1 000 – 3 000
millet	1.0	2 500 – 5 600
winter wheat	1.7	1 700 – 4 000
spring wheat	1.3	1 700 – 4 000
spring oats	2.0	1 100 – 2 900

* If the grain yield is lower than the minimum value in the range, the index (harvest residue/grain yield) will need to be increased. If the yield is greater than the maximum value in the range, the index will need to be reduced

Source: McClellan et al., (2012)

The total annual production of corn grain, from all the municipalities in all regions was 19 000 tons (18 963 t, Tab. 1). The average amount of post-harvest residues which remains after the corn harvest, according to a large number of scientific studies, for the yield of 1 ton of corn grain, the same amount (1 ton) of stems and parts of finished cobs remain as post-harvest residues. The largest amount (7 230 t) of corn post-harvest residue remains in the areas which were planted in Cesinovo – Oblesevo municipality. The least post-harvest corn residues (25 t) remain on the lands in Lozovo municipality.

Table 3. Barley and rice production by municipalities in the Bregalnica region in 2017.

Bregalnica region-municipalities	Barley				Rice			
	Area (ha)		Production		Area (ha)		Production	
	Sown area	Harvested area	Total t (tons)	Yield kg/ ha	Sown area	Harvested area	Total t (tons)	Yield kg/ha
Berovo	130	130	215	1 650	-	-	-	-
Vinica	266	266	532	2 000	95	95	475	5 000
Delcevo	1 008	1 008	1 823	1 809	-	-	-	-
Zrnovci	343	336	841	2 502	158	158	736	4 657
Karbinci	1 077	1 017	989	972	318	150	941	6 270
Kocani	812	812	1 459	1 796	1 124	1 124	5 563	4 949
Lozovo	668	668	1 136	1 700	-	-	-	-
Makedonska kamenica	226	226	361	1 599	-	-	-	-
Pehcevo	121	121	281	2 323	-	-	-	-
Probistip	521	521	1 167	2 241	39	34	204	6 000
Sveti Nikole	3 049	3 049	3 211	1 053	-	-	-	-
Cesinovo - Oblesevo	834	834	1 495	1 793	1 713	1 713	9 162	5 349
Stip	1 182	1 182	853	722	6	-	-	-
Total ha/ t (tons)/ Average (kg/ha)	10 237	10 170	14 363	1 412	3 453	3 274	17 081	5 217

Source: Statistical Review: Agriculture (Statistical Office of the Republic of Macedonia, 2018)

The annual production of barley grain, from all municipalities in the region was 14 363 t (Tab. 3). The average amount of post-harvest residues remaining after barley harvest, for a yield of 1 ton of barley grain, on average about 1,7 t of straw remain as post-harvest residues, similar to wheat. The total average annual production of barley grain in the Bregalnica region was 14 363 t. When this will increase by 70% (1:1,7), on average, barley straw as a post-harvest residue remains about 25 000 tons (24 417 tons) in the territory of the Bregalnica region. The largest amount (5 459 tons) of post-harvest barley residues remains on the areas which were planted in Sveti Nikole municipality. The least post-harvested residues of barley crop (365 tons) remain on the lands in Berovo municipality.

In 2017, on an annual level, the total production of rice paddy in seven municipalities in which the rice is produced in the Bregalnica region was about 17 000 tons (17 081 tons, Tab. 3). If this amount is multiplied by the "post-harvest residues/grain yield" (0,75) the value of an average of 12 814 tons of rice straw post-harvest residue per year will be obtained. This value, as well as the values for the quantities of the other crops described so far, may vary from year to year, and in this paper, they are calculated according to the data for 2017. The largest amount (6 871 tons) of post-harvest residues of rice straw remains on the areas which were sown in Cesinovo – Oblesevo municipality. The least post-harvest residues from rice straw (153 tons) remain in Probistip lands.

Table 4. Rye and oats production by municipalities in the Bregalnica region in 2017.

Bregalnica region-municipalities	Rye				Oats			
	Area (ha)		Production		Area (ha)		Production	
	Sown area	Harvested area	Total t (tons)	Yield kg/ha	Sown area	Harvested area	Total t (tons)	Yield kg/ha
Berovo	870	870	2 443	2 808	120	120	138	1 146
Vinica	87	87	131	1 500	47	47	71	1 506
Delcevo	269	269	466	1 733	259	259	400	1 546
Zrnovci	10	10	19	1 850	3	3	5	1 667
Karbinci	-	-	-	-	15	14	9	671
Kocani	93	93	97	1 046	89	89	94	1 056
Lozovo	-	-	-	-	-	-	-	-
Makedonska kamenica	79	73	129	1 773	23	23	26	1 113
Pehcevo	350	350	976	2 789	100	100	115	1 145
Probistip	62	62	95	1 529	58	40	48	1 206
Sveti Nikole	61	61	85	1 401	-	-	-	-
Cesinovo - Oblesevo	10	10	17	1 740	3	3	5	1 500
Stip	11	11	7	609	15	15	8	511
Total ha/ t (tons)/ Average (kg/ ha)	1 902	1 896	4 465	2 355	732	713	919	1 289

Source: Statistical Review: Agriculture (Statistical Office of the Republic of Macedonia, 2018)

The total annual production of rye grain in the Bregalnica region is amount of 4 465 tons (Tab. 4). According to the data of the Ministry of Agriculture, Forestry and Water Management, P.E Kocani, the index of "post-harvest residues/ grain yield" of cultivated rye plant, grown in the Bregalnica region was 1,7. In Bregalnica region, on average per year, the amount of 4 465 tons multiplied by the "post-harvest residue/grain yield" index (1,7) for rye, amounts 7 590 tons

of post-harvest rye straw residues. In Berovo municipality, the largest amount of post-harvest residues of rye straw was observed (4 136 tons). In 2017, the smallest amount of post-harvest rye straw residues (12 tons) was recorded in Stip municipality, while no rye was produced in Karbinci and Lozovo.

The oat is the least represented cereal crop grown in Bregalnica region and its annual grain production was 919 tons. According to several researches (Tab. 2), the index of "post-harvest residues/grain yield" for the oat plant amount 2. The amount of 919 tons multiplied by the index of "post-harvest residues/grain yield" (2) for oat, amounts 1 838 tons post-harvest oat straw residues, on average per year in Bregalnica region. In Delcevo, the largest amount of post-harvest oat straw residues was recorded (800 tons), while the smallest amount (16 tons) was obtained in Stip municipality. In 2017, in Sveti Nikole and Lozovo, the oat crop was not cultivated.

Table 5. Average grain yield and "plant biomass after harvest" of some cereals grown in the Bregalnica region in 2017.

Plant species	average yield – grain (kg/ha)	average yield – plant biomass after harvest (kg/ha)
Wheat	1 622	2 760
Corn	4 740	4 740
Barley	1 412	2 400
Rice	5 217	3 900
Rye	2 355	4 000
Oats	1 289	2 580

Experiences and practices with the treatment of harvested biomass in the Bregalnica region

Certain unscrupulous growers mostly of rice knowingly break the law and decide on the unpopular agrotechnical measure of burning the after-harvest residues. For that activity they explain their purposes, reasons and justifications.

- PURPOSES: enabling the monocultural cultivation, that is, the non-implementation of the crop rotation;
- REASONS: farmers do the burning because they don't have their own machinery – e.g., baling machines, the lack of manual labour or tractor loaders for loading and unloading the bales;
- JUSTIFICATIONS: destruction of: weed residues; plant residues from the previous crop, in order not to hinder the further cultivation of the soil; harvested crop residues and weeds infected with present residues of plant disease agents; destruction of eggs, larvae and eggs of insects – pests of cultivated crops.

Some harmful consequences of the burning of harvest residues

They are listed below according to the harmful consequences of burning post-harvest vegetable residues:

- significant net source of CH_4, CO, NO_2 and N_2O, which contributes to global warming, where CO and O_3 are indirect greenhouse gases;
- it has been scientifically proven that dioxin emissions increase 150 times when biomass treated with 2,4-D is burned;
- dioxin emission is between 35 and 270 times greater in the case of burning pesticide-treated corn crop residues, compared to the amount of dioxin released when such crop residues are not treated;
- the increased pollution with suspended PM particles and sulphur dioxide (which are released into the atmosphere during the burning of rice straw);
- destruction of aboveground and underground beneficial flora and fauna - the temperatures that develop reach values of 200 to 400°C, and heat transfer entering at a depth of 5 to 20 cm in the soil has been recorded up to a critical 35 - 50°C, which is very harmful or lethal to the flora and fauna in the soil.

Alternatives for the application of agro-ecological activities for the usage of the harvest residues

The management of post-harvest residues from agricultural crops and the possibilities of applying agro-ecological measures, should be considered within a comprehensive view, integrating Best Management Practices – BMPs, in order to reduce the negative impacts that would be caused as a result of any improper treatments with the harvest residues. Integrating - Best Management Practices - BMPs - procedures that are increasing the sustainability of crop residues in the long term and including them in the replacing of the soil nutrients that are extracted with crop yield and residues - a cheap and ecological resource for improving soil properties.

These practices usually include:

- reduced or minimal tillage of the soil,
- application of an appropriate crop rotation with the inclusion of cover crops, as well as the usage of organic fertilizers and siderites.
- Plant post-harvest residues can also be used for energy purposes:
- obtaining liquid and gaseous fuel (biogas and gas generating plants, pyrolysis technology and "fast" pyrolysis = bio-oil from rice straw through the fast pyrolysis process that are taking place in the special reactors in which the "flour" of finely ground rice straw is treated) and production of bioethanol and biodiesel
- processing into pellets or briquettes.

CONCLUDING REMARKS

The general one's conclusions – recommendations for good agricultural practices for the management of harvest residues in the bregalnica region would be:

Prediction, organizing, directing and implementing activities in the direction of**:**

- designing and building processing capacity for the production of some of the products that can be obtained by processing harvested residues (compost, bio-oil, pellets, briquettes, biogas, etc.);
- organizing logistics (baling, loading, transport, unloading), i.e. through agricultural cooperatives;
- application of appropriate technologies for preparation and usage of rice straw and other types of plant residues for animal feed, by enriching them with nitrogen, in order to improve the protein content and meet the requirements for proper animal nutrition;
- education of farmers with positive examples for the usage of the harvest residues (i.e. the usage of microbiological compounds that can accelerate the residues decomposition);
- modification and increasing of the subsidy values for the producers of compost, lumbrihumus, mushrooms and agropellets;
- maximum subsidy for the purchase of appropriate specialized agricultural machinery for mulching, baling, transporting (loaders, special trailers for transport), compost preparation machines, biodigesters, balers, etc.

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МОЖНОСТИ – АЛТЕРНАТИВИ ЗА ПРИМЕНА НА АГРОЕКОЛОШКИТЕ МЕРКИ И КОРИСТЕЊЕ НА ПОЖЕТВЕНИТЕ ОСТАТОЦИ

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Резиме

Во најголем број случаи стандардното одгледување на житните култури од страна на земјоделските производители се спроведува со примена на неодржливи на долг рок земјоделски практики. Особено третманот на пожетвените остатоци (најчесто палење на стрништата од ориз, пченица и јачмен), придонесува за зголемување на степенот на ризик од побрза и најчесто неповратна или многу бавно повратна деградација на природните ресурси.

Постапувањето со пожетвените остатоци задолжително треба да биде во согласност со стратегиите и практиките на добрата земјоделска пракса, кои се компатибилни со стратегијата на ЕУ во доменот на земјоделската политика. Само во Брегалничкиот Регион на годишно ниво на производните земјоделски оранични површини вкупно остануваат околу 100 000 тони (96 082 тони) „пожетвени остатоци". Овие количини се добиваат од вкупно околу 31 100 хектари оранична површина на која се одгледуваат најзастапените житни култури (пченица, пченка, јачмен, ориз, 'рж и овес). Просечно од сите култури на годишно ниво остануваат по околу 3,2 t/ha пожетвена надземна растителна биомаса, што претставува значаен ресурс кој најчесто се искористува нерационално, а понекогаш и штетно за околината од страна на земјоделските производители.

Анализирана е праксата на земјоделските производители на жита и предложени се алтернативни пристапи за користење на пожетвените остатоци. Потенцирани се дополнителните финансиски импликации кај фармерите кои можат да ги остварат со правилен третман на пожетвените остатоци. Образложени се предлог-мерки и активности за одржливо управување со растителните остатоци по жетвата.

Клучни зборови: *жита, земјоделски практики, деградација, одржливо управување.*

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SOIL FERTILITY AS A NECESSARY MEASURE FOR SUSTAINABLE TOBACCO PRODUCTION IN THE AREA OF MUNCIPALITY OF DOLNENI

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Abstract

Republic of North Macedonia has a long history of tobacco production. Therefore, the precise determination of soil fertility parameters is of essential importance. The main purpose of this study was to determine the spatial distribution of tobacco soil properties as a useful strategy for guiding agricultural production and field management on specific sites. Furthermore, diagnosing soil fertility provides with proper and rational fertilization recommendations, which are integral parts of sustainable tobacco production. Following soil properties were monitored: pH, humus content, total nitrogen, available phosphorus and potassium, carbonates and physical clay in 153 of top soil samples (0-30 cm). The samples were collected from the area of municipality of Dolneni, which is part of Pelagonia region and accounts for almost 50% of the total area for tobacco production in the country.

The results show that soil properties exhibit spatial variation. Based on the performed classifications, 54 % of the soil samples have low humus and nitrogen content, 65 % of the samples have low available phosphorus content and only 6.5 % have low available potassium content. The soil reaction varies widely within the limits suitable for tobacco production, and most of the sampled soils are loamy. Thus, the application of mainly complex fertilizers, such as 10:30:20, 6:24:12 and 8:22:20, results with optimal fertility in the investigated area.

Key words: *soil properties, Pelagonia, spatial distribution*

INTRODUCTION

Soil is the basis of agricultural production, an extremely important medium for the cultivation and development of many crops. Soil is a heterogeneous, diverse and dynamic system and its properties change in time and space continuously (Brodsky et al.,2001; Denton et al., 2017). Soil properties vary spatially and they are affected by many factors that include nature of the pedogenic materials, evolution processes of the types of soil, soil management practices, fertility status, crop rotation (Bouma, 1999; Kumhalova & Matejkova, 2017; Jerzy & Boguslaw, 2018). Monitoring and quantifying variations in soil properties are necessary to understand the effects of land use and to provide sustainable agricultural management.

Analyses of spatial distribution and correlation of soil properties represents an important outset for precision agriculture (Boruvka et al., 2002). In the past years introduction of precision agriculture with the use of remote sensing, Geographic Information System (GIS) and Global Positioning Systems (GPS) has resulted in accurate and efficient mapping of fertility parameters (Bouma, 1997; Kuzyakova et al.,2001; Robinson & Metternicht, 2006). The concept of precision agriculture ensures an increase in productivity of agricultural goods while reducing production costs. By determining the most appropriate and localized management practices, precision agriculture helps to improve and align the soil conditions and quality for the effective use of nutrients (Bölenius et al., 2017; Usowicz & Lipiec, 2017). Almost all agricultural data has some form of spatial component that is used for predictive maps which have high

levels of predictive accuracy on spatial variations (Robinson & Metternicht, 2006). Geographic information system technique provides various possibilities in prediction while wisely using the data obtained from agrochemical soil analyses.

Given the long history of tobacco production in Republic of North Macedonia, the precise determination of soil parameters is of essential importance. Controlling, managing and monitoring soil fertility is a long-term process, but it is a prerequisite for sustainable tobacco production. On the other side, diagnosing the soil fertility status using smart tools, leads toward effective management practices. This study was conducted as attempt to explore the fertility of agricultural soils of Municipality of Dolneni using the GIS tools. In this region, most of the arable area is part of the Pelagonia region, that occupies almost 50 % of the total area for tobacco production in Republic of North Macedonia. The main purpose was to map the spatial distributions of soil parameters as a useful strategy for guiding agricultural production and field management at specific sites.

MATERIAL AND METHODS

The study area is the Municipality of Dolneni, which is part of the largest tobacco production area in Pelagonia. The climate in this area is determined as continental with moderate precipitation (Lazarevski, 1993). The Pelagonian massif is a crystalline core with a continental type of Earth's crust, primarily composed of the oldest Precambrian formations (Durmudzanov et al., 2005; Jovanovski et al., 2012; Stafilov & Shajn, 2017). Alluvial and delluvial soil cover the municipality of Dolneni, mainly arable land. Soil samples were collected from 153 locations at fixed depth (0-30 cm) in each field with two replicates in the period from 2017-2021. Samples were collected in fall after crop harvesting. The sampling map (Figure 1) reveals distributional uniformity of samples and represents soil for tobacco cultivation only.

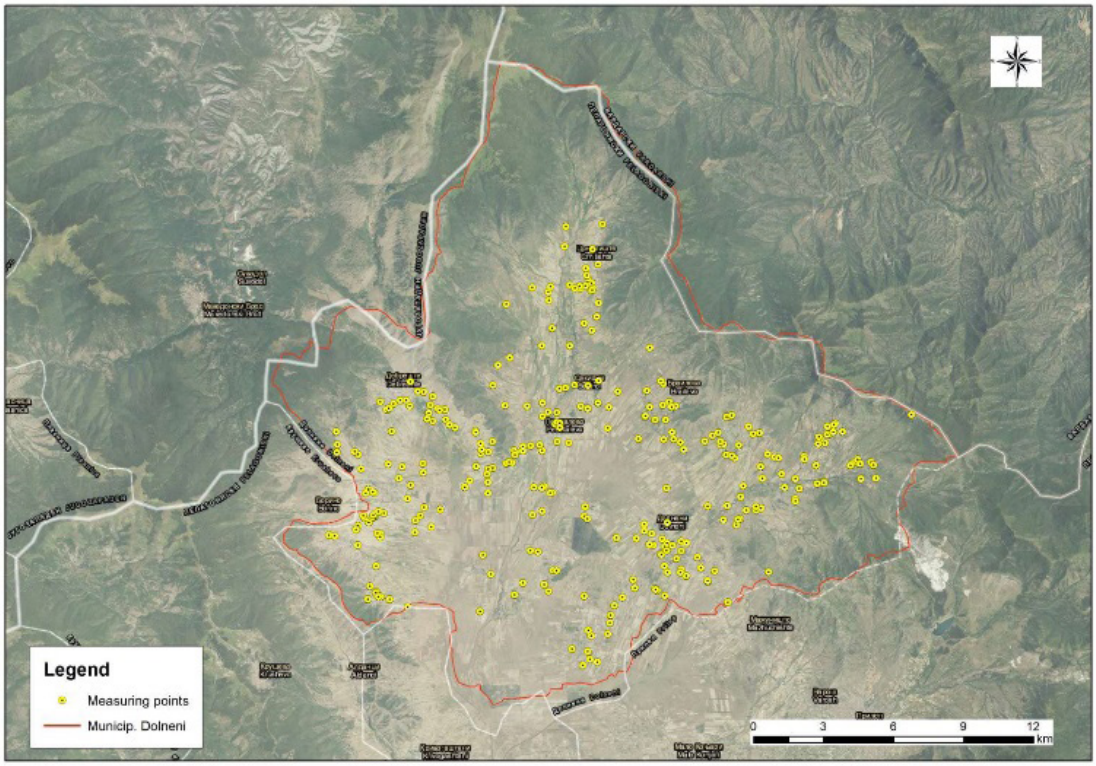

Figure 1. Sampling locations (municipality of Dolneni, n=153)

The variables as soil texture, pH, total nitrogen, organic matter, carbonates, available phosphorus and potassium were determined (Pelivanoska, 2012). The geographical coordinates of the sampling points are plotted on a map using the tools of the Source Geographic Information System - QGIS, software version 3.18.2. Interpolations and analysis of all sampling points were made according to Inverse distance weighting (IDW) parameters. This technique predicts attribute values at unsampled area based on the spatial distance of known observations from these unsampled locations (Wu & Hung, 2016).

RESULTS AND DISCUSSION

The examination of soil fertility through the analysis of average soil samples taken from a unit of production area is the fundamental measure for diagnosing the current state of the soil. Summary statistics of the analysed soil parameters are provided in Table 1. As shown, the coefficient of variation for most of the tested parameters is higher than 25%, indicating the heterogeneity of agricultural soil in the study area. The coefficient of variation for available phosphorus is 131 %, suggesting that this parameter exhibits greater variability compared to the other parameters in the study area. The highest coefficient of variation can be attributed to the carbonate content of the tested samples, as only 4 out of all tested samples contained carbonate (Table 1).

Nitrogen is a very important component of proteins, chlorophyll and nicotine in the tobacco plant. Optimal content of soil nitrogen is required for high yield, good quality and proper development of the tobacco plants. All tested soils have low to medium total nitrogen that varies from 0.01 to 0.20 %. Based on the performed classifications, 58 % of the collected samples have low humus and nitrogen content, 65 % of the samples have low available phosphorus content and only 6.5 % have low available potassium content. The soil reaction shows wide variation, with most of the sampled soils being loamy. According to agrochemical parameters, soils suitable for the production of oriental tobacco should have an average content of humus, low content of available phosphorus, optimal content of available potassium, and a neutral to slightly acid pH reaction (Mitreski, 2012; Pelivanoska et al.2017, 2018). According to these authors, most of the tested samples fall within the suitable limits for producing high-quality tobacco raw materials.

Table 1. Statistical parameters that characterize the frequency distribution of soil properties (n=153).

Soil parameter	X_g	Md	S_a	Skewness	Kurtosis	Minimum	Maximum	CV, %
Humus, %	1.47	1.4	0.5	0.65	0.70	0.5	3.2	33
Total nitrogen, %	0.07	0.1	0.02	0.61	0.59	0.0	0.2	33
pH (H_20)	5.95	5.9	0.6	1.06	2.78	4.6	8.4	11
pH (KCl)	4.94	4.9	0.6	1.57	4.62	3.8	7.7	13
$CaCO_3$	0.63	0.0	4.5	8.63	81.58	0.0	47.7	718
P_2O_5 mg/100 g	11.30	6.6	14.8	4.27	22.72	1.3	115.9	131
K_2O mg/100 g	18.56	16.9	7.2	1.27	2.20	5.6	50.2	39
Clay, %	32.47	31.4	9.3	0.89	0.98	15.7	68.0	29

*Xg – geometrical mean, Md - median, Sa– standard deviation, CV- coefficient of variation

The Pearson correlation coefficients pertaining to the associations between soil properties and the element content of all analysed soil samples are presented in Table 2. Correlations were observed to identify positive or negative influence of the selected variables. The correlation coefficients considered significant were only those with probability level lower than 1% and 5% ($P<0.01$ and $P<0.05$). A strong and positive correlation is observed between humus content and total soil nitrogen ($r=0.999$). For the rest of the analysed variables, moderate correlations were obtained.

Table 2. Pearson's correlation coefficients between selected soil properties.

	Humus	Total nitrogen	pH (H_20)	pH (KCl)	$CaCO_3$	P_2O_5	K_2O
Total nitrogen	0.999**	1					
pH (H_20)	0.298**	0.288**	1				
pH (KCl)	0.380**	0.376**	0.912**	1			
$CaCO_3$	0.206*	0.205*	0.487**	0.550**	1		
P_2O_5	0.154	0.154	0.156	0.261**	0.111	1	
K_2O	0.478**	0.480**	0.272**	0.360**	0.076	0.282**	1
Clay	0.525**	0.524**	0.223**	0.232**	0.007	-0.037	0.135

** Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0.05 level (2-tailed)

Based on the normality tests, most of the observed variables exhibit normal distribution, except available phosphorus and carbonate content. The spatial distributions of the selected soil parameters are presented in Figure 2-8. The maps are interpolated using the inverse distance weighting process, which is a deterministic estimation method. It determines values at unmeasured points by using a linear combination of values at nearby measured points (Wu & Hung, 2016). Various approaches exist for selecting the appropriate interpolation method (Goovaers, 1992, 1999; Robinson & Metternicht, 2006; Zhang et al., 2011; Zhang et al., 2013; AbdelRahman et al., 2018; AbdelRahman et al., 2021). According to the most of the mentioned authors, ordinary kriging and inverse distance weighting are the most frequently used geostatistical techniques for predicting soil parameters. In this research, inverse distance weighting was considered the most reliable interpolation method (Hengl et al., 2007; Sun et al., 2012; Ibrahim et al., 2015).

Based on the soil properties fertilizer application rates were calculated and mainly complex fertilizers were found suitable for tobacco production. For the majority of the tested soils (71%) from the municipality of Dolneni, the recommended fertilizer is mainly complex NPK 10:30:20. For the remaining samples, NPK 6:24:12 and 8:22:20 are suggested. To maintain fertility and prevent soil depletion, it is necessary to apply fertilizer in quantities derived from yields. On very poor and acidic soils, nutrient availability decreases, requiring an increase in the dose of mineral fertilizers. To increase the amount of available phosphorus by 1 mg in 100 grams of soil, 30 kg of pure P_2O_5 is required. It is advised to only apply nitrogen to soils with very high and extremely high levels of phosphorus and potassium.

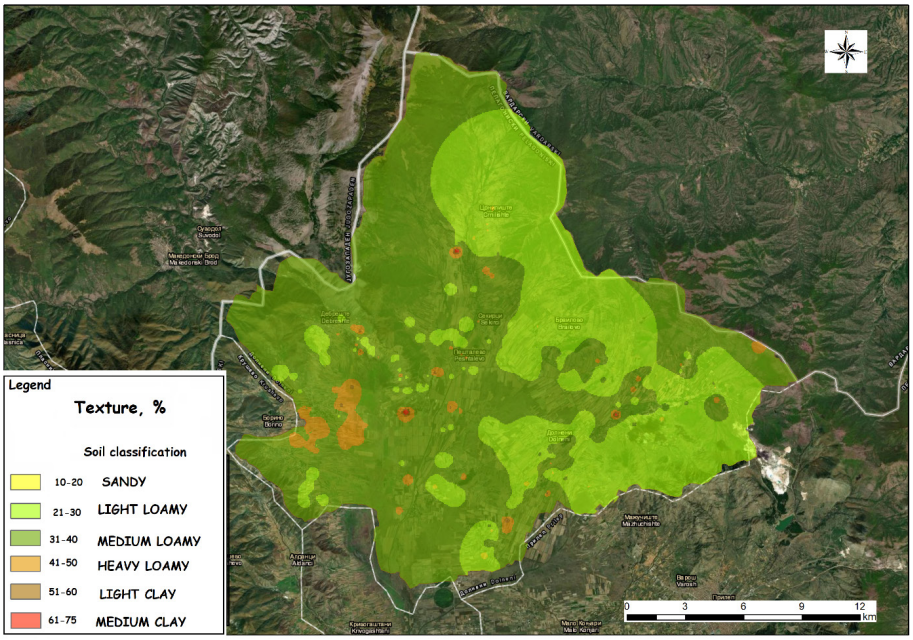

Figure 2. Spatial distribution of physical clay content

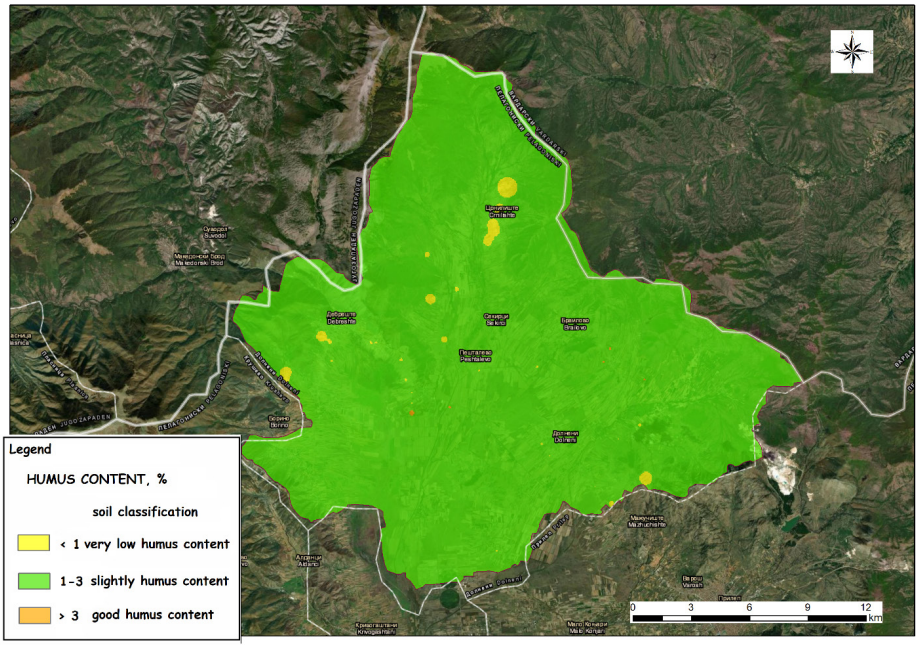

Figure 3. Spatial distribution of humus content

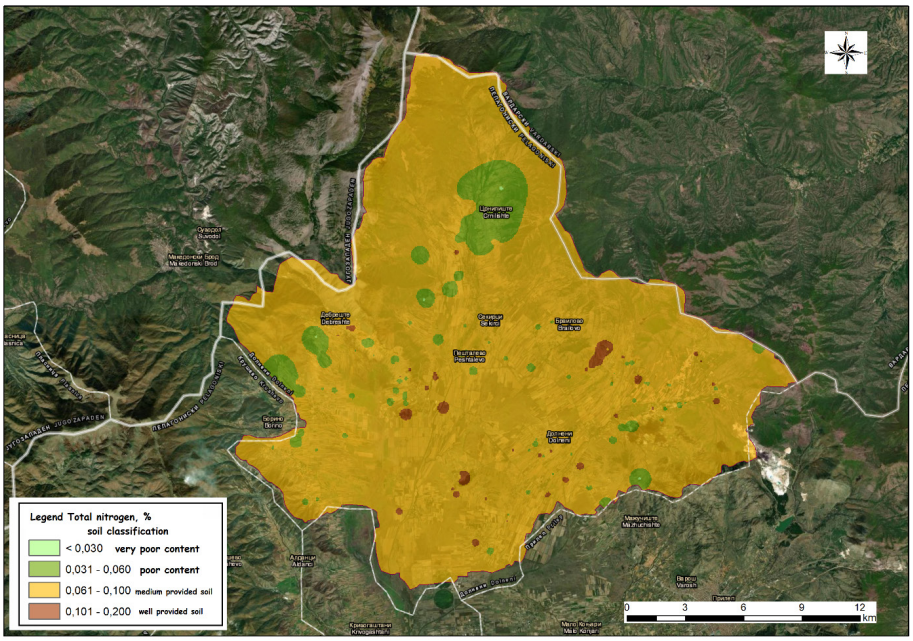

Figure 4. Spatial distribution of total nitrogen content

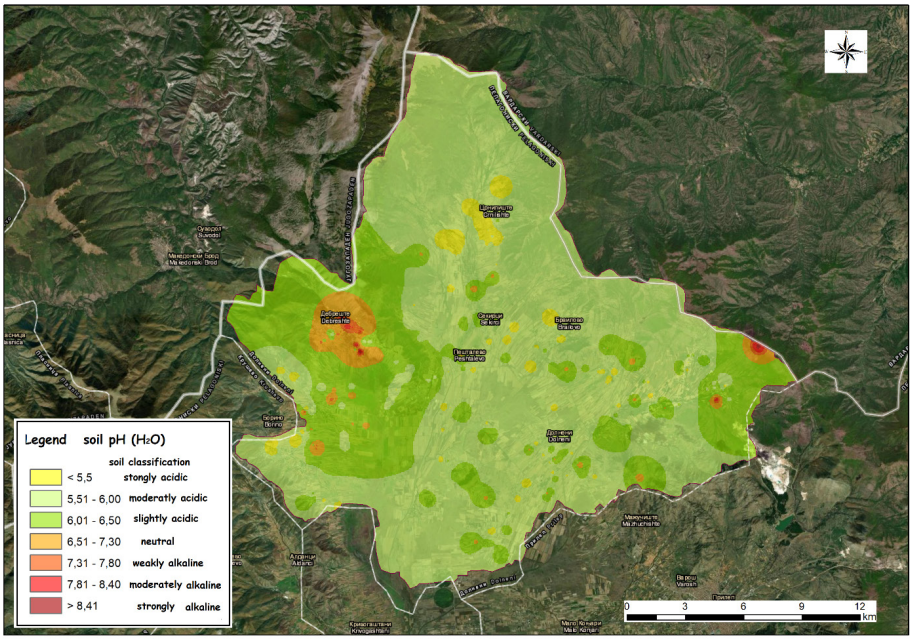

Figure 5. Spatial distribution of soil pH in water

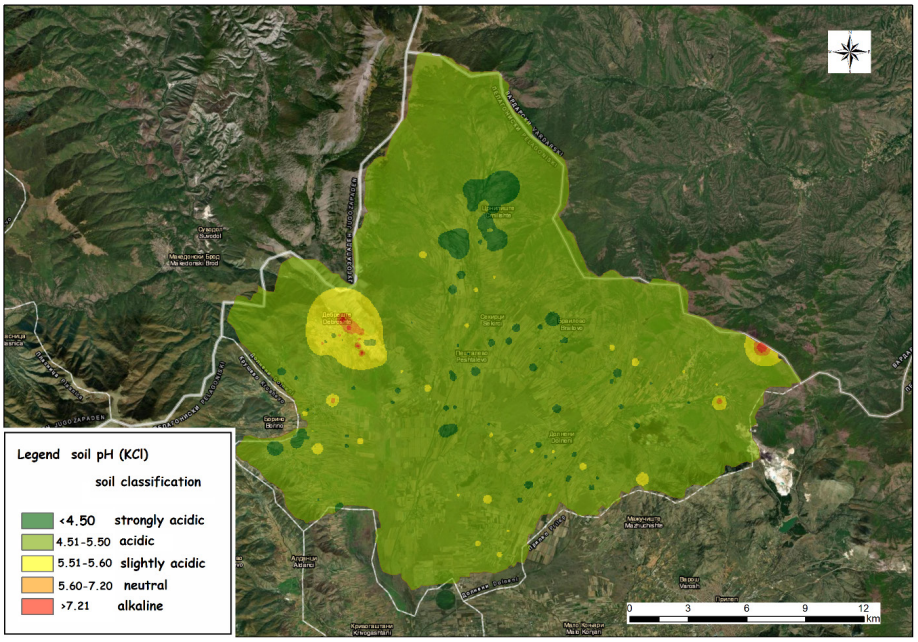

Figure 6. Spatial distribution of soil pH (KCl)

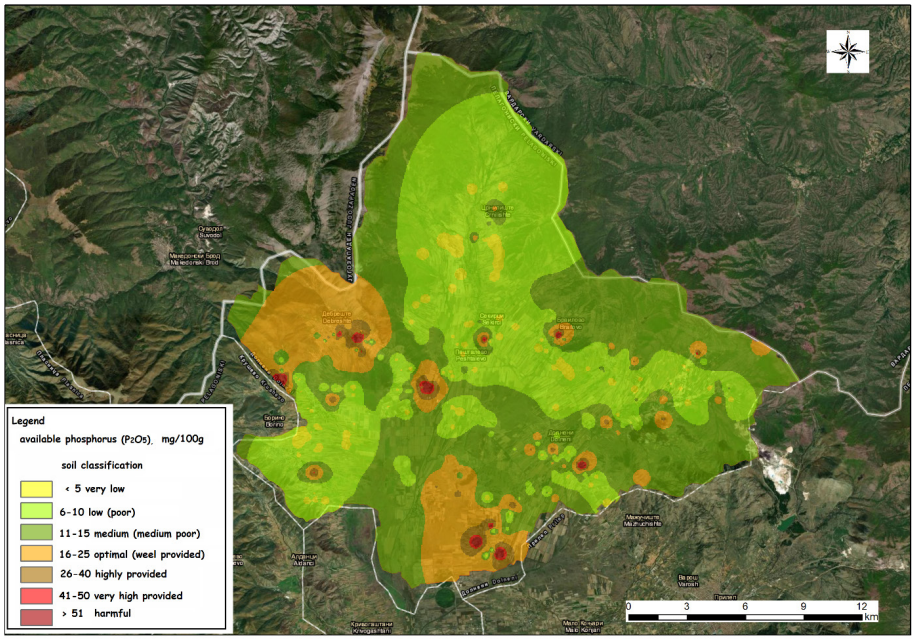

Figure 7. Spatial distribution of available phosphorus content (P_2O_5)

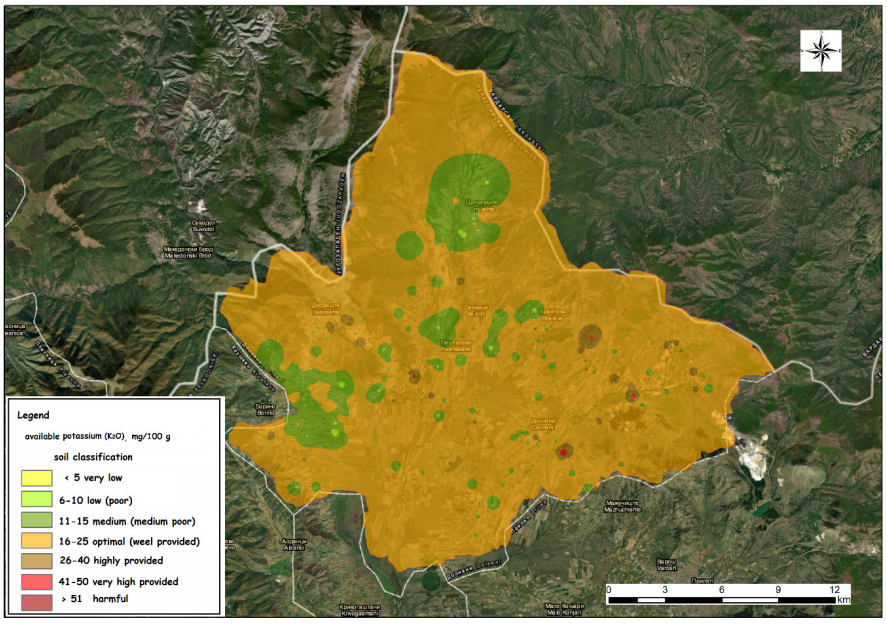

Figure 8. Spatial distribution of available potassium content (K_2O)

CONCLUDING REMARKS

Based on the results, we can conclude that monitoring soil fertility parameters is essential for sustainable tobacco production. Most of the soil samples have low nitrogen content, a slightly acid pH, and a light mechanical composition, making them ideal for the production of high-quality raw tobacco. To maintain fertility and prevent soil depletion, it is necessary to apply fertilizers in quantities derived from yields. It is recommended that soils with very high and extremely high levels of phosphorus and potassium to be fertilized only with nitrogen. This approach will significantly reduce costs per unit of production and avoid environmental burden. Given the fact that soil fertility is a dynamic value, further and extended monitoring is crucial in order to test and validate appropriate interpolation methods for estimating the spatial distribution of soil parameters. It is important to expand the availability of soil resource information maps to facilitate the planning of appropriate tobacco soil management practices, including fertilization for agricultural production and environmental protection.

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ПЛОДНОСТА НА ПОЧВАТА КАКО ПРЕДУСЛОВ ЗА ОДРЖЛИВО ПРОИЗВОДСТВО НА ТУТУН ВО ОБЛАСТА НА ОПШТИНА ДОЛНЕНИ

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Резиме

Производството на тутун во Република Северна Македонија има долга историја, затоа прецизното определување на параметрите кои ја отсликуваат плодноста на почвата е од суштинско значење. Главна цел на оваа студија беше одредување на просторна распределба од почвените параметри за почвите каде се одгледува тутун, како корисна стратегија за насочување на земјоделското производство и управувањето со теренот во одредена област. Како составен дел од одржливото производство на тутун, со дијагностицирањето на плодноста на почвата се обезбедуваат правилни и рационални препораки за ѓубрење. На 153 површински почвени примероци (0-30 cm) беа проучени следните параметри: pH, содржина на хумус, вкупен азот, достапни форми на фосфор и калиум, карбонати и механички состав. Примероците се земени од површината на општина Долени, која е составен дел на Пелагониски тутуно-производен регион кој зафаќа речиси 50% од вкупните површини за производство на тутун во државата.

Резултатите покажуваат дека својствата на почвата просторно се разликуваат. Врз основа на извршените класификации, 54 % од примероците почва имаат ниска содржина на хумус и азот, 65 % од примероците имаат ниска содржина на достапен фосфор и само 6,5 % имаат ниска содржина на достапен калиум. Реакцијата на почвата варира во граници кои се поволни за производство на тутун, а повеќето од анализираните почви се илести. Согласно истражувањето, комплексните ѓубрива како 10:30:20, 6:24:12 и 8:22:20 се најсоодветни за обезбедување оптимални услови за плодноста во истражуваната област.

Клучни зборови: *почвени параметри, Пелагонија, просторна распределба*

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INFLUENCE OF THE TYPE OF FERMENTER ON THE CHEMICAL COMPOSITION OF VRANEC AND MERLOT WINES

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Abstract

In this study, red wines from *V. vinifera* L. cv. Vranec and Merlot (harvest 2021) have been produced by three different fermentation methods, applying classical, roto and punch down fermenters, in order to study their influence on the wine quality. The chemical parameters that confirm the basic wine quality have been determined, including alcohol, dry extract, specific density, reducing sugars, total and volatile acidity, pH, free and total SO_2. The Vranec wines contained relatively higher content of alcohol compared to the Merlot wines, due to the higher content of sugars in the grapes. Wines produced with roto fermenter presented higher values of dry extract and total acidity, compared to the wine produced with classical and punch down fermenters, which confirms that roto fermentation is most suitable for production of complex and structured wines. The alcohol content in all wines ranged from 12.08 to 12.4%, total acidity was between 5.8 to 6.3 g/L and the dry extract from 39 to 45 g/L, while the content of volatile acidity was in range from 0.41 to 0.45 g/L. All wines were dry wines, with content of reducing sugar lower than 4 g/L and all of them were well protected from the oxidation. It was concluded that the type of fermentation affected the wine quality, confirming that applied wine technology is important for production of quality red wines.

Key words: *fermentation, Vranec, Merlot, chemical parameters*

INTRODUCTION

Wine is a beverage obtained by alcoholic fermentation of carbohydrates in the presence of yeast, which converts sugars into ethyl alcohol and carbon dioxide and releasing energy. The production of quality wine is influenced by numerous factors of which grape quality is one of the most important factors. However, the production of quality wine is not possible without good winemaking techniques and effective quality control. The production of red wine involves several procedures, such as grapes harvesting, grapes crushing, alcoholic fermentation (adding sulphur dioxide, yeast and nutrients to start alcoholic fermentation), maceration, addition of enzymes, cold stabilization, filtration, and bottling of the wine (Ivanova et al. 2012). After the addition of SO_2, the grape mash is inoculated with yeast (*Saccharomyces cerevisiae*) to start the alcoholic fermentation. The choice of the type of yeast depends on the grape variety as well as the characteristics of the wine that the producer wants to obtain (Lin et al. 2012).

The alcoholic fermentation is a complex biochemical process which depends on many factors such as: temperature, content of sugars, pH, acidity, presence of phenolic compounds as well as the content of the produced alcohol (Fleet, 2003, Divol et al. 2012, Mamolar-Domenech et al. 2023, Vion et al. 2023). Temperature is one of the most important factors for the start of alcoholic fermentation as well as for its duration. If the temperature is higher than necessary, the alcoholic fermentation starts earlier and lasts shorter. Therefore, the fermentation temperature should not be higher than 32°C. The sugars content in the must also affects the alcoholic fermentation. At low concentrations of sugars, the fermentation takes place slowly, while at concentrations higher than 250 g/L,

the fermentation is laboured and even may stop (Fleet, 2003).

The vessels in which the alcoholic fermentation process is carried out are called fermenters, which have a variety of shapes, sizes and technical designs. Today's fermenters are made of inox, stainless material and have a straight cylindrical or inverted cone shape. Classic fermenters are cylindrical stainless-steel vessels with a conical bottom, into which the crushed grapes enter from the upper opening. These fermenters are supplied with a pump that transports the fluid from the bottom to the top as well as temperature regulation, cooling with circulating water. Roto fermenters are cylindrical vessels in a horizontal position, supplied with a rotation programme. The contact between the must, skins and seeds is better and the maceration is more effective. Punch-down fermenters are cylindrical vessels in a vertical position, equipped with a cap submerging system, which consists of a punch-down agitator, pneumatic cylinder and a control unit. The punch-down mechanism makes it possible to break down the cap that was created during fermentation and submerge it into the must. The pneumatic cylinder continuously pushes the shovel-shaped punch-down agitator up and down, which makes the agitator gently pierce the cap and submerge it in the must.

This study is focused on the production of wines from Vranec and Merlot grapes, with three different types of fermenters: classic, roto and punch-down. The aim was to study the influence of the type of fermentation on the principal chemical parameters of wines from both varieties, such as alcohol, dry extract, specific density, reducing sugars, total and volatile acidity, pH, free and total SO_2.

MATERIAL AND METHODS

Chemicals and reagents

The following chemicals and reagents have been used: NaOH, concentrated H_2SO_4, $Na_2S_2O_3$, phenolphthalein, bromothymol blue, starch, KI, Feling I, Feling II and buffers (pH: 4, 7 and 7), all of them purchased from Alkaloid (Skopje). All reagents used were with analytical grade of purity. Ultra-pure deionized water with 0.0005 μS conductivity was obtained with a membrane filtration unit (Millipore, Molsheim France).

Winemaking

Grapes from *V. vinifera* L. *cv*., Vranec and Merlot were grown in Ovcepole wine region, harvested at optimal technological maturity (Vranec grapes: 24.5° Brix and Merlot grapes: 22.9 °Brix) in September 2021 and transported (~ 5 000 kg of each variety) to the wine cellar IMAKO, Stip, Republic of N. Macedonia. Both grape varieties were processed separately, applying the same technological procedures. Thus, the crushing of the grapes and the separation of the stems was carried out with a mechanical crusher/destimer (Della Toffola Treviso-Italy). The produced grape mash of both varieties was then transported to a cooling system at a temperature of 15 ^{0}C. Cooling was done using a heat exchanger (Della Toffola Treviso-Italy). To prevent oxidation, the cooled grape mash was treated with a 5% solution of sulphur dioxide, to reach a maximum concentration of free SO_2 of 40 mg/L. After cooling and sulfiting, the grape mash was transported to a suitable fermenter: classic, roto and punch down. All three types of fermenters have a volume of 9000 L.

A pectolytic enzyme (Sodinal, Speed up Rouge, 20g/100L) was added to each fermenter. After three hours, the grape mash in each fermenter was inoculated with commercial yeast *Saccharomyces cerevisiae* (Sodinal FERMCTIVE ROUGE, 20g/100L pre-activated and rehydrated in water at 30 ^{0}C), followed by the addition of nitrogen-based nutrients (ammonium dihydrogen phosphate, 10g/100L), necessary for the yeast to carry out the fermentation completely and successfully. Alcoholic fermentation in all three fermenters lasted 9 days for both varieties, and the temperature was constantly controlled at 24 ^{0}C by a cooling system.

The maceration for both grape varieties was carried out in a different way depending on the type of fermenter. In the classical fermenter the contact between the must, skins and seeds was achieved by mixing with a centrifugal pump that pumps the liquid together with the seeds and skins (from the lower part to the upper part of the fermenter) and thus caused mixing and improving the contact between the lees and the hard parts of the grapes. The mixing was programmed by turning on the pump 4 times a day. In the roto fermenter, maceration was performed by rotating the mash 4 times a day.

During this rotation, there was a mixing of the must and the solid parts of the grapes along the entire length of the fermenter. In the punch-down fermenter, the crushing of the formed cap of seeds and skins and their return to the liquid part was performed with the help of a piston that was programmed to push 4 times a day.

After completion of the alcoholic fermentation and maceration (the reducing sugar content was lower than 5 g/L), the wine from each of the three fermenters was separated from the pomace by pressing. The wine obtained from each fermenter was separately transported in a clean stainless-steel tank (7000 L). The wines were decanted and left to rest for a period of two weeks. After completion of the malolactic fermentation, the wines were sulphated again with a 5% solution of SO_2 to a concentration of free SO_2 to 40 mg/L. The wines were then left at a low outdoor temperature for a period of two months (December and January) to carry out natural tartrate stabilization. After stabilization, the wines from both varieties and fermented in the three different fermenters, were bottled in 0.75 L bottles under an inert nitrogen atmosphere to prevent the oxidation process. The wine bottles were stored in a cellar at a temperature of 2-8 °C for a period of one month before the analysis. Table 1 contains the labels of the produced wine samples.

Table 1. Labels of the wine samples.

Type of fermenter	Label of wine	
	Vranec	Merlot
Classical	V-C	M-C
Roto	V-R	M-R
Punchdown	V-P	M-P

Principal chemical composition

The following principal chemical parameters have been determined using the official methods of analysis of wines (OIV, 2022): alcohol (OIVMA-AS312-01 A), dry extract (OIV-MA-AS2-03B), specific density (OIV-MA-AS2-01 A), total acidity (OIV-MAAS313–01), volatile acidity (OIV-MA-AS313–02) and pH (OIV-MA-AS313-15).

Determination of reducing sugars, free SO_2 and total SO_2 was performed according to the methods published by Ivanova-Petropulos & Mitrev, 2014.

RESULTS AND DISCUSSION

The basic chemical parameters that are important for wine quality are: alcohol, dry extract, specific density, reducing sugars, total acidity, volatile acidity, pH, free SO_2 and total SO_2. These parameters determined for wine samples from Vranec and Merlot varieties obtained with three different types of fermenters (classic, roto and punch-down) are shown in Table 2.

Table 2. Basic chemical composition of Vranec and Merlot wines obtained under different fermentation methods.

Wine	V-C	V-R	V-P	M-C	M-R	M-P
Alcohol (%)	12.40	12.28	12.36	12.10	12.08	12.16
Dry extract (g/L)	40	45	40	39	44	39
Specific density	0.9957	0.9956	0.9959	0.9946	0.9946	0.9951
Reducing sugars (g/L)	2.7	2.4	2.6	2.2	2.5	2.4
Total acidity (g/L)*	6.1	6.3	6.2	5.9	6.0	5.8
Volatile acidity (g/L)**	0.44	0.44	0.42	0.45	0.41	0.44
pH	3.3	3.4	3.4	3.2	3.2	3.3
Free SO_2 (mg/L)	42	44	40	42	44	39
Total SO_2 (mg/L)	98	89	92	102	87	94

V-C: wine Vranec produced with classical fermentation, V-R: wine Vranec produced with roto fermentation, V-P: wine Vranec produced with punchdown fermentation, M-C: wine Merlot produced with classical fermentation, M-R: wine Merlot produced with roto fermentation, M-P: wine Merlot produced with punchdown fermentation.

*Total acidity expresses as g/L tartaric acid

**Volatile acidity expressed as g/L acetic acid

The alcohol content in the analysed wine samples from both varieties ranged from 12.08 to 12.4% (mean value 12.23%). The Vranec wines contained a slightly higher content of alcohol compared to the Merlot wines, regardless of the type of fermentation applied, because Vranec grapes had a higher content of reducing sugars than Merlot grapes (24.5° Brix in Vranec grapes and 22.9 °Brix in Merlot grapes). It is already known that according to the sugar content of the grapes/must, the alcohol content expected to be produced during fermentation can be approximately calculated. Thus, a wine with about 10% alcohol (*V/V*) should be obtained from must with 180 g/L of sugar. Considering the influence of fermentation, wines of both varieties obtained by roto fermentation presented the lowest alcohol value, while the highest value was measured for the Vranec wine obtained by the classic fermentation method (V-C) and the Merlot wine obtained by the punch-down method (M-P).

The wine dry extract consists of non-volatile soluble components, including sugars, non-volatile acids, glycerol, 2,3-butyl glycol, and phenols. In this study, the wines of both varieties presented high dry extract values, ranging from 39 to 45 g/L, which indicates that produced wines are rich and structured. Regarding the influence of the type of fermentation, the wines obtained by roto fermentation have the highest extract content, regardless of the variety, while the wines obtained by classical fermentation and punch-down fermentation have lower extract values. In fact, by the roto method greater extraction of the non-volatile components from the grapes in the wine has been achieved, confirming that the most complex and structured wines, more stable and richer wines have been obtained applying the roto fermenter.

Regarding the specific gravity of the wines, values ranging from 0.9946 to 0.9959 were obtained. These values are expected for red wines, according to the alcohol content and dry extract.

The main carbohydrates in grapes are glucose and fructose, which are usually called "reducing sugars". The content of sugars in grapes depends on the variety, ripeness, health of the grapes and the growing conditions. During alcoholic fermentation, the content of sugars decreases as a result of their conversion to ethyl alcohol, which usually results in dry wines with a low sugar content (< 5 g/L) (Neceva & Ivanova Petropulos, 2016). In this study, the analysed wines had a low content of reducing sugars ranging from 2.2 g/L (M-C) to 2.7 g/L (V-C), which means that all wines were dry and in all of them the fermentation ended successfully.

According to the wine acidity, it is very important to distinguish between the several types of wine acidity: total acidity, pH and volatile acidity, because all of them affect the wine's sensory characteristics. Total acidity is the sum of "non-volatile and volatile acidity", and includes all types of acids, such as formic acid, organic acids (tartaric, malic and citric), as well as amino acids. Since the tartaric acid is the predominant component in must and wine, the total acidity of the wine is expressed in tartaric acid equivalents. In this study, the concentration of total acidity ranges from 5.8 (M-P) to 6.3 g/L (V-R). These values are relatively high and sufficient to ensure the chemical and microbiological stability of the wines, as well as a sufficiently optimal freshness. Red wines are stable even at lower acidity due to the presence of phenols that increase acidity and enable stability of the wines during maturation. Taking into account the influence of fermentation, the wines obtained by the roto method presented slightly higher values of total acidity compared to the wines obtained by classical and punch-down fermentation.

Another factor that protects the wine and influences its stability is the pH. The pH values ranged between 3.2 to 3.4, typical for red wines. It is generally known that the lower the pH, the more difficult it is for microorganisms to survive. Comparing to Montenegrin wines (Pajović-Šćepanović, et al. 2016), the content of total acidy was slightly higher, and the value of pH was slightly lower in the analysed Vranec and Merlot wines.

Volatile acidity of wine represents a very significant physico-chemical parameter, which should be monitored during the winemaking. Increased volatile acidity in wine negatively affects its organoleptic characteristics, as well as its overall quality. The occurrence of increased content of volatile acids is related to the process of formation of acetic acid in a relatively high concentration (Neceva & Ivanova Petropulos, 2016). All wines, regardless of variety and type of fermentation, contained low and similar volatile

acidity in the range of 0.41 to 0.45 g/L, with no influence on quality. These values were expected, considering the fact that the temperature of fermentation was controlled and the content of added SO_2 in the wines was sufficient to protect the wines from oxidation and also from the creation of a higher concentration of acetic acid. The content of acetic acid is a parameter that is legally controlled and therefore constant and regular monitoring during production is necessary. The maximum allowed content of volatile acidity is 1.2 g/L acetic acid for red wines (Official Gazette of the Republic of Macedonia, 2012).

The use of SO_2 in winemaking is due to its ability to act as an effective antioxidant and antimicrobial agent. SO_2 has the ability to bleach pigments as well as the ability to eliminate unpleasant odours in wine. Moreover, SO_2 can selectively act against wild yeasts originating from grape skins and block their activity. Sulphur dioxide can be added into wine as a solution (5% solution of sulfuric acid) or as a potassium metabisulfite salt ($K_2S_2O_5$) which is ionized in an acidic environment releasing gaseous SO_2 (Ivanova-Petropulos & Mitrev, 2014). In this study, the free SO_2 content ranged from 39 to 44 mg/L, while the total SO_2 content ranged from 87 to 102 mg/L, which is a sufficient SO_2 content for the wines to be protected from oxidation.

Understanding the relationship between pH and SO_2 is of particular importance for winemaking. The effectiveness of the antimicrobial activity of SO_2 depends on the pH of the wine. The higher the pH of the wine, the less SO_2 will be need in a free form. Therefore, it is very important for wines to have a lower pH value and a higher content of total acidity, because in this way the microbiological and chemical stability of the wine would be achieved. If SO_2 is added in a higher concentration, it causes an unpleasant sharp smell of SO_2, and strengthening the metallic taste in the mouth, which has negative effects on the organoleptic characteristics of the wine, and thus on its the quality.

CONCLUDING REMARKS

Vranec and Merlot wines were produced by three types of fermentation, applying classical, roto and punch-down fermenters and the chemical composition was determined. Wines presented relatively high values of alcohol, dry extract, total acidity and low values of volatile acidity. This means that wines from both varieties had satisfactory quality, presenting good chemical and microbiological stability. Alcoholic fermentation was finished in all wines. Wine produces with roto fermenter contained highest dry extract and total acidity, which confirms that this roto fermentation was most suitable for production of more complex and structural wines.

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ВЛИЈАНИЕ НА ТИПОТ НА ФЕРМЕНТАТОР НА ХЕМИСКИОТ СОСТАВ НА ВИНА ОД СОРТИТЕ ВРАНЕЦ И МЕРЛО

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Резиме

Во ова истражување произведени се црвени вина од *V. vinifera* L. *cv*. сортите Вранец и Мерло (година на берба 2021) со примена на три ферментациони методи, со примена на класичен ферментатор, рото ферментатор и ферментатор со потиснување, со цел да се проучи нивното влијание врз квалитетот на винаta. Беа определени хемиските параметри со кои се потврдува основниот квалитет на виното, вклучувајќи алкохол, сув екстракт, специфична тежина, редуцирачки шеќери, вкупна и испарлива киселост, pH и слободен и вкупен SO_2. За винаta од сортата Вранец беше забележано дека имаат повисока содржина на алкохол споредено со винаta од сортата Мерло, поради повисоката содржина на шеќери во грозјето од сортата Вранец. Винаta произведени со рото ферментатор имаат повисока содржина на вкупен екстракт и вкупна киселост, споредено со винаta произведени со класичен ферментатор и ферментатор со потиснување, со што се потврдува дека рото ферментацијата и посоодветна за производство на комплексни и структурни вина. Содржината на алкохол во сите вина се движеше во граници од 12,08 до 12,4 %, вкупната киселост беше во опсег од 5,8 до 6,3 g/L и сувиот екстракт во граници од 39 до 45 g/L, додека содржината на испарлива киселост беше во граници од 0,41 до 0,45 g/L. Сите вина беа суви, со содржина на редуцирачки шеќери пониска од 4 g/L, и сите беа соодветно заштитени од оксидација. Беше заклучено дека начинот на ферментација влијае на квалитетот на виното, при што беше потврдено дека е многу важно каква технологија ќе се примени за производство на квалитетно вино.

Клучни зборови: *ферментација, Вранец, Мерло, основни хемиски параметри.*

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