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INTORDUCTION

In the past twelve years the educational, research and applicative 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 2nd International Meeting Agriscience & Practice (ASP 2019), giving an opportunity to the participants for presentation and discussion of original scientific and practical results in different fields of agriculture.

The 2nd International Meeting Agriscience & Practice (ASP 2019), heled on 12 April 2019 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 2019 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. 17, No. 2 mostly contains the presented papers from the 2nd International Meeting Agriscience & Practice (ASP 2019).

Editorial Board, **Editor in chief,**

December, 2019 **Prof. Liljana Koleva Gudeva, PhD**

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QUANTITATIVE CHARACTERISTICS OF RABBIT HYBRIDS

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Abstract

Flemish giant rabbit and New Zealand white rabbits (group ON), were used as a material for the study. Rabbits were fed ad libitum with commercial pelleted feed, with the addition of small amounts of green feed and hay. The experiment included 6 rabbits, 3 males and 3 females. Previously defined live weight of 1800 to 2500 g was achieved within 77 days and after that they were slaughtered. Study results presented in this paper show fattening and slaughter properties of Flemish giant rabbit and New Zealand white rabbit. The achieved average weight of male rabbits was 2792 g and meat-to-bone ratio was 47.92%, 2597 g and 48.61% respectively in female rabbits. Average participation of the back part of the carcass in male/female rabbits was 28.02% and 27.34%, dorsal part was 20.93% and 22.65%, front of the carcass was 25.26% and 25.39%.

Key words: *rabbit, hybrids, live weight*

INTRODUCTION

The food of animal origin still constitutes one of the most important components of human nutrition, because it is a source of numerous essential nutrients that a person needs for growth, development and normal functioning.

Rabbit meat products can be evaluated according to carcass quality and to meat quality as for other livestock. Carcass quality has to satisfy economic objectives, such as saleable meat yield and attractiveness to consumer (Gjurič, 1985; Dalle Zotte, 2002). The dressing percentage is a very important economic variable in the rabbit market. Some combinations of measurements such as retail cut weights or length measurements are necessary to predict lean percentage in the carcass (Yalçın et al., 2006).

The meat of rabbit belongs to the group of meat rich with protein, significantly low in fat and cholesterol and it contains essential nutrients: essential amino and fatty acids, calcium, copper, iron, vitamins from the B complex, low energy value, and less connective tissue (Koch & Pavčič, 2000; Wood et al. 2003; Dal Bosko et al. 2001; Pogány Simonová, et al. 2010; Chrenek, et al. 2012; Para et al. 2015). The meat has a mild taste and is easily digestible therefore it is known as a dietary food. It is recommended for children in growth, for a diet of patients with arteriosclerosis and enlarged lipids, as well as for patients with stomach illness and elderly people (Grün, 2002; Lah, 2006).

World rabbit meat production amounted to 1.56 million tonnes in 2014. The leading world producer of rabbit meat is China with 762,627 t year, while, in Europe, the main producer is Italy (268,980 t), followed by Spain (63,790 t), France (53,292 t), Czech Republic (38,602 t) and Germany (34,253 t year). Unfortunately, for most Balkan countries we have not managed to find data, except for Greece (6,799 t), Bulgaria (6,629 t) and Romania (143 t year) (FAOSTAT, 2014; Belichovska et al. 2017).

The purpose of cultivating the hybrids is to obtain the meat, that is, they are kept solely for slaughter. When creating animals - hybrids, two (or more) selected lines are crossed along by

either the father or the mother. During crossing two lines hybrids are created, which in turn will give the best features from both lines. The mother line brings fertile properties (increased number of offspring), while the father gives racial qualities (ability to grow rapidly, good food utilization, good slaughter quality and meatiness) (Grün, 2002).

The influences in the nutrition over the parts of the trunk, the fattening and slaughtering properties of rabbits have been studied by many authors (Panić & Petrović, 1989; Ozimba & Lukefahr 1991; Dalle Zotte et al. 1996; Skandro et al. 2004; Metzger et al. 2006; Omojola 2007; Baiomy & Hassanien 2011; Kostovska 2012). Chemical composition of rabbit meat has been especially investigated (Dalle Zotte et al. 1996; Hernández et al. 1998; Gondret et al. 1998; Nizza & Moniello 2000; Metzger et al. 2003; Wood et al. 2003; Pascual et al. 2004; Polak et al. 2006; Ali, 2007; Nistor et al. 2013).

The purpose of this paper is to present the fattening and slaughtering properties of the meat of crossed hybrids based upon our results and data.

MATERIAL AND METHODS

Six rabbit's hybrids (3 male and 3 female), that is, crossed units from the so-called Belgium oriash and New Zealand's white rabbits (ON) fed ad libitum with industrially pelleted food (containing alfalfa, barley, corn, wheat, soy, granules sunflower premixes, salt, vitamins and minerals) were the research material for our study.

The rabbits reached the defined weight from 1800 to 2500 g in 77 days kept in separate wire cages. 24 hours before slaughter, their feeding was stopped. Slaughtering and primary processing of rabbits was performed in the usual way. The slaughter was done after the veterinary examination and looting. 24 post mortem cool carcasses were cut in basic peaces and measured on an electronic scale, there in each organ individually, and then cut to the front, the middle and the back part (Bivolarski et al., 2011).

Meat-to-bone ratio (slaughter weight expressed in percentage) was calculated in compliance with the recommended processing procedure (Gjurič, 1985; Caklovica et al. 1986; Omrčen, 1995; Skandro et al. 2004; Ali, 2007).

Statistical analyses were made by using the statistical software SPSS ver. 21 (SPSS Inc, Chicago, IL, 2012).

RESULTS AND DISCUSSION

The middle values of live weight of male/female rabbits are shown following the cycle of 77 days of their age. The table shows that the live weight of male rabbits on average was 2792 grams while at female rabbits it was 2579 grams. The results that we found in relation to the live weight of male vaccines are in line with the results obtained by Kostovska (2012), while in relation to female units the results obtained by Kostovska (2012) compared to our results are higher for 191 grams.

The values we found in relation to the live mass compared to the values of live mass found by Ali (2007) and Skandro et al. (2008) are higher. Within our study the male rabbits achieved a higher body mass compared to females (for 213 grams), while in the studies by Skandro et al. (2008) the female rabbits achieved a higher body mass than the males for 10 grams.

Table 1. Meat-to-bone ratio (%) - slaughter and live weight (g) of male and female rabbits.

	ON Male	ON Female
Live weight (g)	2792±140	2597±90
Mass slaughter (g)	1338±90	1280±50
Meat-to-bone ratio (%)	47.92	49.29

$\bar{x}$ – mean, Sd – standard deviation; number of pieces = 3

Meat-to-bone ratio (%) of male rabbits is 47.92% while at female rabbits it is 49.29%. The results that we obtained in relation to the meat-to-bone ratio (%) are in line with the allegations of other researchers (Kovačević and Rašeta, 1983; Urošević et al. 1986; Skandro et al. 2008). Tafro et al. (1989); Skandro et al. (2004), Ali (2007) and Kostovska (2012) referred to meat-to-bone ratio (%) values for rabbits between 40 and 53%.

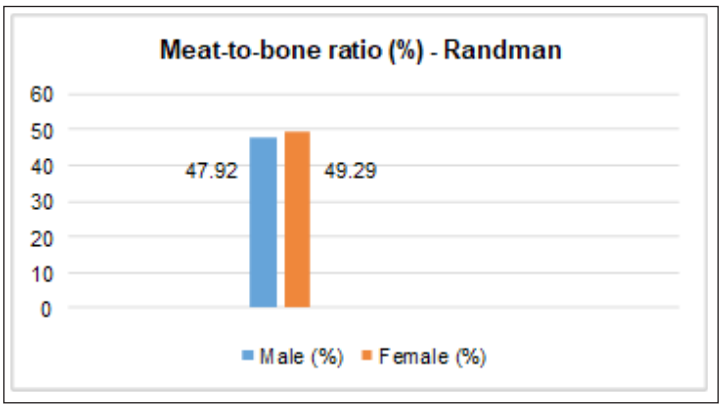

Figure 1. Meat-to-bone ratio (%) of male and female rabbits.

The average level of blood in the total body mass is 3.34%; at male rabbits it is 3.22% and at female rabbits it is 3.46%. A bit lower results in relation to the results obtained by us in terms of the prevalence of blood in the live weight of rabbits is stated by Kostovska (2012). The same author found blood levels in relation to the live weight of male rabbits of 2.95% and at female rabbits of 2.97%.

Skandro et al. (2008) found blood levels in relation to the live weight of rabbits, which is 7.20%, that is 7.70% at male rabbits and 6.70% at female rabbits. The results they received were greater than the results obtained by us probably because they also calculated the crack of cutting.

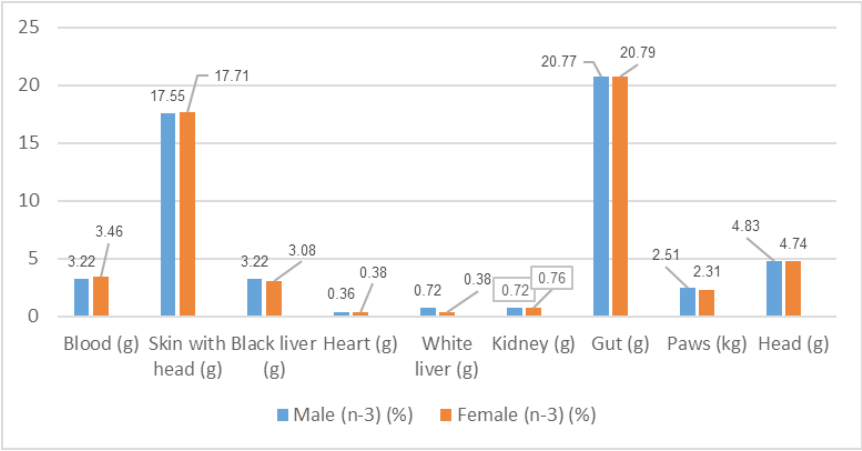

Figure 2. Participation of major meat categories in carcass composition of male and female rabbits.

The head with the total body weight at male rabbits is 4.83% and at female rabbits it is 4.74%. The results that we obtained are lower compared to the results obtained by Skandro et al. (2008); Kostovska (2012). Compared to our results, Kostovska (2012) found a slightly higher participation of the head in the body weight of the fuselage of 5.71% at male rabbits and 5.34% at female blacks, probably as a result of researching a larger number of rabbits (n = 18; 9 male / 9 female).

Skandro et al. (2008) determined the participation of the head in the final body mass of the body 8.68% (at male rabbits 8.71%, at female rabbits 8.65%). Our values were lower than the results obtained by Skandro et al. (2008) and Kostovska (2012) probably due to the larger number of male and female individuals (n = 24 rabbits; 12 male / 12 female).

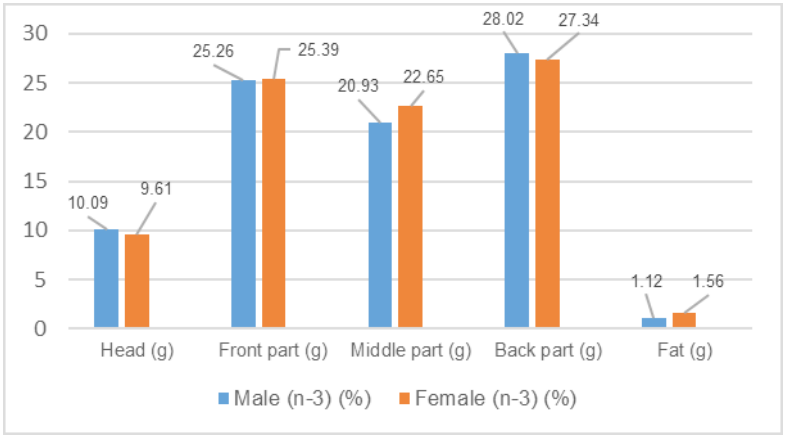

Figure 3. Participation of fat and major meat categories in carcass composition of male and female rabbits

The average participation of internal organs in the total body mass of male and female rabbits is 25.79%. Our results were lower than the results obtained by Ali (2007) who found a share of internal organs of 30.72%, and those were greater than the values obtained by Skandro et al. (2008) and Kostovska (2012), who established a share of internal organs of 23.84% at male rabbits and 23.48% at female rabbits, that is, 24.39% at male rabbits and 24.83% at female rabbits.

CONCLUSIONS

Based on results of investigation of quantitative characteristics of meat of Flemish giant rabbit and New Zealand white rabbit, it may be concluded as follows:

- Average carcass dressing percentage, without head, was higher in female rabbits,
- Participation of hind and back parts, considered as the qualitative parts of meat in dressed and cooled carcass, was higher in male rabbits.

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КВАНТИТАТИВНИ КАРАКТЕРИСТИКИ НА ХИБРИДИТЕ ЗАЈАЦИ

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Резиме

Како материјал за ова истражување ни послужија хибриди на вкрстени единки од т.н. белгиски оријаши и новозеландски бели зајаци (ОН), кои беа хранети ад либитум со индустриски пелетирана храна. Во истражувањето беа опфатени шест зајаци и тоа три машки и 3 три женски. Дефинираната жива маса од 1800 до 2500 g зајаците ја постигнаа за 77 дена, по што се изврши колењето. Во трудот се изнесени резултатите од истражувањето на товните и клавните својства, како и утврдување на составот на месото од зајаци. Достигната е просечна жива маса на машките зајаци од 2792 g, и рандман од 47,92%, додека кај женските зајаци просечната жива маса беше 2695 g и рандман од 48,61%. Просечен удел на задниот дел од трупот кај машките/женските зајаци 28,02% и 27,34%, на грбниот дел 20,93% и 22,65%, додека на предниот дел на трупот 25,26% и 25,39%.

Клучни зборови: *зајаци, хибриди, жива маса.*

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CONSUMER PREFERENCES AS DETERMINANT OF THE SUCCESSFUL QUALITY MANAGEMENT OF DAIRY PRODUCTS

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Abstract

The strategic goal of managing the quality of milk is a premium product with a high level of internal and external quality characteristics. In order to succeed, most of the activities in the management process should focus on those quality features that are recognizable for the average consumer of milk. For this purpose, was conducted an indicative survey of a sample of 1130 respondents from the young population was conducted. The results show that young consumers from the internal quality characteristics mostly value the taste and quantity of milk fats in the category 2.8% -3.2%, while from the external quality characteristics they value the price and origin of the product or the origin of the milk. The origin of the milk is not fully utilized in the promotional efforts of the producers in its promotion, especially in the sub-segment of urban young consumers, which would clearly differentiate the producers, but they would also have a competitive advantage on the domestic and international market.

Key words: *consumer preferences, market, quality, quality management, milk*

INTRODUCTION

Quality is often described as a set level of certain properties of a product or service that indirectly or directly affects the use value or usefulness of products or services. Therefore, quality becomes an important factor in non-price competition, which largely depends consumer satisfaction in the domestic and international markets. Although the term quality is emphasized as the basis for achieving comparative advantages, quality management, as a kind of new form of management, becomes one of the most important factors in the design, development and growth of the market competitiveness of business entities.

This puts in focus the qualitative growth of each manufacturer, the high quality of the product and its maintenance, as well as the tendency for continuous improvement of the internal and external quality characteristics.

Cow's milk is a biological product whose composition depends on many factors, such as race, individual characteristics and genetic factors, animal nutrition, lactation phase, environmental conditions, and animal health conditions (Šimundić, 2008; Cashman, 2006). The quality of milk is determined by organoleptic analyses that determine the colour, consistency, smell and taste; then it is determined by physical and chemical methods that determine the specific weight, the degree of acidity, the amount of milk fat, the amount of dry substance etc., as well as by applying microbiological tests to determine the total number of microorganisms and the type and number of pathogens (Zamberlin et. al., 2012).

But in practice, data on water content, milk fat, protein, milk sugars (lactose) and total non-fat dry matter are the most frequently used (Bosnić, 2003). Quality characteristics can be divided into interior, that are closely related to physical performances and cannot be separated from the product during consumption (taste, smell, composition), and external that are bound to the product, but are beyond its physical shape

(price, country of origin, image, guarantee) and are becoming an important point of reference in the decision-making process for purchasing (Jiménez-Guerrero et.al., 2012).

The indicated characteristics of milk can also be seen as key words by which potential and actual milk consumers identify quality, because understanding and managing customer needs is one of the main areas of the concept of quality improvement (Lazibat et al., 2007) i.e. the buyer is which should be the starting point and the final point in the quality management (Pešić, 2009; Filipović et. al., 2008).

As the perception, that is, the subjective quality assessment differs from consumer to consumer (Weatherell e t al., 2003) so the various marketing efforts should focus on those quality characteristics that a particular consumption group recognizes and at the same time values them. Consumers should be informed in an appropriate manner how the milk from a simple, generic product shifts into a value-added product based on different quality characteristics (Pažek et al., 2014).

Young consumers, of course, do not represent a segment that has the highest consumption of milk, but it should not be forgotten that it is this young population that at the end of its adolescence forms its views, opinions and preferences that will remain for their entire life. Which means exactly this age group should be directed large promotional efforts in creating a culture of drinking milk.

Distinguishing one's own product from that of a competitive milk producer can be achieved by emphasizing the country of origin, certain areas of production, or at best through a distinguishable national producer (Wolf et al., 2011).

Straete and Marsden define two dimensions of spatial product quality: non spatial where the land and place of origin do not have any importance and a localized dimension where the degree of importance for highlighting the place of origin is very high (Stræte et al., 2006).

Milk belongs to the group of localized quality with a prominent importance in emphasizing the place of origin. The origins and the image have proven to have a positive effect on the subjectivity of consumers in the decision-making process (Tempesta et al., 2013, Taglioni et al., 2011).

In the perception of the consumer, there is a difference between domestic and imported milk that is in favor of domestic milk (Chambers et al., 2007). This was one of the reasons why in 2013 in Republic of Macedonia started a project aimed at stimulating higher consumption of milk by domestic producers, which resulted in the creation of a recognizable sign for consumers of the packaging of Macedonian products. The introduction of this promotional sign 'Macedonian sun' for the quality of the export products, but also for the products that are being put into circulation on the domestic market, means building a national recognizable brand. Over time, products become recognizable both nationally and internationally.

The importance of such 'designer' markings on quality is certainly reflected in the determination of the country of origin of the product, increased demand by customers, but also the reduction of consumer sensitivity in terms of price (Fotopoulus et al., 2009).

In any case, the strategic goal for quality management of milk should be a top product whose internal and external characteristics are roughly equal, but high-quality level is what requires continuous research and monitoring of specific changes in consumer perceptions.

The main goal of this paper is to investigate and determine the preferences of young consumers in terms of external and internal characteristics of milk through their demographic and socio-economic characteristics. The information obtained from this research can serve as a framework for future research on consumer preferences on a sample of the entire population of the Republic of Macedonia, as well as for other individual market segments of milk consumers.

MATERIAL AND METHODS

The research used a method of collecting primary data as a research tool, and the survey was conducted in the period from February to May 2018. The entire questionnaire contains 34 closed-type questions divided into several groups that refer to milk consumption, milk quality, price significance, distribution and promotion, as well as the attitudes of the respondents with respect to milk and dairy products. Finally, are given questions concerning the socio-demographic characteristics of the respondents.

Due to the size of the research, the paper presents only sections related to the attitudes of consumers in relation to certain determinants of the quality of milk. Target group of respondents are young people aged 18 to 25 years old, and the sample is intended and includes 1,130 respondents from Republic of Macedonia, out of which 1,060 reported that they consume milk (91,6%) and their responses are considered relevant. Of the total number of respondents, 63.2% are female, and 36.8% male. Almost half of the respondents are from urban areas (47.3%), of which 14.1% are from the suburbs and 38.6% from the rural areas.

A total of 13.1% of the respondents have monthly household incomes of less than 29,999.99 denars, 18.6% of them 30,000.00-44,999.99, then 19% from 60,000.00-84,999.99, and 14.5% with monthly income over 85,000.00 denars, while 21.8% of the respondents did not want to report or did not know how to answer the question about monthly income in the household. The income of their households is derived from non-agricultural activities in 72.4% of the respondents, while in 6.4% of the respondents; household income is generated only from agriculture as an economic activity.

The data collected in the research were processed with statistical software SPSS, while in the research a method of descriptive statistical data analysis was used to describe the sample (frequency, percentage, arithmetic mean, standard deviation). Inferential statistics procedures also are used to determine the probability that the conclusions based on the information are valid. Of the nonparametric tests, a chi-square test (χ^2-test) was used, to determine whether there are differences in the preferences among the respondents in view of the socioeconomic and demographic variables. From parametric tests, independent t-tests and simple variance analysis (one-way ANOVA) were calculated to check the differences in individual views among the examinees.

RESULTS AND DISCUSSION

Quality milk on the market should be more identifiable primarily through the quality of its basic qualitative characteristics, which in practice is often not the case. The perception of consumers in terms of quality is far ahead of the simple perception of product quality with regard to the perception of the quality of the entire production process, i.e. of the quality management (Rijswijk et. al., 2008). The main reason for this is different attitudes and preferences of individual consumer groups, on which depends or should depend on the creation of the elements of the marketing mix of each product when they are present on the domestic or international market.

The preferences of certain consumer groups or subgroups are sometimes relevant data to producers rather than the preferences of the total population (Bayarri et al., 2011). Young consumers are particularly important in milk consumption (Yayar, 2012). Watanabe points out that young people from larger families and people with calcium deficiency drink milk very often and are therefore particularly interesting as a market segment (Watanabe, 1996). In order to determine how much milk quality is at all a significant consumption group called 'young consumers', it is also asked about the most important elements that are taken into account when making the purchase decision, as well as the consumption of milk. Using the descriptive statistic methods, the frequencies (n) and percentages of answered questions are calculated as shown in Table 1.

Table 1. Distribution of responses given to the most important factors when making a decision to purchase milk.

	N	Participation (%)
Design and packaging	66	5.7
Quality	242	20.92
Favorable price	426	36.82
Brand of the product	133	11.5
Milk fat percentage	426	36.82
Producer (origin)	291	25.15
Friend recommendation	34	2.94
Aroma and smell	287	24.81
Taste	607	52.46
Macedonian product	245	21.18
Better promotion	13	1.12

More than half of the respondents (52.46%) answered that the taste is the most important and largely important for them are the price of milk and the percentage of milk fat. The taste and content of milk fat are essential characteristics whose dominance is also reflected in the researches of certain authors who have proven the connection between young consumers and the high appreciation of taste as a qualitative characteristic. It should not be ignored that the price (36.82%), origin (25.15%) and Macedonian product (21.18%) are the most important external features, while the promotion of products is completely marginalized and is placed in the last place (1.12%).

The origin and the Macedonian product can be perceived as one group, thus indirectly crossing into the category of the most important external characteristics, which corresponds to the results of the researches of Tempesta and Vecchiata (Tempesta et al., 2013), which have proven that the country of origin for Italian consumers is the most important external feature, followed by price, and at the very end is the economic propaganda as an element of the promotional mix. The increasing importance of external features (Bernués et al., 2012; Hovhannisyan et al., 2012) is also reflected in many studies of foreign authors, especially the country of origin (Ehmke et.al., 2008; Fotopoulus et. al., 2009) in the category of milk (Taglioni et al., 2011; Kapaj et al., 2013; Cernea, 2011; Braghieri et al., 2014).

In order to reveal the essential characteristics of the quality of milk that the young consumers group perceived as the most significant, they asked the question "With which words do you link the quality of milk?". The distribution of responses is shown in Figure 1.

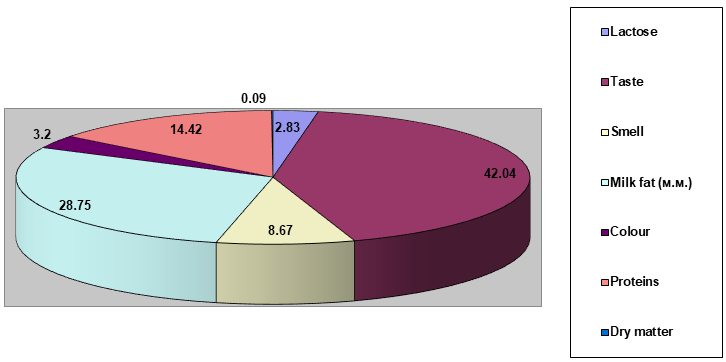

Figure 1. Distribution of responses related to milk quality.

The quality of milk is mostly related to the taste (42.0% of the respondents), but also the amount of milk fat (28.7%). There is a statistically significant difference between sexes related to the term they associate with milk quality ($p < 0.01$). Male examinees more closely associate quality with protein (16.7% compared to 13.0% women) and taste (44.7% compared to 40.5% women), while female respondents more closely associate with the amount of milk fat (30.7% compared to men 25.3%) because of the reason women consume products with less milk fat.

In order to determine the trends in milk consumption in Republic of North Macedonia, the questionnaires are asked questions about consumption depending on the amount of milk fat, whereby the obtained answers are shown in Table 2. The results of the research shown in Table 2 show that 55.5% of the examinees, even more than half, usually consume milk with 2.8-3.2% milk fat, 15.2% consume milk with less than 2% milk fat, 23.4% in the subcategory 2-2.79 % milk fat, and 5.9% of the examinees consume milk with milk fat greater than 3.2%.

Considering the demographic and socio-economic factors (Yayar, 2012), statistically significant differences exist in the area as category ($\chi^2 = 16.04$, df = 6, $p < 0.05$) and household income ($\chi^2 = 39.95$, df = 15, $p < 0.01$), which corresponds to the research of Andersen and Smed, which on a sample of 1347 households in Denmark showed statistical differences in the same socio-demographic categories (Andersen et al., 2013).

Table 2. Distribution of responses considering with consumption of milk depending of different milk fat content (in %).

		N	<2 %	2-2.79 %	2.8-3.2 %	>3.2 %	sig (p)
Total		100	15.19	23.40	55.47	5.94	
Gender	Male	36.51	14.21	20.41	58.66	6.72	>0.05
	Female	63.49	15.75	25.11	53.64	5.50	
Area	Urban	46.89	17.91	24.75	53.92	3.42	<0.05
	Suburban	14.34	13.82	23.03	55.26	7.89	
	Rural	38.77	12.41	21.90	57.42	8.27	
Income per household	To 29.999	16.69	10.07	20.14	64.03	5.76	<0.01
	30.000-44.999	23.04	14.06	20.84	51.04	14.06	
	45.000-59.999	25.21	15.71	29.05	51.91	3.33	
	60.000-84.999	16.69	17.99	21.58	56.12	4.31	
	More than 85.000	18.37	14.38	20.92	60.13	4.57	
Source of income	Non-agricultural activities	72.08	14.92	23.82	56.68	4.58	>0.05
	Non-agricultural and agricultural	21.41	15.42	21.14	55.07	8.37	
	Only agricultural	6.51	17.39	26.09	43.48	13.04	

Note: In order to determine the difference in milk consumption considering the categories of responses to demographic variables, chi-square tests (χ^2) were calculated.

Table 3. Respondents attitudes regarding to the quality and origin of milk.

Claims	N	M	SD
Higher milk fat in milk indicates that it is better milk	1130	3.25	1.062
Domestic producers produce milk with better quality than foreign (Proposition 2)	1130	3.56	1.058
When buying milk, it is very important for me to be a domestic producer or a home-made milk (Proposition 3)	1130	3.27	1.16
Milk from Macedonian farms is healthy milk (Proposition 5)	1130	3.67	0.921

Note: N = total number of respondents; M = arithmetic mean; SD = standard deviation

Table 3 shows that young consumers believe that our domestic producers produce more quality milk than foreign producers and that milk is healthy milk but are not well informed with the promotional sign 'Macedonian sun' that should additionally valorise milk as a product. Markings about the quality or origin of the product worldwide become a significant reference value for consumers (Bernués et al., 2012) who perceive such products as superior quality products (Hovhannisyan, et al., 2012) for which people are willing to pay a higher price (Liu et al., 2013).

Braghieri has again proved that milk consumers in Southern Europe with the quality are most closely related to traditions and heritage, while consumers from Central and Northern Europe are related with the impact on health and availability of products (Braghieri et al., 2014). In order to see if there is a difference in the individual views of the respondents regarding the established claims for the quality of milk and the origin of the product mentioned in the previous table, considering their socio-economic and demographic characteristics is used a t-test and a simple analysis of the variance (ANOVA) as described in Tables 4,5,6 and 7. There are statistically significant differences in the attitudes of the respondents regarding the area from which they come, the magnitude of the income and the source of income. Compared to the respondents coming from the cities, respondents coming from the village more agree with the claim 'Domestic producers produce better milk than foreign producers' ($F = 4.269$, $df = 2$, $p < 0.014$). 'When buying milk, it is very important to me whether it is from a domestic producer i.e. from domestic milk' ($F = 4.349$, $df = 2$, $p < 0.013$) and 'Milk from Macedonian farms is healthy milk' ($F = 5.631$, $df = 2$, $p < 0.004$).

Respondents with higher incomes agree with the claim 'I am well acquainted with the trademark 'Milk from Macedonian farms' ($F = 7.142$, $df = 2$, $p < 0{,}000$). 'The milk of the Macedonian farms is healthy milk' ($F = 5.047$, $df = 2$, $p < 0.007$), 'The higher is the milk fat in the milk, the milk is better' ($F = 3.350$, $df = 2$, $p < 0.035$) and 'When buying milk, it is very important to me whether it is a domestic producer i.e. domestic milk' ($F = 13.290$, $df = 2$, $p < 0.000$).

From the stated results it emerges that producers and distributors of milk and dairy products should, in the process of creating a production strategy for market appearance (Olynk, 2012), also orient themselves to preferences of consumers divided into certain market segments, but also sub-segments.

Even within the market segment of young consumers, there is a distinction between the rural population that is more valued by domestic products, the Macedonian origin of milk, and a greater amount of milk fat in milk. Therefore, the segment of young consumers can be divided into sub-segments of rural young and urban young consumers that require a differentiated appearance on the market by the producers.

Table 4. Testing the differences in the arithmetic meanings of the claims by gender.

	Gender					
	Male		Female			
Attitudes	M	SD	M	SD	t-test	P
Claim (1)	3.24	1.046	3.26	1.072	0.310	0.757
Claim (2)	3.56	1.064	3.56	1.055	0.070	0.944
Claim (3)	3.27	1.192	3.27	1.142	0.032	0.974
Claim (4)	2.85	1.179	2.92	1.189	0.840	0.401
Claim (5)	3.67	0.918	3.67	0.923	0.054	0.957

Note: To determine the differences in the arithmetic meanings of the claims in relation to the gender category, a t-test for independent samples was used (*p <0.05; **p <0.01).

Table 5. Testing the differences in the arithmetic meanings of the claims in relation to the area.

	Where do you come from?								
	Urban		Suburban		Rural				
Attitudes	M	SD	M	SD	M	SD	df	F-ratio	p
Claim (1)	3.19	1.048	3.22	1.094	3.34	1.065	2	2.440	0.088
Claim (2)	3.49	1.022	3.48	1.027	3.67	1.103	2	4.269	0.014*
Claim (3)	3.18	1.141	3.23	1.179	3.40	1.168	2	4.349	0.013*
Claim (4)	2.89	1.167	2.91	1.206	2.89	1.202	2	0.017	0.983
Claim (5)	3.58	0.872	3.70	0.950	3.78	0.958	2	5.631	0.004**

Note: To determine the differences in the arithmetic meanings of the claims in relation to the area category, a simple analysis of the variance - ANOVA was used (*p <0.05; **p <0.01).

Table 6. Testing differences in the arithmetic meanings of claims in relation to household incomes.

	How much is the monthly income of your household (net amount)?												
	<29.999		30.000-44.999		45.000-59.999		60.000-84.999		>85.000		df	F-ratio	p
Attitudes	M	SD	M	SD	M	SD	M	SD	M	SD			
Claim (1)	3.39	1.10	3.19	1.02	3.25	1.08	3.23	1.09	3.36	1.14	2	1.427	0.212
Claim (2)	3.55	1.10	3.41	1.08	3.52	1.06	3.55	1.08	3.62	1.12	2	1.663	0.141
Claim (3)	3.09	1.21	3.20	1.08	3.33	1.23	3.47	1.15	3.31	1.21	2	1.967	0.081
Claim (4)	2.77	1.23	2.58	1.22	3.00	1.13	3.13	1.19	3.17	1.12	2	7.142	0.000**
Claim (5)	3.69	0.94	3.53	0.99	3.73	0.86	3.62	0.93	3.77	0.90	2	1.821	0.106

Note: To determine the differences in the arithmetic meanings of the claims in relation to the income's category, a simple analysis of the variance - ANOVA was used (* p <0.05; ** p <0.01).

Table 7. Testing the differences in the arithmetic meanings of the claims about the sources of income

	Who is the main source of income in the household?								
	Non-agricultural activities		Agriculture and non-agriculture		Exclusively agricultural activities				
Attitudes	M	SD	M	SD	M	SD	df	F-ratio	p
Claim (1)	3.22	1.059	3.27	1.098	3.56	0.927	2	3.350	0.035*
Claim (2)	3.53	1.045	3.61	1.091	3.73	1.076	2	1.514	0.220
Claim (3)	3.18	1.176	3.44	1.102	3.81	0.952	2	13.290	0.000**
Claim (4)	2.91	1.159	2.73	1.277	3.30	1.068	2	6.447	0.002**
Claim (5)	3.63	0.902	3.74	0.966	3.96	0.924	2	5.047	0.007**

Note: To determine the differences in the arithmetic meanings of the claims in relation to the source of incomes category, a simple analysis of the variance - ANOVA was used (*p <0.05; **p <0.01).

CONCLUDING REMARKS

Trends in food consumption are constantly changing, depending on the specific characteristics of various consumer groups. The results show that for young consumers, a basic indicator of the quality of milk is the amount of milk fat and taste that satisfy their needs and desires, during consumption. However, the importance of the country of origin must be emphasized as an external characteristic of quality, which would awaken the awareness of a recognizable and quality Macedonian product, as well as using emotional notes that would develop the relationship and connectivity of the buyers with the product.

Manufacturers and distributors certainly need to know their customers well so that they can make decisions based on accurate information, create new products, but also to be able to specify marketing activities to individual market segments that can differentiate quality.

The survey shows that there is a significant difference between young consumers from urban and rural areas, where the rural young population more values the amount of milk fat,

then the domestic production and the origin of the product. Orientation and creation of added value in relation to each market segment i.e. sub-segment, education for autochthonism and tradition, as well as development of a culture of drinking of domestic milk especially in the sub-segment of the young urban population are certainly the correct procedures in creating a framework and promoting the activities in the process of quality management of milk.

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ПРЕФЕРЕНЦИИТЕ НА ПОТРОШУВАЧИТЕ КАКО ДЕТЕРМИНАНТА НА УСПЕШНОТО УПРАВУВАЊЕ СО КВАЛИТЕТОТ НА МЛЕКОТО

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Резиме

Стратешката цел на управувањето со квалитетот на млекото, секако, е премиум производ со високо ниво на внатрешни и надворешни карактеристики на квалитетот. Со цел да се успее во тоа, најголем дел од активностите во процесот на управување треба да се фокусираат на оние карактеристики на квалитетот кои се препознатливи за просечниот потрошувач на млеко. За таа цел, спроведено е индикативно истражување на примерок од 1130 испитаници од младата популација. Резултатите покажуваат дека младите потрошувачи од внатрешните карактеристики на квалитетот најмногу ги вреднуваат вкусот и количината на млечни масти во категорија 2,8%-3,2%, додека од надворешните карактеристики на квалитетот ги вреднуваат цената и потеклото на производот или потеклото на млекото. Потеклото на млекото не е до крај искористено во промотивните напори на производителите при негова промоција, особено во потсегментот на урбани млади потрошувачи, со што производителите јасно би се диференцирале, но и би остварile конкурентска предност на домашниот и на меѓународниот пазар.

Клучни зборови: *потрошувачки преференции, пазар, квалитет, управување со квалитетот, млеко.*

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THE INFLUENCE OF ORGANIC FERTILIZERS ON THE GROWTH AND YIELD OF BROCCOLI (*Brassica oleracea* L. var. *italica*)

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Abstract

The experiment was conducted in order to determine the influence of organic fertilizers on the growth dynamics and yield of broccoli grown in the open field. The variety Verdija F1 cultivated in Skopje region during one year (2018) was used in the experiment. The treatments were as follows: Ø control - no use of organic fertilizer, V-1 - foliar treatment with Biohumus, V-2 - foliar treatment with Orgalife, V-3 - foliar treatment with Bio – Vital. The treatments were conducted every 10 days, starting on 19.08.2018, three times during vegetation period. The following traits were examine: dynamics of growth and broccoli yield. According to the results, the V-2 foliar treatment with the organic fertilizer Orgalife resulted in considerable higher yield on the central flower (6.98 t/ha) as compared to the control treatment (5.41 t/ha), proven with statistically significant difference, at the level of 0.05 with the LSD test. The results obtained for the average height of the plants in the three treatments showed that there are highly statistically significant differences at the level of 0.01 between the V-2 treatment (55.6 cm) and the control (48.7 cm). According to the overall results of the experiment, it can be concluded that the V-2 foliar treatment has given the best results in both traits as the plant height and the yield obtained.

***Key words**: growth dynamics, Biohumus, Orgalife, Bio – Vital*

INTRODUCTION

Broccolis belong to the group of leafy vegetables from the family Brassicaceae; they are an important vegetable crop with high nutritional and good commercial value (Yoldas et al., 2008). They are food with a low concentration of salts, without fats and calories, rich in vitamin C and a good source of vitamin A, vitamin B2 and calcium (Decoteau, 2000). According to Apahidean (2011), the broccoli inflorescences (flowers) have great therapeutic value in the battle against cancerous diseases. Today, broccolis attract more attention because of their variety of uses and excellent nutritional value (Salunkhe and Kadam, 1998; Talalay and Fahey, 2001; Rangkadilok et al., 2002;). The broccoli production, along with the application of organic fertilizers, could serve as an alternative to mineral fertilizers (Gupta et al., 1988; Wong et al., 1999; Yoldas et al., 2008; Farahzety and Siti Aishah, 2013) as well as to improve the soil structure (Bin, 1983;) and microbiological biomass (Suresh et al., 2004). However, it has been shown that liquid organic fertilizer as compost tea contains nitrogen mainly in an inorganic form such as ammonia (Price and Duddles, 1984; Gross et al., 2007) and make the nutrients immediately available to the plants. Bio-fertilizers play a multifunctional role, not only in the soil improvement process with microorganisms, but also as nutrients, stabilizers, hormones and isolates (Mohapatra et al., 2013). The application of organic (bio) fertilizers in the production of vegetables leads to higher plant productivity, quality improvement, early fruit growth and greater fruit durability.

MATERIAL AND METHODS

The experiment was set up near Skopje, in the village of Jurumleri, on alluvium soil type, during the cultivation season in 2018. Broccoli (*Brassica oleracea* L. var. *italica*), hybrid Verdia F1 was used as research material. The broccoli was cultivated from seedlings produced in cold beds, transplanted on open field. During the vegetation, the plants were treated with organic fertilizers Biohumus - organic fertilizers made from California worms in liquid (V-1 treatment), Orgalife – organic fertilizers made from California worms in liquid (V-2 treatment) and Bio - Vital microbiological fertilizer which contains bacteria in its composition (nitrifying microorganisms, phosphate-solubilizing microorganisms and cellulolytic microorganisms) (V-3 treatment). All fertilizers were used in concentration of 1% solution.

The treatments were set in three replications in the following order:

1. Ø control – no use of organic fertilizer,
2. V-1 – foliar treatment with Biohumus,
3. V-2 – foliar treatment with Orgalife,
4. V-3 – foliar treatment with Bio – Vital.

Each treatment was applied every 10 days. The beginning of the treatments started on 19.08, the second application of fertilizers was on 29.08 and the third application was performed on 09.09.

During the vegetation, dynamics of plant growth and yield were monitored. The dynamics of plant growth were monitored by measuring the height of the plants before each treatment.

The plant height was determined using a metro and the height was measured from the base to the top of plant. The height measurement was performed before each application of the organic fertilizers.

The yield per plant was determined only by the central inflorescence, by measuring them on an analytical scale and determining the arithmetic mean.

An analysis of - variance (ANOVA) for the two factors, the application timing and the fertilizer type for plant height and a single yield factor, was performed to determine the data obtained, and the smallest significant data ratios were determined by the LSD test at the level of $p = 0.05$ and $p = 0.01$.

RESULTS AND DISCUSSION

Plant height

It is widely known that the broccoli height is usually from 50 to 100 cm with spirally arranged leaves with an elongated shape, in the base more or less cut off depending on the type. In general, the plant is permeable, and the inflorescence is formed at the tip of the elongated trunk. In the generational phase, the broccoli tree grows from 50 to 90 cm (Lešić et al., 2002).

The results for the average plant height after applied treatments are given in Table 1. According to the results, the average plant height ranged from 32.4 cm in V-3 treatment at the first measurement to 67.6 cm, in V-2 treatment at the third measurement. The difference between the control (no use of organic fertilizer) and the treatments ranged from 6.9 cm (V-2 treatment) to 0.7 cm (V-3 treatment). The absolute dispersion was the highest in the control treatment and V-3 treatment, while the lowest was found for the V-1 treatment. The relative placement of treatment data was the highest for the V-2 treatment with the highest plants (8%) and the lowest for the control treatment.

Statistical significance at the level of p=0.01 was obtained only in the V-2 treatment. While insignificant differences between the treatments were obtained in V-1 in terms of control and the V-3 variant, while the V-2 secondary variant varies considerably with all variants (V-2 and V-1) and control. The V-3 treatment did not show any significance regarding the treated, and the data was closest to the control variant.

The average height of plants ranged from 48.7 cm in control, at a V-3 height of 49.4 cm, at a V-1 height of 49.7 cm, and the highest average height had plants in V-2 of 55.6 cm.

The results obtained for average plant height from all three treatments showed high statistically significant differences of 0.01 between control and V-2.

Table 1. Average plant height (cm) after three applications of different treatments.

Treatments		Ø	V-1	V-2	V-3
Application	I	32.8	33.7	39.7	32.4
	II	49.6	53.3	59.5	52.6
	III	63.7	62.2	67.6	63.3
±		48.7a	49.7ab	55.6c**	49.4a
+ - од Ø		Ø	1.0	6.9	0.7
SD		15.5	13.6	14.3	15.7
CV (%)		7.5	6.9	8.0	7.7
LSD 0.05		2.82			
0.01		4.27			

SD – standard deviation; CV – coeficinet of variation
*Significant differences at level *p=0.05, **p=0.01, for treatment and a, b, c for applications.*

The differences on plant height between the treatments and the control are shown in Figure 1. The plants in treatment V-2 were averagely 14% higher as compared to the plants in the control, 11% of treatment V-1 and 13% of the V-3 treatments. Treatments V-1 showed 3% higher plants than the control and 2% of the treatments V-3, while plants of treatments V-3 were 1% higher than the control.

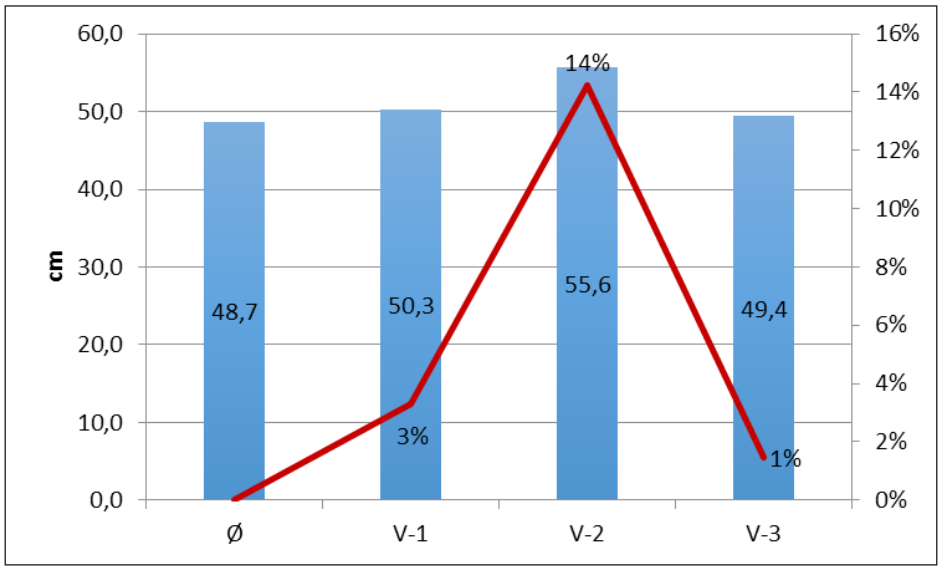

Figure 1. Average plant height (cm) and percentage of difference among different treatments.

Yield of central inflorescence in broccolis

Adding organic matter enhanced the soil structure conditions, creates conducive conditions for good root development (Arisha et al., 2003; Togun- and Akanbi, 2003) and mineralization by microorganisms. Hence, plants are able to get nutrients for higher yield (Radwan et al., 1993; El-Mansi et al., 1999; Wong et al., 1999; Abdelrazzag 2002; Al-Nasir 2002; Togun & Akanbi, 2003) upon application of organic fertilizers.

The harvesting of the inflorescences of the broccoli should be done before the inflorescences begin to open. Harvesting can be one-off or multiple. According to Đurovka et al. (2008), the yield can range from 15 to 30 t/ha, including main and lateral inflorescence.

According to the results from our research regarding the central inflorescence yield, the highest average yield was obtained in V-2 treatment (6.98 t/ha) which was 1.57 t/ha higher yield as compared to the control. The lowest yield was recorded in V3 treatment (5.18 t/ha) and it was 0.23 t/ha lower than control. The standard deviation in all treatments was low, while the relative distribution was the highest in control (13.82%) and the lowest in V-3 treatment (7.54%).

Table 2. Broccoli yield (t/ha) after three applications of different treatments.

Variants		Ø	V-1	V-2	V-3
Repetitions	I	6.25	7.05	6.96	4.64
	II	5.54	5.36	7.77	5.56
	III	4.43	6.84	6.19	5.34
±		5.41a	6.42b	6.98c*	5.18a
+ - Ø		Ø	1.01	1.57	-0.23
SD		0.75	0.75	0.65	0.39
CV (%)		13.82	11.75	9.25	7.54
LSD 0.05		1.55			
0.01		2.25			

SD – standard deviation;CV – coeficinet of variation
*Significant differences of level *p=0.05, **p=0.01, for treatment and a, b, c for applications.*

Significant difference (p = 0.05) between the organic fertilizer treatments and control was noted only for V-2 treatment. A significant difference was showed for V-1 treatment in relation to V-3 treatment and the control.

The average yield from all treatments and the control in our research was 6.01 t/ha, higher results showed V-2 (0.97 t/ha) and V-1 (0.41 t/ha), while the V-3 and control showed lower results than the average yield of all treatments (Figure 2).

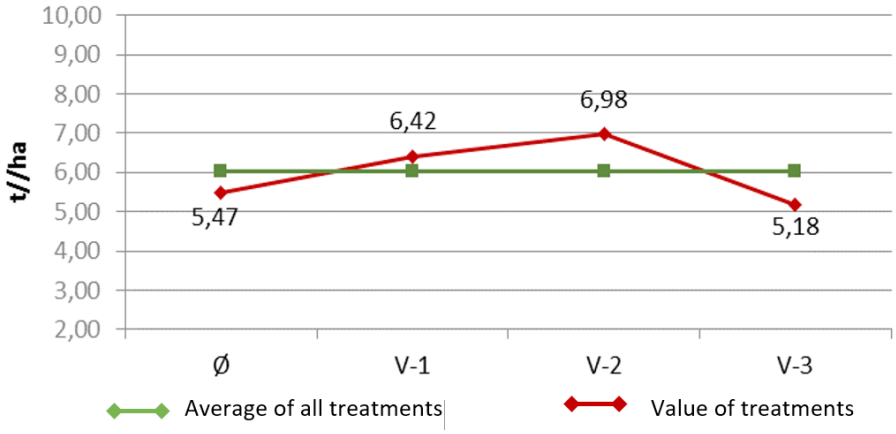

Figure 2. Differences between the average yields in different treatments.

CONCLUSIONS

The application of organic fertilizers in the production of broccoli gave positive results, both for average plant height and yield per hectare. The application of the liquid organic fertilizer based on California worms, Orgalife, gave the best results based on the two examined parameters.

Organic fertilizer treatments gave averagely higher plants up to 14% in V-2, 3% in V-1 and 1% in V-3 as compared to the control plants.

According to the results, it can be concluded that the use of Orgalife contributed to a significantly higher yield on the central flower in treatment V-2 (6.98 t/ha) as compared to control (5.41 t/ha), treatment V-1 (6.42 t/ha) and treatment V-3 (5.18 t/ha). gave the best results based on the two examined parameters.

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ВЛИЈАНИЕТО НА ОРГАНСКИТЕ ЃУБРИВА НА РАСТОТ И ПРИНОСОТ НА БРОКУЛА (*Brassica oleracea* L. var. *italica*)

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Резиме

Експериментот беше спроведен со цел да се утврди влијанието на органските ѓубрива врз динамиката на растот и приносот на брокула одгледувана на отворено. Беше користена сортата *Verdija F1*, која е одгледувана во Скопскиот регион во текот на 2018 година. Беа користени следниве третмани со органски ѓубрива: Ø контрола - без употреба на органско ѓубриво, V-1 - фолијарен третман со Biohumus, V-2 - фолијарен третман со Orgalife, V-3 - фолијарен третман со Bio - Vital. Третирањето е изведувано на секои 10 дена, почнувајќи од 19.8.2018 г. и тоа три пати во текот на вегетацијата. Целта на истражувањата беше да се испита влијанието на соодветните третмани врз динамиката на растот и приносот кај брокулата. Според добиените резултати може да се утврди дека со користење на органски ѓубрива Orgalife е добиен значително повисок принос на централниот цвет со третманот V-2 (6.98 t/ha) во споредба со контролниот третман (5.41 t/ha). Статистички значајна разлика, на ниво од 0,05 со LSD-тест, беше добиена помеѓу V-2 и контролата. Резултатите добиени за просечната висина на растенијата, за време на трите третмани, покажаа дека постои високостатистички значајна разлика на ниво од 0,01 помеѓу V-2 (55.6 cm) и контролата. Според добиените резултати може да се заклучи дека V-2 даде најдобри резултати и во зголемувањето на растот на брокулата и во приносот.

Клучни зборови: *динамика на раст, Biohumus, Orgalife, Bio – Vital.*

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Review paper

IMPROVEMENT OF THE PRODUCTION TECHNOLOGY OF MEAT PRODUCTS WITH THE ADDITION OF FUNCTIONAL COMPONENTS

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Abstract

Functional food is a food where a new ingredient has been added to a food and the new product has a new function (often one related to health-promotion or disease prevention). Functional foods are one of the most important segments of the food industry. Many food products including fruits, vegetables, wine and cheese have been found to contain components with potential health benefits. In addition to these foods, new foods are being developed to enhance or incorporate these components.

The paper presents research related to the improvement / enrichment of meat products, produced in the meat industry in the Republic of N. Macedonia. The research relates to durable and semi-durable sausages and their production technologies in which various functional components are added: vegetable oils, antioxidants, probiotic. From the obtained research results, it can be concluded that the addition of functional components has contributed to improving the quality without changing the sensory properties of the products, the health benefits of consumers and the expansion of the assortment of meat products.

Key words: *functional food, quality, sausages, meat industry*

INTRODUCTION

Scientific progress in understanding the relationship between nutrition and health has an increasingly profound impact on consumer's approach to nutrition which has resulted in the development of the concept of functional foods. It is a practical and new approach to achieve optimal health status by promoting the state of well-being and possibly reducing the risk of disease. The term "functional foods" comprises some bacterial strains and products of plant and animal origin containing health-promoting physiologically active compounds in addition to the traditional nutrients which are beneficial for human health and reducing the risk of chronic diseases (Bhat & Bhat, 2011).

There are three basic requirements for a food to be regarded as functional: (i) it should be a food derived from naturally occurring ingredients; (ii) it should be consumed as a part of the daily diet; and (iii) once ingested, it must regulate specific processes such as enhancing biological defence mechanisms, preventing and treating specific diseases, controlling physical and mental conditions, delaying the ageing process etc. (Goldberg, 1994; quoted by Verma & Banerjee, 2009).

Meat and meat products have many disease-preventing, health-promoting benefits, which according to a research makes them a viable option to be used as functional foods.

Meat contains many important nutrients, including bioactive compounds such as taurine, L-carnitine, creatine, conjugated linoleic acid (CLA) and endogenous antioxidants. Meat also contains unique endogenous antioxidants including carosine, anserine and others, along with iron and zinc, nutrients often lacking in the average diet and also contains a significant

source of vitamin B-12 (Leroy, 2006). Meat and meat products could be made more functional with some modifications. Modification of fatty acid and cholesterol levels in meat may be influenced by selection of breeds and genetic lines, changes in animal feeding practices and additional ingredients added during meat processing (Leroy, 2006).

Adding probiotics to fermented meat products (i.e. sausage) may lead to health benefits, although this application is still marginal.

FUNCTIONAL COMPONENTS AND SUPPLEMENTS THAT FULFILL A HEALTH EFFECT INTO MEAT PRODUCTS

Numerous studies have sought to demonstrate the possibility of changing the image of meat and meat products from the traditionally accepted image to one of healthy living thanks to the addition (vegetables, extracts, fibers, and so forth), elimination (fats), and reduction (additives) of different ingredients (Fernàndes-Ginès et al., 2006).

When incorporating supplements and functional components into meat products, they may be declared as functional foods if they on one hand attain and fulfill the expected effect and it is also necessary to ensure that the products would ultimately preserve their specific properties, moreover the sensory properties, on the other hand. Functional components and supplements that fulfill a health effect can affect the flow of production and storage changes in different ways. Some of these supplements reduce and some increase the pH value of fermented sausages (Vasilev, 2009), while vegetable oils do not actually affect the pH changes. The physico-chemical and sensory characteristics of the meat products depend on the intensity of the change in pH. The pH value significantly affects the colour, firmness and aroma of fermented sausages. The presence of bioactive peptides, the application of functional starter cultures that produce antimicrobial compounds or probiotic strains, are significant potential during the production of fermented meat products, primarily fermented sausages. The above-said compounds and microorganisms can contribute to improving the quality of health and safety products. Most of these compounds contain substances that are not naturally active, but upon release from the rest of the complex protein molecule, these compounds become biologically highly active (presenting antihypertensive, antioxidant, immuno-stimulatory, antimicrobial and antimicrobial activity).

Grujić et al. (1988, 1989, 1990a1990b) investigated the impact of soy protein and brewer's yeast protein on the technological properties (rheological and color properties) and health effects of semi-durable sausages. Research has led them to conclude that the addition of these proteins to meat products has a significant effect on the increase of essential minerals and essential amino acids (tryptophan and arginine). The addition of fiber in meat products, affects the reduced energy value of the finished product, and thus the prevention of many chronic diseases (colon cancer, obesity, cardiovascular disease, etc.).

Sadri and Mahjub (2006) found a positive correlation between the addition of fiber and oils and colon cancer. Several studies have shown that dietary fiber has the ability to affect lowering LDL cholesterol in the blood, the risk of type 2 diabetes mellitus, the risk of coronary heart disease, the risk of blood pressure, the risk of obesity and the risk of colon cancer, (Willet et al., 2002; Liu et al., 2003; Schatzkin et al., 2007).

Jimenez-Colmenero et al. (2003) investigated the nut supplement in a restructured steak and found that the supplement affects changes in product properties during heat treatment, but also in color, texture, and sensory properties, making the product softer and providing better water binding.

Potential probiotic strains *Lb. rhamnosus LG*, *Lb. rhamnosus L-705*, *Lb. rhamnosus E-97800* and *Lb. plantarum E-98098* have been successfully used in the production of traditional fermented sausages (Erkilä, 2001).

Vukovich et al. (2009) in their research used a probiotic of the genus *Lb. chasse* (LH 01) in functional fermented beef and pork sausages. The prebiotic used reached more than 8 log CFU/g, fermenting the sugars, creating favorable ripening conditions. The same authors conclude that functionally fermented sausages have a high biological value and a positive effect on human health.

Concerns about dietary sodium depletion are an important issue for the meat processing

industry (Lilić & Matekalo-Sverak, 2011). Decreasing the amount of sodium chloride affects the reduction of water retention and emulsifying properties of proteins in meat.

Procedures during meat processing that lead to increased fat content, saturated fatty acids, salt and nitrite have attracted particular attention from researchers investigating the impact of human nutrition on health and the examination of functional foods.

CURRENT CONDITION OF THE MEAT PROCESSING INDUSTRY IN REPUBLIC OF NORTH MACEDONIA

According to the Food and Veterinary Agency data there are 50 meat and meat processing facilities registered in Macedonia. 12 of them are completely out of work, and some of them do not operate out of incompletely clarified reasons. 38 facilities still operating and 15 are registered to produce semi-durable and durable products with a capacity of 45,000 tonnes per year in one shift. As of the installed production facilities, 94% are owned by companies. With a total of 11,500 tons of processed meat last year, only 28% of the potential production capacity of 45,000 tons of semi-durable and durable meat is used, which is shocking. Out of the total processed meat, only 1,700 tons, or only 15%, are from domestic producers, i.e. meat from domestic producers. The rest comes from imports. In 2018, 2,936,495 kg of meat products were exported, and in 2017 2,070,904 kg, which is an increase of 41.7%, while imports last year amounted to 6,039,749, and in 2017 was 6,744,566 kg, which is decreased by 10.4% (Petrovski, 2017).

Macedonian meat processing companies offer mainly durable and semi-durable meat products. At the same time, the domestic meat industry is characterized by large investments, new facilities, machinery and processing technologies. It is in this context that, given the increasingly stringent requirements regarding the composition and nutritional value of food, meat industries need to set up and develop a new generation of meat preparations with the addition of functional components that will have high biological value and be accepted on the domestic market for its quality. From the range of processed meats mainly durable and semi-durable sausages can be modified to avoid the standard recipe for production of animal fat and spices. In order to meet the pre-set consumer demands and achieve greater competitiveness, the meat industry is facing a major challenge in finding new recipes with the addition of functional components.

Current condition of the meat products functional components produced in Macedonian meat factories

There are several studies on the addition of functional components into the meat products, produced in our meat factories. Various vegetable oils, antioxidants and probiotics were mainly added from the functional components.

Olive oil is a vegetable oil with the highest level of monounsaturated fatty acids (MUFA) and has attracted attention as a fat replacement in meat preparations. The oil has a high biological value due to the favorable blend of predominantly monounsaturated fatty acids (MUFAs) and naturally occurring antioxidants including vitamin E, vitamin K, carotenoids and polyphenols.

Kuzmanovski (2018) investigated the effects of different concentrations of added olive oil on the quality and sensory properties of semi-durable coarse-chopped sausages - from a range of Kranj sausages and National sausages, produced according to the Product Specification, in the domestic meat industry in the country. Four groups of these two types of sausages have been produced. The first group was produced without addition of olive oil (control group), the second one with addition of 3g/kg, and the third one with addition of 4g/kg and the fourth group with addition of 5g/kg olive oil. After production, the groups of sausages were vacuumed and stored in refrigerator at temperature from 0 to +4°C. From the performed examinations and the obtained results, it could be concluded that the used concentrations of cold-pressed oil in the groups of sausages do not have impact on their chemical composition. Used concentrations of cold-pressed oil do not have impact on the oxidative changes of the examined groups of sausages, which means that olive oil fills antioxidant, antibacterial and functional properties. The ratio of PUFA / SFA in these sausage batches is 0.4%, which means that this group also satisfies the lipid content requirements of the product.

Sunflower (*Helianthus anuus* L.) besides soybeans, rapeseed and peanuts is one of the four most important oilseed crops worldwide. This oil has a high biological-nutritional value because of its beneficial effect on the functioning of the heart and cardiovascular system and on the maintenance and improvement of the general health of the body (Škorić et al., 2000; Lepšanović & Lepšanović, 2000) quoted by Premović et.al (2015). Pumpkin (*Cucurbita pepo* L.) is used for human and livestock nutrition. Pumpkin seeds are a source of protein, phytosterols, vitamins, glyceride oil, carotenoids, tocopherols microelements (K, Mg, Mn, Zn, Se, Co, Cr, Mo).

Malinov (2019) investigate the impact of cold-pressed sunflower and pumpkin oil on the chemical composition and microbiological quality of Bacon Folk sausages produced in local domestic meat industry. For this purpose, eight groups of Bacon Folk sausages have been produced. The first group was produced without addition of sunflower oil (control group), the second one with addition of 3g/kg, and the third one with addition of 4g/kg and the fourth group with addition of 5g/kg olive oil. According to the same order were produced the sausage groups with the addition of pumpkin oil. Sausage groups with cold pressed sunflower oil and pumpkin, have a slight decrease in the water content on the sixtieth day of production compared to the first day of production. The water content of the first day of production was (52.86% to 54.73% for sunflower sausages group; 52.24% to 54.61% for sausages with addition of pumpkin oil) and (52.71% up to 54.48%; 51.59% to 53.58%) on the sixtieth day of production. Protein content in cold pressed sunflower oil and pumpkin sausages group ranged from (10.85 to 11.54%; 11.65 to 11.95) on the first day and (11.52% to 12.01%; 11.52% to 12.01%) sixtieth day of production. The content of fats and minerals in both produced sausage groups were increasing to the end of production. Pathogenic bacteria were not detected. Used concentrations of cold-pressed sunflower and pumpkin oil in the groups of sausages do not have statistically significant impact on the chemical composition and microbiological quality of the sausages and full field the anti-oxidative effect.

Numerous microorganisms have been studied in the past sixty years, of which only a few have commercial use in the meat industry. Today, several companies that produce starter culture provide pure cultures of *Lactobacillus* spp.*, Pedococci acidilacetic, P. Pentosaceus, Staphylococcus flusus* and *S. carnosus.*

When selecting starter cultures, their impact on the sensory properties of the product should be primarily determined. Incorrect selection of starter cultures and inappropriate technological processes often lead to the accumulation of undesirable metabolic products that can greatly impair the quality of the product and thus pose a health threat to consumers. In order for them to be used in the production of fermented sausages at all, it is necessary to select them. One possibility is to test existing strains that are part of starter cultures in terms of probiotic properties. Thus, for example, co-infected *L. sakei Lb3* and *P. acidilacetic* acid PA-2 have shown the ability to survive in conditions in which the intestinal tract is simulated, (Obradović & Vesković-Moračanin, 2007). In order to be a probiotic property, it is considered measurable that consuming one gram of fermented sausages should bring into the body at least one million probiotic bacteria (Vuković et al., 2009).

Joshevska (2013) evaluated the influence of probiotic and prebiotic on the quality of three production batches fermented sausages produced as functional food in the meat industry in Macedonia. As probiotic is used *Bifidobacterium longum BB536* and inulin in powder form. The results of physico-chemical and functional parameters, microbiological quality and sensory evaluation can be used towards the introduction and implementation of functional components in the technology of production of this type of sausage in most meat industries in our country, in order to improve the quality and increase market competitiveness. From the investigation, it can be observed that the probiotic bacteria *Bifidobacterium longum BB536*, are intensively developed in the production batches until the 7th day of maturation. The added probiotic culture proved to be well reproduction during the fermentation of the fermented sausage, as it reached 6.63 / 6.03 log CFU/g. According to the adopted standards, the number of probiotic bacteria in functionally fermented products should be greater than 6 log CFU/g (Vukovic et al., 2009).

Goji berry fruits contain a wide spectrum of phyto compounds, vitamins B1, B2 and B6, minerals- Fe, Zn and Cu, amino acids (proteins), fatty acids and specific antioxidants so that many health experts call them "super food of berries".

Mitev (2018) investigate the influence of dried and minced Goji berries over the oxidative changes (acid level, peroxide value), microbiological status and chemical properties on semi-durable bacon folk sausage vacuumed and kept at a temperature of +4°C. The study was carried out on the 1, 10, 25, 35, and 50 day of the production of the sausage groups. The obtained results indicate that the low levels of the acid and the peroxide values are probably a result of the anti–oxidative activity of the applied minced Goji berry fruits and vacuuming of the sausages. The fact that in none of the tested sausage groups bacteria (*Listeria monocitogenes*, *Salmonella species*, *Escherichia coli* and *Staphylococcus aureus*) were not determined is due to the good hygiene practice where they are produced. The fruits of Goji berry have no statistical proven effect on the chemical composition (water, fats, proteins, ash) of the sausage during storage at +4°C. This functional compound it can be used towards the introduction and implementation of functional components in the technology of production of this type of sausage in order to improve the quality of the product.

CONCLUSION

As of the aforementioned research regarding the usage of the functional components in meat products produced in meat processing factories in Macedonia, it can be stated that each addition proved to be successful because it affects the increase of the product's biological value thus to the health benefits of the consumers, however not deteriorating the physico-chemical composition and the sensory features of the products themselves. Those researches can stimulate the producers to adjust their assortment and increase their market value.

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ПОДОБРУВАЊЕ НА ТЕХНОЛОГИЈАТА ЗА ПРОИЗВОДСТВО НА МЕСНИ ПРОИЗВОДИ СО ДОДАТОК НА ФУНКЦИОНАЛНИ КОМПОНЕНТИ

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Резиме

Функционална храна е храна во која е додадена нова состојка (функционална компонента) и новиот производ има нова функција (честопати поврзана со унапредување на здравјето или спречување на болести). Функционалната храна е еден од најважните сегменти на прехранбената индустрија. Откриено е дека многу прехранбени производи, вклучително овошје, зеленчук, вино и сирење содржат компоненти со потенцијални здравствени придобивки. Покрај овие намирници, се развива

нова храна за зајакнување или вклучување на овие компоненти. Во трудот се дадени истражувања поврзани со подобрување / збогатување на месни производи произведени во месната индустрија во Република Северна Македонија. Истражувањето се однесува на трајни и полутрајни колбаси и нивните технологии за производство, во кои се додаваат различни функционални компоненти: растителни масла, антиоксиданси, пробиотици, пребиотици. Од добиените резултати од истражувањето може да се заклучи дека додавањето на функционалните компоненти придонесува за подобрување на квалитетот без промена на сензорните својства на производите, здравствени придобивки на потрошувачите и проширување на асортиманот на месни производи.

Клучни зборови: *функционална храна, квалитет, колбаси, месна индустрија.*

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THE SIZE AND NUMBER OF MIDDLE BELT LEAVES IN SOME VARIETIES OF WILD TOBACCO

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Abstract

In contrast to the cultivated type *Nicotiana tabacum*, which has haploid number of chromosomes (24), wild tobacco species differ in the number of chromosomes and in morphological properties also, such as: stalk, leaf number, flowers and plant height. Some wild tobaccos species have the same number of chromosomes as the cultivated tobacco and can be easily used for crossbreeding. This should be emphasized because the most important reasons for crossing are plant resistance to diseases and inheritance of some morphological traits, e.g. higher leaf number, shorter vegetation period, etc. The trial was set up in the experimental field of the Scientific Tobacco Institute – Prilep in 2014 and it included the following species: *N. rustica, N. alata, N. longiflora, N. petunia hybrida, N. repanda, N. glutinosa, N. miarsii, N. undulate*. The obtained results were compared with the standard variety P 12-2/1.

The aim of the study was to analyze some morphological traits (length, width and number of middle belt leaves) of tobaccos grown in the region of Prilep and to compare them with the cultivated species *N. tabacum*. The results of the study will be of benefit to tobacco breeders. Data on morphological measurements were statistically processed using the following parameters: mean error of the average ($c\bar{x}$), standard deviation (σ) and coefficient of variation CV%.

Key words: *length, width, number of middle belt leaves*

INTRODUCTION

Tobacco plant is classified in the genus Nicotiana, tribe Cestineae, family Solanaceae, order Symetale, class Magnoliopsida (dicotyledons).

There are two classifications of the genus Nicotiana: after Doncho Kostov and after Goodspeed. The classification of the Bulgarian scientist Doncho Kostov is based on morphological characteristics, but also on cytogenetic differences (Uzunoski, 1985). According to Kostov, all Nicotiana species are divided into two groups:

1. American tobacco
2. Australian tobacco

The classification of Goodspeed is based, in addition to morphological differences, on geographical origin, cytological characteristics and number of chromosomes. The author classifies the genus Nicotiana into three subgenera, subdivided into sections and species. Wild species are a real treasure. By their crossbreeding, a great number of forms with different morphological and biological characters can be created and used in selection. N. glauca species can survive at a temperature of - 10^0C, which can be used to create varieties that can resist low temperatures. *N. silvestris* does not contain nicotine and can be used to create varieties with less nicotine content (Uzunoski, 1985). Some species are resistant to drought and some species of the Attenuata section are used to create varieties with shorter vegetative cycle.

MATERIAL AND METHODS

The trial was set up in the field of the Scientific Tobacco Institute – Prilep, in randomized block design with 3 replications. Eight wild species were included in the trial: *N. rustica* - Fig. 1, *N. alata* - Fig. 2, *N. longiflora* - Fig. 3, *N. petunia hybrida* - Fig. 4, *N. repanda* - Fig. 5, *N. glutinosa* - Fig. 6, *N. miersii* - Fig. 7, *N. undulata* - Fig. 8 and the cultivated tobacco variety P-12-2/1 was used as a standard. Seedlings were grown in the seedbeds of Tobacco Institute – Prilep and the same cultural practices and protective measures were applied for all wild species included in the study, both in seedbeds and in field. Morphological measurements were made in the stage of full bloom, on five stalks of each species. Data from the morphological measurements were processed by variational statistical method, using the following parameters: mean error ($c\bar{x}$), standard deviation (σ) and coefficient of variation (CV%), estimated according to Najcheska (2002), Filiposki (2011) and Korubin-Aleksoska (2017).

Figure 1. *N. rustica*

Figure 2. *N. alata*

Figure 3. *N. longiflora*

Figure 4. *N. petunia hybrida*

Figure 5. *N. repanda*

Figure 6. *N. glutinosa*

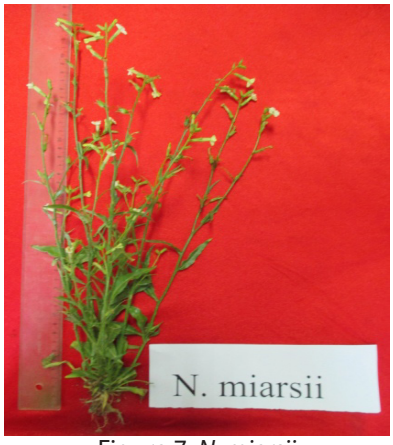

Figure 7. *N. miersii*

Figure 8. *N. undulata*

RESULTS AND DISCUSSION

Morphological properties are among the most important characteristics for recognizing different types and varieties of tobacco and other plant species. Under the influence of external factors, plants are subject to certain changes. Thus, in conditions of abundant precipitation, the plant habitus increase and in drought the habitus decreases, but still, the typical form of the plant is retained. Rudolf (1973) reported that under the influence of external environment, some characters undergo only insignificant changes and others are subject to large variations, such as the quantitative characters plant height, number of leaves and thickness, as well as some qualitative characters (chemical composition). Our investigations were focused on the dimensions of the middle belt leaves and the number of leaves of some wild species in the region of Prilep.

Length of the middle belt leaves

The average length of the middle belt leaves is presented in Table 1. Leaf length was measured in the stage of full bloom. The highest average length of the middle belt leaf was recorded in the check variety P-12-2 / 1 (26.6 cm ± 0.64) and it has the lowest standard deviation (0.64), with insignificant coefficient of variation (5.71%), which shows that this variety is stable. The smallest length of the middle belt leaves was recorded in *N. miersii* (2.3 cm ± 0.12), with coefficient of variation 11.90%, which indicates medium variability of this character. Najcheska (2002) reported that variability of a character is insignificant if the coefficient of variation (CV) is not exceeding 10%, medium if CV is between 10 and 20% and significant if CV is higher than 20%. For the length of middle belt leaves, the highest standard deviation of 2.17 and coefficient of variation of 17.77% was calculated in *N. repanda*. The lowest standard deviation was calculated in *N. miersii* (0.27) and the lowest coefficient of variation in *N. rustica* (3.29%).

Table 1. Average length of the middle belt leaves.

Variety/Species	Length (cm)		
	$\bar{x} \pm c\bar{x}$	σ	CV%
N. tabacum P-12-2/1	26.6 ± 0.64	0.64	5.71
N. rustica	14.4 ± 0.21	0.47	3.29
N. alata	19.2 ± 0.58	1.30	6.79
N. longiflora	19.2 ± 0.58	1.30	6.79
N. petunia hybrida	13.5 ± 0.35	0.79	5.85
N. repanda	12.2 ± 0.97	2.17	17.77
N. glutinosa	8.9 ± 0.37	0.82	9.23
N. miersii	2.3 ± 0.12	0.27	11.90
N. undulata	19.2 ± 0.58	1.30	6.79

Width of the middle belt leaves

The average width of the middle belt leaves (Table 2) is the highest in *N. rustica* (11.3 cm ± 0.46), with standard deviation being 1.04 and coefficient of variation 9.18%. *N. myersii* is characterized by the smallest width of the leaves (2.3 cm ± 0.12), with the lowest standard deviation (0.22) and the highest coefficient of variation (20.33%). According to the data obtained, the standard deviation is low, which is an indication of stability of the investigated wild species, including the check variety. According to Najcheska (2002) and Filiposki (2011), the coefficient of variation has significance if it has a positive value. For leaf width, the tested wild species are characterized by medium variability, except for *N. myersii*, which coefficient of variation of 20.33% indicates that this species has significant variability. According to the obtained data, it can be stated that the tested wild species are characterized by small size of the leaves, both in length and in width.

Table 2. Average width of the middle belt leaves.

Variety/Species	Width (cm)		
	$\bar{x} \pm c\bar{x}$	σ	CV%
N. tabacum P-12-2/1	10.5 ± 0.57	0.65	12.27
N. rustica	11.3 ± 0.46	1.04	9.18
N. alata	8.5 ± 0.63	1.41	16.64
N. longiflora	8.5 ± 0.63	1.41	16.63
N. petunia hybrida	8.6 ± 0.19	0.42	4.86
N. repanda	10.0 ± 0.67	1.50	15.00
N. glutinosa	7.6 ± 0.19	0.42	5.50
N. miersii	1.1± 0.10	0.22	20.33
N. undulata	8.5 ± 0.63	1.41	16.64

Number of leaves

The average leaf number is presented in Table 3. The largest number of leaves (44) was counted in *N. petunia hybrida*, with standard deviation of 2.86 and coefficient of variation 6.48%. The lowest number of leaves (8-9) was recorded in *N. undulate*, with standard deviation of 2.95 and coefficient of variation 8.88%. Kochoska (2006) reported that in the same semi-oriental varieties and lines tested, the number varied 1 - 3 leaves compared to the check, depending on the conditions of breeding (non-irrigated check and irrigated trial).

Table 3. The average number of leaves.

Variety/Species	Leaf number		
	$\bar{x} \pm c\bar{x}$	σ	CV%
N. tabacum P-12-2/1	38.0 ± 1.61	1.27	9.49
N. rustica	10.0 ± 4.06	9.08	18.92
N. alata	33.2 ± 1.32	2.95	8.88
N. longiflora	33.2 ± 3.97	2.95	8.88
N. petunia hybrida	44.2 ± 1.28	2.86	6.48
N. repanda	23.6 ± 0.87	1.95	8.26
N. glutinosa	32.2 ± 1.85	4.15	12.88
N. miersii	19.8 ± 1.39	3.11	15.73
N. undulata	8.5 ± 3.97	2.95	8.88

CONCLUDING REMARKS

The largest length of the middle belt leaf was measured in the check variety P-12-2 / 1 (26.6 ± 0.64), with coefficient of variation 5.71%, and the smallest length in wild species *N. myersii* (2.3 ± 0.12), with coefficient of variation 11.90%.

For the character length of the middle belt leaves in the wild species tested, the highest standard deviation of 2.17 and coefficient of variation 17.77% was found in *N.repanda*. The lowest standard deviation was recorded in *N. miersii* (0.22), and the lowest coefficient of variation in *N. rustica* (3.29%). From the obtained data it can be stated that the variability of the character length of the middle belt leaf is medium.

The largest average width was measured in the leaves of *N. rustica* (11.3±0.46), with standard deviation 1.04 and coefficient of variation 9.18%. The wild species *N. miersii* has the smallest width of the leaves (2.3±0.12), with lowest standard deviation (0.22) and the highest coefficient of variation (20.33%).

The largest number of leaves (44) was counted in the wild species *N. petunia hybrid*, which is characterized by standard deviation of 2.86 and coefficient of variation 6.48%. The lowest number of leaves was counted in the wild species *N. undulata* (8-9), which had standard deviation 2.95 and coefficient of variation 8.88%.

According to the obtained data, it can be stated that wild species grown in the region of Prilep are characterized by insignificant to medium variability of the investigated characters.

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ДИМЕНЗИИ И БРОЈ НА ЛИСТОВИ ОД СРЕДНИОТ ПОЈАС КАЈ НЕКОИ ДИВИ ВИДОВИ ТУТУН

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Резиме

За разлика од културниот вид тутун *N. mabacum* кој се одликува со хаплоиден број на хромозоми (24), дивите видови тутун се одликуваат со различен број на хромозоми и со многу различни морфолошки својства на растението, и тоа во стеблото, бројот на листови, цветовите и висината на растенијата. Одреден број диви видови имаат ист број на хромозоми со културниот вид тутун и лесно може да се искористат за вкрстување. Ова го нагласуваме бидејќи една од поважните причини за вкрстување во селекцијата е и својството отпорност на растенијата од болести, како и наследувањето на некои морфолошки особини, на пример поголем број на листови, добивање на растенија со краток вегетациски период и др. Опитот беше поставен во опитното поле на Научниот институт за тутун – Прилеп во 2014 година, при што беа испитувани дивите видови: *N. pustica*, *N. alata*, *N. longiflora*, *N petunija hibrida*, N. *repanda*, *N. glutinosa*, *N. miarsii*, *N. undulate*, чии вредности беа споредувани со контролната сорта тутун П 12-2/1. Целта на испитувањето беше да се анализираат морфолошките својства на тутуните одгледувани во Прилепскиот регион (должина, ширина и број на листови од средниот појас) и да се споредат со културниот вид *N. tabacum*, а добиените податоци да бидат придонес за селекцијата. Податоците од морфолошките мерења беа варијационо-статистички обработени со следниве статистички параметри: средна грешка на средната вредност (сx̄), стандардна девијација (σ) и варијационен коефициент CV%.

Клучни зборови: *должина, ширина, број на лисја, среден појас.*

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ACTIVITY OF ENZYME CATALASE IN ALFALFA (*Medicago sativa* L.) AS AN INDICATOR FOR ABIOTIC STRESS

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Abstract

To understand the adaptability of alfalfa (*Medicago sativa* L.) to environmental stress, the activity of antioxidant enzyme catalase in alfalfa shoots were analyzed at three slopes, subjected to drought stress during vegetation. The presence of the enzyme catalase is a signal that changes occur in plants, due to certain environmental abiotic stress factors. Catalase is a ferment that intensely catalyzes the decomposition of hydrogen peroxide into water and oxygen, which is produced under stressful conditions. Owing to the toxic nature of H_2O_2 to living plant cell, the importance of catalase is of great essence for the plants to adapt to stressful environmental conditions. Therefore, increased antioxidant activity of the enzyme catalase is considered as an indicator of abiotic stress conditions in plants.

In this paper, the activity of catalase enzyme at alfalfa (*Medicago sativa* L.) was investigated, depending on the climatic conditions in three regions of Tetovo, Skopje and Ovche Pole, in 19 different locations in the Republic of North Macedonia. The results showed that in the Ovche Pole region, where the arid period is the longest, the activity of the catalase enzyme is highest in all three slopes. The enzyme catalase is an indicator of abiotic stress in the Ovche Pole region.

Key words: *antioxidant activity, Walter climate diagram, water deficit, hydrogen peroxide*

INTRODUCTION

Catalase activity is one of the most important indicators of antioxidant activity of plants. Results of numerous studies indicate increased catalase activity in drought conditions and increased soil salinity, i.e. vegetation of culture on halophyte soil. Hydrogen peroxide occurs in stressful conditions, as do other reactive oxygen radicals, and it occurs at heat shock, metallic stress, at pathogenic infections in plants, at photooxidative processes in plants induced by abiotic stress conditions such as cold, drought, salinity and ozone stress.

The three main mechanisms that reduce alfalfa yield due to water deficiency are: (1) reduction of absorption in photosynthetic radiation, (2) reduction of radiation efficiency, and (3) reduction of harvest index (Earl and Davis, 2003). The results of Clarke and Siddique's research in 2004 showed that low temperatures, wind and water erosion also had a negative impact, contributing to oxidative stress in alfalfa and increased catalase activity.

Wang et al., (2009) performed an analysis of antioxidant enzyme activity during alfalfa germination in drought conditions and increased salinity. The results indicated that tolerance of varieties in saline and dry stress conditions, during germination, was associated with increased activity of antioxidant enzymes. The alfalfa response, due to water deficit, depends immensely on the severity of stress, growth phase and the physiological state. This results in a 49% reduction in biomass and an 18% increase in leaf-to-tree ratio (Bouizgaren et al., 2013). Water deficiency caused in the alfalfa, as in other legumes, to decrease leaf

area, to reduce leaf number, to close the stoma, so that thereby limit the CO_2 assimilation, the photosynthetic activity and the growth (Butleska Gjoroska et al., 2016; Yousfi et al., 2016; Tardieu et al., 2014).

The research in this paper aims to determine the activity of enzyme catalase in alfalfa (Medicago sativa L.) from three slopes, on 19 investigated locations in three regions of Tetovo, Skopje and Ovche Pole in the Republic of North Macedonia. The climatic conditions in the studied regions are determined with climate diagrams by Walter, and the results clearly indicate that the arid period, observed with water deficit and soil salinity in the Ovche Pole region, confirms the increased activity of the catalase enzyme.

MATERIAL AND METHODS

Plant material

Alfalfa plant material (Medicago sativa L.) was collected from three different regions in the Republic of North Macedonia: the Skopje region, the Ovche Pole region and the Tetovo region, from 19 different locations in three slopes (Table 1). The material was collected during the vegetative cycle (from June to August) in 2013. In the first, second and third slope, plants were collected from 15 to 17 June, from 16 to18 July and from 17 to 19 August, respectively.

Table 1. Description of the locations of the examined locations altitude (m), latitude (^{0}N) and longitude (^{0}E) with the dates of first, second and third slope.

Nr	Location	Region	Altitude (m)	Latitude (°N)	Longitude (°E)	Firs slope (date)	Second slope (date)	Third slope (date)
1	Bogovinje	Tetovo	531,50	41,9236809	20,9168772	15.06.2013	16.07.2013	17.08.2013
2	Vrutok	Tetovo	682,41	41,7665300	20,8381550	15.06.2013	16.07.2013	17.08.2013
3	Dzepchiste	Tetovo	474,48	42,0331690	21,0001650	15.06.2013	16.07.2013	17.08.2013
4	Galate	Tetovo	600,73	41,8381370	20,8813700	15.06.2013	16.07.2013	17.08.2013
5	Zelino	Tetovo	1605,94	41,9006530	21,1175770	15.06.2013	16.07.2013	17.08.2013
6	Peckovo	Tetovo	991,87	41,7843700	20,8311530	15.06.2013	16.07.2013	17.08.2013
7	Jegunovce	Tetovo	658,34	42,1245655	21,0875064	15.06.2013	16.07.2013	17.08.2013
8	Avtokomanda	Skopje	246,68	42,0006868	21,4536642	16.06.2013	17.07.2013	18.08.2013
9	Sopiste	Skopje	1017,16	41,8638490	21,3083500	16.06.2013	17.07.2013	18.08.2013
10	Dracevo	Skopje	264,41	41,9352675	21,5098515	16.06.2013	17.07.2013	18.08.2013
11	Saraj	Skopje	424,88	42,0017493	21,2815977	16.06.2013	17.07.2013	18.08.2013
12	Radishani	Skopje	392,32	42,0732769	21,4479917	16.06.2013	17.07.2013	18.08.2013
13	Vlae	Skopje	256,07	42,0072938	21,3801924	16.06.2013	17.07.2013	18.08.2013
14	Glumovo	Skopje	274,74	41,9817742	21,3103747	16.06.2013	17.07.2013	18.08.2013
15	Cheshinovo	Ovche Pole	294,00	41,8735350	22,2905610	17.06.2013	18.07.2013	19.08.2013
16	Karbinci	Ovche Pole	342,98	41,7882100	22,2622460	17.06.2013	18.07.2013	19.08.2013
17	Oblesevo	Ovche Pole	297,63	41,8639320	22,2622460	17.06.2013	18.07.2013	19.08.2013
18	Lozovo	Ovche Pole	277,86	41,7806752	21,8995629	17.06.2013	18.07.2013	19.08.2013
19	Mustafino	Ovche Pole	289,18	41,8407190	22,0789350	17.06.2013	18.07.2013	19.08.2013

Determination of climate conditions according to climate diagrams by Walter

During the examination, the meteorological data for the investigated regions were monitored at the National Hydrometeorological Department of the Republic of North Macedonia. The data for Tetovo region were obtained from Tetovo meteorological station, the data for Skopje region from meteorological station at Zajchev Rid, and for Ovche Pole region the meteorological data were obtained from meteorological station in Stip.

From the mean monthly temperatures and the sums of rainfall presented in the climate diagrams by Walter (1957), the humid and arid nature of the climate during the vegetation period of the study in the three regions examined is determined (Figure 1, 2 and 3).

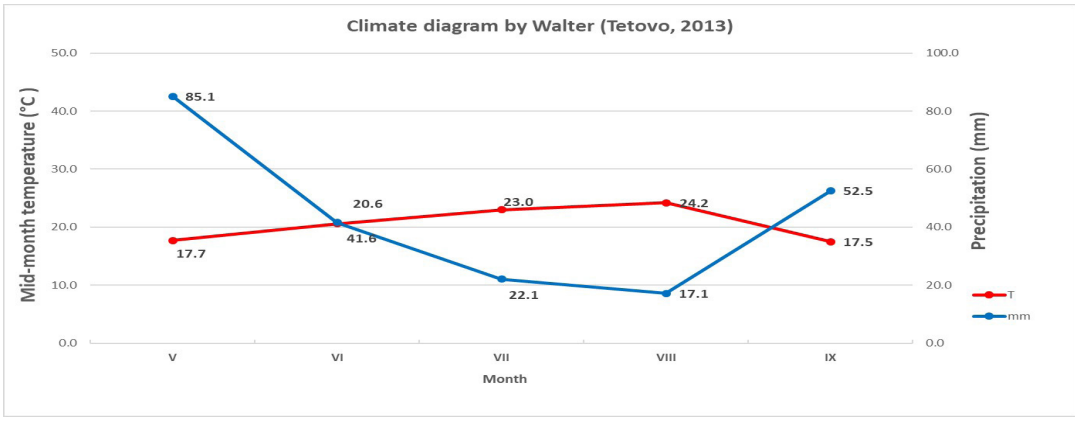

Figure 1. Climate Diagram by Walter for the Tetovo region, 2013.

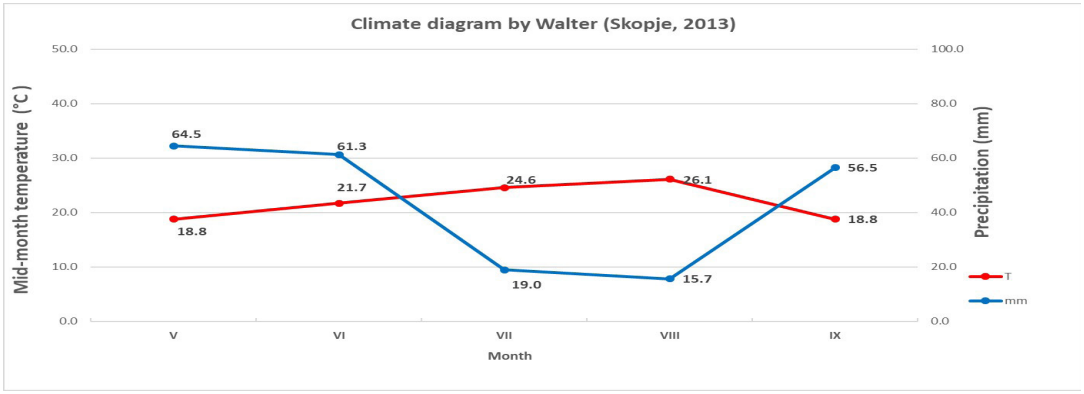

Figure 2. Climate Diagram by Walter for the Skopje region, 2013.

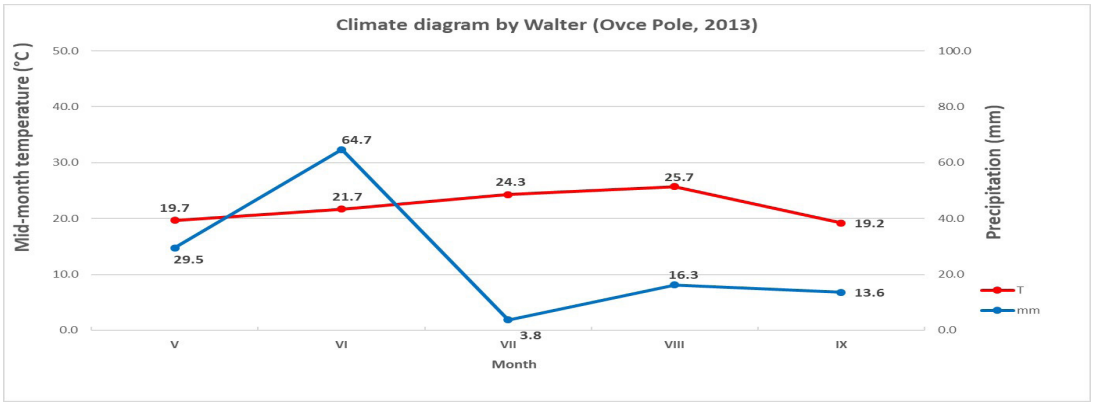

Figure 3. Climate Diagram by Walter for the Ovche Pole region, 2013.

Determination of antioxidant activity of the enzyme catalase

The catalase determination was titrimetric done by the method of Bach and Oparin (1923). The quantitative determination of catalase is based on the properties of H_2O_2, which remains dissolved after the catalase effect, to react with $KMnO_4$ to form free oxygen. The equation resulting this reaction is as follows:

$$2KMnO_4 + 5H_2O_2 + 4H_2SO_4 \longrightarrow 2MnSO_4 + 2KHSO_4 + 8H_2O + 5O_2$$

Based on the difference in milliliters of $KMnO_4$ solution used for the titration of the control and the test sample, the amount of H_2O_2 is obtained, which is dissolved by the fermenter. In addition to $KMnO_4$, that serves as a means of decomposing the extra H_2O_2 after incubation, J_2 is used. The reaction that occurs is presented in the following equation:

$$H_2O_2 + 2KJ + H_2SO_4 \longrightarrow K_2SO_4 + J_2 + 2H_2O$$

The excess of iodine is determined with $Na_2S_2O_3$.

To determine the content of the catalase enzyme, 2 g of dry plant material was weighed on analytical scales. The material in the mortar was macerated with a small amount of quartz sand and distilled water. The mixture was poured into a 100 ml flask and stirred well. Then 2-3 drops of toluene were added and the flask was filled up with distilled water up to the indicated mark, and the mixture was left at room temperature for 2 hours. After extraction, the content was filtered and the filtrate was used as the catalase extract.

A volume of 20 ml of the filtrate was placed (divided) into four flasks. Then, two flasks (1 and 2) were heated until boiling and boiled for 5 minutes to inactivate the enzyme. After boiling, the flasks were left to cooled at room temperature. Flasks 3 and 4 were not heated since they were used as a control. After heating and cooling, in the test samples (1 and 2), as well as in control samples (3 and 4), a volume of 20 ml of distilled water and 5 ml of H_2O_2 solution (1%, v/v), previously neutralized with NaOH solution, were added and left at room temperature for 30 minutes, followed by addition of 5 ml of H_2SO_4 (10%, v/v) and titration with 0.02 M $KMnO_4$ solution. Catalase activity (Ac) was calculated by the amount of H_2O_2 in mg, dissolved over 30 minutes, using catalase containing 1 g of test material (1 ml of 0.02 M $KMnO_4$ is equivalent to 1.7 mg of H_2O_2)

$$Ac = \frac{(a - b) \times 1.7}{g}$$

where:

a – 0.02 M $KMnO_4$ spent for the control test (ml),

b – 0.02 M $KMnO_4$ spent for the test trial (ml),

g - quantity of the test plant material in grams (g).

Statistical data processing

One-way analysis of variance (ANOVA) was used to statistically analyze the results in order to determine significant differences ($p < 0.05$ and $p < 0.01$) between the arithmetic mean of the samples. All analyzes were performed in three repetitions, presented as mean value.

To determine the significance of the difference between the investigated parameters and their rankings at the level of 0.05 and 0.01, the results were post-hoc analyzed using Duncan's multiple range test. The statistical package for the Social Sciences software (IBM SPSS Statistics Software v. 23) was used for statistical result processing.

RESULTS AND DISCUSSION

According to the climatic conditions during the investigated period, it was noticed that they were moderately favorable for cultivation of alfalfa in certain different locations in the Tetovo and Skopje regions, but in the Ovche Pole region the climate condition was with extremely low annual precipitation, which reflects the yield of the culture studied. During the survey in the Tetovo region, the Walter's climate diagram in figure 1, shows that the highest average monthly air temperatures were recorded in July and August. The highest amount of monthly precipitation was in May (85.1 mm) and September (52.5 mm). The surface that is located by both curves when the precipitation curve is below the temperature curve at the climate diagram by Walter, gives the arid (dry) period. The arid period of the investigated period is June, July and August.

The climatic conditions for the Skopje region are shown on the Walter climate diagram, in figure 2. The highest average monthly air temperature was measured in July and August, and the highest amount of monthly precipitation was measured in May and June. The arid period is from July to September.

During the research period in the Ovche Pole region, from Walter's climate diagram in figure 3, it can be noticed that the highest average monthly air temperature was measured in July and August, and the mean monthly maximum temperatures were also in July and August, and the minimum were in May and September. The arid period is from June to September, and is still growing to the following month of October. The arid period is dominant in this region, as evidenced by lower rainfall, especially in July (3.8 mm precipitation).

In table 2, the mean value of the content of the enzyme catalase, in the first, second and third percentiles, at 19 locations in the three regions studied, are shown. The content of enzyme catalase (%) deployed by the regions, in the three slopes, in dry plant material from alfalfa is presented in the table 3.

Table 2. Content of enzyme catalase (%) in the examined locations, in the three slopes, in dry plant material from alfalfa.

Location	First slope		Second slope		Third slope		All slopes together	
	Catalase %	*p<0.05 **p<0.01	Catalase %	*p<0.05 **p<0.01	Catalase %	*p<0.05 **p<0.01	Catalase %	*p<0.05 **p<0.01
Bogovinje	33.3±1.7	*g **g	37.3±1.7	*k **h	35.5±2.4	*k **j	35.4±2.4	*i **l
Vrutok	13.8±0.4	*a **ab	13.3±0.1	*a **a	14.5±1	*ab **ab	13.8±0.8	*a **a
Dzepciste	12.9±0.5	*a **a	14.1±1.7	*ab **a	15.9±2.8	*abcd **abcd	14.3±2.1	*a **ab
Galate	21.2±0.3	*cde **cde	19±1.4	*cdef **abcd	15.8±2	*abcd **abcd	18.7±2.7	*bc **cdef
Zelino	20.9±0.7	*cd **cde	24±1.5	*ghi **def	22.9±1	*efgh **defgh	22.6±1.7	*def **efgh
Peckovo	16.2 ±1.5	*abc **abcd	17.1±0.5	*abcd **abc	17.8±2.3	*abcde **abcde	17±1.6	*ab **abcd
Jegunovce	22.2±2	*def **cde	23±2.9	*fghi **cdef	20.7±0.4	*cdefg **bcdefg	22±2	*de **efgh
Avtokomanda	15.2±1.1	*ab **abc	15.2±2.1	*abc **ab	12.8±2.4	*a **a	14.4±2.1	*a **ab
Sopiste	32.2±3	*g **fg	32.1±3.3	*j **gh	25.9±2.9	*ghi **fghi	30.1±4.1	*h **k
Dracevo	20.1±2.5	*bcd **bcde	22.1±1	*efgh **cdef	21.2±0.4	*defg **bcdefg	21.1±1.6	*cd **defg
Saraj	22.5±9.7	*def **de	18.1±1.9	*bcde **abcd	14.6±8.8	*ab **abc	18.4±7.4	*bc **bcde
Radisani	13.2±2.4	*a **ab	17.2±2.4	*abcd **abc	15.4±1	*abc **abcd	15.3±2.5	*a **abc
Vlae	26.8±2.2	*f **ef	22.9±0.4	*fghi **cdef	19.1±4.5	*bcdef **abcdef	22.9±4.2	*def **fghi
Glumovo	22.6±0.6	*def **de	26.4±6.2	*hi **ef	28.1±2.3	*hij **ghi	25.7±4.1	*fg **hij
Cesinovo	26.3±0.9	*ef **ef	26.9±1.9	*i **fg	28.7±1	*ij **hij	27.3±1,6	*gh **jk
Karbinci	9.6±2	*bcd **abcde	20.6±0.7	*defg **bcde	19±3.4	*bcdef **abcdef	19,7±2.1	*bcd **def
Oblesevo	24.8±1.2	*def **e	25.7±2.7	*hi **ef	23.8±3.3	*fghi **efgh	24.7±2.4	*efg **ghij
Lozovo	20±3	*bcd **bcde	20.5±3.1	*defg **bcde	22.1±1	*efg **cdefgh	20.9±2.4	*cd ** defg
Mustafino	24.7±1.6	*def **e	23.9±3	*ghi **def	32.1±1.8	*jk **ij	26.9±4.4	*g ** ijk

*The mean value in each column marked with the same letter does not differ significantly after the Duncan test for p <0.05
**The mean value in each column marked with the same number does not differ significantly after the Duncan test for p<0.01

Table 3. Content of enzyme catalase (%) by regions, in the three slopes, in dry plant material from alfalfa

Region	Slopes			
	First *p<0.05, **p<0.01	Second *p<0.05, **p<0.01	Third *p<0.05, **p<0.01	All slopes together *p<0.05, **p<0.01
Tetovo	20.1, *a, **a	21.1, *a, **a	20.4, *a, **a	20.5, *a, **a
Skopje	21.8, *a, **a	22.0, *a, **a	19.6, *a, **a	21.1, *a, **ab
Ovche Pole	23.1, *a, **a	23.5, *a, **a	25.2, *b, **a	23.9, *b, **b

*The mean value in each column marked with the same letter does not differ significantly after the Duncan test for p <0.05
**The mean value in each column marked with the same number does not differ significantly after the Duncan test for p<0.01

On location level, the highest content of the catalase enzyme, in all three slopes separately and in all three slopes together, is at the location Bogovinje, at the Tetovo region. For Bogovinje, the value of 33.3±1.7% for the first slope was measured, 37.3±1.7% for the second slope, 35.5±2.4% for the third slope, and 34.5±2.4% for all slopes together (Table 2).

Analysis by regions showed that in the first and second slope, there was no significant difference in the mean value of catalase according to Duncan's test for both p<0.05

and $p<0.01$. In the third slope, the Duncan test showed that the Ovche Pole region was significantly different from the mean value of catalase enzymes of the Tetovo and Skopje regions for $p<0.05$, but for the $p<0.01$ Duncan test showed no significant difference in mean value at the level of regions (Table 3).

When the mean value of catalase was analyzed by regions for all three slopes together by Duncan for $p<0.05$, the Ovche Pole region is significantly different from the mean value of catalase at the Tetovo and Skopje regions, while the Tetovo and Skopje regions do not differ significantly from each other. The same analyzes with Duncan's test for $p<0.01$ showed that only Tetovo and Ovche Pole differ significantly.

The significant difference between regions is primarily due to environmental factors. This suggests that certain factors (temperature shock, agrochemical factors, air pollution, metal stress, high soil salinity, pathogenic infections and other biotic and abiotic stress factors) could be the cause of the increased production of H_2O_2. For these reasons there is an increased activity of catalase, measured by the amount of dissolved H_2O_2, in alfalfa at Ovche Pole region. The presence of enzyme catalase is a signal that changes occur, as a result of certain abiotic environmental stressors.

Increased activity of enzyme catalase also indicates for higher resistance of alfalfa, which means that the Ovche Pole region, which has the highest catalase content, has the highest resistance, and is the result of adaptive alfalfa ability in this region. Tetovo region has the lowest resistance, and thus the lowest value of the catalase enzyme.

Results of numerous studies indicate increased catalase activity under drought and increased salinity (Antolínlara et al., 2010; Rubio et al., 2001). Low temperatures, wind and water erosion also have a negative impact, which contributes to the occurrence of oxidative stress in alfalfa and increased catalase activity (Huang et al., 2007).

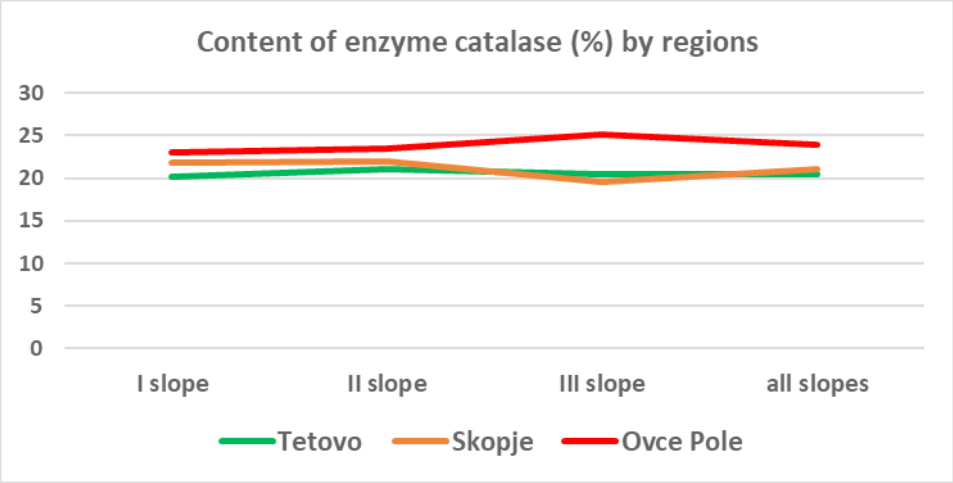

Figure 4. Content of enzyme catalase (%) by regions in first, second and third slope, and in all slopes together.

Drought stress conditions in the Ovche Pole region are also confirmed in this research, where the highest catalase content was recorded in all three slopes in this region. The highest content of catalase enzymes by regions was recorded in the third slope in the Ovche Pole region, which is undeniable evidence that drought and stress conditions were most obvious in the third slope in August (Figure 4).

CONCLUDING REMARKS

Based on the results obtained from this research paper, the following explicit conclusions can be ascertained:

- At all 19 investigated locations, the highest content of catalase in all slopes separately and in all slopes together, is in the location Bogovinje, in the Tetovo region, the highest measured value is in the second slope (37.3 ± 1.7%);
- In the first slope the lowest content of the catalase is measured at the location Karbinci, Ovche Pole region, in the second

slope at the location Vrutok, Tetovo region, in the third slope at the location Avtokomanda, Skopje region, and for all three slopes the lowest value is registered again in the Tetovo region at location Vrutok;

- The existence of drought stress conditions and high soil salinity in the Ovche Pole region lead to the highest catalase content recorded in all three slopes in this region. In the Ovche Pole region the highest content of the enzyme catalase was registered in the third slope 25.2%, which is certain evidence that drought, as abiotic stress, was most pronounced in August;
- According to the measured values for the activity of the enzyme catalase, the most favorable location for alfalfa cultivation in the Tetovo region is Dzepciste, in the Skopje region is the location Avtokomanda and in the Ovche Pole region is the location Karbinci;
- The most favorable region for growing alfalfa is Tetovo region, followed by Skopje region and Ovche Pole region, as a region in which unfavorable abiotic stress conditions are registered;
- The results of the influence of climate factors on catalase activity in alfalfa allow us to conclude that catalase may serve as an indicator of abiotic stress.

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АКТИВНОСТА НА ЕНЗИМОТ КАТАЛАЗА ВО ЛУЦЕРКА (*Medicago sativa* L.) КАКО ИНДИКАТОР ЗА АБИОТСКИ СТРЕС

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Резиме

За да ја разбереме способноста за адаптација на луцерката (*Medicago sativa* L.) на стрес во животната средина предизвикан од суша ја анализиравме активноста на антиоксидативниот ензим каталаза во три откоси за време на вегетацијата. Присуството на ензимот каталаза е сигнал дека се случуваат промени во растенијата, кои се резултат на одредени абиотски стрес фактори на средината. Каталазата е фермент кој интензивно го катализира разложувањето на водородниот пероксид на вода и кислород, кој се создава во стресни услови. Со оглед на токсичниот карактер на H_2O_2 за живата клетка, значењето на каталазата е многу големо за растенијата за адаптација на стресните услови на животната средина. Затоа, зголемена антиоксидативна активност на ензимот каталаза се смета како индикатор за абиотски стресни услови кај растенијата.

Во овој труд е испитувана активноста на ензимот каталаза кај луцерка (*Medicago sativa* L.) во зависност од климатските услови во три региони Тетово, Скопје и Овче Поле, на 19 различни локации во Република Северна Македонија. Резултатите покажаа дека во Овчеполскиот регион, каде што аридниот период е најдолг, активноста на ензимот каталаза е највисока во сите три откоси. Ензимот каталаза е индикатор за абиотскиот стрес во Овчеполскиот регион.

Клучни зборови: *антиоксидативна активност, клима-дијаграми по Walter, воден дефицит, водород пероксид.*

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EVALUATION OF BACTERICIDAL ACTIVITY OF SELECTED WILD MACROFUNGI EXTRACTS AGAINST *Escherichia coli*

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Abstract

The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microorganisms has led to the evaluating of novel sources for their antimicrobial potential. Nature is a generous source of compounds with antimicrobial activity. However, a large number of natural products with the potential to act as antimicrobials still await further investigation. In this study, antimicrobial activities of the extracts from four wild mushrooms: *Amanita echinocephala*, *Russula medulata*, *Ishnoderma benzoinum* and *Laetiporus sulphureus* were evaluated against Gram-negative bacterium *Escherichia coli*. The antimicrobial potential of the methanolic mushroom extracts was investigated by the microdilution method. Mild inhibitory activity was observed in 3 out of 4 mushroom species included in the study. The extracts were further tested for bactericidal activity and minimum bactericidal concentration (MBC) values were determined. The tested microorganism was most sensitive to the examined extracts of *Laetiporus sulphureus* (MBC=62.5 mg/mL).

Even though the results revealed in this study may suggest that tested wild macrofungi extracts possess mild antimicrobial activity, their antimicrobial potential against other microorganisms need to be further elucidated.

Key words: *mushroom, antimicrobial activity, microdilution method, minimum bactericidal concentration (MBC)*

INTRODUCTION

Antimicrobial resistance exhibited by pathogenic microorganisms is a significant public health concern (Walsh & Amyes, 2004). The World Health Organization presented the global report which indicates that *Escherichia coli* is among the three most resistant strains posing a real risk in the community (WHO, 2014). As a result, exploration of natural sources for novel bioactive compounds with antimicrobial properties is an emerging field of science over the last decades.

Nature is a generous source of compounds with antimicrobial activity. However, a large number of natural products with the potential to act as alternative antimicrobials still await further investigation. Previous studies have indicated that mushrooms are rich sources of natural antibiotics (De Silva et al., 2013). Various taxonomic mushroom groups have been investigated for their antimicrobial activities and many low- and high- molecular weight compounds with antimicrobial properties were identified. Many secondary metabolites, such as terpenes, steroids, anthraquinones, benzoic acid derivatives, quinolines; and primary metabolites such as oxalic acid; and high- molecular weight compounds mainly peptides and proteins are among the identified antimicrobial compounds in mushrooms (Alves et al., 2012).

The aim of this study was to evaluate the antimicrobial activities of the extracts from four wild mushrooms: *Amanita echinocephala*, *Russula medulata*, *Ishnoderma benzoinum* and *Laetiporus sulphureus* against Gram-negative bacterium *Escherichia coli*.

MATERIAL AND METHODS

Fruiting body selection

Samples of the wild macromycetes *Amanita echinocephala, Russula medulata, Ishnoderma benzoinum* and *Laetiporus sulphureus* were collected from different locations and habitats in Macedonia. Geographical location and natural habitat of the mushroom specimens are shown in Table 1. Taxonomic identification was made in the Mycological Laboratory at the Institute of Biology, Faculty of Natural Sciences and Mathematics in Skopje, by implementing standard methods of microscopic and chemical techniques (colouring of fruit bodies and spores), as well appropriate literature. The representative voucher specimens were deposited at the Macedonian Collection of Fungi (MCF) at the Institute of Biology (Tab. 1).

Table 1. Geographical location and natural habitat of the mushroom species studied for antimicrobial potential.

Species	Habitat	Geographical location	Collection number
Amanita echinocephala	mycorrhizal (*on ground in park*)	Botanical garden, Skopje	MAK 10/13309
Ishnoderma benzoinum	saprotrophic (*on stump of pine trees*)	Suva Gora Mt.	MAK 11/13252
Laetiporus sulphureus	parasitic (*on living black locust trunks*)	Kozle, Skopje	MAK 11/13361
Russula medulata	mycorrhizal (*on ground in park*)	Gazi Baba, Skopje	MAK 10/13305

In vitro antimicrobial assay

Test microorganism. Antimicrobial activities of methanol extracts were tested against Gram-negative bacterium *Escherichia coli* ATCC 8739. The microorganism was provided from the collection held by the Microbiology Laboratory, Faculty of Natural Sciences and Mathematics in Skopje.

Microbial suspension was prepared by the direct colony method. The turbidity of initial suspension was adjusted by comparison with 0.5 McFarland's standard (Andrews, 2005). The initial suspension contained about 10^8 colony forming units (CFU)/mL. Additionally, 1:100 dilutions of initial suspension were prepared into sterile 0.9% saline.

Microdilution method. The antibacterial activities of the mushroom extracts were assessed using the microdilution method with resazurin as an indicator of microbial growth (Sarker*et al.*, 2007). The antimicrobial assay was performed by using a sterile 96-well plate and the minimum inhibitory concentration (MIC) values were determined. The test plates were prepared by dispensing 50 µL of Mueller-Hinton broth into each well. A volume of 50 µL from the stock solution of tested mushroom extracts was added into the first row of the plate and then two-fold serial dilutions of extracts were performed. Each test plate included growth control and sterility control. MIC was defined as the lowest concentration of tested extracts that prevented a resazurin colour change from blue to pink. All tests were performed in triplicate and MIC values were constant.

The extracts that demonstrated inhibitory activities were further tested for bactericidal activity. A sample from each well that tested positive for inhibitory activity was inoculated on fresh sterile Mueller-Hinton agar (MHA) plates and incubated additional 24 h at 37°C. Absence of colonies was regarded as positive for bactericidal activity, while growth of colonies was regarded as negative. MBC was defined as the lowest concentration of the mushroom extract that results in microbial death. All tests were performed in triplicate and MBC values were constant.

RESULTS AND DISCUSSION

The antimicrobial activity of the tested extracts was quantitatively assessed and the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) were used as a measure of the antibacterial activity of the mushroom extracts included in the study. The antibacterial activity of the mushroom extracts is shown in Table 2.

Table 2. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of methanolic extracts from mushroom samples.

Samples	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC ratio
Amanita echinocephala	200	200	1
Ishnoderma benzoinum	125	125	1
Laetiporus sulphureus	62.5	62.5	1
Russula medulata	-	-	-

(-) No antibacterial activity was observed at the highest working concentration of 200 mg/ml.

The results of the study demonstrated that the mushroom extracts possess either relatively weak or none antibacterial activity at all. Three (*Amanita echinocephala, Ishnoderma benzoinum* and *Laetiporus sulphureus*) out of four extracts demonstrated mild inhibitory and bactericidal effects against the tested microorganism. The methanol extract of *Russula medulata* showed no antibacterial activity against *Escherichia coli* at the concentration used. Our results revealed that the examined extracts from *A. echinocephala, I. benzoinum* and *L. sulphureus* possess bactericidal activity with MBC/MIC ratio=1. However, in all cases relatively high concentrations of extracts, ranging from 62.5 to 200 mg/mL, were required to achieve the antibacterial effect.

The potent bactericidal activity of the polypores is well documented in the literature (Demiri & Yamaç, 2008; Zjawiony, 2004). In this study, the antimicrobial potential of two polypore fungi *I. benzoinum* and *L. sulphureus* has been examined. The results demonstrated that the tested microorganism was most sensitive to the extracts of the examined polypore fungi. The highest bactericidal activity was obtained in the extract from the species *L. sulphureus* with the MBC value of 62.5 mg/ml. This result is in accordance with earlier reported data which confirm moderate antimicrobial potential of *L. sulphureus* against *Escherichia coli* (Nowacka et al., 2014; Demiri & Yamaç, 2008; Turkoglu et al., 2007). Higher MBC value of 125 mg/ml and 200 mg/ml was obtained in the extracts from *I. benzoinum* and *A. echinocephala,* respectively that corresponded to lower bactericidal potential against tested *Escherichia coli*.

The present study was a continuation of our previous studies in which antibacterial activity of the same mushroom species have been tested against Gram-positive bacterium *Staphylococcus aureus* (Nikolovska Nedelkoska et al., 2017) and Gram-negative bacterium *Pseudomonas aeruginosa* (Nikolovska Nedelkoska et al., 2018). Comparison of the results obtained shows that *S. aureus* and *P. aeruginosa* were more sensitive to the examined macrofungi extracts than the tested *Escherichia coli*. These findings are also in accordance with earlier reported data from our first research on antibacterial screening of the selected Macedonian wild mushrooms (Nikolovska Nedelkoska et al., 2013), which showed that the tested mushroom extracts possess either none (*Boletus lupinus, Flammulina velutypes*) or relatively weak (*Phellinus igniarius, Sarcodon imbricatus, Tricholoma aurantium, Xerocomus ichnusanus*) antibacterial activity against *E. coli* (MIC=10-50 mg/mL) compared with other tested bacteria. The cell wall structure might be the reason for the relatively high resistance towards antimicrobial agents that was observed for the microbial species tested in this study. It is known that Gram-negative bacteria possess an outer membrane and a periplasmic space, both of which are absent from Gram-positive bacteria, and that differences in the cell wall structure can produce differences in antibiotic susceptibility of the cells (Martínez de Tejada et al., 2012). Several studies have also shown that the Gram-positive bacteria are generally more sensitive to the antimicrobial effect of the macrofungi extracts compared to Gram-negative bacteria, but this relationship does not hold for every mushroom species (Alves et al., 2012; Suay et al., 2000).

Even there are many studies on phytochemical characterisation of cultivated and wild mushrooms, only little information is available on chemical characterisation of specific classes of antimicrobial compounds in tested mushrooms. According to the available literature data, few antimicrobial secondary metabolites have been identified in mushroom

extracts from *I. benzoinum* and *L. sulphureus*. The antibiotic 1-hydroxy-2-nonyn-4-one has been isolated from the submerged cultures of *I. benzoinum* (Anke et al., 1982). Another example of antimicrobial secondary metabolites is a cyclodepsipeptide, beauvericin, produced by *L. sulphureus* (Zjawiony, 2004).

CONCLUDING REMARKS

The present study was undertaken to quantitatively assess the antimicrobial potential of methanolic extracts from fruiting bodies of four wild macromycetes (*Amanita echinocephala, Russula medulata, Ishnoderma benzoinum* and *Laetiporus sulphureus*) against the tested *Escherichia coli*. Even though the results revealed in this study may suggest that tested wild macrofungi extracts possess mild antimicrobial activity, their antimicrobial potential against other microorganisms need to be further elucidated.

The evaluation of the mushroom extracts against other microorganisms may demonstrate a stronger effect and will be a promising field for assessing their potential as novel antibiotics. Considering that the extracts used in the study were crude mixtures rather than pure substance, further studies are needed for the chemical characterization of specific classes of antimicrobial compounds of the selected mushrooms.

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ЕВАЛУАЦИЈА НА БАКТЕРИЦИДНАТА АКТИВНОСТ НА СЕЛЕКТИРАНИ ДИВИ МАКРОФУНГИ ВО ОДНОС НА *Escherichia coli*

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Резиме

Зголемената неефикасност на медикаментната терапија и евидентната резистентност на патогените микроорганизми ја наметна потребата од евалуирање на нови извори за алтернативни антимикробни соединенија. Природата е дарежлив извор на компоненти со антимикробна активност. Сепак, голем број природни продукти со антимикробен потенцијал сè уште не се доволно истражени. Во оваа студија антимикробната активност на екстрактите добиени од четири видови на диви печурки: *Amanita echinocephala*, *Russula medulata*, *Ishnoderma benzoinum* и *Laetiporus sulphureus* беа евалуирани во однос на Грам-негативната бактерија *Escherichia coli*, со примена на микродилуциониот метод (метод на последователни разредувања). Умерена антимикробна активност беше утврдена кај три од четирите анализирани видови. Исто така, беше испитана бактерицидната активност на екстрактите и беа определени минималните бактерицидни концентрации (МБЦ). Тест-микроорганизмот покажа најголема сензитивност кон екстрактот добиен од габата *Laetiporus sulphureus* (МБЦ=62,5 mg/mL).

И покрај тоа што резултатите од оваа студија укажуваат на релативно слаба антимикробна активност на видовите печурки опфатени во истражувањето, сепак за конечна антимикробна евалуација на овие видови потребно е да се испита нивниот антимикробен потенцијал и во однос на други микроорганизми.

Клучни зборови: *печурки, антимикробна активност, микродилуционен метод, минимална бактерицидна концентрација (МБЦ).*

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Presentation

PERSPECTIVES AND POSSIBILITIES FOR DEVELOPMENT OF TOBACCO PRODUCTION IN THE REPUBLIC OF NORTH MACEDONIA

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Abstract

Tobacco production, with its socio-economic significance, is a source of subsistence, engagement and income for a large part of the population, as well as income from the export of the state. Over the past decade, the World Health Organization has made efforts to reduce tobacco areas through the FCTC (Framework Convention on Tobacco Control), but it succeeds only in developed EU member states, while in other parts of the world is not so. That production is maintained at a stable level.

The Republic of North Macedonia does not envisage measures to reduce tobacco production because of the sensitivity and socio-economic aspect, this issue has been left on after Macedonia's EU entry in the EU when tobacco production plans align with the EU rules.

Tobacco production in Macedonia in the last few years is around 25000 tons per year, whose real increase requires more human resources, which in the next period is not certain (there will be an aging of the population and the emigration of young people in the cities and abroad). Threats always exist, which are different developments on the external market, competition from neighboring countries producing oriental tobacco (Turkey, Greece and Bulgaria), as well as from some far-eastern countries. The spread of some new tobacco products that are not very dependent on tobacco production in the field, such as so-called electronic cigarettes and similar products, are also a real threat.

Keywords: *strategy, subsidies, price policy, production trends, sustainable development*

INTRODUCTION

What is the meaning of tobacco for Macedonia?

The production with its socio-economic significance is a source of existence, engagement and income of a large part of the population, as well as income from the export of the state.

Over the past decade, the World Health Organization has made efforts to reduce tobacco areas through the FCTC (Framework Convention on Tobacco Control), but it succeeds only in developed EU member states, while in other parts of the world is not so. That production is maintained at a stable level.

This Framework Convention is aimed at addressing some of the world's important issues such as: illegal tobacco and tobacco products trade, control of harmful ingredients in cigarettes and tobacco smoke, retail, wholesale and international trade.

The Republic of North Macedonia does not envisage measures to reduce tobacco production because of the sensitivity and socio-economic aspect, this issue has been left on after Macedonia's EU entry in the EU when tobacco production plans align with the EU rules. Tobacco production in Macedonia in the last few years is around 25000 tons per year, whose real increase requires more human resources, which in the next period is not certain (there will be an aging of the population and the emigration of young people in the cities and abroad

Threats always exist, which are different developments on the external market, competition from neighboring countries producing oriental tobacco (Turkey, Greece and Bulgaria), as well as from some far-eastern countries. The spread of some new tobacco products that are not very dependent on

tobacco production in the field, such as so-called electronic cigarettes and similar products, are also a real threat.

In this context of facts about the meaning of tobacco for our country, it is interesting to mention the history and origin of tobacco in the Republic of North Macedonia.

Namely many examples, theories, facts, objects, devices and products indicate that tobacco has been part of life not only in these areas, but also much wider. Tobacco in Macedonia has been reached during the presence of the Ottoman Empire and since then it has been deeply embedded in the life of the Macedonian who shaped it according to its needs.

Wider in the world, tobacco has been discovered since Columbus, but his focus was more on gold, while he was not yet aware of the financial implications of other yellow gold. In Europe, the first cigarette was burnt in 1506 when unfortunate Fragamon Panee wanted to demonstrate to the citizens of Seville what the Indians looked like while smoked tobacco. While he smoked, the Inquisition arrested him and tortured him to admit that he had a pact with the devil, after which he was sentenced to seven years in prison. After he left the prison, he was amazed when he saw that everyone was smoking through the streets, and the tobacco was called by Spanish craziness.

The value of tobacco is confirmed by another argument, in the homeland of tobacco in the US, in the states of Virginia and Maryland, even for centuries tobacco is used as a means of payment. The first law in free America was the Tobacco Law, and not the Constitution, the first standard was the tobacco standard and not the gold, making tobacco a very important and respected culture; it was luxury, prestige and power. The first scientific book on tobacco is Tobacco by Johannes Neander of 1626, when tobacco is still seen as a medicinal herb and when almost all diseases were treated with tobacco.

If the Indians were a symbol of peace, in our country through centuries up to now, tobacco is an opportunity for existence.

In Macedonia, tobacco arrives later from the rest of the world, in 1873, and became the center of spiritual life. Not so much as a crop that yields per unit area, because tobacco is considered a labor intensive culture, but because still tobacco cultivation carried large financial returns and was paid off. Even the local, folklore architecture was defined according to the needs for drying tobacco strings. The picturesque verandas were adapted for natural storage and drying of tobacco.

MATERIAL AND METHODS

During the preparation of this paper, statistical information from the World Bank, the State Statistical Office of the Republic of Macedonia, the Ministry of Agriculture, Forestry and Water Economy of the Republic of Macedonia were used. Macedonia, data published by magazines: Tobacco journal, International Year Book, Statistics-addresses-brands, Tobacco, World Markets and Trade and wider literature

The data processing was used: analytical, mathematical-statistical and comparative method, as well as tabular and graphical representation.

We used the linear trend method for analyzing and predicting the results.

RESULTS AND DISCUSSION

Tobacco production is gaining more strategic importance for the state economy and is a significant item in filling the state budget. On the positive balance sheets after the purchase of last year's harvest, the upward trends in the value of the exported tobacco are added.

The Republic of North Macedonia is on the 30th place in terms of the quantity of tobacco produced in the world, as well as on the 15th place according to the value of the realized tobacco export worldwide. The largest importers of tobacco from Macedonia are the USA and Germany, that is, they are multinational companies that have factories in the countries mentioned above.

Thus, tobacco annually provides around 100 million euro foreign exchange inflow. The growing interest in tobacco production, as well as the growing planted areas, is motivating the state to provide increased subsidies, and since the last year's harvest, the subsidy model has been introduced at three levels, for the first

class 80, for the second 70 and 60 denars per kilogram for a third, fourth and for additional classes.

The production is of economic importance and our country is a well-known producer of high-quality oriental type tobacco. The economic and social significance of tobacco production are supported by the number of about 40 thousand families for which it is the main source of existence. The entry of large cigarette companies like Philip Morris and Imperial mean a lot to Macedonia. These companies did not come here by chance, they believe that Macedonia has perspectives for production of quality tobacco, especially Oriental.

Table 1. Production, surfaces and average yield (kg/ha) of tobacco in the Republic of North Macedonia

	Year	Tobacco production in kg	Index-base (1953)	Average yield per ha (kg/ha)	Index base (1953)	Planted hectares	Index base (1953)	Number of signed contracts of tobacco growers	Planting hectares according to the manufacturer
1	1970	23.643.000	1,72	820	1,04	28.833	1,66	69.586	0,41
2	1971	21.589.000	1,57	800	1,01	26.986	1,55	65.392	0,41
3	1972	28.983.000	2,11	1.000	1,27	28.983	1,67	69.770	0,42
4	1973	32.437.000	2,37	1.120	1,42	28.962	1,67	71.886	0,40
5	1974	27.978.000	2,04	930	1,18	30.084	1,73	69.913	0,43
6	1975	34.126.000	2,49	1.030	1,30	33.132	1,91	78.776	0,42
7	1976	33.721.000	2,46	1.030	1,30	32.739	1,89	79.408	0,41
8	1977	32.296.000	2,35	1.030	1,30	31.355	1,81	74.313	0,42
9	1978	31.154.000	2,27	1.070	1,35	29.116	1,68	67.536	0,43
10	1979	29.447.000	2,15	1.090	1,38	27.016	1,56	59.677	0,45
11	1980	23.587.000	1,72	890	1,13	26.502	1,53	55.355	0,48
12	1981	31.294.000	2,28	1.230	1,56	25.442	1,47	50.831	0,50
13	1982	34.000.000	2,48	1.260	1,59	26.984	1,55	60.259	0,45
14	1983	22.490.000	1,64	830	1,05	27.096	1,56	58.757	0,46
15	1984	30.719.000	2,24	1.185	1,50	25.923	1,49	53.692	0,48
16	1985	30.728.000	2,24	1.078	1,36	28.505	1,64	71.033	0,40
17	1986	35.020.000	2,55	1.159	1,47	30.216	1,74	80.256	0,38
18	1987	28.648.000	2,09	1.125	1,42	25.465	1,47	57.826	0,44
19	1988	22.259.000	1,62	1.201	1,52	18.534	1,07	54.440	0,34
20	1989	27.537.000	2,01	1.126	1,43	24.456	1,41	49.135	0,50
21	1990	16.452.000	1,20	790	1,00	20.825	1,20	38.809	0,54
22	1991	25.195.000	1,84	1.375	1,74	18.324	1,06	40.750	0,45
23	1992	26.502.000	1,93	1.178	1,49	22.497	1,30	49.348	0,46
24	1993	24.002.000	1,75	1.123	1,42	21.373	1,23	53.809	0,40
25	1994	18.862.000	1,38	1.269	1,61	14.864	0,86	35.416	0,42
26	1995	15.683.000	1,14	1.440	1,82	10.891	0,63	24.752	0,44
27	1996	15.412.000	1,12	1.313	1,66	11.738	0,68	27.110	0,43
28	1997	25.308.000	1,85	1.312	1,66	19.290	1,11	33.050	0,58
29	1998	32.746.000	2,39	1.309	1,66	25.016	1,44	54.661	0,46
30	1999	29.368.000	2,14	1.189	1,51	24.700	1,42	44.822	0,55
31	2000	22.175.000	1,62	973	1,23	22.790	1,31	37.617	0,61
32	2001	23.217.000	1,69	1.157	1,46	20.067	1,16	33.906	0,59
33	2002	22.911.000	1,67	1.116	1,41	20.530	1,18	26.971	0,76
34	2003	23.986.000	1,75	1.332	1,69	18.008	1,04	32.000	0,56
35	2004	21.630.000	1,58	1.221	1,55	17.715	1,02	27.343	0,65
36	2005	27.691.000	2,02	1.498	1,90	18.485	1,06	38.000	0,49
37	2006	25.036.000	1,83	1.436	1,82	17.507	1,06	29.230	0,60
38	2007	22.056.000	1,61	1.287	1,63	17.183	1,06	29.771	0,58
39	2008	17.087.000	1,25	1.001	1,27	17.064	1,06	30.519	0,56
40	2009	24.122.000	1,76	1.355	1,72	17.809	1,06	38.710	0,46
41	2010	30.280.000	2,21	1.492	1,89	20.300	1,06	40.743	0,50
42	2011	26.537.000	1,93	1.348	1,71	19.693	1,06	33.234	0,59
43	2012	27.333.000	1,99	1.392	1,76	19.656	1,06	29.090	0,68
44	2013	27.859.000	2,03	1.453	1,84	19.178	1,06	42.367	0,45
45	2014	27.578.000	2,01	1.553	1,97	17.758	1,06	34.445	0,52
46	2015	24.237.000	1,77	1.503	1,90	16.126	1,06	28.454	0,57
47	2016	25.443.000	1,86	1.554	1,97	16.373	1,06	27.380	0,60
48	2017	23.559.000	1,72	1.643	2,08	14.342	1,06	29.132	0,49

Based on these data, using the linear trend method, we determined the following absolute and estimated values by 2022 .

Table 2. Tobacco production (absolute/estimated values) by 2022 in the Republic of North Macedonia.

	Year	Tobacco production - absolute values	Tobacco production - estimated values		Years	Tobacco production - absolute values	Tobacco production - estimated values
1	1970	23.643.000	24.295.090,37	28	1997	25.308.000	25.393.366,44
2	1971	21.589.000	24.335.767,27	29	1998	32.746.000	25.434.043,33
3	1972	28.983.000	24.376.444,16	30	1999	29.368.000	25.474.720,22
4	1973	32.437.000	24.417.121,05	31	2000	22.175.000	25.515.397,11
5	1974	27.978.000	24.457.797,94	32	2001	23.217.000	25.556.074,00
6	1975	34.126.000	24.498.474,83	33	2002	22.911.000	25.596.750,90
7	1976	33.721.000	24.539.151,72	34	2003	23.986.000	25.637.427,79
8	1977	32.296.000	24.579.828,61	35	2004	21.630.000	25.678.104,68
9	1978	31.154.000	24.620.505,50	36	2005	27.691.000	25.718.781,57
10	1979	29.447.000	24.661.182,40	37	2006	25.036.000	25.759.458,46
11	1980	23.587.000	24.701.859,29	38	2007	22.056.000	25.800.135,35
12	1981	31.294.000	24.742.536,18	39	2008	17.087.000	25.840.812,24
13	1982	34.000.000	24.783.213,07	40	2009	24.122.000	25.881.489,14
14	1983	22.490.000	24.823.889,96	41	2010	30.280.000	25.922.166,03
15	1984	30.719.000	24.864.566,85	42	2011	26.537.000	25.962.842,92
16	1985	30.728.000	24.905.243,74	43	2012	27.333.000	26.003.519,81
17	1986	35.020.000	24.945.920,63	44	2013	27.859.000	26.044.196,70
18	1987	28.648.000	24.986.597,53	45	2014	27.578.000	26.084.873,59
19	1988	22.259.000	25.027.274,42	46	2015	24.237.000	26.125.550,48
20	1989	27.537.000	25.067.951,31	47	2016	25.443.000	26.166.227,37
21	1990	16.452.000	25.108.628,20	48	2017	23.559.000	26.206.904,27
22	1991	25.195.000	25.149.305,09	49	2018		26.247.581,16
23	1992	26.502.000	25.189.981,98	50	2019		26.288.258,05
24	1993	24.002.000	25.230.658,87	51	2020		26.328.934,94
25	1994	18.862.000	25.271.335,77	52	2021		26.369.611,83
26	1995	15.683.000	25.312.012,66	53	2022		26.410.288,72
27	1996	15.412.000	25.352.689,55				

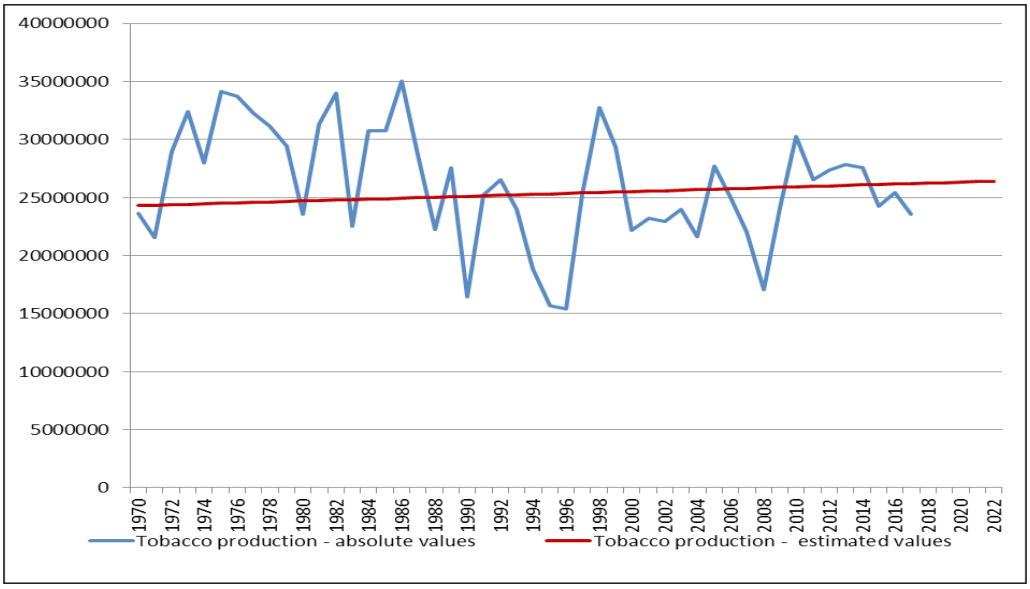

Figure 1. Tobacco production (absolute/estimated values) by 2022.

Table 3. Concluded contracts (absolute/estimated values) by 2022 in the Republic of North Macedonia.

Years	concluded contracts		Planted hectares of tobacco		years	concluded contracts		Planted hectares of tobacco	
	absolute values	estimated values	absolute values	estimated values		absolute values	estimated values	absolute values	estimated values
1970	69.586	73.912,63	28.833	26.435,41	1997	33.050	44.591,23	19.290	21.159,65
1971	65.392	72.826,65	26.986	26.240,01	1998	54.661	43.505,25	25.016	20.964,25
1972	69.770	71.740,67	28.983	26.044,61	1999	44.822	42.419,27	24.700	20.768,85
1973	71.886	70.654,69	28.962	25.849,22	2000	37.617	41.333,30	22.790	20.573,45
1974	69.913	69.568,72	30.084	25.653,82	2001	33.906	40.247,32	20.067	20.378,05
1975	78.776	68.482,74	33.132	25.458,42	2002	26.971	39.161,34	20.530	20.182,65
1976	79.408	67.396,76	32.739	25.263,02	2003	32.000	38.075,36	18.008	19.987,25
1977	74.313	66.310,78	31.355	25.067,62	2004	27.343	36.989,39	17.715	19.791,85
1978	67.536	65.224,81	29.116	24.872,22	2005	38.000	35.903,41	18.485	19.596,46
1979	59.677	64.138,83	27.016	24.676,82	2006	29.230	34.817,43	17.507	19.401,06
1980	55.355	63.052,85	26.502	24.481,42	2007	29.771	33.731,45	17.183	19.205,66
1981	50.831	61.966,87	25.442	24.286,03	2008	30.519	32.645,48	17.064	19.010,26
1982	60.259	60.880,89	26.984	24.090,63	2009	38.710	31.559,50	17.809	18.814,86
1983	58.757	59.794,92	27.096	23.895,23	2010	40.743	30.473,52	20.300	18.619,46
1984	53.692	58.708,94	25.923	23.699,83	2011	33.234	29.387,54	19.693	18.424,06
1985	71.033	57.622,96	28.505	23.504,43	2012	29.090	28.301,56	19.656	18.228,66
1986	80.256	56.536,98	30.216	23.309,03	2013	42.367	27.215,59	19.178	18.033,27
1987	57.826	55.451,01	25.465	23.113,63	2014	34.445	26.129,61	17.758	17.837,87
1988	54.440	54.365,03	18.534	22.918,23	2015	28.454	25.043,63	16.126	17.642,47
1989	49.135	53.279,05	24.456	22.722,84	2016	27.380	23.957,65	16.373	17.447,07
1990	38.809	52.193,07	20.825	22.527,44	2017	29.132	22.871,68	14.342	17.251,67
1991	40.750	51.107,10	18.324	22.332,04	2018		21.785,70		17.056,27
1992	49.348	50.021,12	22.497	22.136,64	2019		20.699,72		16.860,87
1993	53.809	48.935,14	21.373	21.941,24	2020		19.613,74		16.665,47
1994	35.416	47.849,16	14.864	21.745,84	2021		18.527,77		16.470,08
1995	24.752	46.763,19	10.891	21.550,44	2022		17.441,79		16.274,68
1996	27.110	45.677,21	11.738	21.355,04					

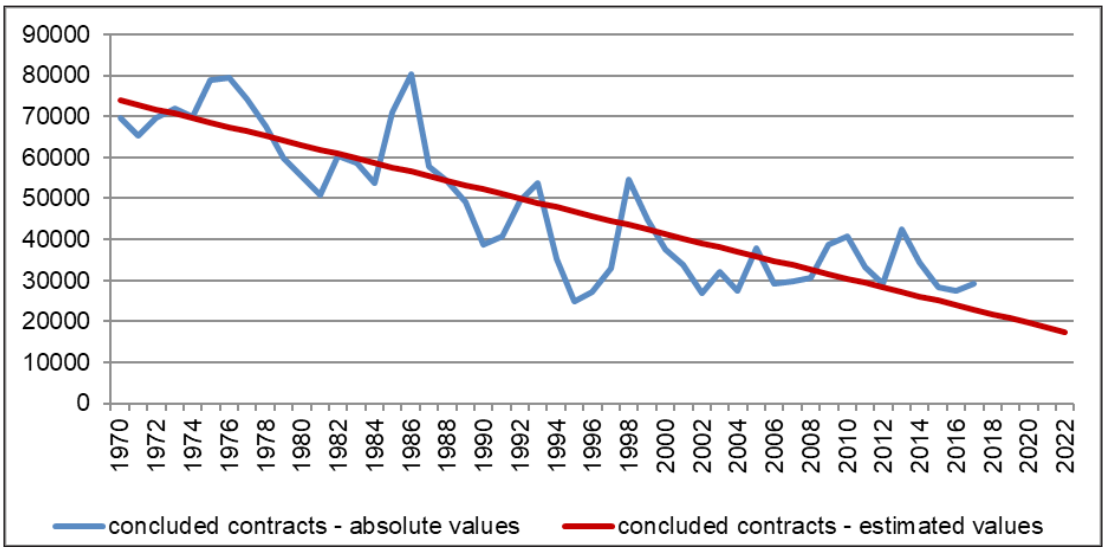

Figure 2. Concluded contracts (absolute/estimated values) by 2022 in the Republic of North Macedonia.

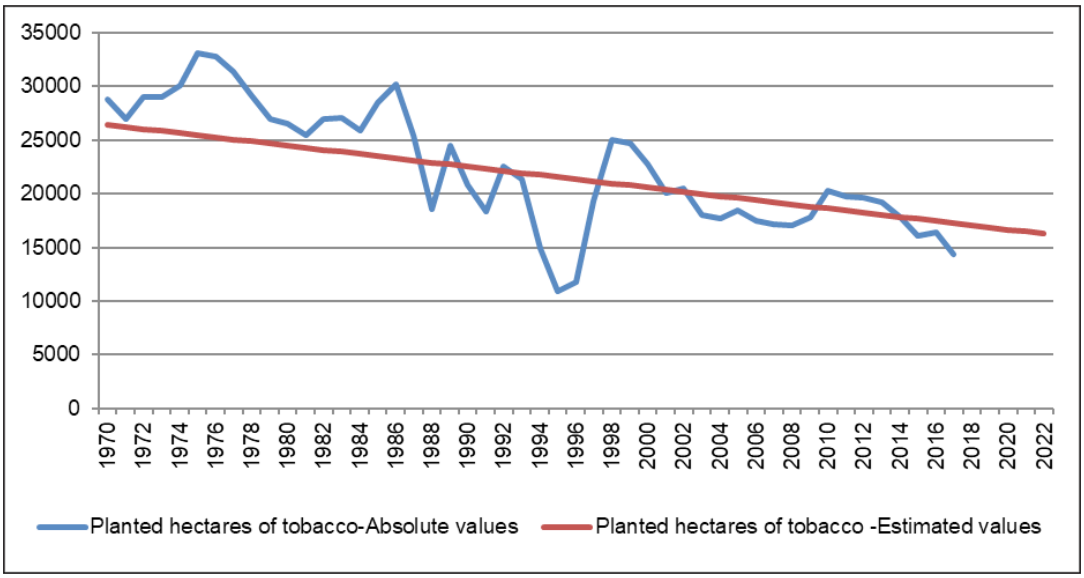

Figure 3. Planted hectares of tobacco(absolute/estimated values) by 2022 in the Republic of North Macedonia.

Which direction will take the tobacco production in Republic of North Macedonia?

Starting from the requirements of the world market, the scientific and technological achievements of the world and the available natural, production and human (labor) potentials, it can be freely stated that today, and even more in the future, the need will be expressed:

- Raising the quality of tobacco production at a much higher level, taking into account the requirements of buyers.
- Efficient and effective production, where the emerging and potential problems will be identified in an easier way and in an efficient way organism, an entity will be able to handle them.
- Raising the motivation and satisfaction in the work of the employed (engaged) people, and in particular better use of the technical and technological benefits, greater use of resources and mechanization that greatly replace the physically invested labor and reduce the time production operations and processes; and Fig.
- Continuous development of research, educational and advisory activities for raising the proper implementation of Good Agricultural Practices (GAP).
- Raising awareness of the quality of the overall performance of all engaged individuals in the firm or farm.
- Guaranteeing the quality of tobacco and tobacco products production and secure placement;
- integrating economic and environmental goals, on the one hand increasing income, and on the other hand preserving the environment for healthy plants and favorable livelihoods of all living beings.
- Developing opportunities for creating alternative (combined) revenues through the expansion of tobacco production and other additional products or services.
- Taking care of environmental protection through a publicly declared policy about it.
- Establishing ways to monitor tobacco production (developing monitoring) for protection.

CONCLUDING REMARKS

In order to contribute to the clarification of the views towards determining the strategy for development of tobacco production in the Republic of Macedonia in the following period, the activities in the field of tobacco production should be directed towards:

- Soil management and irrigation (conditions with arable and non-cultivated areas, water potential, proper soil and water management, etc.);

- Preserving the purity of tobacco purity (tobacco selection);
- Yield management;
- Integrated pest management;
- Agrochemical Management;
- Properly manipulating and storing tobacco;
- Development of farm tobacco production, family business, small enterprises with basic activity for tobacco production;
- Farmers' training;
- Socio-economic issues (problems);
- Developing sustainable tobacco production by unifying the knowledge of the utilization of free surfaces, applying the correct management activities and the preference of the good agricultural practices;
- Promoting tobacco production and raising public awareness for the preservation of the environment, as well as other areas that are in the interest of clarifying the aspects of promotion of tobacco production in the Republic of Macedonia in accordance with the relevant development policy in the European Union and the positive global (world) policy.

Tobacco producers often face the question of why they most often decide to produce tobacco, and no other crops. Below are some of the strongest arguments in favor of tobacco:

- Tobacco is a legally traded agricultural crop, for which global demand remains a major demand.
- Tobacco grows on low fertility soil and some tobacco, such as Oriental type, is best grown in dry environments. Such conditions are always inadequate for the successful production of other crops.
- In many areas where tobacco is grown, crops are grown after tobacco cultivation, benefiting from residual fertilizers in the soil.
- As it is less perishable than most other alternative alternative cultures, tobacco can be easily stored.
- Tobacco is characterized by relative price stability.
- As a rule, sales are guaranteed, the price is agreed and it is most often respected.

Alternative cultures an extremely difficult task, because tobacco is an alternative to tobacco.

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ПЕРСПЕКТИВИ И МОЖНОСТИ ЗА РАЗВОЈ НА ТУТУНОПРОИЗВОДСТВОТО ВО РЕПУБЛИКА СЕВЕРНА МАКЕДОНИЈА

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Резиме

Тутунопроизводството со своето социоекономско значење претставува извор на егзистенција, ангажираност и приход на голем дел од населението, како и приход од извоз на државата. Во последнава декада Светската здравствена организација прави напори да се намалат површините под тутун, преку Рамковната конвенција за контрола на тутунот (FCTC – Framework Convention on Tobacco Control), но тоа успева само во развиените земји членки на ЕУ, додека во останатите делови во светот тоа не е така. Таму производството се одржува на стабилно ниво.

Република Северна Македонија не предвидува мерки за намалување на производството на тутун бидејќи, поради осетливоста и социоекономскиот аспект, тоа прашање е оставено за понатаму, по евентуалниот влез на Македонија во ЕУ кога плановите за производството на тутун се усогласуваат со правилата на ЕУ.

Производството на тутун во Македонија во последните неколку години се движи околу 25.000 тони годишно за чие реално зголемување се потребни поголеми човечки ресурси, што во наредниот период не е извесно дека ќе ги има (стареење на популацијата и иселувањето на младите луѓе во градовите и во странство). Закани секогаш постојат, а тоа се различни случувања на надворешниот пазар, конкуренцијата од соседните земји производители на ориенталски тутуни (Турција, Грција и Бугарија), како и од некои далекуисточни држави. Ширењето на некои нови тутунски производи кои не се многу зависни од производството на тутун на нива, како на пример таканаречените електронски цигари и слични производи, исто така се реална закана.

Клучни зборови: *стратегија, субвенции, политика на цени, производствени трендови, одржлив напредок.*

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