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

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ABSTRACT

The mode of inheritance was studied in ten F_1 crosses between five parental genotypes: MV-1, P 76/86, Adiyaman, Basma-Djebel and P 66 9 7 and following quantitative traits: number of leaves per stalk, length of leaves from the middle belt, yield of green leaf mass per stalk and per hectare, in 2018-2019 year.
The aim of this work was to study the mode of inheritance of the quantitative traits, to detect heterosis and to assess its economic viability.
The hybrids MV-1 x Adiyaman, P 76/86 x Basma-Djebel, P 76/86 x P 66 9 7 and Basma-Djebel x P 66 9 7 have a positive heterotic effect on the length of the leaves. Oriental hybrids where one of the parents is variety P 66 9 7, have positive heterosis for yield of green leaf mass per stalk. The investigation provides very useful guidance for future successive selection activities.

Key words: tobacco, inheritance, dominance, intermediality, partial dominance.

ХЕТЕРОТИЧЕН ЕФЕКТ НА НЕКОИ КВАНТИТАТИВНИ СВОЈСТВА КАЈ F_1 ДИЈАЛЕЛНИ ХИБРИДИ ОД РАЗЛИЧНИ ТИПОВИ ТУТУН

Овој труд го проучува наследувањето kaj десет F_1 крстоски добиени меѓу пет родителски генотипови: МВ-1, П 76/86, Adiyaman, Басма-Џебел и П 66 9 7, за следниве својства: број на листовите по страк, должина на листовите од средниот појас и приносот на зелена лисна маса по страк и по хектар, во периодот од 2018 и 2019 година.
Целта на истражувањата беше да се проучи начинот на наследување на квантитативните својства, да се открие хетерозис и да се процени неговата економска оправданост.
Крстоските МВ-1 x Adiyaman, П 76/86 x Басма-Џебел, П 76/86 x П 66 9 7 и Басма- Џебел x П 66 9 7 располагаат со позитивен хетеротичен ефект за должината на листовите. За приносот на зелена лисна маса по страк и по хектар, позитивен хетерозис имаат ориенталските крстоски каде еден од родителите е сортата П 66 9 7. Истражувањата даваат многу корисни насоки за идната сукцесивната селекциона дејност.

Клучни зборови: тутун, наследност, доминантност, интермедијарност, парцијална доминантност.

INTRODUCTION

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

The topic of this paper is focused on the mode of inheriting of the most important quantitative traits in F_1 offspring of hybrids obtained by diallel crossing between varieties of different tobacco types. In the last ten years, a number of authors – breeders have worked on this subject: (Aleksoski, 2010), in a two-year study of four parental genotypes and their six diallel F_1 hybrids, obtained a different way of inheriting of leaf length and a weak heterotic effect without economic justification. (Gixhari and Sulovari, 2010) conducted three-year research at two locations in a genetically diverse population of eight oriental tobacco and their one-way diallel hybrids and obtained a dominant and partial-dominant mode of inheritance and heterosis in leaf size and yield. Aleksoski and Korubin – Aleksoska (2011) conducted three-year studies for green and dry mass yields in a one-way diallel on three oriental and one large-leaf variety and their six F_1 crosses and obtained positive and negative heterosis. Dimanov and Dyulgerski (2012a) found heterosis with varying strength in number of leaves in ten F_1 crosses obtained from parent pairs of local and introduced Burley tobacco varieties. Dyulgerski and Dimanov (2012b) performed examinations of the dimensions of 7-8 leaves and 13-14 leaves in the P1, P2 and F_1 populations at ten crosses originating from local and introduced varieties of Burley tobacco and obtained a high heterotic effect of significant economic importance. Aleksoski et al. (2013) found weak heterosis in the inheritance of leaf number in a three-year study of four parental tobacco genotypes of different types and their six diallel F_1 hybrids. In an experiment in Khan Gari, Mardan, Pakistan, with seven Virginia flue-cured genotypes and their 42 two-way diallel crosses, Imtiaz et al. (2014) found heterosis with high heterotic effect, with the possibility of using it on leaf number per stalk, leaf length and weight of green leaf mass per plot. Dyulgerski and Radoukova (2015) studied the mode of inheritance, the coefficient of inheritance and the expression of heterosis and transgression in seven Burley-type crosses and seven Virginia-type crosses of local and introduced origin in F_1 and F_2 offspring and found a dominant and partial- dominant inheritance in the length of the leaves, always in the direction of the parent with longer leaves, and the resulting heterosis had no economic importance. Ramachandra et al. (2015) obtained hybrids superior to the control variety in number and length of leaves, in genetic studies on the yield and quality of 62 genotypes (six lines of different types of tobacco, eight testers and their crosses), at the Agricultural Research Station, Nipaniat Belgaum, Karnataka - India. In the investigation with hybrids obtained with semi diallel hybridizations of Xanthi-2A, Nail, Gümüşhacıköy, Taşova, Katerini, Canik and Erbaa in Tokat Province, Turkey, Kinay and Yilmaz (2016) found an average heterosis of 28.4%. The heterotic effect on dry leaf yield was 4%. Shah et al. (2017) found highly significant differences in the number of leaves per stalk in a two-year study of ten Virginia flue-cured hybrids. The field experiment was set up in agro-ecological conditions at Khan Gari in Mardan, Pakistan. Aleksoski (2018) examined four oriental varieties and their diallel F_1, F_2, BC_1 (P1) and BC_1 (P2) crosses in the number and length of leaves per stalk, and found low variability in F_1 generation, indicating uniformity of populations due to homozygosity of parent pairs. Dyulgerski and Docheva (2018) analyzed ten introduced Burley tobacco varieties for biological, economic, and chemical traits and found that Kentucky 908 and Banquet 102 could be used in selection programs as donors to increase leaf number, leaf length, and yield. Ganachari et al. (2018) examined six flue- cured tobaccos and their 30 crosses in order to assess heterosis in dry leaf yield and its components at ZAHRS (Zonal Agricultural and Horticultural Research Station) College of Agriculture - Shivamogga, Karnataka - India. The authors discovered five hybrids that showed a highly significant heterotic effect. In research for inheritance of leaf number per stalk on six flue-cured tobacco and their 30 diallel crosses in ZAHRS, Karnataka, India, Ganachari et al. 2018) did not obtained significant differences between genotypes, but obtained heterosis with highly significant heterotic effect on leaf number and yield. In relation to the mode of inheritance of dimensions of the leaves from the middle belt, Korubin – Aleksoska and Dojcinov (2018) found intermediate and partial dominant mode of inheritance without heterosis in five parental varieties- four oriental in the role of a mother and one large-leafed flue-cured variety in the role of a father and their four F_1 crosses. In a one-way diallel of four small-leaf aromatic and one large-leaf flue-cured variety and their 10 F_1 crosses, Aleksoski (2019) discovered partial dominance and negative heterosis in leaf number per stalk, all possible inheritance modalities and positive heterosis in leaf dimensions and yield. In relation to leaf number and yield in eight Burley newly created hybrid combinations from first-generation, Dyulgerski (2019) found that all of them are better than the standard variety Pliska 2002, which indicates the possibility of exploiting heterosis in Burley tobacco. Dyulgerskи and Radoukova (2019) investigated the inheritance and manifestation of transgression and heterosis in seven Burley hybrids in F_1 and F_2 generation and found dominance of the parents with more leaves, the existence of all modes of dominance in inheriting leaf dimensions, in the direction of the stronger parent. Qaizar et al. (2019) did research on seven flue-cured varieties and lines and their complete diallel crosses, in the Mardan and Mansehra regions of Pakistan, and found positive and negative heterosis in the inheritance of some agronomic and biochemical traits. The best hybrids with favorable significant heterotic effect on yield were: KHG24 x Spt G 28; KHG21 x NC606 and Spt G 126 x KHG24. In a semi- diallel of seven genotypes and 21 F1 hybrids, tested in two locations in the Black Sea region of Turkey, Kinay et al. (2020) obtained positive heterosis in yield without economic justification. Dyulgerski (2021) found a positive-dominant and partial-dominant way of inheriting in dimensions of the leaves in local and introduced varieties of Burley and seven hybrids of F_1 and F_2 generation, indicating effective selection in later hybrid generations. The resulting heterosis had no economic significance.

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

MATERIALS AND METHODS

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

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

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

In 2019, a trial was set up using the same method, in which the same set of 15 genotypes was planted.

The total area of the trial (working area - 405m^2 + area of paths - 477 m^2) was 882 m^2.

826 plants (770 plants + 56 plants as protection) were planted to fill the surface of one repetition. A total of 3304 plants (stalks) were planted in the experiment.

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

Processing of results

Statistical data processing was performed using the measurements of the subject traits of each variant. The mode of inheritance of traits is determined on the basis of test-significance from the mean value of the F1 generation in relation to the average of both parents, according to Borojevic (1981). Intermediate mode of inheritance (i) occurs when the mean value of a trait in the offspring is equal to the parent average. There is a partial-dominant mode (pd) when the mean value of the hybrid offspring approaches one of the parent varieties. Dominance in inheritance (d) - positive or negative, occurs when the mean value of the cross coincides with the mean value of one of the parents (+d - when the parent with a higher mean value dominates, -d - when the parent with a lower mean value dominates). Positive heterosis (+h) occurs in the hybrid with a significantly higher value than that of the parent with a higher mean value, while negative heterosis (-h) occurs in the hybrid with a significantly lower value than the one from the parent with a lower mean value.

Meteorological data

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

In 2018:

- The average monthly air temperature from May to September was 22.12 ºC (May 19.8 ºC, June 22.4 ºC, July 24.4 ºC, August 23.9 ºC, September 20.1 ºC).
- The average monthly maximum air temperature from May to September was 25.82 ºC (May - 22.3 ºC, June -25.9 ºC, July - 27.5 ºC, August - 29.8 ºC, September -23.6 ºC).
- The average monthly minimum air temperature from May to September was 13.4 ºC (May- 11 ºC, June – 12.9 ºC, July – 15.8 ºC, August -15.3 ºC, September – 12 ºC).
- The average monthly air humidity from May to September was 78.8 % (May - 81 %, June - 80%, July - 78 %, August - 75 %, September - 80%).

- The total rainfall from May to September was 169 mm (May – 18 mm, June – 20 mm, July – 21 mm, August – 20 mm, September – 90 mm).

In 2019:

- The average monthly air temperature from May to September was 22.43 ºC (May 15.77 ºC, June 22.77 ºC, July 24.26 ºC, August 27.39 ºC, September 21.97 ºC).
-The average monthly maximum air temperature from May to September was 27.2 ºC (May – 20.0 ºC, June – 28.0 ºC, July – 29.0 ºC, August – 32.0 ºC, September – 27.0 ºC).
- The average monthly minimum air temperature from May to September was 14.8 ºC (May – 8.0 ºC, June – 16.0 ºC, July – 14.0 ºC, August – 22.0 ºC, September – 14.0 ºC).
- The average monthly air humidity from May to September was 58.7% (May – 71.30%, June – 67.17 %, July – 59.42 %, August – 42.61%, September – 53%).
- The total rainfall from May to September was 404.8 mm (May – 124.1 mm, June – 139.9 mm, July – 91.8 mm, August – 9.5 mm, September – 39.5 mm).

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

Soil conditions

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

RESULTS AND DISCUSSION

The number of leaves is a trait that is directly related to yield. Because of that, it is the most common subject of research in all selection programs for tobacco breeding.
In 2018 the most common way of inheriting of the number of leaves per stalk in the F_1 generation is the partial-dominant. Intermediate inheritance occurs only in Adiyaman x P 66 9 7. The hybrid P 76/86 x P 66 9 7 has heterosis with a negative heterotic effect, which means that the offspring in the F_1 generation has less leaves than both parents.

In 2019 we found an identical scheme for this trait. This indicates the fact that these are stable homozygous parents whose offspring forms the first investigated generation. The number of leaves is a highly inherited trait on which environmental factors have limited influence.
Table 1. shows the mean values of the number of leaves per stalk in the parental genotypes and their diallel F_1 offspring, as well as the mode of inheritance of this trait in 2018 and 2019.

Table 1. Mode of inheritance of the number of leaves per stalk in diallel F_1 hybrids in 2018 and 2019

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

In the inheritance of length of leaves from the middle belt of the F_1 offspring in 2018, all the modalities are present. There is intermediate inheritance in Adiyaman x P 66 9 7. There is a partial-dominant way of inheritance in MV-1 x Basma-Djebel, MV-1 x P 66 9 7, P 76/86 x Adiyaman and Adiyaman x Basma-Djebel. Positive dominance occurs in MV-1 x P 76/86. Positive heterosis in inheritance of this trait occurs in MV-1 x Adiyaman, P 76/86 x Basma-Djebel, P 76/86 x P 66 9 7 and Basma-Djebel x P 66 9 7, which means that the F1 generation has longer leaves than the leaves of the both parents. The same mode of inheriting in crosses was obtained in 2019, which means that the trait is highly inherited and it is a varietal characteristic. Table 2 shows the mean values of the leaf length of the middle belt of the stalk in the parental genotypes and their diallel F_1 offspring, as well as the mode of trait inheritance in 2018 and 2019.

Table 2. Mode of inheritance of the length of the leaves from the middle belt of the stalk in diallel F_1 hybrids in 2018 and 2019

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

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

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

Table 3. shows the mean values of the yield of green leaf mass per stalk in the parent genotypes and their diallel F_1 offspring, as well as the mode of inheritance of this trait in 2018 and 2019.

Table 3. Mode of inheritance of green mass yield per stalk in diallel F_1 hybrids in 2018 and 2019

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

In 2018, the mode of inheritance of the yield of green leaf mass per hectare in F_1 offspring is partially-dominant and positively dominant. Negative heterosis is present in crosses where one parent is MV-1 (with the exception of MV-1 x Basma-Djebel in which there is partial dominance in the direction of the weaker parent). The occurrence of positive heterosis has the Oriental crosses where one parent is P 66 9 7.

In 2019, there are changes in the way of inheritance of the yield of green leaf mass per hectare and the yield of green leaf mass per stalk (P 76/86 x Adiyaman - positive dominance and Adiyaman x Basma-Djebel - partial dominance). The analysis of the heredity of the yield of green leaf mass per stalk and the yield of green leaf mass per hectare indicates drastic differences and therefore it is necessary to indicate the reasons.

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

Here, instead of partial dominance, negative heterosis occurs (with the exception of MV-1 x Basma-Djebel, where instead of intermediate, partial dominance occurs). The reason for this outcome is in the different planting distance of the plants in the experiment. Oriental parents and oriental hybrids are planted with a row spacing of 45 cm and plant spacing of 15 cm, while the large-leaved MV-1 and its crosses are planted with a row spacing of 90 cm and plant spacing of 50 cm, which means that the calculation of yield per hectare is different, depending on the plant composition of the genotype.

Table 4. shows the mean values of the yield of green leaf mass per hectare in the parent genotypes and their diallel F_1 offspring, as well as the mode of inheritance of the trait in 2018 and 2019.

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

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

CONCLUSIONS

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

- All the modalities in the two years of investigations can be found in the length of the leaves from the middle belt of the stalk. Positive heterosis in this trait occurs in MV-1 x Adiyaman, P 76/86 x Basma-Djebel, P 76/86 x P 66 9 7 and Basma-Djebel x P 66 9 7.
- Inheritance of the number of leaves per stalk and the length of the leaves from the middle belt of the stalk in F_1 hybrids shows identical values for both years of research, which indicates the fact that the parents in the diallel are stable and homozygous and the environmental factors have a limited impact on these traits.
- The mode of inheritance of the yield of green mass per stalk is partially-dominant in the direction of the parent with higher yield and positively-dominant (only in MV-1 x Basma-Djebel there is an intermediate mode). Positive heterosis occurs in Oriental crosses where one parent is P 66 9 7.
- The mode of inheritance of the yield of green leaf mass per hectare is partially-dominant and positively dominant. Negative heterosis is present in hybrids where one of the parents is MV-1 (with the exception of MV-1 x Basma-Djebel where there is partial dominance in the direction of the weaker parent). The occurrence of positive heterosis has the Oriental hybrids where one of the parents is P 66 9 7.
- The picture of the heritability of the yield detects minimal differences compared to the two years of research, which indicates the fact that it is a trait that is greatly influenced by the environmental factors, but also that there is a professional approach to the overall work.
- From the analysis of the inheritance of the yield of green leaf mass per stalk and the yield of green leaf mass per hectare, it is concluded that there are no differences with the oriental hybrids, but there are differences with the hybrids where one of the parents is MV-1, and that is a consequence of the different planting distance of plants in the experiment.
- The results of this paper are a good original source material for further successive selection activity.

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APPLICATION OF THE EM TECHNOLOGY IN TOBACCO SEEDLING PRODUCTION

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ABSTRACT

Modern EM technology has a particular contribution to environmental management and sustainable agricultural production. The aim of the research was to determine the effect of the application of the probiotic EMa 5 or the combination EMa 5+ EmFarma, as well as the best model of application (on the soil before sowing; after the emergence of tobacco seedlings; on the seeds) and with/without herbicide application.
Probiotics have a positive effect on tobacco seedlings. The best development of the root system, as well as the total length of the tobacco seedling, was determined in the variant - application of EmFarma Plus + Emma 5 on the soil, before sowing and during the application of herbicide. The length of the root system of the seedling in the standard treatment is lower than that of the seedling of all variants of probiotic application.
Application of probiotics is possible under standard sowing conditions, that is, when herbicide is used.
The results of these researches create a good perspective for their application in the production of tobacco seedlings.

Key words: tobacco, EM technology, probiotics, EmFarma Plus, Ema 5, development, root

ПРИМЕНА НА ЕМ ТЕХНОЛОГИЈАТА ВО ПРОИЗВОДСТВОТО НА ТУТУНСКИ РАСАД

Современата ЕМ технологија има особен придонес во управувањето со животната средина и оддрживото земјоделско производство.
Целта на истражувањето беше да се утврди ефектот од примената на пробиотикот ЕМа 5 или комбинацијата ЕМа 5+ EmFarma, како и најдобриот модел на апликација (врз почвата пред сеидба; по поникнување на тутунскиот расад; врз семето) и со/без примена на хербицид.
Пробиотиците имаат позитивен ефект врз тутунскиот расад. Најдобар развој на корeновиот систем, како и вкупна должина на тутунскиот расад е утврдена кај варијантата - апликација на EmFarma Plus + Ema 5 врз почвата, пред сеидба и при примена на хербицид. Должината на корeновиот систем од расадот при стандардниот третман е помала од таа на расадот од сите варијанти на примена на пробиотици.
Примена на пробиотиците е можна при стандардни услови на сеидба, односно при употреба на хербицид.
Резултатите од овие истражувања создаваат добра перспектива за нивна примена во производството на тутунски расад.

Клучни зборови: тутун, ЕМ технологија, пробиотици, EmFarma Plus, Ema 5, развој, корен

INTRODUCTION

The integrated concept of sustainable agricultural production is based on the principles of ecology and the relationships between organisms and the environment.

EM technology is one of the basic ways to manage the environment in the direction of sustainable agricultural production. The basic principle of this technology is application and increase of the population of efficient and beneficial microorganisms in the soil.

Increasing the population of EM in the soil with application models increases the development of existing soil microorganisms. Thus, the microflora in the soil becomes rich and the soil develops a well-balanced microbial system (Nayak et al., 2020). Degenerative microorganisms, especially soil pathogens, are pushed out. This creates a healthy environment for the plants where the effective microorganisms continue to coexist in the rhizosphere.

But plant roots secrete substances such as carbohydrates, amino and organic acids and active enzymes. Effective microorganisms use these secretions for their development. But, during those processes they also secrete and provide amino and nucleic acids and many vitamins and hormones that are used by plants. Thus, EM coexists with the plant root in the rhizosphere (Higa and Wididana, 1989).

Thanks to all the microbiological processes in which the soil, and especially the rhizosphere microflora participates, the plants are provided with all the nutrients. Thus, they have an outstanding opportunity for proper development. Modern EM technology, or generally accepted term "probiotics", includes four very important groups of microorganisms - lactic acid bacteria, photosynthetic bacteria, actinomycetes and yeasts, mixed in a carbohydrate medium. Depending on the purpose, certain preparations have a "fortified" formula in which, in addition to the basic components, other bioactive components are represented, e.g., plant extracts. Such is the case with the preparation EMa 5 (SCD Probiotics, 2012).

Application of probiotics doesn't mean only improvement of soil properties and proper development of plants, but also prevention of disease attack. In that regard, the preparation EMa 5 is particularly noteworthy, which is why it is mainly used as a biofungicide.

Therefore, our aim was to investigate the possibility of using probiotics in tobacco seedling production and determine the effect of the application of EMa 5 alone (given its promotion as a biofungicide) or in combination with the probiotic EM Farma. Specifically, our aim was to confirm their multifunctionality and promising role in the production of tobacco seedling.

The examinations also ensure the determination of the best application model. What is most important, it is adapted for application during usual cultural practices in the production of tobacco seedling.

MATERIAL AND METHODS

The examinations were carried out in tobacco seedbeds in 2020. The soil treatment, which was planned when defining the variants, was carried out the previous day. The seedbeds were sown with tobacco seeds of variety P -66-9/7. Seed amount of 6.75 g/10 m^2 was used.

Each variant was tested in three replications, and the area for each replication was 3.33m^2.

The preparation EmFarma was applied in a dose of 1000 ml/100 m^2, i.e., 30 ml / 3.33 m^2.

The preparation Ema 5 was applied in a dose of 300 ml /100 m^2, i.e., 10 ml / 3.33 m^2.

Tobacco seeds (2.25 g/3.33 m^2) were soaked in 3 ml prepared solution and kept for 24 hours before sowing. For this purpose, a solution with EM farm was previously prepared in a dose of 100 ml/10m^2. An overview of the variants with the respective treatments is given in Table 1.

Table 1. Variants and treatments

Application of probiotics	Mark	Variant	Treatment		
	1	Control, without any treatment			
	2	Standard treatment with herbicide, fungicide and supplement			
on the soil	3	EmFarma Plus +Ema 5 Herbicide		EmFarma Plus + Ema 5	EmFarma Plus + Ema 5
	5	Ema 5 Without herbicide		Ema 5	Ema 5
	7	EmFarma Plus+ Ema 5 Without herbicide		EmFarma Plus + Ema 5	EmFarma Plus + Ema 5
after emergence	4	Herbicide Ema 5 after emergence	after emergence Ema 5	Ema 5	Ema 5
	6	Herbicide EmFarma Plus + Ema 5 after emergence	after emergence EmFarma Plus + Ema 5	EmFarma Plus + Ema 5	EmFarma Plus + Ema 5
on the seed	8	Seed treated with EmFarma Plus + soil treated with Ema 5 Without herbicide		EmFarma Plus + Ema 5	EmFarma Plus + Ema 5
	9	Seed treated with EmFarma Plus + soil treated with Ema 5 Herbicide		EmFarma Plus + Ema 5	EmFarma Plus + Ema 5

The herbicide Gamit 4 EC was applied in a dose of 0.07 ml/m^2 in the respective variants.
The treatment after seedling emergence was performed only in the respective variants (variants marked 4 and 6).
The second treatment was performed in all variants. The same day, the second control i.e., standard, received treatment with the fungicides Top M (0.1%) and Ridomil (0.25%). Fertilizing with 15 g/m^2 of ammonium nitrate was carried out the previous day.
The third treatment with probiotics was performed at the beginning of the vigorous growth phase.
During the research, the seedling was observed daily, and the growth and condition of the damping off disease was monitored.
Seedling length was measured twice, first time before the third treatment and second time in the phase of vigorous growth, before pulling for transplanting.

RESULTS AND DISSCUSION

Tables 2 and 3 present the results about the size of tobacco seedling in the three replications of each variant. However, for better conclusions about the impact of probiotics on the development of tobacco seedling, the average values were analyzed (Table 4 and 5).
Seedling have the lowest growth in the control (without any treatment), which is quite expected (Table 4). However, the largest length of the root system, as well as the total length, has the seedling of the 4th variant, i.e., Ema 5 after emergence. They are larger than the dimensions in the standard treatment with herbicide, fungicide and fertilizer. Ema 5 is a probiotic with a fungicidal effect. In addition to the basic formulation characteristic of EM technology, it also

contains plant extracts (SCD Probiotics, 2012). According to the multiple effect (including antagonistic effect against fungal and bacterial pathogens and detoxifying effect on the soil from pesticide residues) it is expected that the composition of all its active components will act very stimulative.

Acting against fungal and bacterial pathogens, it detoxifies the soil of pesticide residues. Starting from the base (probiotic i.e., EM technology) and its additional effect, it is expected that the composition of all its active components will act not only fungicidally, but also stimulating.

Good development of the whole plant and, what is of particular importance, the root system, has the seedling of variants 7 and 8. Although there are different ways of application (after emergence and on seeds), both variants are without any use of herbicide

Table 2. Impact of probiotics on the development of tobacco seedling - 1 evaluation

Application of probiotics	Mark	Variant	replication								
			I			II			III		
			root	stalk	Total length	root	stalk	Total length	root	stalk	Total length
	1	Control, without any treatment	3.25	9.50	9.75	2.60	11.50	14.10	2.00	9.16	11.16
	2	Standard treatment with herbicide, fungicide and supplement	2.62	16.37	18.99	2.50	12.50	15.00	3.00	15.33	18.33
on the soi	3	EmFarma Plus +Ema 5 Herbicide	3.12	14.12	17.24	2.66	17.00	19.66	3.33	13.66	16.99
	5	Ema 5 Without herbicide	2.62	14.00	16.22	3.00	12.33	15.33	3.66	13.16	16.82
	7	EmFarma Plus+ Ema 5 Without herbicide	3.25	15.00	18.25	3.33	15.33	18.66	3.00	17.50	20.50
after emergence	4	Herbicide Ema 5 after emergence	3.75	15.25	19.00	2.66	13.16	15.82	4.16	18.33	22.49
	6	Herbicide EmFarma Plus + Ema 5 after emergence	3.00	14.75	17.75	3.33	15.83	19.16	2.66	15.66	18.32
on the seed	8	Seed treated with EmFarma Plus + soil treated with Ema 5 Without herbicide	3.33	15.00	18.33	3.16	17.83	20.99	3.16	15.16	18.32
	9	Seed treated with EmFarma Plus + soil treated with Ema 5 Herbicide	3.25	14.62	17.87	3.33	14.50	17.83	3.00	13.50	16.50

It is known that the herbicide has a harmful effect on the soil as well as on the rhizosphere microflora (Ayansina and Oso, 2006). Such a negative effect can be long-lasting and affect the entire microflora-plant system. The soil in which there is no harmful effect of the herbicide is a good substrate for the reproduction and development of the effective microflora and its activity, thus providing the root system with various nutrients.

Table 3. Impact of probiotics on the development of tobacco seedling - 2 evaluation

Application of probiotics	Mark	Variant	replication								
			I			II			III		
			root	stalk	Total length	root	stalk	Total length	root	stalk	Total length
	1	Control, without any treatment	3.50	12.10	15.60	3.10	12.40	15.40	3.30	12.20	15.50
	2	Standard treatment with herbicide, fungicide and supplement	3.37	19.25	22.62	3.16	17.33	20.49	2.50	18.66	21.16
on the soil	3	EmFarma Plus +Ema 5 Herbicide	3.40	14.20	17.60	4.20	18.20	22.40	4.60	35.00	39.60
	5	Ema 5 Without herbicide	4.25	15.25	19.50	5.50	16.33	21.83	2.66	15.33	17.99
	7	EmFarma Plus+ Ema 5 Without herbicide	3.87	17.50	21.37	4.66	19.33	23.99	3.66	20.66	24.32
after emergence	4	Herbicide Ema 5 after emergence	4.37	16.75	21.12	4.66	18.66	23.32	4.23	17.13	21.36
	6	Herbicide EmFarma Plus + Ema 5 after emergence	3.75	21.25	25.00	3.33	19.66	22.99	3.33	21.00	24.33
on the seed	8	Seed treated with EmFarma Plus + soil treated with Ema 5 Without herbicide	3.25	18.75	22.00	2.83	15.00	17.83	4.00	23.66	27.66
	9	Seed treated with EmFarma Plus + soil treated with Ema 5 Herbicide	4.50	19.50	24.00	2.83	13.66	16.49	2.83	14.33	17.16

The effect of the seedling treatment with probiotics is best seen in the stage of vigorous growth. According to Table 5, the length of the root system of the seedling in the standard treatment is the smallest. It is even smaller than the control - without any treatment, which can certainly be explained by the special conditions of water supply that favor the development of the root in length. But it lags behind all variants treated with probiotics.

Tobacco seedling of the variants 3 and 7 had the best development, especially of the root system, but also of the whole plant, when EmFarma Plus + Ema 5 was applied to the soil, before sowing.

The obtained results are in accordance with the statements of Ashraf et al. (2004). According to them, the introduction of effective microflora can activate nitrogen fixation, phosphorus solubility, increased release of siderophores, phytohormones, exopolysaccharides, known microbial products that increase plant development and protect them from abiotic stress.

Also, Combant et al (2005) claims that proliferation of microorganisms that are promoters of plant growth in the soil, especially in the rhizosphere, stimulates plant development. At the same time, through the competition for space and food and creation of inhibitory substances, they induce systemic resistance of plants.

Table 4. Impact of probiotics on the development of tobacco seedling - (1 evaluation - average values)

Application of probiotics	Mark	Variant	length		
			root	stalk	whole plant
	1	Control, without any treatment	**2.61**	10.05	11.67
	2	Standard treatment with herbicide, fungicide and supplement	2.70	14.73	17.44
on the soil	3	EmFarma Plus +Ema 5 Herbicide	3.03	14.92	17.96
	5	Ema 5 Without herbicide	3.09	13.16	16.25
	7	EmFarma Plus+ Ema 5 Without herbicide	3.19	15.94	19.13
after emergence	4	Herbicide Ema 5 after emergence	**3.52**	15.58	19.10
	6	Herbicide EmFarma Plus + Ema 5 after emergence	2.99	15.41	18.41
on the seed	8	Seed treated with EmFarma Plus + soil treated with Ema 5 Without herbicide	3.21	15.99	**19.21**
	9	Seed treated with EmFarma Plus + soil treated with Ema 5 Herbicide	3.19	14.20	17.40

Increasing soil microbial diversity improves plant health and productivity (Okorski et al., 2008). The same value of the root system length in these two variants (without and with the use of herbicide) confirms the possibility of using probiotics under standard sowing conditions i.e., when using herbicide.

Also, the application of probiotics on the soil confirms the positive effect of probiotics on plant development and at the same time represents a good application model and perspective for their application in the production of tobacco seedling.

The seedling from the variants where the seeds were treated with EmFarma Plus and the soil with Ema 5 have a poorly developed root system and a similar average value of the total length of the plant as the seedling from the standard treatment. There are certain dilemmas whether probiotics applied to seeds increase the percentage of germination, whether they develop a strong secondary root system and a healthy stalk, and whether it is a good application method, (Olle and Williams, 2013).

Table 5. Impact of probiotics on the development of tobacco seedling - (2 evaluation - average values)

Application of probiotics	Mark	Variant	length root	length stalk	length whole plant
	1	Control, without any treatment	3.30	12.23	15.50
	2	Standard treatment with herbicide, fungicide and supplement	**3.01**	18.41	21.42
on the soil	3	EmFarma Plus +Ema 5 Herbicide	**4.06**	22.40	**26.53**
on the soil	5	Ema 5 Without herbicide	4.13	15.63	19.77
on the soil	7	EmFarma Plus+ Ema 5 Without herbicide	**4.06**	19.16	23.22
after emergence	4	Herbicide Ema 5 after emergence	4.23	17.13	21.36
after emergence	6	Herbicide EmFarma Plus + Ema 5 after emergence	3.47	20.63	**24.10**
on the seed	8	Seed treated with EmFarma Plus + soil treated with Ema 5 Without herbicide	3.36	19.13	22.49
on the seed	9	Seed treated with EmFarma Plus + soil treated with Ema 5 Herbicide	3.38	15.83	19.21

Bharti et al. (2007) present facts that the rhizosphere is a microbial center where there are different but at the same time significant interactions between microorganisms and the soil together with the plants. The nature of these interactions is entirely determined on a molecular basis. These positive beneficial interactions significantly affect plant development and growth, and subsequently result in a progressive impact on yield and production.

These data confirm the results of our research that the best development of the root system as well as the whole plant is determined when probiotics are applied to the soil, before sowing (at the same time highlighting it as the best application model). The soil is a natural environment for these groups of microorganisms, in which they best manifest their activity (specific processes). At the same time, early interactions occur in the soil (among microorganisms, but also microorganisms-plants) which lead to an increase in their activity. All this leads to stimulation of plant growth.

CONCLUSIONS

- Probiotics have a positive effect on the development of tobacco seedling, especially on the root system.
- The length of the root system of the seedling with standard treatment was smaller than the length of the seedling treated with all the probiotic variants.
- Tobacco seedling had the best development especially of the root system, but also of the whole plant, when EmFarma Plus + Ema 5 was applied to the soil, before sowing. At the same time, it represents a good application model of probiotics in the production of tobacco seedling.
- Application of probiotics is possible under standard conditions of sowing, i.e., when using herbicide.
- Probiotics have a good perspective for their application in the production of tobacco seedling.

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Original Scientific Paper

ASSESMENT OF FACTORS INFLUENCING THE TRACE ELEMENT AVAILABILITY IN TOBACCO PLANTS GROWN IN THE PELAGONIA PRODUCTION AREA

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The chemical composition and bioaccumulation of trace elements by plants is determined by number of soil characteristics, plant properties and the environment in which they are grown. The subject of this research was study on the soil properties that influence the availability of trace elements to oriental tobacco grown in the Pelagonia production area. Soil properties were obtained by the following parameters: soil acidity (pH), humus, total nitrogen, available forms of macronutrients (P_2O_5, K_2O) and physical clay. Total and extractable anthropogenic and crustal trace elements content was analyzed by atomic emission spectrometry with inductively coupled plasma (ICP-AES). The objective was to determine the distribution and intensity of accumulation of the trace element content in the oriental tobacco. Relationship with site-specific soil properties and chemical composition of tobacco plants was studied by statistical models (factor and correlation analyses).

Key words: tobacco, trace elements, distribution, availability, Pelagonia

ПРОЦЕНКА НА ФАКТОРИТЕ КОИ ВЛИЈААТ НА ДОСТАПНОСТА НА ЕЛЕМЕНТИТЕ ВО ТРАГИ ВО ТУТУНСКИ РАСТЕНИЈА ОДГЛЕДАНИ ВО ПЕЛАГОНИСКОТО ПРОИЗВОДНО ПОДРАЧЈЕ

Хемискиот состав и биоакумулацијата на елементите во траги во растенијата се одредени од голем број почвени карактеристики, својствата на самите растенија и околината во која се одгледуваат. Предмет на ова истражување е проучувањето на почвените својства кои влијаат врз достапноста на елементите во траги за ориенталниот тутун одгледан во Пелагонискиот приозводен регион. Почвените својства се проучени преку следните параметри: pH на почвениот раствор, хумус, вкупен азот, достапни форми на макронутриентите (P_2O_5, K_2O) и механички состав. Вкупната и достапната содржина на елементите кои имаат човечко и природно потекло е анализирана со атомска емисиона спектрометрија со индуктивно спрегната плазма (ICP-AES). Целта беше да се одреди дистрибуцијата и интензитетот на акумулација на елементите во траги од страна на растенијата тутун. Поврзаноста меѓу специфичните својства на почвата и хемискиот состав на растенијата тутун е проучена преку статистички модели (факторна и коорелациона анализа).

Клучни зборови: тутун, елементи во траги, дистрибуција, достапност, Пелагонија

INTRODUCTION

The absorption and accumulation of trace elements by plants has a strictly specific character, both in relation to the types of plants and to the specific element. One of the main factors related to the accumulation of these elements by tobacco plants are their content and their chemical forms in the growing medium itself. Most of the mineral nutrients contained in the soil are bound in the solid phase and are unavailable to plants (Kabata-Pendias, 2011). Only in certain portions they are dissolved in the aqueous phase of the soil solution and their soluble form is absorbed by plants through the root system. Phyto availability (uptake into plants) depends entirely on the mobility and availability of the elements in the soil. How much content will accumulate in plants is of interest for two reasons. The first is their toxic effect on plants, which is usually reflected in the yield, and the second is the transfer of heavy metals from the soil, through the plants to the food chain.

The content of different elements in tobacco plants depend on both natural and anthropogenic factors. Trace elements of anthropogenic origin are more mobile than those of lithogenic origin (pedogenetic processes), (Han and Singer, 2007). Compared to the elements of anthropogenic origin, lithogens correlate better with clay minerals, organic matter, oxides, and carbonates in soil (Burt et al., 2003). The way of their binding, and thus their bioavailability, depend on several soil properties such as: mechanical composition, soil pH, organic matter content, ion exchange capacity, content of macro and micro nutrients, oxidation-reduction potential, the activity of microorganisms and sorption capacity (Adamu et al., 1989; Prasad and Freitas, 2003; Geoffrey, 2004; Golia et al., 2007; Nouri et.al, 2009). All these factors, during the processes of sorption, complexation or immobilization of soil particles affect the content of trace elements that will accumulate in the plant material.

The influence of basic soil characteristics on the behavior of trace elements in the tobacco plant is individual for most elements and generalizations are not possible. One of the main factors affecting the content of various elements in tobacco leaves is the soil pH (Khan et al., 1992; Bell et al., 1992; Gondola and Kadar, 1993; Mitsios et al., 2005; Golia et al., 2009; Zaprijanova, 2010). According to these authors, the content of clay and organic matter have an indirect and direct influence on the content of heavy metals in tobacco, and the different accumulation is conditioned by the varieties of tobacco. The Berley and Virginia varieties are the most commonly researched.

The subject of this research was to determine the influence of some soil parameters on the trace element content of oriental tobacco. The main goal was to survey the availability of these elements for the plants grown in particular production area, as essential for achieving sustainable tobacco production. Correlation and factor analyses was used in order to determine the relationships between the elements, thus obtaining certain associations of elements that reflect the geological or anthropogenic origin and to examine the possibility of differentiating the production sites.

MATERIALS AND METHODS

The study area is Pelagonija, the largest tobacco production area in the Republic of North Macedonia, that consists the following municipalities: Prilep, Mogila, Krusevo, Krivogastani, Bitola, Novaci, Demir Hisar, Doleni and Resen. Samples were taken from all listed municipalities, with the exception of Resen, because of the insignificant tobacco production. Soil samples were taken from 20 sampling locations from municipalities of Dolneni and Prilep and 10 samples per municipality for the rest, 100 samples total. Soil samples were collected after tobacco harvesting, and at the same sites, leaf samples were collected from all three primings, root, stems, leaves, blossoms and seeds.

Soil properties such as: clay, pH, total nitrogen, humus content, carbonates, available phosphorus and potassium were determined (Pelivanoska, 2011). For total digestion, soil samples (0.2500 g) were placed in a Teflon digestion vessel and were digested on a hot plate (ISO 14869-1:2001) and measured by ICP-AES (Varian, 715-ES). Total content of the elements in plant material was Digested in Teflon vessels

with HNO_3 and H_2O_2 using the Mars microwave system (CEM, USA) measured by ICP-AES with ultrasonic nebulizer CETAC (ICP/U-5000 AT).

Correlation and factor analyzes were used for the statistical processing of the data (Pearson correlation, two-tailed) with the use of the SPPS software (IBM SPSS v 19). Parametric, non-parametric analysis and descriptive statistics were made from all types of samples and all analyzed elements. Bivariate statistics (linear correlation coefficients, *r*) was used to determine the degree of correlation between certain elements and the influence of soil parameters on the elemental composition of plant samples.

RESULTS AND DISCUSSION

Soil parameters

Main soil properties are presented in Table 1. As it can be can be seen, most of the tested samples from most municipalities have a low humus content. Similar to the humus content, average total nitrogen content is low. The average soil pH in water is within the range of is 6.01-6.83.

Table 1. Average soil properties in the Pelagonia producing area (n=100)

Muncipality	Humus	Total nitrogen	pH (H_20)	pH (KCl)	Clay	Available P	Available K
	%	%			%	mg/100 g	
Prilep	1.68	0.08	6.61	5.49	38.04	28.03	21.44
Mogila	1.33	0.07	6.54	5.40	36.61	14.23	20.59
Krushevo	1.56	0.08	6.01	4.97	39.18	21.60	20.09
Krivogastani	2.12	0.10	6.83	5.71	54.70	15.88	17.11
Bitola	1.70	0.09	6.04	4.85	44.96	9.77	17.44
Novaci	1.37	0.06	6.55	5.46	32.05	20.10	23.81
Demir Hisar	2.27	0.13	6.72	5.65	49.40	18.1	33.5
Dolneni	1.46	0.08	6.56	5.51	1.46	9.6	19.6

Available phosphorus and potassium content vary differently (Table 1). According to the presented data, it can be concluded that most of the samples from all municipalities are well provided with these nutrients. Some anomalies are recorded in the few samples from municipalities of Novaci, Demir Hisar and Prilep with available phosphorus and potassium of over 60 mg/100 g, and they were excluded as outliers in the average content of nutrients in Table 1. Based on the above, diversity of this nutrients may be conditioned by the geochemical origin of the different type of soil, but certainly the long period of cultivation is not excluded.

Descriptive statistics of obtained chemical composition of the soil samples is given in Table 2. According to our research, all data is comparable to the data obtained from the study on agricultural soils of Europe (Salminen et al., 2005; Soriano-Disla et al., 2013).

The content of Fe in soil is inherited from the natural phenomena, from the parent rocks and natural processes, mostly found in the range from 0.5 to 5 %. Values of 5 % Fe were measured in the samples from Bitola and Mogila. These values are higher than the Fe content in European topsoil average of 2.2 % (Salminen et al., 2005). The high contents are due to the "Suvodol" coal mine, which is part of the Pelagonian massif. On the European scale, the mean value of Mn content in the surface layers of the soil is 524 mg/kg (Salminen et al., 2005), and in our samples 551 mg/kg (Table 3). As it can be seen in the Table 3, higher deviations are detected in the content of Cr, Ni, Cu and Zn that are due to the higher values obtained in some samples from municipality of Doleni.

Table 2. Descriptive statistics of total chemical composition in soil samples (*n*=100).
The values for Ca, Fe, K, Mg and Na are given in %, for the rest of the elements in mg/kg,
LOD – limits of detection, Xg is geometrical mean, Sa-standard deviation

	LOD	Pelagonia area (*n*=100)		
	mg/kg	X_g	s_a	Min - Max
Ag	0,2	0,5	0,2	0,2 – 1,1
Al	0,9	5	0,9	2,8 – 6,8
Ba	10	358	121	137 - 764
Ca	10	1,1	0,6	0,2 - 3
Cr	1	48	31	15 - 292
Cu	0,5	17	9	5 - 49
Fe	1	2	0,8	0,9 - 5
K	2	1	0,3	0,4 - 2
Li	0,25	14	7,6	3,4 - 53
Mg	0,5	1	0,2	0 ,4 - 2
Mn	0,5	551	171	218 - 1268
Na	1	1	0,3	0,1 - 2
Ni	0,5	22	13,6	7 - 124
P	1	475	320	140 - 2098
Pb	10	14	4,8	10 - 30
Sr	10	151	89	26 - 537
V	1	66	32	27 - 217
Zn	1	59	84	16 - 593

Plant analyses

The descriptive statistics of the content of analyzed elements in the tobacco leaves is given in Table 3. In comparison with other literature sources, concentration of all studied elements is within the range for oriental tobacco leaves (Tso 1990; Gondola and Kadar 1994; Metsi et al., 2002; Golia et al., 2009; Pelivanoska et al., 2011). Only Fe, Cr, Ni, Sr and Ba had higher mean concentration than values reported by Tso 1990, but they are all below the contamination limit for plants (Kabata-Pendias 2011).

Table 3. Descriptive statistics of average chemical composition of tobacco leaves (*n*=100).
The values are given in mg/kg, LOD – limits of detection, Xg is geometrical mean, Sa-standard deviation

	LOD	Pelagonia area		
	mg/kg	X_g	s_a	Min-Max
Al	0,01	1426	849	158-3686
Ba	0,03	48	16	16-103
Ca	0,025	14287	2836	7707-19914
Cd	0,005	0,35	0,17	0,09-0,89
Cr	0,05	2,1	1,9	0,3-16
Cu	0,02	11	4	4-35
Fe	0,006	820	492	147-2367
K	5	7306	2430	2575-13292
Li	0,05	13	10	2-49
Mg	0,025	1332	224	749-1883
Mn	0,0015	64	27	28-172
Na	2,5	66	22	38-150
Ni	0,25	2	1	1-6
P	0,5	795	213	439-1529
Pb	0,5	0,9	0,6	0,5-4
Sr	0,025	53	15	16-109
V	0,05	3	2	0,2-9
Zn	0,003	20	6	8-48

Influence of soil parameters on the elemental composition of tobacco samples

According to the correlations of environmental soil properties and their influence on the composition and intensity of trace element accumulation in oriental tobacco, generally weak relationships were established (Table 4). Soil mechanical composition has moderate to strong correlations with Al and Fe content in oriental tobacco leaves. One of the major factors influencing the element concentration in tobacco leaves is soil pH (Bell et al., 1992; Golia et al., 2009; Khan et al., 1992; Zaprijanova, 2010). The pH of the soil solution from our findings has moderate to strong correlations with the content of Ni, Cr and V in tobacco samples. Humus is moderately correlated with Mn in tobacco.

In order to determine certain associations of the elements that reflect the geological or anthropogenic origin factor analyses was done. The purpose of factor analysis is to process a large amount of information from the original set of variables and convert them into smaller ones with minimal loss of information. The matrix of rotated factor loadings is given in Table 4. Six components were extracted explaining the 75.80 % of the total variance. According to factor analyses, three geogenic, two mixed (geogenic-anthropogenic) and one anthropogenic association were divided (Table 5). Similar to findings from the production in whole tobacco producing area in the Republic of North Macedonia (Jordanoska et al., 2018a; 2018b).

Table 4. Pearson's correlation coefficients between trace elements in tobacco leaves and other selected soil properties

	Humus	clay	pH (H_2O)	Available P	Available K
Ag	0.176	0.019	0.141	0.178	0.077
Al	0.161	**0.65**	0.146	0.235	0.225
Ba	0.195	**0.025**	-0.011	0.269	*0.167*
Ca	0.176	**0.286**	**0.4**	0.28	**0.547**
Cr	0.161	**0.303**	0.094	*0.282*	0.072
Cu	0.195	0.032	0.091	*0.434*	0.123
Fe	0.19	**0.488**	0.032	*0.415*	0.046
K	*0.188*	0.014	0.045	0.11	0.014
Li	0.156	**0.308**	0.13	*0.385*	*0.209*
Mg	0.157	**0.339**	**0.212**	*0.303*	0.155
Mn	**0.495**	*0.125*	-0.008	*0.432*	0.017
Na	*0.26*	*0.196*	-0.062	0.266	*0.226*
Ni	0.202	**0.399**	**0.293**	0.366	0.112
P	*0.214*	0.01	-0.005	0.123	0.015
Pb	*0.243*	*0.122*	0.132	*0.226*	0.115
Sr	*0.205*	0.069	-0.058	0.16	0.061
V	0.061	*0.349*	-0.029	*0.259*	-0.086
Zn	*0.163*	*0.214*	0.049	*0.226*	0.046

Bold -correlation is significant at the 0.01 level (2-tailed);
Italic-Correlation is significant at the 0.05 level (2-tailed).

Using all determined contents of the elements in the different organs of tobacco (root, stem, leaf, flower and seed), the biological accumulation factor (BAF) was calculated. This factor is defined as the ratio between the total content of the specified element in all tobacco organs and its total content in the corresponding soil (growing medium). Obtained values are presented in Figure 1. In the same figure, in addition to the biological accumulation factor, the biological transfer factor (BTF) is presented. This factor indicates the efficiency of the transfer of elements in the aerial parts of the plants and it represents the ratio of the content of the examined element in the above-ground tissues of the plant and the root.

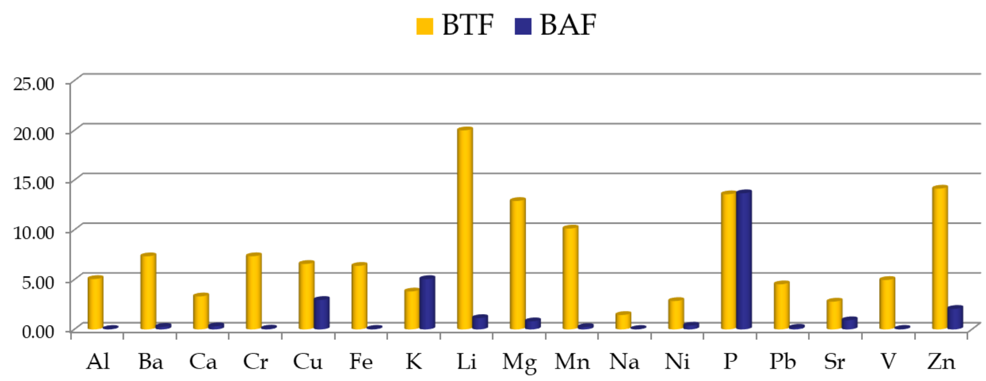

Figure 1. Biological transfer factor (BTF) and biological accumulation factor (BAF)

Table 5. Rotate component matrix (*n*=100) for selected elements in tobacco leaves Com - Communality (%), Var- Variance (%)

Element	F1	F2	F3	F4	F5	F6	Com
Fe	**0.95**	0.03	0.05	-0.02	0.15	0.02	94.80
V	**0.93**	0.13	0.03	-0.06	0.11	0.04	88.98
Al	**0.92**	0.19	0.16	0.05	0.08	0.06	93.84
Pb	**0.66**	0.00	0.17	0.22	0.04	-0.08	50.34
Sr	-0.04	**0.92**	-0.04	0.08	-0.04	0.15	85.87
Mg	0.12	**0.76**	0.30	-0.11	0.15	-0.19	76.93
Na	0.21	**0.62**	0.13	0.34	-0.07	0.01	60.03
Ba	0.15	**0.62**	0.16	0.09	0.02	0.43	66.07
Zn	0.25	0.11	**0.82**	0.26	0.09	0.01	78.56
Cd	0.02	0.03	**0.80**	0.11	0.13	0.33	72.26
Cu	0.18	0.25	**0.73**	-0.10	0.08	0.04	67.09
P	0.00	-0.02	-0.14	**0.89**	-0.01	-0.02	76.03
K	0.18	0.04	0.26	**0.75**	0.01	0.22	69.60
Ca	0.00	0.37	0.19	**0.68**	0.01	0.02	60.75
Cr	0.20	-0.03	-0.01	-0.02	**0.95**	-0.09	90.76
Ni	0.13	0.06	0.31	0.04	**0.90**	0.09	90.75
Mn	0.12	0.08	0.02	-0.02	0.05	**0.85**	67.65
Li	-0.20	0.04	0.27	0.19	-0.09	**0.77**	59.65
Var	27.34	14.90	9.95	9.40	7.43	5.94	75.80

CONCLUSIONS

From the obtained results, it can be concluded that tobacco samples from Pelagonia producing area leaves contain a relatively high content of iron, which is dependent by its content in the soil. According to the correlations of environmental soil properties and their influence on the composition and intensity of trace element accumulation in the oriental tobacco, generally weak relationships were established. Soil mechanical composition has moderate to strong correlations with Al and Fe content in oriental tobacco leaves. Soil pH has moderate to strong correlations with tobacco Ni, Cr, and V content, while humus has a moderate correlation with tobacco Mn content. According to factor analyses trace elements are found in three geogenic and two mixed geogenic-anthropogenic associations. Mixed factors are presented by Fe-V-Al-Pb and P-K-Ca associations. Calculated biological accumulation factor shows that oriental tobacco accumulates greater amount of P, K and Cu and the transfer of the elements in the antenna parts of the plant follows the sequence: Li > Zn > P > Mg > Mn > Cr > Ba > Cu >Fe > Al > V > Pb > K > Ca > Ni > Sr > Na.

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Original Scientific Paper

PRODUCTION OF SECONDARY BIOMOLECULES IN TOP INSERTION AT DIFFERENT TOBACCO TYPES (*Nicotiana tabacum* L.) IN PRILEP REGION

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ABSTRACT

Flavonoids are the largest and most widely spread group of phenolic compounds. Flavonoids are known for their anti-inflammatory and anti-allergic properties, antithrombotic and vasoprotective properties. The paper presents results of total content of flavonoids in dry tobacco, from several varieties of tobacco from the Prilep region.
Tobacco raw material from the top harvests (harvest 1 - Lower top leaves and harvest 2-Top leaves), obtained during one vegetation period, was analyzed. Total flavonoids in tobacco are determined by the aluminum chloride colorimetric method after extraction of tobacco by the ultrasound.
The obtained results emphasize that the highest concentration of total flavonoids calculated as μg/g of dry mass is found in Prilep Basma type, Prilep Basma 82 (63.12 μg/g) and Prilep Basma 2 (64.13 μg/g) varieties. The variety P-12-2/1 which belong to the type Prilep has the lowest content of flavonoids (32.40 μg/g). The results indicate the presence of flavonoids as secondary biomolecules in all analyzed tobacco varieties.

Keywords: flavonoids, tobacco, polyphenols, extraction, top harvest.

ПРОДУКЦИЈА НА СЕКУНДАРНИ БИОМОЛЕКУЛИ ВО ГОРНА ИНСЕРЦИЈА КАЈ РАЗЛИЧНИ СОРТИ НА ТУТУН (*Nicotiana tabacum* L.) ВО ПРИЛЕПСКИОТ РЕГИОН

Флавоноидите се најголема и најшироко распостранета група фенолни соединенија. Флавоноидите се познати по антифламаторни и антиалергиски својства, антитромботични и вазопротективни својства. Во трудот презентирани се резултати од вкупни флавоноиди во сув тутун, од повеќе сорти на тутун од Прилепскиот регион. Анализирана е тутунската суровина од врвните берби (берба 1-подврв и берба 2-врв), добиена во еден вегетациски период. Вкупните флавоноиди во тутунот се одредуваат со колориметриски метод со алуминиум хлорид по екстракција на тутунот со ултразвук.
Добиените резултати покажуваат дека најголема концентрација на вкупни флавоноиди пресметана како μg/g сува маса има кај сортите Прилеп басма 82 (63,12 μg/g) и Прилеп басма 2 (64,13 μg/g) од типот Прилеп басма. Кај типот прилеп сортата П-12-2/1 има најмала содржина на флавоноиди (32,40 μg/g). Резултатите укажуваат на присуство на флавоноиди како секундарни биомолекули во сите анализирани сорти тутун.

Клучни зборови: флавоноиди, тутун, полифеноли, екстракција, горна берба.

INTRODUCTION

Tobacco is grown in more than 119 countries in the world (Majdi et al., 2012). The production of raw materials from tobacco leaves doubled in the year 2000, compared to the last century.

Great progress has been made in the analysis of secondary metabolites in plants during the last years (Okada et al., 2010). The use of modern analytical techniques such as chromatography, electrophoresis, analysis with isotopes and enzymology enabled the elucidation of a large number of structural formulas and important biosynthetic pathways (Harborne, 1998; Balunas and Kinghorn, 2005).

Flavonoids have recently attracted much attention due to their antioxidant and antiradical activities. The researches themselves show that they are good scavengers of free radicals, and they are also important in the pharmaceutical and food industry; that is why a large number of analyzes are done to determine the concentration of flavonoids in different plants.

A large number of researches that indicate the presence of flavonoids in tobacco have been done, and therefore due to the abundance of bioactive ingredients such as polyphenols, proteins, aromatic compounds in tobacco leaves they are considered as economically valuable (Zhang et al., 2012; Ruiz et al., 1998; Theng et al., 2012).

Wang et al. (2008) identified the presence of polyphenols in tobacco leaves and their antioxidants, investigating antimicrobial activities. Wang (2008) designates the rutin and chlorogenic acid as two major polyphenolic compounds in tobacco leaves. It has been confirmed that some polysaccharides are associated with phenols which can be separated using polar solvents (Galanakis, 2010).

Qiao-Mei Ru et al., (2012) indicate that flavonoids are effective in removing free radicals and have great potential to be powerful antioxidants. So, tobacco leaves are considered as a potential source of natural antioxidants for food, cosmetics, etc.

An important factor for the aroma, smell, and resistance to insects of a certain variety of tobacco is the presence of different polyphenolic components on the surface of the leaf (Georgieva, 1998).

Guardia et al., (2001) and Hertog et al., (1995), highlight the activity of flavonoids as anti-allergic, anti-cancer, antioxidant, anti-inflammatory and antiviral compounds.

A group of authors emphasize again that the group of flavonoids is known for its anti-inflammatory and anti-allergic actions, for its antithrombotic and vasoprotective properties, for inhibition of tumors and for the protection of the gastric mucosa (Montanher et al., 2017, Serafini et al., 2010).

A large number of the analyzes and studies have been made on tobacco, which prove that it has a complex chemical composition that changes depending on the age of the plant, the variety, the meteorological and soil conditions in which is grown.

The chemical composition of tobacco leaves is influenced by factors such as ripening, drying, fermentation, treatment processing, and storage. The quality of the tobacco leaf does not depend so much on the individual chemical components it contains as on their mutual ratio, on the conditions of cultivation, the method of drying, and the harvest (Bajlov, 1965).

Accumulation of secondary metabolites in general is extremely dependent on weather conditions, since they are response of plants to their environment (Fu et al., 2016).

The aim of our research is to determine the presence of flavonoids in dry tobacco, and to determine the concentration of flavonoids in the samples.t

This work reveals the differences of flavonoids among seven tobacco varieties and provides comprehensive information about their phenolic composition and distribution as well as the possibilities of using tobacco in different industries.

MATERIAL AND METHODS

Materials

The research was done on dry leaves of seven varieties of tobacco *(Nicotiana tabacum* L.*)*, Yaka YV 125/3, Yaka YV 48, Prilep P 12-2/1, PP 66-7/9, Djebel DJ 48, Prilep Basma 82, Prilep Basma 2, grown in agrotechnical practice for oriental tobacco at the experimental field of the Scientific Institute for Tobacco - Prilep.

The tobacco raw material for analysis is from one vegetative period, top harvests (harvest 1 and harvest 2). Tobacco is produced on a colluvial deluvial type of soil, with a slightly acidic pH reaction, low content of nitrogen and organic matter, and medium supplied of easily available phosphorus and potassium. The tobacco raw material for analysis is properly collected, dried, stored and fermented for analysis.

Extraction of Total flavonoids from tobacco leaves

The total flavonoids in tobacco are extracted by ultrasound in an ultrasonic bath (DU-22, Clifton, UK) for 20 minutes at a temperature of 25 °C and power 40 kHz. One g of tobacco powder was extracted with 30 mL of methanol:water solution (70:30, *v/v*).

Determination of Total flavonoid

The total flavonoid content was measured by the aluminum chloride colorimetric method. The tobacco extract (0.5 mL) was mixed with 0.5 mL of 10 % aluminum chloride and 0.1 mL of 1M sodium acetate (water solution) in a small eprouvette, followed by 30 min. of incubation at 25 °C. The absorbance at 415 nm was measured and quercetin methanolic solution (0–50 µg/mL) was added for standard curve generation. Each sample was processed in triplicate, and the result was presented in mg quercetin equivalents.

RESULTS AND DISCUSSION

A greater percentage of tobacco row material are discarded along processing as waste, which can pollute the environment. The discarded tobacco leaves are economically valuable due to the abundance of bioactive ingredients such as polyphenols, proteins, and aromatic compounds. Tsaballa et al. (2020) emphasize that tobacco contains various phenolic compounds. These plant metabolites are synthesized mainly through shikimic acid and malonic acid (Azmir et al., 2013) and provide protection against abiotic stress and pathogenic infection.

Analyzes and the results from the first harvest show that the highest content of total flavonoids can be observed in the variety Basma 2, Basma 82 (Figure 1). It can be noted that the lowest concentration of flavonoids was observed in the variety P 12-2/1, compared to the rest of the analyzed tobacco varieties. Figure 1 shows that the content of flavonoids in the variety Dj 48 was found to have a higher concentration of flavonoids compared to YV 125/3, YV 48.

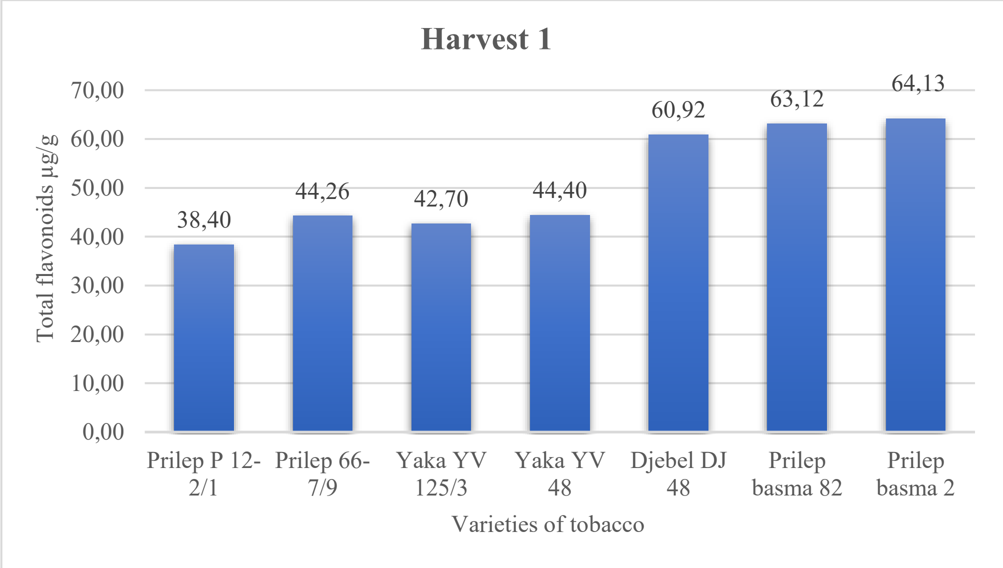

Figure 1. Content of total flavonoids in tobacco calculated in µg/g of dry mass - Harvest 1, Lower top leaves

Small differences can be observed when analyzing the second harvest of tobacco among tobacco varieties, compared to the first harvest. Again, the highest content of flavonoids is found in the variety Basma 2, Basma 82, Dj 48, the lowest content again in the variety P 12-2/1 (Figure 2, Fugure 3). Huge variations between individual varieties of a certain type of tobacco are not observed, especially in the Yaka type with the varieties YV 125/3 and YV 48. In the Prilep type and in both examined harvests, differences are noticeable between the varieties P 12-2/1 and PP 66-7/9

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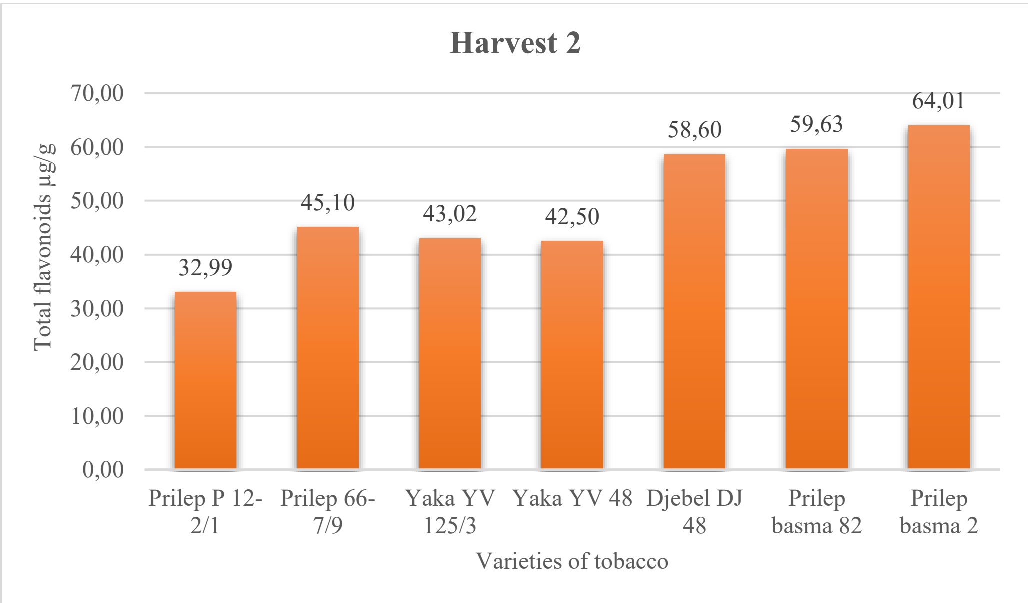

Figure 2. Content of total flavonoids in tobacco calculated in µg/g of dry mass – Harvest 2, Top leaves

According to Tsaballa et al. (2020), the expressed intensity of certain genes determines the difference in the concentration of flavonoids in different tobacco varieties. Meanwhile, the content of flavonoids in tobacco depends on temperature, disease, light intensity and other factors (Fu et al., 2016).

It is characteristic of the oriental type Prilep that it is adapted to light, poor soils, which are not rich in nutrients. This type of tobacco has a specific leaf shape that distinguishes it from other types of tobacco.

Zou et al. (2021), when researching several varieties of tobacco, emphasize that all analyzed varieties of tobacco contain a wide range of polyphenols and are able to effectively clean free radicals, showing a strong antioxidant potential.

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All identified polyphenols reveal the potential value of tobacco by-products. The project by Zou et al. (2021) promotes the recycling of tobacco by-products and offers new raw materials for the food industry and pharmaceutical companies.

Additional studies on tobacco extraction, toxicological, bioavailability as well as animal studies are needed to develop tobacco by-products as commercial ingredients

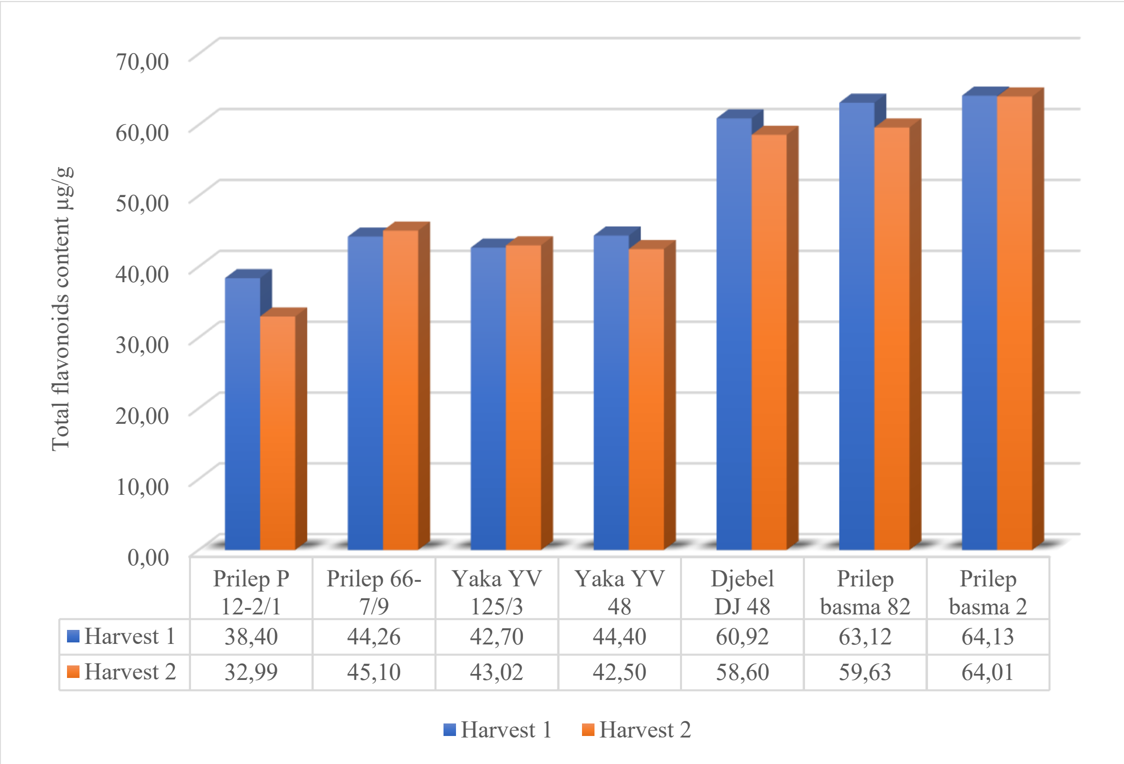

Figure 3. Comparison of content of total flavonoids content in dry tobacco (µg/g) between the analyzed tobacco varieties from Harvest 1 and Harvest 2

Docheva et al. (2014) conducted research on obtaining flavonoid-enriched extracts from tobacco crops and waste products, purified from nicotine and phenolic acids, and evaluating their potential as free radical scavengers. The results obtained by Docheva et al. (2014) support the results of this research. The extracts obtained with the appropriate procedure are enriched with flavonoids with high potential for scavenging free radicals, and can be considered as potential biopharmaceuticals.

Qiao-Mei Ru et al. (2012) investigated flavonoids and polysaccharides in tobacco leaves, total phenolic content of flavonoid and polysaccharide fractions was investigated, the results supporting this paper.

A large number of researches have been carried out on the presence of flavonoids in tobacco, which has been proven, but all of them are in the direction of the prominent effect in the flavonoid's antioxidant activity.

CONCLUSIONS

The results indicate that in all analyzed tobacco varieties from the top harvests (Harvest 1 and Harvest 2); there is presence of flavonoids in all seven analyzed tobacco varieties, with differences in the total concentration among different types, i.e., varieties.

The obtained results show that the lowest content of flavonoids was identified in the variety P-12-2/1.

The highest content of flavonoids was observed in the Basma type, namely the Prilep Basma 2 and Prilep Basma 82 varieties.

When analyzing the results of the two harvests for analysis (Harvest 1 and Harvest 2), it is observed that there are no major differences in the content of total flavonoids, except for the variety P-12-2/1. Harvest 1 has a higher content of flavonoids compared to Harvest 2.

From the analyzed varieties of Yaka type (YV 125/3, YV 3-7, YV 48), it can be seen that they have approximately similar content of flavonoids, varying in the frames from 42.2136 μg/g to 45.3416 μg/g.

According to the results, the content of total flavonoids in Djebel type (variety DJ 38) rebounds compared to other tobacco varieties, so their results are close to the varieties Prilep Basma 2 and Prilep Basma 82.

Since flavonoids are effective in removing free radicals, they have the potential to be powerful antioxidants. That is why tobacco leaves are considered as a potential source of natural antioxidants for food, medicine, cosmetics.

Flavonoids show a more prominent effect in antioxidant activity than polysaccharides. Therefore, in the future, it is important to extract natural antioxidants from tobacco leaves, which can be a good way to intensively utilize tobacco resources

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MARKETING AND DEMARKETING ACTIVITIES IN TOBACCO INDUSTRY

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ABSTRACT

Marketing and demarketing activities in the tobacco business today are imposed as a necessity, because the use of tobacco products (especially smoking) is increasingly condemned by the medical profession, as well as by the legislative bodies of different countries. A large amount of information, either through various scientific studies, media presentations, bans, preferring the WHO tobacco control framework convention, etc., points to the harmfulness of tobacco, thus putting tobacco producers in an uncomfortable position. Despite numerous warnings about the harmfulness of tobacco, however, the popularity of consumption itself is not declining, but on the contrary, smoking continues and it is estimated that by 2025 there will be around 1.3 billion smokers. In the paper, in addition to presenting part of the marketing and demarketing activities, a survey was also made with certain questions of smokers and non-smokers. The collected data are tabularly, graphically and computationally displayed and processed.

Keywords: tobacco business, marketing, demarketing, cigarettes, promotion

МАРКЕТИНШКИТЕ И ДЕМАРКЕТИНШКИТЕ АКИВНОСТИ ВО ТУТУНСКАТА ДЕЈНОСТ

Маркетиншките и демаркетиншките акивности во тутунската дејност денес се наметнуваат како неопходност, бидејќи, употребата на тутунските производи (особено пушењето) сè повеќе се осудува и од медицинската фела, како и од законодавните тела на различни земји. Голем број на информации било преку различни научни студии, медиумски презентаци, забрани, преферирање на рамковната конвенција за контрола на тутунот на СЗО и слично, упатуваат на штетноста на тутунот, со што се доведуваат тутунопроизводителите во непријатна положба. И покрај многубројните предупредувања за штетноста на тутунот, сепак, популарноста на самата консумација не опаѓа, туку напротив и понатаму се пуши и се проценува дека до 2025 година во светот ќе има околу 1,3 милијарди пушачи. Во трудот покрај прикажување на дел од маркенгтиншките и демаркетингшките активности, направено е и анкетно истражување со одредени прашања на лица пушачи и лица непушачи. Собраните податоци се табеларно, графички и пресметковно прикажани и обработени.

Клучни зборови: тутунска дејност, маркетинг, демаркетинг, цигари, промоција

INTRODUCTION

Changed conditions in business operation of the business entities, especially of those in tobacco industry, require appropriate changes in the marketing approach to consumers, as well as application of demarketing strategies. Full commitment, engagement and responsibility of all employees is required in the process of formulating a proper marketing strategy, in order for the marketing to be a factor that will encourage the progress of a certain business entity in the tobacco industry. All activities should be in line with applicable business policies and effective and comprehensive tobacco control policies of the World Health Organization Framework Convention on Tobacco Control (WHO FCTC). Therefore, all stages in the marketing process (starting from planning of marketing activities, organizing, coordinating and managing the financial, technological and human resources to their implementation and control of the achieved marketing results) should take place in a way that will enable stable growth of the company's profit in conditions of efficient use of market opportunities. That includes creating offer that meets those needs and requirements of customers, that the company can satisfy better than competitors.

Of great importance is the ability of the company to satisfy customer superiorly, by providing additional benefits, differentiating the offer, introducing innovations and everything that maintains and increases its competitive advantage. Only in this way, the company can gain their loyalty from which both, consumers and the company will benefit.

In order to achieve the set marketing goals, several national and international marketing strategies are available to the business entities. The strategy that will be applied depends on the desired marketing goals, the capabilities and potential of the respective business entity and on the opportunities that exist on the national or international market.

RESULTS AND DISCUSSION

Today, marketing plays an increasing role in the survival, competitiveness and development of the company, both nationally and internationally. Renowned professor of management and marketing Peter Drucker says that the aim of marketing is to make selling superfluous (Drucker, 1973).

Also, the term marketing is often defined as: a social and managerial process by which individuals and groups obtain what they want and need through creating and exchanging products, information and values with others. Marketing is the performance of business' activities that direct the flow of goods and services from producer to consumer or user Marketing is the sale of goods that are not returned by the buyers, but the buyers return to buy (Lewis et al.,1998).

In fact, marketing is focused on the following inseparable, influential components (Miceski, 2013):

- needs, desires and requirements;
- products and services;
- benefits (values), satisfaction and quality;
- exchanges, transactions, relationships and
- markets.

The idea of satisfying human needs, desires and requirements is the main concept on which marketing is based. Consumers often make purchasing decisions based on their perceived value (benefit) of a particular good. Perceived value is the customers' evaluation of the merits of a product or service, and its ability to meet their needs. Often, consumers do not evaluate product accurately or objectively. They are guided by the perceived value, according to the degree of satisfaction of their needs. In this regard, customer satisfaction is expressed through the degree to which the perceived value of the product matches the expectations of the customer. Most often, customer satisfaction is closely related to the quality of both, the product itself and its packaging design. Therefore, in the last two or three decades, many companies have adopted Total Quality Management (TQM) programs, designed to continuously improve the quality of their products, the appearance of their packaging, their services and marketing processes i.e., to improve the quality through overall operation of the organization.

Many tobacco companies are developing new, more interesting forms of tobacco products for the younger population. New cigarettes that change the taste by pressing the filter or by adding different new flavors, have also appeared on the market. Tobacco companies try to increase the demand for products, especially from the new consumers. Marketing creates consumer demand, by giving reasons why someone would like to smoke cigarettes or to use other tobacco products.

The ban on advertisements that make smoking appear to be appealing, is being circumvented by other methods. Tobacco companies sell cigarettes in many different packaging designs, which can increase adolescents' desire to smoke. Thus, advertisements can be seen in magazines and in other unexpected places, such as night clubs, cafes, through promotion of striking minimal packages, lighters and similar products. The absence of explicit advertising can be seen as a form of covert advertising.

Namely, there is a possibility that consumers are not aware that such an approach to them is marketing communication of a certain brand of cigarettes or similar products (Zanot, 1983).

Tobacco companies find creative ways to market their products, through attractive packaging with various warnings about the dangers of smoking, i.e., through so-called "corporate social responsibility" campaigns. They are trying to present themselves as contributors to environmental protection. Despite increasing restrictions, tobacco industry is still trying to promote its products, especially to the youngest, who are the consumers of the future. Experiential marketing is used for that purpose. Experiential marketing is a kind of technique of encouraging consumers to experience or interact with a particular tobacco brand in recreational places and events, such as concerts, bars or nightclubs.

Most tobacco companies have used this type of marketing for decades, to encourage young people to see what cigarette smoking looks like, and thus to increase cigarettes use by infiltrating their social life. Thus, friendly social atmosphere in the pub or social club contributes a lot to the enjoyment of smoking, and also encourages smokers to smoke more than usual.

Several studies have examined the tobacco industry's efforts to link tobacco use to alcohol use, and found that 74.5% of all young adult smokers said they enjoyed smoking while drinking.

Scientific evidence shows that advertising and promotion of tobacco companies influences young people to start using tobacco (Perks et al., 2018).

- Adolescents who are exposed to cigarette advertising often find the advertisements attractive.
- Tobacco advertisements make smoking look attractive, which may increase adolescents' desire for smoking.

Tobacco industry has always argued that its advertisements are not intended to attract children. However, it fails to hide its marketing activities from young adults. They see a large number of adults and their peers smoking cigarettes every day.

Tobacco companies invest millions of dollars a year in experiential marketing, spending nearly 122 million dollars on experiential marketing of cigarettes and smokeless tobacco in 2016. Experiential marketing remains an important tactic for tobacco companies to reach new customers and retain existing ones

DEMARKETING

The goal of demarketing is to temporarily or permanently reduce demand over a shorter or longer period of time - the goal is not to destroy demand, but only to reduce or divert demand. In this regard, there are several definitions and opinions on what the term demarketing means. Kotler and Levy first devised the term 'demarketing" to describe intentional demand-reducing activities. Thus, in simple terms, demarketing refers to marketing aimed at limiting growth. Demarketing by the governments in many countries is undertaken primarily to modifying socially unacceptable behavior. The main aim is to discourage consumption of products or service that is against social interest, against ecology or even in the interest of the consumer itself. Tobacco and alcohol companies face increasing pressure to prevent smoking and drinking among consumers, (Yang et al., 2013), by using demarketing strategies. In fact, demarketing is a type of strategy that is well-suited to harmful products such as: alcohol, drugs, cigarettes, cigars and other tobacco products, although they may have economic benefits. Many people consume cigarettes and chew gutka, which is a tobacco product, as a habit or as a relief from stress or style.

According to medical views, consumption of tobacco products causes negative side effects on the smokers' health, such as lung cancer and oral diseases. In accordance with the recommendations of the World Health Organization (WHO) and the specific guidelines of the World Health Organization Framework Convention on Tobacco Control (WHO FCTC), the purpose of the demarketing articles in the tobacco industry is to reduce the consumption of tobacco products, especially cigarettes. The most used demarketing activity is constant emphasis that tobacco consumption, especially cigarette smoking, has a great negative impact on human health. World Health Organization (WHO) estimates that by 2030 there will be more than 8 million tobacco-related deaths per year, which is 10% of annual deaths worldwide, Mathers et al. (2006). In addition to the daily indications on the harmfulness of smoking, a particularly pronounced warning is seen on the standardized packages of cigarettes with large graphic health warning labels. Health warnings about the dangers of tobacco use are shown in the photos below on some cigarette packages sold in Macedonia.

The package of MARLBORO (touch) cigarettes (Figure 1) contains a warning: Smoking harms you and others around you and Smoking during pregnancy harms your unborn baby

.

Figure 1. Appearance of MARLBORO (touch) cigarette package

The package of DAVIDOFF cigarettes (Figure 2) contains a warning: Cigarette smoke contains benzene, nitrosamine, formaldehyde and hydrogen cyanide

Figure 2. Appearance of DAVIDOFF cigarette package

On the package of JADĖ cigarettes (Figure 3) it is stated with proper pictures: Smoking can kill and Smoking can reduce fertility.

Figure 3. Appearance of JADĖ cigarette package

The package of CHESTERFIELD cigarettes (Figure 4) states: Smoking kills and Protect children: do not allow smoke to be inhaled, with a photo of a child whose mouth and nose are covered with smoke.

Figure 4. Appearance of CHESTERFIELD cigarette package

On the package of RODEO cigarettes (Figure 5), it is written: Smoking harms you and others around you and ask for help to quit smoking.

Figure 5. Appearance of RODEO cigarette package

The package of LUCKY STRIKE cigarettes (Figure 6) contains a warning: Smoking harms you and others around you and Smoking can cause a slow and painful death. A photo of throat disease has been added

Figure 6. Appearance of LUCKY STRIKE cigarette package

On the package of CAMEL cigarettes (Figure 7), it is stated: Smoking kills/smoking can kill and Smoking is an addiction disease, with a photo of a prison cell, which means captivity.

Figure 7. Appearance of CAMEL (silver) cigarette package

The package of PALL MALL cigarettes (Figure 8) contains a warning: Smoking harms you and others around you and Smoking causes skin aging, with a photo of wrinkled hands.

Figure 8. Appearance of PALL MALL (silver) cigarette package

In fact, demarketing is applied in different ways. Thus, Myanmar is the third Asian country among the twenty in the world, that have implemented standardized packaging, after Thailand and Singapore. The new regulation requires the surface of the package of tobacco products to be in unattractive, dark brown colour and to be flat and smooth, without conspicuous designs or decorative elements. This complements the vivid health warnings that must be printed on the front and back of each package and cover 75 % of the packaging, Nicotine and Tobacco Research (2022).

Standardized packaging and health warnings with photos are part of a comprehensive strategy to reduce tobacco use in order to strategically discourage its use.

EMPIRICAL RESEARCH

The main purpose of the research in this paper is to emphasize the role of unstressed and discreet marketing activities as well as of direct and audio-visual demarketing activities on the consumption of tobacco products, and especially on cigarette smoking. Empirical research has been done with both, smokers and non-smokers. The aim is to assess cigarette smoking, how much do people smoke and how much demarketing activities affect the smokers. It also assesses the impact of the written and photography warning on the harmfulness of smoking in accordance with applicable business policies and effective and comprehensive tobacco control policies by WHO FCTC. The basic hypothesis of the empirical research is based on the claim that cigarette smoking depends on the determination and desire of the person, without significant influence by discrete marketing activities and public and direct demarketing activities. But the basic hypothesis would not be complete if both auxiliary hypotheses related to cigarette smoking are not met. The first auxiliary hypothesis indicates that smoking or non-smoking of cigarettes depends on the self-determination of each person. The second auxiliary hypothesis was set in the direction that public, written and photography demarketing activities that warn about the harmfulness of smoking on the health of smokers have no impact on drastically reducing cigarette smoking. The main instrument used in the empirical research was the survey conducted with respondents in the municipality of Prilep. A structured interview, intended for smokers, with questions and short answers, was used as an additional tool. In order to obtain information, 34 people were interviewed, while the survey included 100 people who smoke cigarettes-smokers and 100 people who do not smoke, non-

smokers. More specifically, the questionnaire for smokers was given to smokers, and the questionnaire for non-smokers was given to non-smokers, randomly, in ten days, while the survey was conducted.
The first question was: **Do you actively smoke cigarettes**? The following answers have been received: **Yes**, answered 89% of the respondents - smokers. **No**, answered 0% of the respondents, and **Sometimes/From time to time** answered 11 smokers. Their answers are presented in Figure 9.

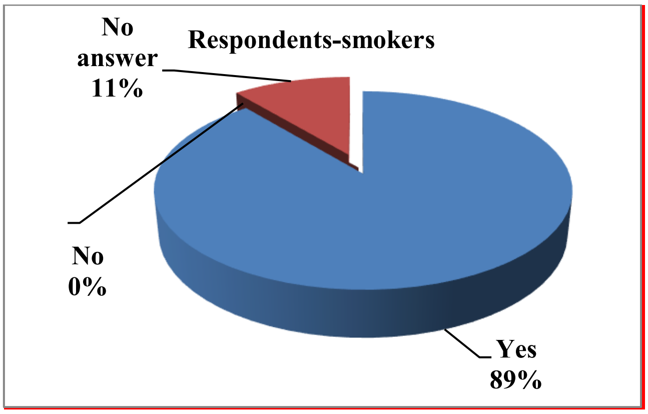

Figure 9. Answers to the first question by the respondents-smokers

The same question was answered by the respondents - non-smokers. **Yes** answered 0 % of them, **No** answered 78% non-smokers, while **Sometimes/From time to time** answered 22 respondents - non-smokers (Figure 10).

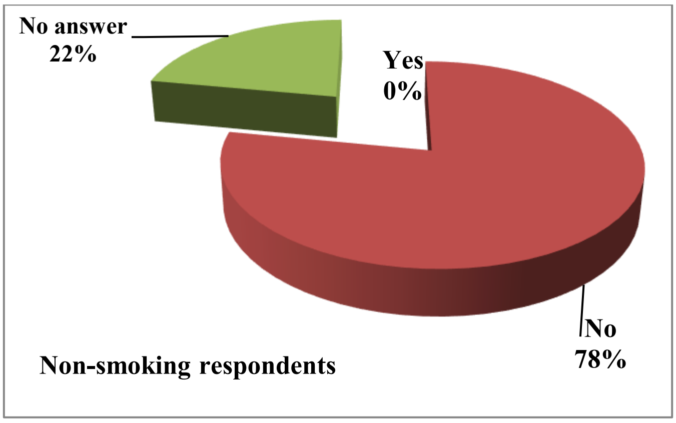

Figure 10. Answers to the first question by the respondents - non-smokers

Correlation dependence tests were performed and a calculated value of 170.67 was obtained for the X^2 test. Contingency coefficient (C) was 0.579.

Calculated value for the X^2 test and the contingency coefficient is:

$$X^2_{0.05\ (calculated\ value)} = 170,67 < X^2_{0.05\ (tabular\ value)} = 5.991,\ C= 0.679$$

From the calculated values of the X^2 test, it can be seen that its calculated value 170.67 is greater than its tabular (theoretical) value, which concludes that the survey was conducted between completely opposite examiners, smokers and non-smokers. Hence, the received answers were completely different, as expected. The contingency coefficient 0.679 shows that there is a dependence between the answers of the respondents, i.e., they interact in the opposite direction, according to the questions asked in different groups.

The second question was: **Are you familiar with the warnings about the harmfulness of cigarette smoking on human health?** The following answers were received: 86% of the respondents-smokers answered **Yes**, which means they were familiar with the warnings about the harmfulness of smoking on human health. **No** answered 2 % of the respondents, and **No answer/I know, but I do not pay attention** answered 12 % of the respondents. Their answers are graphically presented in Figure 11.

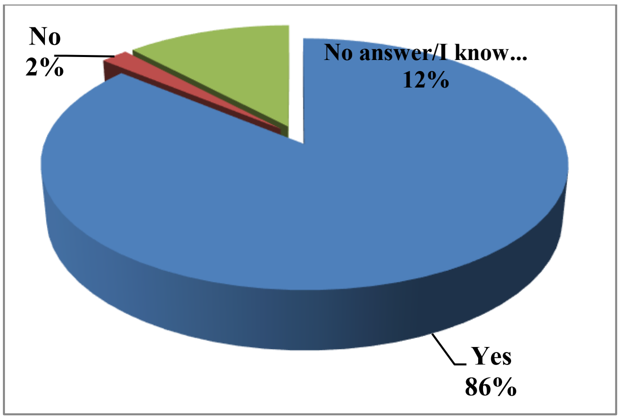

Figure 11. Answers to the second question by the respondents – smokers

75% of the respondents non-smokers answered **Yes**, which means they were familiar with the warnings about the harmfulness of smoking on human health. **No** answered 9 % of the respondents, and **No answer/I know, but I do not pay attention** answered 16 % of the respondents (Figure 12).

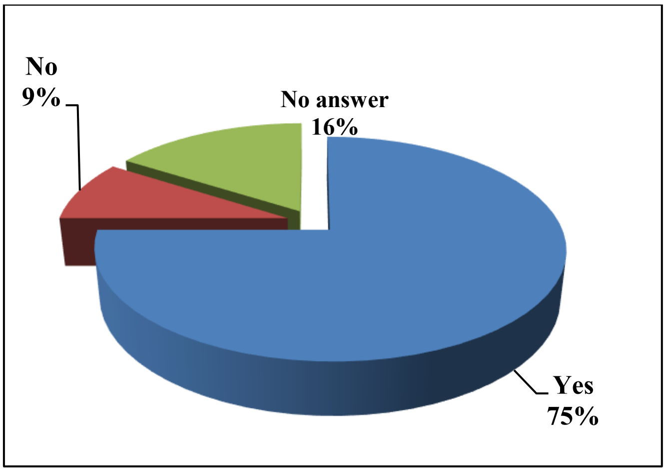

Figure 12. Answers to the second question by the respondents - non-smokers

Calculated value for the X^2 test and the contingency coefficient is:

$$X^2_{0,05\ (calculated\ value)} = 5.778 < X^2_{0.05\ (tabular\ value)} = 5.991,\ C = 0.168$$

From the calculated values of the X^2 test, it can be seen that its obtained value 5.778 is less than its tabular (theoretical) value (5.991), which means that the answers of the respondents, smokers and non-smokers are the same i.e., they are familiar with the warnings on the boxes.

The contingency coefficient is 0.168 which shows that there is no dependence on the answers of the respondents, i.e., their answers do not correspond, because the questions were asked in different groups of respondents.

34 smokers were interviewed at the same time. As previously mentioned, the interview consisted of 4 questions, and all respondents were randomly selected.

The first question was: **Why do you smoke cigarettes?** Most of the interviewees answered identically: I like it, it gives me pleasure, I am attached to smoking, it calms me down, it relaxes me, I do not feel very hungry, habit, I feel relaxed or I do not even know how to explain it myself, I just cannot do without cigarettes and so on.

The answers to the question **How did you start smoking cigarettes?** where: by watching people older than me, by watching movies, at parties, with friends, I tried and it went well for me, we were trying to be cool at that time, almost everyone around me smoked and made figures with the smoke, and so on.

To the question **Do you see and follow the warnings about the negative impact of smoking on human health?** most smokers answered yes i.e. They read and heard a lot about the harmful effects of smoking on human health, but it did not affect them to quit smoking. They explained that they did not take the smoke in their chests, but in their mouths and immediately let it out. Also, many smokers do not believe the written words about the negative effects of cigarettes because they know many people who smoked for more than 80 or 90 years.

To the question **Does Smoking Affect Your Health?** most of the interviewed smokers answered no. They did not feel any health changes. Some dare to say that they felt worse when they did not smoke. When we asked if they cough in the morning, the answer was: Like any other person, I cough sometimes.

After processing the empirical data from the answers, through analysis, modelling and simultaneous tabular and graphical presentation, as well as calculating the value of the x2-test, it can be concluded that most of the surveyed smokers are not affected by visible marketing activities or by highly offensive demarketing activities.

Interviewees started smoking cigarettes accidentally, by watching their peers and older people while smoking. The older generations born in the previous century started smoking by watching movies, where the main characters smoked, then at parties, gatherings and the similar events. This fully confirms the general hypothesis that smoking or non-smoking depends on the self-determination of each person, how he perceives smoking. Most of the respondent smokers stated that smoking has a positive effect on their feelings, it provides them with better concentration, a better ability to focus on their activities and strengthens work motivation.

CONCLUSIONS

Marketing activities are an integral part of every business because they are a process that connects production or services with customer requirements and satisfaction. They enable products or services from manufacturers or service providers to reach consumers (customers) and information about products/services (satisfaction or dissatisfaction with products or services, their characteristics, quality and requirements) from consumers to reach the manufacturers. The purpose of marketing is to attract new customers, and at the same time to retain existing customers and cause satisfaction in them, by delivering quality i.e., acceptable products. Thus, as a process of conceptualized activities thatwill enable tobacco companies to market their products to consumers, it must be performed discreetly, through unobtrusive and direct approaches to consumers, because a large amount of information from various scientific studies, media presentations, bans, preference of the World Health Organization Framework Convention on Tobacco Control (WHO FCTC), point to the harmfulness of tobacco. All of the information puts tobacco producers in an awkward position as a result of developing strong offensive demarketing activities.

In fact, demarketing activities are a kind of marketing for temporary or permanent reduction of demand in tobacco products or cigarettes, due to medical indications for the harmful effects of smoking on the smokers' health. In accordance with the recommendations of the World Health Organization (WHO) and the specific guidelines of the World Health Organization Framework Convention on Tobacco Control (WHO FCTC), the purpose of demarketing articles in tobacco industry is to reduce the consumption of tobacco products i.e., cigarettes. Despite the daily reports on the harmfulness of smoking, by the specially expressed warning on the standardized packaging of cigarettes with written recommendations and warning photos still no drastic reduction of smoking can be noticed. Thus, most of the respondents in our research, stated that smoking has a positive effect on their feelings, provides them with better concentration, its strengths their work motivation and ability to focus on their activities. They emphasize that they will quit smoking if they notice that it affects their health.

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