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Original Scientific paper

QUANTITATIVE GENETIC INVESTIGATIONS ON SOME IMPORTANT TRAITS IN TOBACCO VARIETIES AND THEIR DIALLEL ONE-WAY AND BACK-CROSS GENERATIONS

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ABSTRACT

Investigations were made on stalk height with inflorescence, leaf number and dry mass yield per stalk in four parental tobacco genotypes and their one-way diallel crossing in F_1, F_2, $BC_1 – P_1$ and $BC_1 – P_2$. Diallel analysis allows better understanding of the genetic character of parents and their hybrids. The trial was set up in the Experimental field of the Tobacco Institute–Prilep in 2014 and 2015, in randomized block design with four replications.

The aim of investigations was to get important information on the heredity of quantitative traits, through genetic components of variance for F_1, F_2 and two BC_1 generations.

Investigations show that additive variance is more effective in inheritance of the investigated traits in the above generations. The interaction between additive and dominant genes for stalk height and leaf number per stalk in BC_1 is different from that in other generations, whereas for dry mass yield the interpretation is the same in all generations. The ratio of the total number of dominant and recessive genes in BC_1 differs from other generations regarding the first two traits, whereas for dry mass yield all generations are interpreted identically. The heritability is very high, indicating that the studied traits are highly heritable.

Keywords: tobacco (*Nicotiana tabacum L.*), diallel analysis, variance, interaction, heritability.

КВАНТИТАТИВНИ ГЕНЕТСКИ ИСТРАЖУВАЊА ЗА НЕКОИ ВАЖНИ СВОЈСТВА КАЈ ТУТУНСКИ СОРТИ И НИВНИТЕ ДИЈАЛЕЛНИ ЕДНОНАСОЧНИ И ПОВРАТНИ ГЕНЕРАЦИИ

Трудот опфаќа истражувања за висината на стракот со соцветие, бројот на листови по страк и приносот на сува маса по страк, кај четири родителски генотипови и нивните дијалелни F_1, F_2, $BC_1 – P_1$ и BC1 – P_2 генерации. Дијалелната анализа нуди подобри сознанија за генетскиот карактер на родителите и нивните крстоски. Опитот беше поставен на Опитното поле при Научниот институт за тутун – Прилеп во 2014 и 2015 година, по случаен блок – систем во четири повторувања.

Целта на истражувањата беше да се добијат информации во врска со наследувањето на поважните квантитативни особини, преку одредувањето на генетските компоненти на варијансата за F_1, F_2 и двете BC_1 генерации.

Истражувања покажаа дека предност има адитивната компонента на варијансата во наследувањето на спомнатите својства во испитуваните генерации. Интеракцијата помеѓу адитивните и доминантните гени за висината на стракот и бројот на листови по страк во BC_1 е различна од онаа во другите генерации, а за сувата маса по страк е иста во сите генерации. Сооднос на вкупниот број доминантни спрема рецесивни гени кај BC_1 се разликува од оној на другите генерации за првите две особини, додека за приносот на сува маса сите генерации се толкуваат идентично. Вредностите за херитабилноста се многу високи, што покажува дека испитуваните особини се високонаследни

Клучни зборови: тутун (*Nicotiana tabacum L.*), стари сорти, комерцијални сорти, квантитативни својства, анализа на варијанса.

INTRODUCTION

For successful selection and breeding of the existing tobaccos and creation of new varieties with higher yield and improved quality, it is necessary to have a good knowledge on the available genotypic material. The application of diallel analysis and estimation of the genetic components of variance allow better understanding of the genetic nature of parents and their hybrids.

Genetic components of variance have been studied by many geneticists and breeders. Here we cite results of recent authors, whose subject of the work is tobacco. Chaubey et al. (1990) investigated 72 genotypes of N. rustica L. for hookah tobacco and found high heritability for leaf number and yield per stalk. Butorac (1999) investigated four parental varieties of Burley tobacco and their diallel F_1, F_2, BC_1 and BC_2 progenies and reported that their broad-sense heritability was higher than the narrow-sense heritability. The highest heritability was estimated in the inheritance of leaf area and sixth leaf weight. Peksuslu et al. (2002) obtained a high broad-sense heritability - over 80% in the length of tobacco leaves. Masheva (2008) found that the leaf length in hybrids of oriental tobaccos successively involves additive, additive-additive and dominant-dominant gene effects, and the sign determining the width is a fundamental part of the dominant gene effect. Korubin–Aleksoska and Aleksoski (2009) in three oriental and one semi-oriental variety and their F_1 and F_2 progenies obtained very high index for both types, with predominance of the broad-sense heritability. Aleksoski and Korubin–Aleksoska (2011) investigated four types of oriental tobacco and their one–directional diallel F_1 hybrids for dry mass yield per stalk and received high results for h^2, where the values of broad-sense heritability were higher than those of the narrow-sense heritability. Dyulgerski and Dimanov (2013) in six hybrid combinations of Virginia parental tobacco varieties found that the inheritance of length and width of the middle-belt leaves is overdominant and always in direction of the parent with higher values. The phenotypic manifestation of leaf length is strongly influenced by the negative epistatic interactions. Aleksoski et al. (2013), in his three-year investigations on stalk height, number of leaves and yield in parental genotypes of oriental tobacco and their F_1 crosses, found high genetic variance for the investigated quantitative traits, which can be noted from the high percentage values for heritability. Korubin–Aleksoska (2014) in her studies on the inheritance of nicotine content and yield of oriental varieties and their diallel F_1 and F_2 crosses, found that additive genetic component had higher values for dry yield than the dominant one. It can be concluded from interaction that in F_1 progeny genes from the lower yielding-parents prevail, while F_2 progeny is controlled by the genes from parents with higher yields. Low values of E show limited impact of the environment on the modification of this trait. Heritability in both generations has high values, indicating that this is a highly heritable trait. Masheva (2014) estimated the gene effects for plant height, number of leaves per plant and flowering time in two crosses (Plovdiv 50 x Basma Xanti 101 and Krumovgrad 90 x Basma Xanti 101) and found additive and non-additive gene effects, but the additive effect was predominant in the expression of the estimated traits.

Investigations were made on the following traits: stalk height, leaf number per stalk and dry mass yield per stalk in parental genotypes and their diallel crosses.

The aim of investigations was to study the mode of inheritance of the above quantitative traits through genetic components of variance for F_1, F_2, $BC_1 - P_1$ and $BC_1 - P_2$ generations and to give some instructions for further selection work.

MATERIAL AND METHODS

Four tobacco varieties were used as material for work: P10-3/2, A 42/3, YV 125/3 and FL-1. From these parental genotypes, 24 diallel crosses were made (six from F_1, F_2, backcross BC_1 –P_1 and reciprocal backcross BC_1 – P_2 generation, respectively). The trial was set up in 2014 and 2015, in the Experimental field of Scientific Tobacco Institute - Prilep, using randomized blocks design with four replications.

Data on stalk height with inflorescence and leaf number per stalk were obtained by measuring 400 plants in flowering stage for each generation (except for F_1, in which 100 plants were measured). Harvesting of tobacco was performed manually by insertions and dry mass yield was weighed on fermented tobacco prior to manipulation.

Genetic components of variance and broad-sense heritability for all combinations in F_2, BC_1 – P_1 and BC_1 – P_2 generations were estimated by the methods of Mather and Jinks (1985), according to the formula:

$$h^2 = \frac{\frac{1}{2}D+\frac{1}{2}H1-\frac{1}{4}H2-\frac{1}{2}F}{\frac{1}{2}D+\frac{1}{2}H1-\frac{1}{4}H2-\frac{1}{2}F+E}$$

Parental genotypes:

Prilep P 10-3/2 – originating from the local tobacco variety Djumaj–bale from Gorna Djumaya–Bulgaria, created in Tobacco Institute – Prilep by Rudolf Gornik and put into production in the 30–ies of the last century. Characterized by cup–like habitus, average stalk height 50 cm, with 30–36 sessile leaves, dry mass yield averages 1200 kg/ha (Fig. 1).

Djebel A 42/3 – created in Tobacco Institute–Prilep by Ana Korubin–Aleksoska, recognized by the Ministry of Agriculture, Forestry and Water Management of R. Macedonia in 2010. It is characterized by cylindrical habitus. The average stalk height is 110 cm. It has approximately 45 sessile leaves (17 cm x 9 cm), with very pleasant aroma. The dry mass yield is 1400-1700 kg/ha (Fig. 2).

YV 125/3 – created by Mile Uzunoski, through hybridization and selection; recognized in 1984 in former Yugoslavia. Its habitus is elliptical to cylindrical and the average stalk height is 120 cm. It has approximately 40 sessile leaves (21.6 cm x 11.2 cm), with very pleasant aroma. The dry mass yield is 1500-1800 kg/ha (Fig. 3).

Floria FL-1 – breeding line adopted in Tobacco Institute – Prilep by Bolsunov in 1977. Its average stalk height is 115 cm and has approximately 20 very soft leaves (35 cm x 22 cm). The dry mass yield is 1700-1900 kg/ha (Fig. 4).

Fig. 1. Prilep P 10-3/2

Fig. 2. Djebel A 42/3

Fig. 3. Yaka YV 125/3

Fig. 4. Floria FL-1

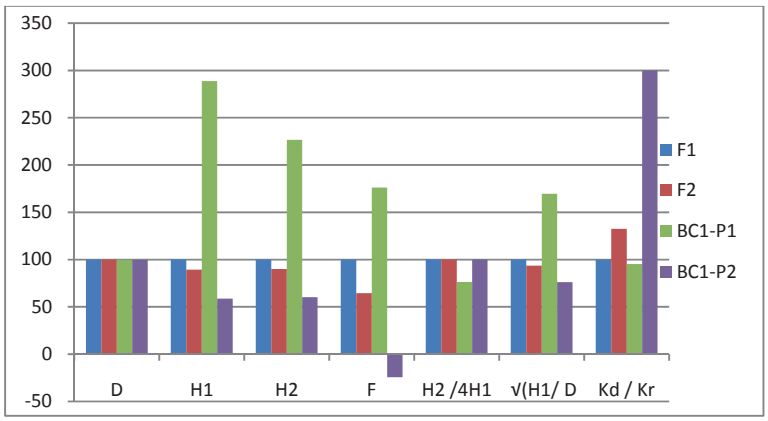
FRESULTS AND DISCUSSION

Stalk height with inflorescence

Genetic analysis showed higher presence of additive genetic variance (D) over the dominance variance (H_1 and H_2) in all generations investigated. Negative values of the interaction F in F_1, F_2 and $BC_1 – P_1$ indicates the dominance of genes from parents with lower stalk height. In $BC_1 – P_2$ generation, interaction F has a positive value, which denotes domination of genes from parents with higher stalk height. The values for the expression $H_2/4H_1$ in all generations were below 0.25, resulting in asymmetry of the locations of dominant and recessive alleles. The average degree of dominance ($\sqrt{(H1/D)}$) in all generations was less than one, indicating partial dominance in the inheritance of this trait. Ratio of the total number of dominant and recessive alleles (Kd/Kr) in F_1, F_2 and $BC_1 – P_1$ was less than one, indicating prevalence of recessive alleles. In $BC_1 – P_2$ this ratio was higher than one, which denotes that dominant alleles prevail. All crosses in the investigated generations show high heritability (h2), indicating a highly heritable trait and its fast fixation. The results of genetic components on height of the stalk with inflorescence are presented in Table 1. Graph 1 shows the percentage values of genetic components for F_2, $BC_1 – P_1$ and $BC_1 – P_2$, compared to the first uniform heterogeneous generation – F_1 for height of the stalk with inflorescence.

Table 1. Genetic components of variance for the height of the stalk with inflorescence in F_1, F_2, $BC_1 – P_1$ and $BC_1 – P_2$ generations

Generations	Components of genetic variance								
	D	H_1	H_2	F	E	$H_2/4H_1$	$\sqrt{H1/D}$	Kd / Kr	h^2
	Height of the stalk with inflorescence								
F_1	841,05	177,33	146,84	– 307,7	2,80	0,21	0,46	0,43	
F_2	840,58	158,60	132,30	– 198,1	3,27	0,21	0,43	0,57	0,994
$BC_1 – P_1$	838,55	512,12	332,84	– 541,8	5,30	0,16	0,78	0,41	0,994
$BC_1 – P_2$	838,18	104,24	88,38	74,8	5,67	0,21	0,35	1,29	0,986



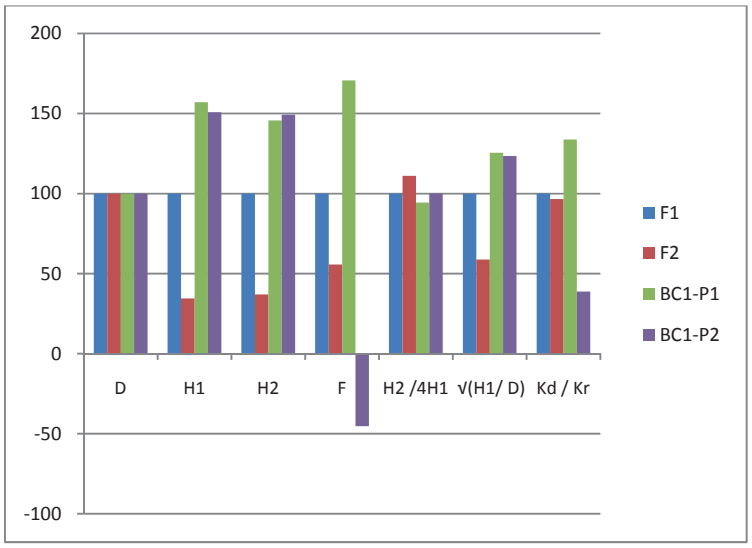
Graph 1. Graphical presentation to the percentage values of genetic components for F_2, $BC_1 – P_1$ and $BC_1 – P_2$, compared to F_1 for height of the stalk with inflorescence

Number of leaves per stalk.

Genotypic analysis of leaf number per stalk shows higher presence of additive component compared to the dominant in all generations studied. The positive value of interaction F in F_1, F_2 and $BC_1 - P_1$ shows dominance of genes from parents with higher number of leaves. In $BC_1 - P_2$ generation, interaction F has a negative value, indicating domination of genes from parents with lower number of leaves. The values for the expression $H_2/4H_1$ in all generations were below 0.25, resulting in asymmetry of the locations of dominant and recessive alleles. The average degree of dominance ($\sqrt{(H1/D)}$) in all generations was less than one, which denotes partial dominance in inheritance of the trait. Ratio of the total number of dominant and recessive alleles (Kd/Kr) in F_1, F_2 and $BC_1 - P_1$ was higher than one, indicating prevalence of dominant alleles. In $BC_1 - P_2$ this ratio was less than one, denoting dominance of recessive alleles in inheritance of the trait. Heritability (h^2) in all crosses is very high, which points out to a highly heritable trait and its fast fixation in successive selection (Table 2). Graph 2 shows the percentage values of genetic components for F_2, $BC_1 - P_1$ and $BC_1 - P_2$, compared to the F_1 for leaf number per stalk.

Table 2. Genetic components of variance for the leaf number per stalk in F_1, F_2, $BC_1 - P_1$ and $BC_1 - P_2$ generations

Generations	Components of genetic variance								
	D	H_1	H_2	F	E	$H_2/4H_1$	$\sqrt{H1/D}$	Kd / Kr	h^2
	Number of leaves per stalk								
F_1	100,69	26,32	19,42	34,63	0.17	0,18	0,51	2,01	
F_2	100,54	9,10	7,20	19,30	0,32	0,20	0,30	1,94	0,993
$BC_1 - P_1$	100,55	41,33	28,30	59,10	0,31	0,17	0,64	2,69	0,991
$BC_1 - P_2$	100,52	39,69	29,00	- 15,70	0,34	0,18	0,63	0,78	0,995



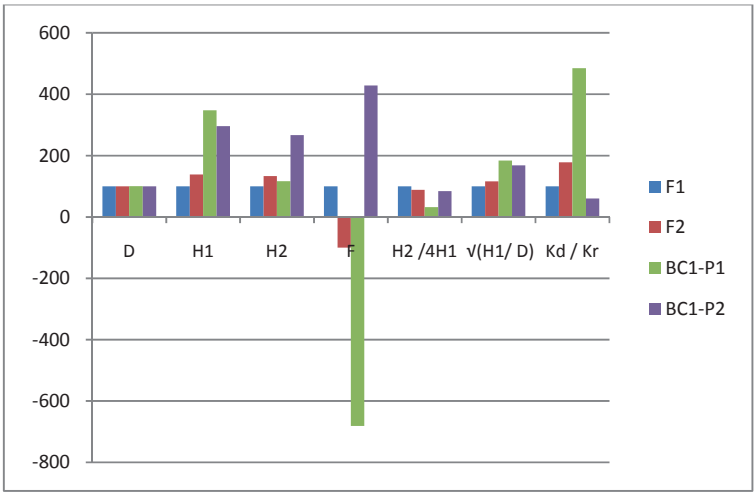
Graph 2. Graphical presentation to the percentage values of genetic components for F_2, $BC_1 - P_1$ and $BC_1 - P_2$, compared to F_1 for leaf number per stalk

Dry mass yield per stalk

The value of additive genetic variance D is significantly higher than the dominant H_1 and H_2 in all generations tested, indicating that major part in inheritance of this trait belongs to the additive component. Interaction F in F_1 and $BC_1 – P_2$ has a negative value, indicating dominance of the parent with lower dry yield. F in F_2 and $BC_1 – P_1$ has a positive value, indicating dominance of genes from parents with higher dry mass yield. The values for $H_2/4H_1$ denote asymmetrical distribution of dominant and recessive alleles, except for F_1 (= 0,25), which has symmetrical distribution of alleles. The average degree of dominance ($\sqrt{(H1/D)}$) in all generations is less than one, indicating partial dominance in the inheritance of this trait. Ratio of the total number of dominant and recessive alleles (Kd/Kr) in F_2 and $BC_1 – P_1$ is higher than one, which denotes prevalence of dominant alleles, whereas in F_1 and $BC_1 – P_2$ it is less than one, indicating prevalence of the recessive alleles. Heritability (h^2) of dry mass yield per stalk in all generations is very high, from which it can be concluded that this is a highly heritable trait and its stabilization will be provided in the shortest possible selection period (Table 3). Graph 3 shows the percentage values of genetic components for F_2, $BC_1 – P_1$ and $BC_1 – P_2$, compared to the F_1 for dry mass yield per stalk.

Table 3. Genetic components of variance for the dry mass yield per stalk in F_1, F_2, $BC_1 – P_1$ and $BC_1 – P_2$ generations

Generations	Components of genetic variance								
	D	H_1	H_2	F	E	$H_2/4H_1$	$\sqrt{H1/D}$	Kd / Kr	h^2
	Dry mass yield per stalk								
F_1	36,72	1,27	1,20	- 2,10	0,12	0,25	0,19	0,73	
F_2	36,73	1,76	1,60	2,10	0,11	0,22	0,22	1,30	0,994
$BC_1 – P_1$	36,74	4,42	1,40	14,30	0,10	0,08	0,35	3,54	0,992
$BC_1 – P_2$	36,63	3,76	3,20	- 9,00	0,21	0,21	0,32	0,44	0,991



Graph 3. Graphical presentation to the percentage values of genetic components for F_2, $BC_1 – P_1$ and $BC_1 – P_2$, compared to F_1 for dry mass yield per stalk

CONCLUSIONS

The results obtained for genetic components of three investigated traits reveal that dominant components of variance are much lower than the additive component, indicating that their inheritance is dominated by recessive genes.

Interaction F for stalk height has a positive value in $BC_1 – P_2$, indicating that the inheritance of this trait is dominated by the higher parent, while the negative value in F_1, F_2 and $BC_1 – P_1$ points out to dominance of the shorter parent; leaf number per stalk has a positive value in F_1, F_2 and $BC_1 – P_1$, which denotes dominance of the parent with higher number of leaves, while the negative value for this trait in $BC_1 – P_2$ denotes dominance of the parent with lower number of leaves; dry mass yield per stalk has a positive value in F_2 and $BC_1 – P_1$, indicating dominance of the parent with higher yield, while negative value in F_1 and $BC_1 – P_2$ denotes dominance of the parent with lower dry yield.

Low values of E show limited impact of the environment on the modification of investigation traits.

The value for $H_2/4H_1$ indicates asymmetrical distribution of dominant and recessive alleles.

The average degree of dominance shows partial dominance in inheritance of these traits.

Ratio of the total number of dominant and recessive alleles for the character height of the stalk in F_1, F_2 and $BC_1 – P_1$ showed dominance of the recessive alleles while in $BC_1 – P_2$ dominant alleles were prevailing; leaf number per stalk in F_1, F_2 and BC1 – P_1 was governed by dominant alleles, while $BC_1 – P_2$ by recessive alleles; dry mass yield per stalk in F_2 and $BC_1 – P_1$ showed prevalence of dominant alleles and in F_1 and $BC_1 – P_2$ prevalence of recessive alleles.

Results for broad-sense heritability showed a very high percentage of genetic variance which leads to a conclusion that the investigated traits of diallel crosses are highly heritable and their fixation will be achieved in early generations of the selection.

REFERENCES

1. Aleksoski J., Korubin–Aleksoska, A., 2011. Degree of Inheritance and Heritability of Yield in Parental Genotypes and F1 Hybrids of Tobacco. Journal of Agricultural Sciences, Belgrad, 3, 165-172; DOI: 10.2298/JAS1103165A
2. Aleksoski J., Dimitrieski M., Korubin–Aleksoska, A., 2013. Investigations of heritability as an indicator of the inheritance of quantitative characters in tobacco. Tutun/Tobacco, 7-12, 54-62.
3. Butorac J., 1999. Nasljednost nekih parametara lista duhana tipa burley. Poljoprivredna znanstvena smotra 2, 87-96.
4. Chaubey C.N. Mishra S.K., Mishra A.P., 1990. Study of variability and path analysis for leaf yield components in Hookah tobacco. Tob. Res. 16-1, 47-52.
5. Dyulgerski Y., Dimitar D., 2013. Inheritance of the leaf size in Virginia tobacco crosses. Tutun / Tobacco, 7-12, 15-19.
6. Masheva V., 2008. Study on the inheritance of some characters in agro-morfologichni selection of oriental tobacco. Rastinievadni nauki, 2, 107-109.
7. Masheva V., 2014. Analysis of gene effects and inheritance of some quantitative parameters

in oriental tobacco varieties. Tutun / Tobacco, 1-6, 12-18.
8. Mather K., Jinks J.L., 1985. Biometrical Genetics. Chapman and Hall Ltd, London.
9. Korubin–Aleksoska A., Aleksoski J., 2009. Heritability of quantitative traits in F_1 and F_2 progenies of some domestic and foreign tobacco varieties. Proceedings of the 44th Croation and 4th International Symposium on Agriculture - Opatija, 330-334.
10. Korubin–Aleksoska A., 2014. Components of genetic variance for yield and nicotine inheritance in tobacco. Turkish Journal of Agricultural and Natural Science, Special Issue 2, 1396-1401.
11. Peksuslu A., Sabanci C.O., Kücüközden R., Sekin S., 2002. Genotype x environment interactions and heritabilities of some important agronomic traits in tobacco. The second Balkan scientific conference quality and efficiency of the tobacco production, treatment and processing, Plovdiv; 80-85.

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Original Scientific paper

RESULTS OF THE INVESTIGATIONS ON SOME CHARACTERISTICS OF VIRGINIA TOBACCO VARIETIES AND LINES IN 2010 AND 2011

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ABSTRACT

Two-year investigations (2010 – 2011) were made on some qualitative and quantitative characteristics of seven Virginia tobacco varieties and lines. The obtained results showed some dominance of male-sterile hybrid lines over the fertile varieties with regard to yield and quality and the achieved economic effect. The best average results were obtained in lines V-88/09 CMS F_1 and V-82/07 CMS F_1.
Key words: climate, tobacco, variety, Virginia, yield, quality.

РЕЗУЛТАТИ ОД ИСПИТУВАЊЕТО НА НЕКОИ СВОЈСТВА КАЈ ВИРЏИНИСКИ СОРТИ И ЛИНИИ ТУТУН ВО 2010 И 2011 ГОДИНА

Во текот на 2010 и 2011 година беа извршени испитувања со 7 вирџиниски сорти и линии на тутун со цел да се оценат некои квалитативно – квантитативни карактеристики кај истите. Добиените резултати од истражувањата покажаа извесна доминација на машкостерилните хибридни линии над фертилните сорти како по принос така и по квалитет и постигнати економски ефекти. Со најдобри севкупни резултати се истакнаа линиите V-88/09 ЦМС F_1 и V-82/07 ЦМС F_1.
Клучни зборови: клима, тутун, сорта, вирџинија, принос, квалитет.

INTRODUCTION

Virginia tobacco accounts for 50–60% of the composition of blend cigarettes and, along with Burley tobacco, its participation exceeds 80%. This confirms the importance of this type and the need for its further investigation. According to Kalamanda (2009), Virginia tobaccos are basically skeletal, with higher sugar content, lower nitrogen content and good technological properties (high yield in cigarette manufacture).

In R. Macedonia, this type of tobacco has not been produced since 2002 and all the needs are met by imports, which consumes substantial foreign exchange assets.

Initiatives to restart the production of this tobacco come from the Scientific Tobacco Institute – Prilep and are supported by the results of comparative trials with varieties and lines created in the Institute. The results show that in most of the characters, the new creations are not behind some renowned varieties from abroad. In their multi-annual research, breeders of the Institute concluded that male-sterile hybrid varieties give better results than pure varieties and lines. According to Beljo (1996), F_1 hybrids have a better respond to stress,

faster root growth, higher uniformity and higher yield compared to both parents.

MATERIAL AND METHODS

Two-years investigation (2010, 2011) was carried out with 7 varieties, three of which were fertile (K - 326 from USA, Virginia SKR from Zimbabwe and V-972 from Germany) and four male sterile hybrids (V-88/09 CMS F_1, V-63/04 CMS F_1, V-78/07 CMS F_1, V-82/07 CMS F_1) created in Tobacco Institute – Prilep.

The American variety K - 326 was used as a check. The trial was set up in the field of Tobacco Institute on colluvial soil. The first ploughing was done at a depth of 40 cm. In spring, the plots were fertilized with 300 kg/ha NPK (8:22:20) and then ploughed two more times. Before transplanting, the plots were treated with selective herbicide and healthy seedlings were planted in randomized block design with 4 replications at 90 × 50 cm planting density. Prior to second hoeing, 3 g/26 % KAN was applied for nutrition of plants. In the periods of extended drought and in growth stages when tobacco requirements for water were higher, additional irrigations were made. The plants were also treated with chemicals for their protection from pests and diseases. Harvested tobacco leaves were stringed, matured and then cured. Qualitative assessment of cured tobacco was conducted according to the Rules for unique measurement of the quality of leaf tobacco of the type Virginia (Published in the Official Gazette of Republic of Macedonija no.16/07). The obtained results were statistically processed using the method analysis of variance and tested with LSD test.

Climate characteristics

Climate and soil conditions are two very important factors that determine the yield and quality of Virginia tobacco. It is very important to have favorable climate conditions (precipitation, temperature and relative humidity) during the growing season from May to September. Data on mean monthly temperature, relative humidity and total precipitation for 2010 and 2011 are presented in Table 1.

Table 1. Meteorological data in 2010/2011 (May – September)

Meteorological factors	Year	Month					X
		May	June	July	August	September	
Mean monthly air temperature, ^{0}C	2010	15.3	18.8	21.3	23.1	15.9	18.9
	2011	13.9	18.5	21.3	21.9	19.6	19.1
Mean monthly relative air humidity, %	2010	57.0	59.0	53.0	50.0	61.0	56.0
	2011	55.0	51.0	44.0	45.0	48.0	49.0
Total rainfall, mm	2010	64.0	87.0	55.0	45.0	47.0	∑ = 298.0
	2011	63.0	51.0	17.0	11.0	38.0	∑ = 180.0

As can be seen from the Table, the mean monthly air temperature in 2010 ranged from 15.3^{0}C (May) to 23.1^{0}C (August), i.e. in average 18.9^{0}C, which is slightly lower than the optimal needs for this type of tobacco. In 2011, the temperature varied from 13.9 ^{0}C (May) 21.9^{0}C in August, or in average 19.1^{0}C. These data reveal that temperature records during the growing season were close to the lower limit of temperature that ensures normal growth and development of tobacco. According to Hawks (1978), this tobacco is best developed when night temperatures during the vegetation range between 18 and 21^{0}C and daily temperatures from 29 to 32^{0}C.The relative air humidity in 2010 varied from 50% (August) to 61% (September) and in 2011 from 44% (July) to 55% (May). These data are in close relation with the total amount of rainfall during the growing season, which in 2010 ranged from 45 mm/m^2 (August) to 87 mm/m^2 (June), (in average 298 mm/m^2), while in 2011 they were lower (in average 180 mm/m^2), ranging from 11 mm/m^2 (August) to 63 mm/m^2 (May). The above data on the amount of rainfall and relative humidity of the air and their impact on growth and development of tobacco should be taken conditionally, because in times when plants needed more water, they were abundantly watered, so the above values were significantly increased.

RESULTS AND DISCUSSION

According to Dražić (1986), tobacco yield is quantitative character that depends on the impact of genotype and interaction between the genotype and environmental conditions. Basically, however, the yield depends on the number and size of tobacco leaves per stalk. The quality of the obtained raw material, just as the yield, has a strong impact on the final economic effects. The main drivers of these two characters are middle belt leaves along with the color of dried tobacco and leaf size. Thus, variety (line) is not the main and only bearer of yield and quality. According to Beljo (1996), to obtain maximum quality and yield of a variety, there is a chain of actions that have to be carried out (proper fertilization, irrigation, time of harvest, yellowing, curing, etc.

Data on yields, quality and economic impact of the investigated varieties and lines are presented in Tables 2, 3, 4 and 5.

Corrected yield per stalk

According to the data in Table 2, the highest average yield per stalk was obtained in line V-88/09 CMS (161.6 g) and the lowest in variety Virginia SKR (113.5 g), compared to the check variety K-326 (126 g/stalk). The yield in other varieties ranged from 131.9 g/stalk in V-972 to 154.7 g/stalk in line V-82/07 CMS. Analysis of yield by years reveals that all varieties and lines (except for the check) achieved higher yields in the more humid 2010. Statistically significant difference at 1% in both years of investigation was recorded in lines V- 88/09 CMS and V-82/07 CMS, while the line V-63/04 CMS reached such significance only in 2010. Statistically significant difference at 5% was achieved only in line V-78/07 CMS in 2010. Dražić et al. (2012), studying 13 domestic and foreign varieties and lines at various locations in Serbia during 2011 concluded that the yield per stalk varied depending on the variety and location from 105 g to 257 g in Nova Pazova and from 101 g to 298 g in Starchevo. Risteski (1999) reported that variety MV-1 planted at nutritional area of 0.25 m2 gave a yield of 79.7 g/stalk and those planted at 1.0 m^2 achieved 198.2 g/stalk.

Table 2 Corrected yield g/stalk

Variety	Year	Yield g/stalk	Average 2010/2011	Difference from the average		Rank
				Absolute	Relative	
K-326 Ø	2010	123.6	126.8	/	100.00	6
	2011	130.0				
Virginia SKR	2010	116.5	113.5	-13.3	89.5	7
	2011	110.5				
V-972	2010	136.4	131.9	+5.1	104.02	5
	2011	127.4				
V-88/09 (CMS)	2010	168.0**	161.6	+34.8	127.44	1
	2011	155.2**				
V-63/04 (CMS)	2010	160.4**	149.4	+22.6	117.82	3
	2011	138.5				
V-78/07 (CMS)	2010	154.7*	144.1	+17.3	113.64	4
	2011	133.5				
V-82/07 (CMS)	2010	163.5**	154.7	+27.9	122.00	2
	2011	146.0**				

2010 LSD 5 % = 25.85 g/stalk 2011 LSD 5 % = 10.43 g/stalk
1 % = 35.45 g/stalk 1 % = 14.31 g/stalk

Yield per hectare, kg/ha

Yield per hectare is a measurable category closely related to the yield per stalk and along with quality it is very useful in making assessment of a variety and its economic value. Figures on this character in varieties and lines included in the investigation are presented in Table 3.

Table 3. Corrected yield per hectare, kg/ha

Variety	Year	Yield per hectare, kg/ha	Average 2010/2011	Difference from the average		Rank
				Absolute	Relative	
K-326 ø	2010	2748	2819	/	100.00	6
	2011	2890				
Virginia SKR	2010	2590	2523	-296	89.49	7
	2011	2457				
V-972	2010	3031	2931	+112	103.97	5
	2011	2832				
V-88/09 (CMS)	2010	3732**	3591	+772	127.38	1
	2011	3451**				
V-63/04 (CMS)	2010	3565**	3321	+502	117.80	3
	2011	3078				
V-78/07 (CMS)	2010	3437*	3187	+368	113.05	4
	2011	2938				
V-82/07 (CMS)	2010	3631**	3541	+722	125.61	2
	2011	3451**				

2010 LSD 5 % = 575 kg/ha 2011 LSD 5 % = 285 kg/ha
1 % = 789 kg/ha 1 % = 391 kg/ha

According to Table 3, the highest average yield per hectare was obtained in line V-88/09 CMS (3591 kg) and the lowest in variety Virginia SKR (2523 kg). Check variety K-326 was sixth-ranked, with an average yield of 2819 kg/ha. In other varieties and lines, the yield ranged from 2931 kg/ha in variety V-972 to 3541 kg/ha in line V-82/07 CMS. In both years of investigation, statistically significant differences at 1% level compared to the check were recorded in lines V-88/09 CMS and V-82/07 CMS, while in line V-63/04 CMS significance at 1% was achieved only in 2010. Statistically significant difference at 5% was achieved only in line V-78/07 and CMS in 2010. Devčić et al (1982) reported that, in conditions of good cultural practices, hybrid varieties H-30, H-31, H-32 can yield over 2000 kg/ha. Risteski et al. (2012) reported that Virginia tobacco line V-53 SMS F_1 created in Tobacco Institute - Prilep reached a yield of up to 3549 kg/ha.

Average price, denars/kg

Average price achieved with the investigated varieties and lines is actually an indicator of quality expressed in monetary value. The amount of the average price is closely dependent on a chain of properly performed agro-technical measures in the field during harvesting, yellowing curing, etc. Data on average price of the investigated varieties and lines are presented in Table 4.

The highest average price (67.83 denars/kg) was achieved with line V-88/09 CMS and the lowest (55.80 denars/kg) with variety V-972. The check, with an average price of 58.77 denars/kg was third-ranked variety. In other varieties and lines, this character ranges from 59.57 denars/kg in variety Virginia SKR to 64.94 denars/kg in line V-82/07 CMS. Statistically significant difference at 1% compared to the check was recorded only in 2010 in lines V-88/09 CMS, V-63/04 CMS, V-78/07 CMS and V- 82/07 CMS. In 2011 statistically significant difference at 5% was recorded only in line V-88/09 CMS. Investigations carried out in the region of Prilep with six Virginia varieties in 2002 and 2003 revealed that the highest average price was obtained with line V-53 (65.83 denars/kg) and the lowest with line V-69 (57.08 denars/kg) (Kocoska et al., 2004).

Table 4. Average price, denars/kg

Variety	Year	Average price denars/kg	Average	Difference from the average		Rank
			2010/2011	Absolute	Relative	
K-326 ø	2010	52.99	58.77	/	100.00	6
	2011	64.56				
Virginia SKR	2010	51.66	59.57	+0.80	101.36	5
	2011	67.48				
V-972	2010	51.17	55.80	-2.97	94.94	7
	2011	60.43				
V-88/09 (CMS)	2010	62.11**	67.83	+9.06	115.41	1
	2011	73.56*				
V-63/04 (CMS)	2010	55.90**	62.22	+3.45	105.87	4
	2011	68.54				
V-78/07 (CMS)	2010	57.48**	62.37	+3.60	106.12	3
	2011	67.27				

V-82/07 (CMS)	2010	59.09**	64.94	+6.17	110.49	2
	2011	70.80				

2010 LSD 5 % = 0.71 denars/kg 2011 LSD 5 % = 6.64 denars/kg
1 % = 0.97 denars/kg 1 % = 9.10 denars/kg

Gross income, denars/ha

This character is actually a summarized expression of the average price per 1 kg raw tobacco and yield per hectare achieved by the varieties and lines included in the trial.

Table 5. Gross income, denars/ha (Economic effect)

Variety	Year	Gross income denars/ha	Average	Difference from the average		Rank
			2010/2011	Absolute	Relative	
K-326 ø	2010	145 704	166 030	/	100.00	5
	2011	186 357				
Virginia SKR	2010	133 887	149 872	-16158	90.27	7
	2011	165 857				
V-972	2010	154 992	163 248	-2782	98.32	6
	2011	171 505				
V-88/09 (CMS)	2010	231 808**	242 825	+76795	146.25	1
	2011	253 843**				
V-63/04 (CMS)	2010	199 354**	205 274	+39244	123.64	3
	2011	211 195*				
V-78/07 (CMS)	2010	197 606**	197 595	+31565	119.01	4
	2011	197 584				
V-82/07 (CMS)	2010	214 497**	222 251	+56221	133.86	2
	2011	230 005**				

2010 LSD 5 % = 32 458 denars/ha 2011 LSD 5 % = 23 738 denars/ha
1 % = 44 514 denars/ha 1 % = 32 555 denars/ha

The highest gross income, according to Table 5, was obtained with line V-88/09 CMS (242,825 denars/ha) and the lowest with variety Virginia SKR (149 872 denars/ha). The check K-326 achieved 166,030 denars/ha and is a fifth-ranked variety with regard to this trait. In other varieties the gross income ranges from 163 248 denars/ha in variety V-972 to 222 251 denars/ha in line V-82/07 CMS. In the two years of investigation (2010 and 2011), statistically significant difference at 1% compared to the check was achieved only in lines V-88/09 CMS and V-82/07 CMS. Statistically significant difference at 1% was recorded only in 2010 in CMS lines V-63/04 and V-78/07. Statistically significant difference at 5% was achieved only in line V-63/04 CMS in 2011.

CONCLUSIONS

Based on the results of investigations, the following conclusions can be drawn:

- Additional irrigation is important in attaining higher yield and quality of Virginia tobacco in the region of Prilep.
- The highest yield per stalk and hectare was recorded in line V-88/09 CMS (161.6 g/stalk and 3591 kg/ha) and the lowest in the variety Virginia SKR (113.5 g/stalk and 2523 kg/ha).
- The highest average price of dry tobacco per kg was obtained in line V-88/09 CMS (67.83 denars/kg) and the lowest in variety V-972 (55.80 denars/kg).
- The highest gross income was obtained with the line V-88/09 CMS (242,825 denars/ha) and the lowest with the variety Virginia SKR (149 872 denars/ha).
- Results of the investigations reveal high influence of the genotype on qualitative and quantitative characteristics of tobacco, which can be useful in selection of varieties for production.

REFERENCES

1. Beljo J., 1996. Postupak za identifikaciju kultivara duhana. Izvješće o znanstvenom i stručnom radu u 1994 god. Vol 21, str. 55-67. Duhanski institut – Zagreb.
2. Devčić K. Triplat J., 1982. Neka svojstva novih flue-cured sorti uzgajanih u Duhanskom institutu – Zagreb – Тутун – Tobacco, XXXII, N0 11-12, pp. 5-10.
3. Dražić S., 1986. Prilog proučavanju nasledavanja kvantitativnih svojstava duvana. – Univerzitet u Beogradu - Poljopredni fakultet. Doktorska disertacija.
4. Hawks S. N., Colins W.K., 1994. Načela proizvodnje virginiskog duhana. Zagreb. (Превод)
5. Кочоска К., Ристески И., Димитриески М., Мицеска Г., 2004. Компаративни испитувања на некои новосоздадени вирџиниски сорти тутун. 60 год. Јубилеј на научна конференција с международно участие 60 год. ИТТИ, Зборник на доклади, стр.162-167, Пловдив.
6. Каламанда О., 2009. Употребна вредност на тутунската суровина тип вирџинија од производното подрачје на Република Српска – Докторска дисертација . Научен институт за тутун – Прилеп.
7. Ристески И., 1999. Корелација помеѓу хранливата површина и некои производно-технолошки својства на тутунот од сортата вирџинија МВ-1. Магистерски труд, Скопје.
8. Risteski I., Kocoska K., 2012. Results of broadleaf tobacco breeding in Scientific Tobacco Institute - Prilep International simposium for agriculture and food. XXXVII faculty-economy meeting. IV Maceronian simposium for viticulture and wine production, VII simposium for vegetable and flower production. 12-14 december 2012, Skopje, Republic of Makedonija. SECTION 5: Genetics and plant breeding, Proceedings, pp. 643-648.
9. Филипоски К., 2011. Статистички методи во земјоделските истражувања – одбрани поглавја. Научен институт за тутун – Прилеп.

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Original Scientific paper

CONTRACTION OF LEAVES BY BELTS IN SOME VARIETIES OF BASMAK TOBACCO

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ABSTRACT

The most common types of oriental tobacco in Macedonia are Prilep, Yaka and, to somewhat lower extent, Djebel. In recent years, another oriental type was included in tobacco production – the type Basmak, which bears all the characteristics of oriental tobaccos. Three-year comparative trial (2009, 2010, 2011) was conducted at the experimental field of the Scientific Tobacco Institute - Prilep with three newly created and registered Basmak tobacco varieties (MK-1, MB-2 and MB -3) and one variety of Yaka tobacco (Yaka 7-4/2), which was used as a check.
The main goal was to study the contraction of leaves in some varieties of Basmak tobacco by belts. A number of leaves in green and dry condition were measured in order to estimate the following parameters: contraction of thickness, length, width and leaf area by belts.
The results show that the contraction of leaf thickness in investigated varieties reaches the highest values in the lower belts and the lowest values in the upper belts. The percentage of contraction of leaf length in each variety is lower, while the contraction of leaf width is higher.
Compared to the check, all investigated varieties showed visible differences in contraction of leaves by belts.
Keywords: contraction, oriental tobacco, varieties, Basmak

КОНТРАКЦИЈА НА ЛИСТОВИТЕ ПО ПОЈАСИ КАЈ НЕКОИ СОРТИ ТУТУН ОД ТИПОТ БАСМАК

Од ориенталските тутуни во Р. Македонија најзастапени се типовите прилеп, јака и, во нешто помал процент, типот џебел. Последниве години во производството е воведен и ориенталскиот тип басмак, кој ги има сите карактеристики на ориенталските типови тутун. Во текот на тригодишните испитувања (2009, 2010 и 2011 година), на експерименталното поле при Научниот институт за тутун - Прилеп беше поставен компаративен опит со три новопризнати сорти од типот басмак: МК-1, МБ-2 и МБ -3 и една сорта од типот јака - Јака 7-4 / 2, која ја користевме како контрола.
Целта беше да се добие поцелосна слика за контракцијата на листовите по појаси кај некои сорти од типот басмак. Контракцијата на листовите ја добивме со мерење на одреден број на листови во зелена и во сува состојба, со цел да ги пресметаме следниве параметри: контракција на дебелината, должината, ширината и површината на листовите по појаси.
Добиените резултати покажаа дека контракцијата на дебелината на лисното ткиво кај испитуваните сорти се движи по одредена законитост и е најголема во долниот, а најмала во горниот појас. Процентот на контракцијата на листовите кај секоја испитувана сорта по должина е помал, додека по широчина е поголем. Споредено со контролата, кај испитуваните сорти се видливи разлики во контракцијата на листовите по појаси.
Клучни зборови: контракција, ориентalски тутун, сорти, басмак.

INTRODUCTION

The structure of tobacco production in Macedonia consists of oriental tobacco types Prilep, Yaka and Djebel, presented with several varieties which account for about 95% of total tobacco production. In recent years, new tobacco varieties of the type basmak were included in the production. The yield and quality of tobacco raw material obtained from Basmak varieties meet the criteria and quality standards of both manufacturers and tobacco purchase companies. The contraction of tobacco leaves during the curing process differs and depends on several factors: the intensity of water separation from tobacco, duration of tobacco curing, physiological changes that occur in the composition of tobacco leaf while it is still alive, insertion, applied agro-techniques, etc. Curing of tobacco takes place in two stages: the first one is the process of obtaining yellowish color of the leaves, which represents physiological-biochemical process that takes place in leaves which are still alive and real drying, which is physical and autolytic process that takes place in the dead leaves, so that contraction and its intensity depend on changes that occur during the curing process of raw tobacco.

MATERIAL AND METHODS

Through the three-year research (2009/2010/2011), four varieties of oriental tobacco were used as material for investigation: YK 7-4/2 as a check (ø), and Basmak varieties MK-1, MB-2, and MB-3. The tested varieties were set up in 5 replications by the the method of randomized blocks, transplanted at 45×12 cm density on previously prepared soil. The leaves were picked up manually, by belts, in the stage of technical maturity (Photo 1).

Photo 1. Green leaves

To estimate the extent of contraction in the process of curing of tobacco leaves, thickness of a number of leaves in green condition was measured, after which the thickness of leaves in dry condition was measured in μm, by belts (Photo 1,2,3,4 and 5). The obtained index shows the degree of contraction. Leaf length and width in green and dry condition were also measured and the received index of their ratio shows the degree of contraction of leaf length and width. The contraction of leaf surface was obtained by calculating the surface in green and dry condition using the coefficient 0.6354 (Tso, 1990; Naumoski, 1985).

Photo 2. Dry leaves of JK7-4/2

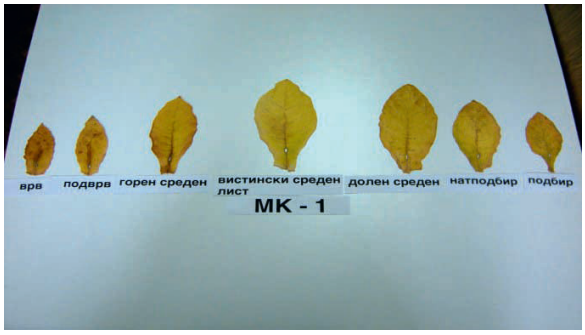

Photo 3. Dry leaves of MK-1

Photo 4. Dry leaves of MB-2

Photo 5. Dry leaves of MB-3

RESULTS AND DISCUSSION

Contraction of tobacco leaves in the process of curing can be monitored from the aspect of their length, width and the main vein. These parameters depend on several factors (Boceski, 2003; Uzunoski, 1989), but the most important factor for the contraction of tobacco leaves are: the structure of leaf tissue, leaf shape, the intensity of water separation from tobacco leaf during the curing process and the duration of tobacco curing. Kabranova (2012) reported the presence of linear contraction in tobacco leaves by their length, width and thickness. All changes are caused by the physiological activity in the cells, the colloid structure of leaf tissue and the quantity of dry matter in the leaf (Boceski, 2003). For these reasons, the changes of different intensity that occur in the process of curing depend on the type of tobacco and the leaf position on the stalk. The following measurements on the contraction of the raw material were made during investigation: contraction of thickness, length, width and surface of the leaves by belts.

Different types of tobacco, as well as various insertions of the same type, have their own specific anatomic structure of the leaf tissue. The best expressed contractions were observed in leaves with a spongy tissue, which contain higher amount of water. Smokvoski et al., (1985) reported that thickness of the lower belt leaves of Prilep tobacco was reduced through the process of curing by 80.6%, whereas that of the top leaves only 58.4%.

Data in Table 1 and Figure 1 reveal the highest thickness of leaf tissue in the lower belt leaves and the lowest thickness in leaves from the middle belt. By belts, the index of contraction of leaf thickness ranges from 18.51 (81.49%) to 38.81 (61.19%) in the upper belt of the check variety. The

highest average contraction of leaf tissue was observed in the lower belt leaves of MK-1 variety (79.32%) and the lowest in the upper belt of the check variety YK 7-4/2 (64.82%).

Table 1. Contraction of leaf thickness by belts (index)

Variety	Year	Belts		
		Lower belt	Middle belt	Upper belt
YK 7-4/2 ∅	2009	20.34	24.78	38.81
	2010	20.91	28.01	32.69
	2011	22.95	30.81	34.05
	Average	21.40	27.87	35.18
	Index	100.00	100.00	100.00
MK-1	2009	19.13	24.79	38.80
	2010	18.66	23.74	26.06
	2011	24.29	29.89	34.91
	Average	20.69	26.14	33.25
	Index	96.68	93.79	94.51
MB-2	2009	18.51	26.95	35.82
	2010	23.79	28.64	31.67
	2011	21.38	27.43	27.61
	Average	21.23	27.67	31.70
	Index	99.21	99.28	90.11
MB-3	2009	21.52	23.00	38.32
	2010	19.80	26.69	30.79
	2011	24.39	29.76	31.99
	Average	21.90	26.48	33.70
	Index	102.34	95.03	95.79

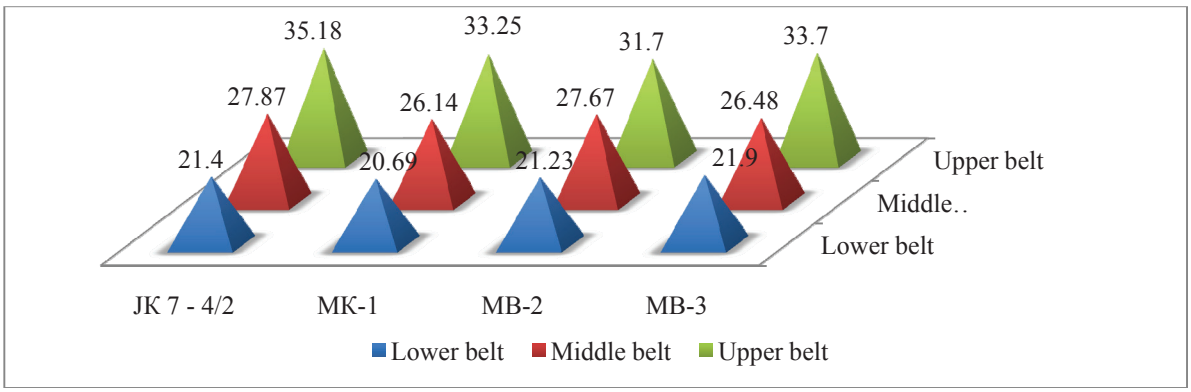

Figure 1. Contraction of leaf thickness by belts (average values)

It can be stated that there is some regularity in relation to contraction of leaves in the varieties tested. The highest contraction of leaf length was measured in the upper belt and the lowest in the middle belt in all of the investigated varieties, except for MB-3, where the lowest contraction was observed in the lower belt. According to the data presented in Table 2, the average index of contraction in leaf length by years ranges from 88.56 with contraction of 11.44% in the upper belt of the variety MK-1 up to 92.40

with contraction of 7.60% in the check variety YK 7- 4/2. The lowest contraction of leaf length was observed in the check variety YK 7-4/2, with 9.66% in the lower belt, 7.60% in the middle belt and 9.72%, in the upper belt. Basmak variety MK-1 is characterized by the highest contraction of 10.24% in the lower belt, 8.51% in the middle belt and 11.44% in the upper belt.

Table 2. Contraction of leaf length by belts (index)

Variety	Year	Belts		
		Lower belt	Middle belt	Upper belt
YK 7-4/2 ∅	2009	93.51	96.26	88.90
	2010	85.18	89.71	90.45
	2011	92.32	91.23	91.50
	Average	90.34	92.40	90.28
	Index	100.00	100.00	100.00
MK-1	2009	89.26	91.60	84.14
	2010	86.41	90.62	89.60
	2011	93.62	92.25	91.95
	Average	89.76	91.49	88.56
	Index	99.36	99.02	98.09
MB-2	2009	90.22	93.13	90.05
	2010	87.50	87.76	90.30
	2011	93.19	93.77	91.25
	Average	90.30	91.55	90.53
	Index	99.96	99.08	100.28
MB-3	2009	94.13	90.67	97.51
	2010	85.35	85.46	80.77
	2011	93.51	93.80	90.66
	Average	90.99	89.98	89.65
	Index	100.72	97.38	99.30

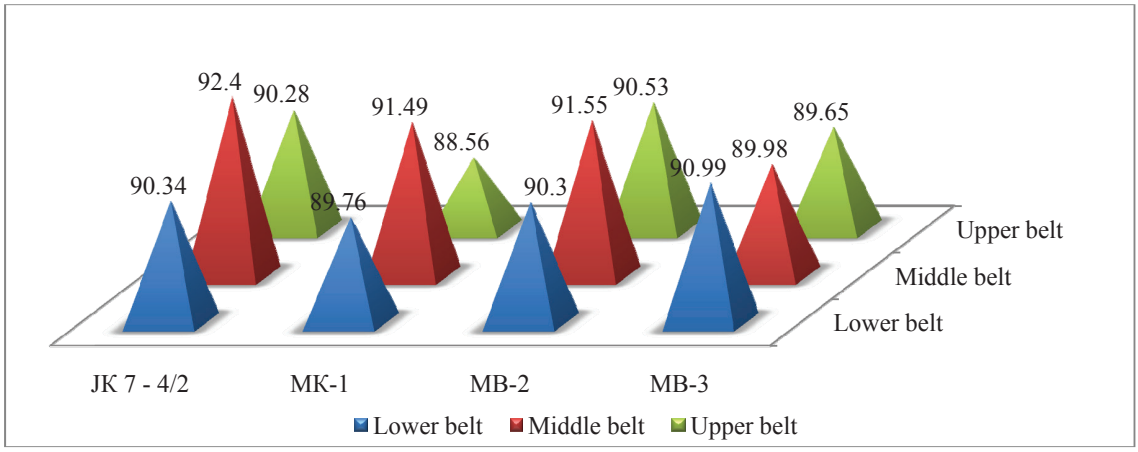

Figure 2. Contraction of leaf length by belts (average values)

According to the data presented in Table 3, the index of contraction of leaf width by belts is variable and there is no regularity by insertions. Thus, the index of contraction of leaf width ranges 91.20 i.e. contraction of 8.80% in the middle belt of the check YK 7-4/2 or 74.17 i.e. 25.83% contraction in the upper belt of the variety MK -1 in 2009. On average, the contraction by belts ranges from 11.13% in the middle belt of the variety MK-1 to 17.43% in the upper belt of the variety MB-3. The highest contraction of width was observed in the leaves from the upper belt.

Table 3. Contraction of leaf width by belts (index)

Variety	Year	Belts		
		Lower belt	Middle belt	Upper belt
YK 7-4/2 ∅	2009	89.00	91.20	82.29
	2010	79.25	86.74	86.21
	2011	84.55	83.94	88.73
	Average	84.26	87.29	85.74
	Index	100.00	100.00	100.00
MK-1	2009	84.94	87.64	74.17
	2010	83.54	89.15	83.80
	2011	88.40	89.81	90.34
	Average	85.62	88.87	82.77
	Index	101.61	101.81	96.54
MB-2	2009	83.97	86.96	88.49
	2010	82.61	84.27	88.79
	2011	88.80	94.35	88.41
	Average	85.12	88.53	88.56
	Index	101.02	101.42	103.29
MB-3	2009	88.54	86.04	85.03
	2010	82.30	82.53	77.80
	2011	87.65	90.65	84.89
	Average	86.16	86.41	82.57
	Index	102.25	98.99	96.30

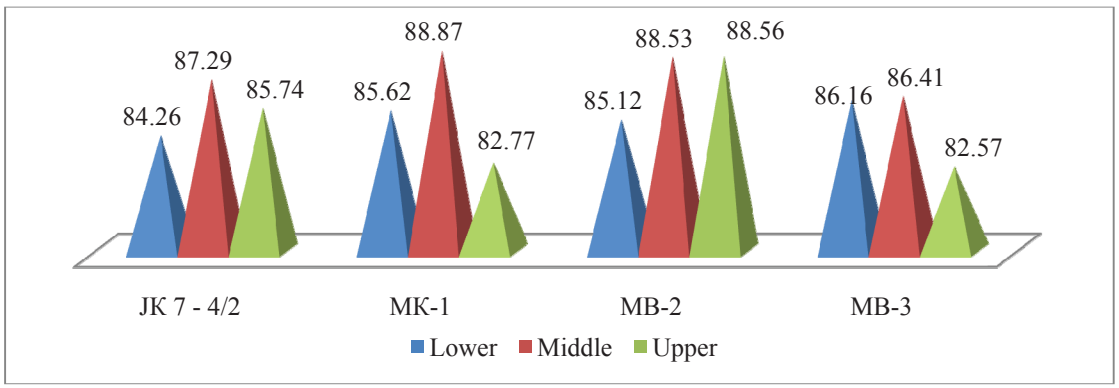

Figure 3. Contraction of leaf width by belts (average values)

The leaf surface in green and dry condition is calculated by multiplication of the length, width and coefficient 0.6354 (Tso, 1990). The contraction of leaf surface by belts is presented in Table 4 and Figure 4. The index of contraction ranges from 67.67 (32.33%) in the lower belt of the check variety YK 7-4/2 in 2010 to 87.79 (12.21%) in the middle belt of this variety in 2009. The average contraction of leaf surface in the varieties tested ranged from 26.48% in the upper belt to 18.71% in the middle belt of the variety MK-1. No regularity was found in the contraction of leaf surface by belts.

Table 4. Contraction of leaf surface by belts (index)

Variety	Year	Belts		
		Lower belt	Middle belt	Upper belt
YK 7-4/2 ∅	2009	83.22	87.79	73.16
	2010	67.67	77.75	77.97
	2011	78.06	76.71	81.20
	Average	76.31	80.75	77.44
	Index	100.00	100.00	100.00
MK-1	2009	75.72	80.21	62.42
	2010	72.22	80.82	75.08
	2011	82.76	82.84	83.06
	Average	76.90	81.29	73.52
	Index	100.77	100.67	94.94
MB-2	2009	75.79	80.97	79.77
	2010	72.25	71.07	72.35
	2011	82.74	85.91	85.99
	Average	76.93	79.31	79.37
	Index	100.81	98.22	102.49
MB-3	2009	83.34	78.03	82.94
	2010	70.30	71.03	73.37
	2011	82.00	85.07	82.53
	Average	78.55	78.04	79.61
	Index	102.94	96.64	102.80

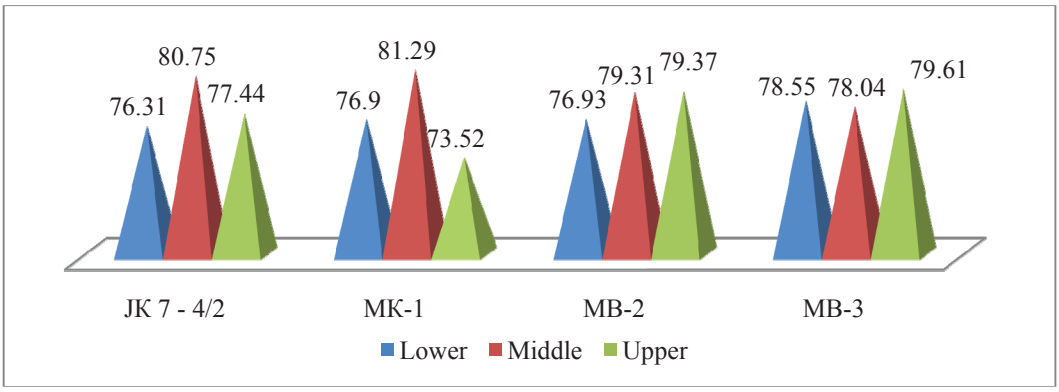

Figure 4 - Contraction of leaf surface by belts (average values)

CONCLUSION

- There is apparent contraction of leaf thickness in the tested varieties of oriental tobacco. The highest contraction was observed in leaves from the lower belts and the lowest in leaves from the upper belts. Thus, the index of contraction of leaf thickness ranges from 18.51 (81.49%) to 38.81 (61.19 %). The highest average contraction of leaf thickness was measured in the lower belt of the MK-1variety (79.32%) and the lowest in the upper belt of the check variety YK 7-4/2 (64.82%).
- The lowest contraction of leaf length was measured in the check variety YK 7-4/2, ranging from 9.66% in the lower belt, 7.60% in the middle belt and 9.72% in the upper belt. The Basmak variety MK-1 was characterized with the highest contraction - 10.24% in the lower belt, 8.51% in the middle belt and 11.44% in the upper belt.
- The index of contraction of leaf width by belts is variable and shows no regularity. It reached 91.20 (8.80%) in the middle belt of the check YK 7-4/2 and 74.17 (25.83%) in the upper belt of the variety MK -1 in 2009.
- On average, the contraction of leaf surface in the tested varieties ranged from 26.48% in the upper belt to 18.71% in the middle belt of the variety MK-1. No regularity was observed in contraction of leaf surface by belts.

The obtained results can contribute to a better knowledge of the contraction of leaves by belts in the tested varieties of Basmak tobacco.

REFERENCES

1. Боцески Д., 2003. Познавање и обработка на тутунската суровина. Институт за тутун – Прилеп, II дополнето издание, 677.
2. Кабранова Р.,2012. Влијание на начинот на производство на тутунски расад врз приносот и квалитетот на тутунот-Докторска дисертација. Факултет за земјоделски науки и храна, Универзитет Св. Кирил и Методиј Скопје. Стр.192.
3. Наумоски К., 1985. Анализа на некои квантитативни својства кај тутунот од аспект на полигено наследување. Тутун/Tobacco, Vol35, N0 7-8, 210-230, Институт за тутун - Прилеп
4. Смоквоски М., Стојкоски С.,1985. Иверзибилна контракција на тутунот од типовите прилеп и отља за време на штавата и сушењето. Тутун бр. 3 - 4. Институт за тутун – Прилеп.
5. Tso, T.C.,1990. Production, physiology, and biochemistry of tobacco plant. IDEALS, Beltsville, MD. pp. 311.
6. Узуноски М., 1989. Производство на тутун, Стопански весник, Скопје. Стр.4.

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Original Scientific paper

INCREASING THE BIOCONTROL ACTIVITY OF Trichoderma spp. WITH THE USE OF AN APPROPRIATE MANURE

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ABSTRACT

The application of a biocontrol agent Trichoderma is an effective and environmentally friendly way in tobacco seedlings protection from the damping off disease. This effect is especially expressed in its greater quantity.
Natural manures have a positive impact on the development of this agent Therefore, the aim of this study was to determine the impact of different manures on the quantitative presence of Trichoderma spp.
The biggest number of the colony units is found in a goat manure (19.21 x 10^4 cfu / g).
Covering the areas sown with tobacco seed with a combination of sheep + goat and sheep + farmyard manure has a significant positive effect. The greatest quantitative presence of Trichoderma spp. is estimated in combination sheep + goat manure (48.20 x 10^4 cfu / g soil).
The right choice of the manure has the positive effect on expression the Trichoderma's biocontrol mechanisms of and of course, increased opportunity for application of biocontrol in tobacco seedlings protection. At the same time, it is a real environmentally friendly way to produce healthy tobacco seedlings.
Keywords: manure, combination, quantity, Trichoderma spp, tobacco seedlings

ЗГОЛЕМУВАЊЕ НА БИОКОНТРОЛНАТА АКТИВНОСТ НА Trichoderma spp. СО УПОТРЕБА НА СООДВЕТНО ЃУБРЕ

Примената на биоконтролниот агенс Trichoderma е ефикасен и еколошки начин за заштита на тутунскиот расад од болеста сечење. Ваквиот ефект е особено изразен при негова поголема застапеност.
Природните ѓубриња имаат позитивно влијание врз развојот на овој агенс Затоа, целта на ова истражување беше да се утврди влијанието на различни ѓубриња врз квантитативната застапеност на Trichoderma spp.
Најголем број на формирани колонии е констатиран кај козјото ѓубре (19,21 x 10^4 cfu / g).
Покривањето на посеаните површини со тутунско семе со комбинација на овчо + козјо и овчо + кравјо ѓубре има значителен позитивен ефект. Најголема квантитативна застапеност на Trichoderma spp. е утврдена во комбинацијата овчо + козјо ѓубре (48,20 x 10^4 cfu / g почва).
Вистинскиот избор на ѓубре за покривање има позитивен ефект врз експресија на биоконтролните механизми на дејство на Trichoderma и секако, зголемена можност за примена на биолошката борба во заштитата на тутунскиот расад. Истовремено, тоа претставува вистински еколошки начин за производство на здрав тутунски расад.

Клучни зборови: ѓубре, комбинација, застапеност, Trichoderma spp, тутунски расад

INTRODUCTION

Biological control is contemporary ecological way for plant protection from diseases which avoids the problems of chemical protection (excessive use of pesticides, resistance to pathogens and negative effects on human health and environment). Therefore, it is incorporated into integrated pest management system (Monte, 2001).

Fungi of the genus *Trichoderma* are among the most famous biocontrol agents. Their effect has been confirmed in numerous pathogens and host plants.

They are present in soil and root ecosystems. Colonizing the root, they use a number of mechanisms by which attack pathogens, but also enhance the development of root and whole plant (Harman, 2004; 2006).

The most *Trichoderma* species are used in plant protection, mainly from soil pathogenic fungi and diseases of seeds, including the causing agents of seed rot, root rot and damping off in plants (Heydari and Pessarakli, 2010).

The main factor for their ecological success is the combination of a very active and efficient mechanisms of defense strategy. There are number of mechanisms involved: mycoparazitizam, antibiosi, competition for food and space, tolerance to stress by increasing root and plant development, improved solubility and adsorption of inorganic substances, induced resistance and enzyme inactivation of the pathogen (Harman, 2000; Monte , 2001).

Many species of the genus *Trichoderma* express their biocontrol effect against pathogenic fungus *Rhizoctonia solani*. *Trichoderma harzianum* is the best antagonistic fungi against to this causing agent of damping off in tobacco seedlings. Its application on a soil before sowing and several times in a growing season of tobacco seedlings has a good result in reducing the intensity of disease (Gveroska and Ziberoski, 2011). Effect is the greater in the larger quantity of a biocontrol agent (Gveroska, 2013). Handelsman and Stabb (1996) estimeted that there is a relation between the population size of biocontrol agent and the suppression degree of the pathogen, too.

Therefore, the development of a suitable technique for the mass production of biocontrol agent is required for each system of biocontrol (Heraux et al., 2005). The commercial application of antagonist *T. harzianum* need maximum production of biomass with minimal economic costs (Jahan et al., 2013).

In general, all studies concerning the use of synthetic substances like glucose, cellulose, starch, etc. According Sargin et al. (2013) solid state fermentation is an effective method for the production of biomass which provides increasing the colony number and production of conidia.

The ability to develop on inexpensive substrates makes Trichoderma isolates suitable for application as biocontrol agents. It actually provides a high degree of ecological adaptability in different environmental conditions and widespread in the world (Harman, 2006).

Many authors examined the possibility of using various "waste" agro-industrial materials (Yadav 2012; Mamo et Alemu, 2012; Duli et al., 2013). However, the use of organic amendments in the culture of the antagonist is considered a very good way to optimize control of plant pathogens through the use of strong potential of conidia producing (Hutchinson, 1999; Heraux et al., 2005; Palanna et al., 2007 , Barakat and Al-Masri, 2009).

Different types of manures influence the development of biocontrol agent *Trichoderma*, which certainly affects its activity. Therefore, the use of certain manures which have a positive effect on the number of colony forming units would have a positive impact on reducing the damping off disease in tobacco seedlings.

The purpose of this research was to determine the influence of different manures on *Trichoderma* spp. quantity.

MATERIAL AND METHODS

Experiment was carried out in a biological laboratory. Soil was prepared in the usual way. 0,3 m^2 was sowed for each variant. The biocontrol agent T. harzianum was previously prepared by sieving the fragment of the pure culture on substrate - rice, according to the method of Soares et al. (2007). Its incubation was performed at 15 days to 25^0C.

Two Erlenmeyer dishes with colony was used by each variant. It was mixed with appropriate manure or their mixture, i.e. combination. Tobacco seed from variety P79 was sown according to the usual sowing norm (0,5 g/m^2). After sowing, soil was covered with appropriate manure or combination (mixing them in an equal ratio). Sheep manure (without application of a biocontrol agent-BCA) was taken as a control, while the same, with application of Trichoderma, as standard. Investigated variants are given in Table 1.

Table 1. Investigated variants

Variants			
Ø	Sheep manure withouth BCA (Check)	5	Sheep + goat manure
1	sheep manure	6	Sheep + farmyard manure
2	goat manure	7	Sheep + poultry manure
3	farmyard manure	8	Goat + farmyard manure
4	poultry manure	9	Goat + poultry manure
		10	Farmyard + poultry manure

The first application of the biocontrol agent was taken with sowing. The second and third application were made in intervals of two weeks. In these cases, biocontrol agent was applied by drenching with suspension prepared from the pure culture.

Total number of colony forming units (cfu/g soil) of *Trichoderma* spp. was recorded 15 days after each application (three estimations).Before sowing, a sample was taken from the soil to determine the possible presence of *Trichoderma* spp.

The medium Rose Bengal Agar with an antibiotic Tetracyclin was used. An average soil sample was taken and made a series dilutions to 10^{-4}. 1ml of the final dilution (10^{-4}) was spilled in petri dishes. 20ml of PDA medium, previously sterilized and

cooled were spilled. For each variant 5 petri boxes were used. They were incubated for 10 days at 28^{0}C. After this period, number of *Trichoderma* spp. colonies was counted. It is expressed in colony forming units in gram of soil (cfu/g soil) multiplied by number of final dilution.

RESULTS AND DISCUSSION

The presence of *Trichoderma* spp. during tobacco seedling vegetation (three estimations) is shown in Table 2. It was presence of 0.3 x 10^4 colonies in the soil sample before sowing.

According to the presented results, the lowest number of Trichoderma spp. colonies at the first estimation is in the check - soil mulched only with sheep manure. This situation is noticeable in the other estimations, too. This is expected because there is no application of biocontrol agent.

Number of colony forming units in the other variants ranged from 2,60 x 10^4 in goat + poultry manure to 27,40 x10^4 in sheep + goat manure and 26,00 x10^4 in sheep + farmyard manure. The number of Trichoderma spp. colonies is the smallest in variants - sheep + poultry, farmyard + poultry and sheep manure.

The highest number of colony forming units was estimated in a combination of sheep + goat as well as in sheep + farmyard manure, followed by goat manure and goat + farmyard combination (Table 2).

Table 2. The presence of *Trichoderma* spp.

Variant	Number of colony forming units x 10^4 (cfu/g soil)		
	1st estimation	2nd estimation	3 rd estimation
Sheep manure withouth BCA (Check)	1,00	1,8	1,8
sheep manure	4,30	8,32	18,48
goat manure	12,2	18,60	19,21
farmyard manure	6,00	10,00	16,34
poultry manure	6,00	7,80	10,23
Sheep + goat manure	**27,40**	**34,60**	**48,20**
Sheep + farmyard manure	**26,00**	**32,44**	**39,62**
Sheep + poultry manure	2,80	7,84	12,63
Goat + farmyard manure	10,60	**24,63**	**28,30**
Goat + poultry manure	2,60	**19,82**	22,67
Farmyard + poultry manure	4,20	16,45	21,40

When considering the results in variants with mulching only with one manure (variants 1-4) (Figure 1), the number of colonies is the largest in soil with goat manure. It certainly influenced on the increasing quantity in combination with sheep, farmyard and poultry manure (Fig 2). The same situation is especially noticeable in the second and third estimation.

Figure 1. Number of colony forming units in soil mulched with different manures

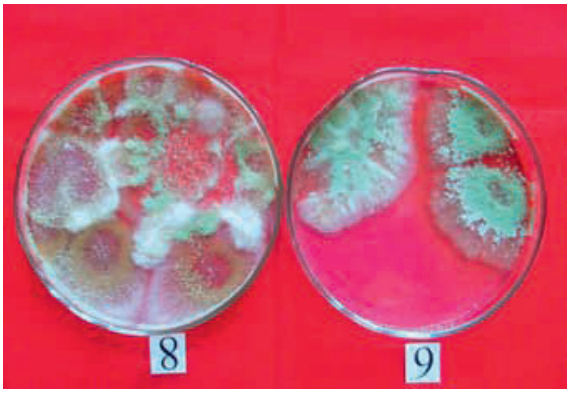

Figure 2. Number of colony forming units in variants goats +farmyard and goat +poultry manure

The number of *Trichoderma* spp. colonies at the second estimation is ranged from 7,80 x10^4 in poultry (and 7,84 x10^4 in sheep + poultry manure) to 34,60 and 32,44 x10^4 in the same variants as in the previous estimation - sheep + goat manure and sheep + farmyard manure.

In all variants, in this estimation there is an increased population of *Trichoderma* than in the first, which is a result of its re-application and the physiological activity of the fungus.

At the third estimation, the quantity of colony forming units is not so distinquished than the previous estimation. There is the highest quantity in the same variants (number 5 and 6). There are noticeable increasing the cfu/g soil in goat +farmyard manure and goat + poultry manure, too. In mulching only with one manure, there is the highest increasing of cgu/g soil in sheep manure, from the lowest value in the first to the second place, in the third estimation. In the sheep manure, the quantity is geting satisfuing value (Table 2).

There is an increasing the quantity of the biocontrol agent over the vegetative period in variants with its application (Table 2). According to Jayalakshmi et al. (2009), Trichoderma spp. are able to use a wide range of substances as a source of carbon or nitrogen and release amount of enzymes which decomposes plant polymers into simple sugars for energy and growth. It allows them utilizing the manures and intensification of development.

According the results, the highest number of colony forming units during the vegetative period was estimated in a combination of sheep + goat as well as in sheep + farmyard manure (Figure 3).

It may be noted that the number of colonies

is the largest when sheep manure is amended, i.e. combined with goat and farmyard manure (Figure 4). Our results are in agreement with the results of Palanna et al., 2007, which examined the effect of five manures and their combinations on *T. viride*. The combination of farmyard + goat has the best effect in increasing the dry mass of the colony, sporulation and fungicidal activity (against *Machropomina phaseolina*). In the case of individual manure, the best effect was noted in farmyard, followed by goat and poultry manure.

In the investigations of Pramodkumar and Palakshappa (2009), the maximum growth (colony diameter and dry biomass) was founded in sterilized farmyard manure. In our investigations, it shows a possitive effect in increasing cfu/g soil, when it is combined with others manures (Table 2, Figure 5).

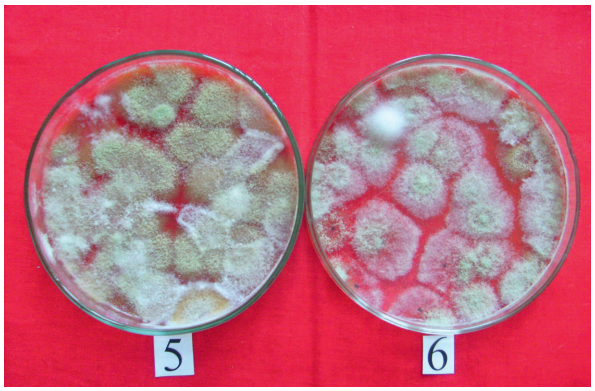

Figure 3.
Number of colony forming units in variants sheep+goat and sheep+farmyard manure

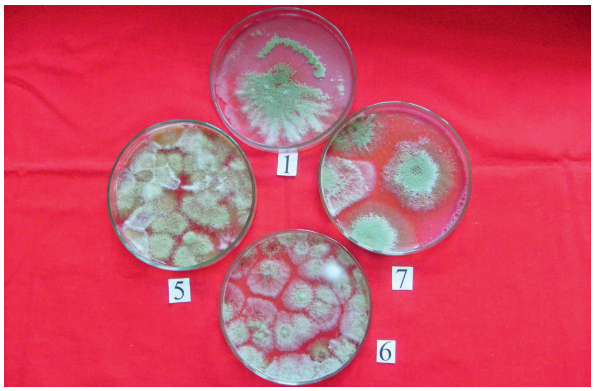

Figure 4.
Number of colony forming units in sheep manure and its combination with other manures

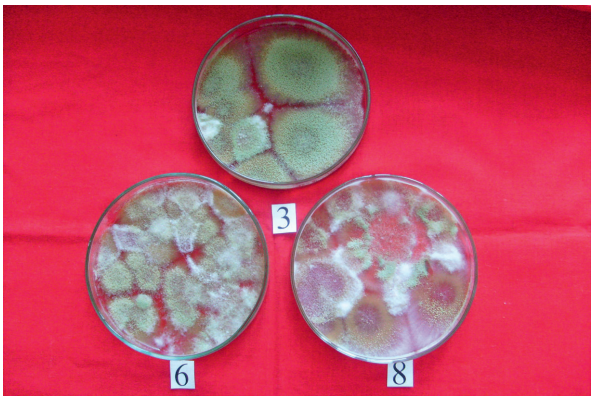

Figure 5.
Number of colony forming units in farmyard manure and its combination

The highest values of cfu/g soil are estimated in combinations sheep + goat and sheep + farmyard manure, as well as goat + farmyard. In combination sheep+poultry manure there was not a positive effect on *Trichoderma* quantity (Figure 4).

In studies of Hutchinson (1999), composted poultry manure inoculated with T.virens can be used as an economical substrate for application of this biocontrol agent in control of weeds. But, in our study, there is not a signifficant result in the application of this manure alone or in combination with others (Table 2).

Quantitative presence of *Trichoderma* spp. during the vegetative period of tobacco seedlings are growing up. There was a low value in the sheep manure at start, but at the end of the vegetation we have a good quantity compared with other varieties. These results are in agreement with those of Barakat and Al-Masri (2009) who stated that the number of *T. harzianum* (10^6 cfu g- 1) significantly increased over time and concentration of organic amendment - sheep manure. It is certainly not disparaging result because increased quantity of *Trichoderma* can be an advantage in rooting of plants during transplantation.

According to Pallana et al., (2007) the use of fertilizers to increase its development is necessary to increase the biocontrol ability of a biocontrol agent. Therefore, the results from these studies filled out an aim - to optimize the control of tobacco seedling from diseases through the application of the biocontrol agent with manure which gives the best effect on increasing its numbers.

CONCLUSIONS

- All investigated manures have a positive effect on increasing the quantitative presence of Trichoderma spp. in soil.
- In the standard way of mulching in tobacco seedbeds, there was a greater increase in the number of biocontrol agent in the third estimation.
- In the case of single manure, there is the highest number of colonies in goat manure.
- Significantly positive effect on increasing the quantity of the biocontrol agent has the mulching of tobacco seed with combinations of sheep + goat and sheep + farmyard manure.
- Increased number of colony forming units has the positive impact on expression of the mechanisms of action in fungi of the genus Trichoderma, which means increased biocontrol activity in plant protection from diseases.
- The application of biocontrol agent in mulching of tobacco seedbeds with manure who gives the greatest effect on increasing its quantity means optimizing the control of tobacco seedlings diseases.
- The real choice of manure also means a bigger possibility for the use of biocontrol in tobacco protection from diseases.
- This way of application the biocontrol agent and use the biocontrol is an ecological way for producin the healtjy tobacco seedlings.

REFERENCES

1. Barakat R.M., Al-Masri M., 2009. Trichoderma harzianum in combination with sheep manure amandment enhances soil suppressiveness of Fusarium wilt of tomato. Phytopathol. Mediterr., 48, pp. 385-395.
2. Duli H.F., Khdiar Y.M., Al-Janaby L.J., 2013. Use Agro-Waste as a culture media for Sporulation and conidia Production of Trichoderma harzianum. Indian Journal of Applied Research, Volume 3, Issue 8, pp. 28-30.
3. Gveroska and Ziberoski, 2011 Gveroska B., Ziberoski J., 2011. The influence of Trichoderma harzianum on reducing root rot disease in tobacco seedlings caused by Rhizoctonia solani. International Journal of Pure and Applied Sciences and Technology. Vol 2 No2, February 2011., pp. 1-11 India.
4. Gveroska B., 2013. Relationships of Trichoderma spp. quantity in soil to reducing the damping off in tobacco seedlings. Bulg. J. Agric. Sci., 19: 666-674, Sofia, Bulgaria.
5. Handelsman J., Stabb E.V. (1996). Biocontrol of Soilborne Plant Pathohens. The Plant Cell, 8, 1855-1869.
6. Harman G.E., 6owell C.R., Viterbo A., Chet I., Lorito M., 2004. Trichoderma species – opportunistic, avirulent plant symbionts. Nature Review Microbiology, 2: 43-56.
7. Harman G., 2006. Overview of Mechanisms and Uses of Trichoderma spp. Phytopathology, 96: 190-194.
8. Heraux F.MG., Hallet S.G., Ragothama K.G., Weller S.C., 2005. Composted Chicken Manure as a Medium for the Production and Delivery of Trichoderma virens for Weed Control. Hort Science, 40 (5): 1394-1397.
9. Heydari A., Pessarakli M.,2010. A Review on Biological Control of Fungal Plant Pathogens Using Microbial Antagonists. Journal of Biological Sciences, 10: 273-290.
10. Hutchinson C.M., 1999. Trichoderma virens- Inoculated Composted Chicken Manure for Biological Weed Control. Biological Control, Volume 16, Issue 2, pp. 217-222.
11. Jahan N., Sultana S., Adhikary S.K., Rahman S., Yasmin S., 2013. Evaluation Of The Growth Performance Of Trichoderma harzianum (Rifai.) On Different Culture Media. IOSR Jpurnal of Agriculture and Veterinary Science (IOSR-JAVS). Volume 3, Ossue 4, pp. 44-50.
12. Jayalakshmi S.K., Raju S., Benagi V.I., Sreeramulu K., 2009. Trichoderma harzianum. L1 as a potential source for lytic enzymes and elicitor of defense responses in chikpea (Cicer arietinum L.) against wilt disease caused by Fusarium oxysporum f.sp. ciceri. Austarlian Journal of Crop Science, 3 (1):44-52.
13. Mamo Z., Alemu T., 2012. Evaluation and optimization of agro-industrial wastes for conidial production of Trichoderma isolates under solid state fermentation. Journal of Applied Biosciences, 54: 3870-3879:
14. Monte E., 2001 Understanding Trichoderma: between biotechnology and microbial ecology. Int. Microbiol., 4: 1-4.
15. Pallana K., Palaiah P., Muthamilan M., 2007. Effect of Manures on Growth, Sporulation and Antifungal Activity of Trichoderma viride. Karnataka J. Agric. Sci. 20 (4): 861-863.
16. Pramodkumar T., Palakshappa M.G., 2009. Evaluation of suitable substrates for on farm production of antagonist Trichoderma harzianum. Karnataka J. Agric. Sci. 22 (1): 115-117.
17. Sargin S., Gezgin Y., Eltem R., Vardar F., 2013. Micropropagule production from Trichoderma harzianum EGE-K38 using solid-state fermentation and a comparative study for drying methods. Turkish Journal of Biology, 37: 139-146.
18. Soares A.C.F., Sousa C.S., Garrido M.S., Perez J.O. (2007): Production of streptomycete inoculum in sterilized rice. Sci. Agric. (Piracicaba, Braz.), 64: 641-644.
19. Yadav S.L., 2012. Antagonistic activity of Trichoderma sp. and evaluation of various agro wastes for mass production. Indian Journal of Plant Sciences, Vol. 1 (1), pp.109-112.

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Original Scientific paper

PHYTOPATHOLOGICAL ASSESSMENT AND SCREENINIG FOR RESISTANCE TO TMV OF VARIETIES AND NEW LINES BURLEY TOBACCO

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ABSTRACT

During the period 2008-2015, is conducted immunological study of reaction to TMV eleven introduced and five bulgarian varieties of varietal group Burley tobacco, appearing output parents of 24 perspective lines tobacco of the same varietal group. Phytopathological assessment is carried out of reaction to virus lines tobacco at different stages of the selection process. The percentage of development of TMV is tracked on genotypes tested. From the results it is seen that under the particular test conditions and the strain composition of the virus, which works immune to TMV of the tested varieties and lines are not established. Phytopathological evaluation of 16 varieties from the group Burley of tobacco showed that resistant to TMV are the majority of genotypes, only 2 of them are susceptible to the virus. Of the tested 24 lines Burley tobacco sensitive to TMV are L.ine 1383, Line 1485 and Line 1488. All other 17 lines have resistance to tobacco mosaic virus. Creating of rich selection material resistant to TMV is success in breeding of Burley tobacco. It is found inconsistency in our results for reaction to TMV of Tennessee 86 variety and the data in the literature.

Keywords: Burley tobacco, phytopathological assessment, new created lines, TMV

ФИТОПАТОЛОШКА ПРОЦЕНА И СЛЕДЕЊЕ НА ОТПОРНОСТА КОН TMV КАЈ НЕКОИ СОРТИ И НОВИ ЛИНИИ ОД ТИПОТ БЕРЛЕЈ

Во периодот 2008-2015 година е спроведена имунолошка студија за реакцијата кон TMV на 11 интродуцирани и 5 бугарски сорти тутун од типот берлеј кои се јавуваат како родители на 24 перспективни линии од овој тип тутун. Извршена е фитопатолошка процена на реакцијата кон вирусот кај некои линии тутун во различни фази на селекциониот процес и следен е процентот на развој на TMV кај испитуваните генотипови. Од добиените резултати, како и од соевиот состав на вирусот, се гледа дека во конкретните услови на испитување ниту една од испитуваните сорти и линии не е имуна кон TMV. Според фитопатолошката оценка на 16 сорти тутун од типот берлеј, поголемиот дел од генотиповите се отпорни на TMV, а само 2 се осетливи на овој вирус. Од испитуваните 24 линии берлејски тутун, Л. 1383, Л. 1485 и Л. 1488 се осетливи на TMV, додека останатите 17 линии поседуваат отпорност кон вирусот. Создавањето на богат селекционен материјал отпорен на TMV е потврда за нашиот успех во селекцијата на типот берлеј. Нашите резултати во врска со реакцијата на сортата Tennessee 86 кон TMV не се совпаѓаат со податоците дадени во литературата.

Клучни зборови: тутун Берлеј, фитопатолошка процена, новосоздадени линии, TMV

INTRODUCTION

Viral diseases are serious problem in tobacco production, causing significant losses to tobacco crop. These losses often make tobacco cultivation unprofitable and sometimes impossible. Currently about 1000 plant viruses are known and most of them have a broad host range.

Plant viruses are one of the most important pathogens of major economic importance. They lead to serious economic losses in many agricultural crops including tobacco, by reducing their yield and quality (Maiss, 2004). Pesticides and herbicides are used in their control, destroying natural reservoirs and vectors of plant viruses. The application of chemical agents is a risk to human health and the environment. To minimize this risk it is necessary to use alternative methods in the control of virus diseases.

The selection method, expressed in creation and use of resistant varieties, is still the cheapest and safest way of reducing losses, which results in ecologically clean production and no environment pollution. (Stoimenova, 2009). To create resistant varieties of tobacco it is necessary to know the reaction of the selection material to the specific economically important pathogens (Bozukov, 2013). Of particular interest are the genotypes carriers of genes for resistance to many diseases - Tobacco mosaic cause (TMV), Potato virus Y (PVY), Tobacco etch virus (TEV) and Tobacco vein mottling virus (TVMV), which occur in complex or alone (Jonchev, 2014), Cucumber mosaic virus (CMV), etc. (Drumeva, Chinchev, 2006).

Important factor in the decision which genes for resistance are suitable for inclusion in plants breeding programs is their stability to high plasticity of the viruses. Factors influencing this stability can hardly be predicted, because there are always new strains of viruses able to overcome the resistance of the varieties used in mass production (Garcia-Arenal & McDonald, 2003; Lecoq, et al., 2004; van den Bosch, et al., 2006).

MATERIAL AND METHODS

During the period 2008-2015, is conducted immunological studies of reaction to TMV eleven introduced and five Bulgarian varieties of group Burley tobacco, appearing output parents of 24 perspective lines tobacco of the same varietal group, created in TTPI (Dyulgerski, 2011). Phytopathological assessment is carried out of response to virus lines tobacco at different stages of the selection process. Percentage of development of TMV on genotypes tested is tracked (Table 1 and Table 2).

Table 1**. Varieties of varietal group Burley tobacco and years of assessment**

Varieties	Origin	Year of assessment
Kentucky 17	USA	2011
Kentucky 907	USA	2011
Kentucky 908	USA	2011
Tennessee 86	USA	2011
Tennessee 90	USA	2011
Coker 46	USA	2011
Banquet 102	USA	2011
Burley NSZ	USA	2011
Burley 21	USA	2011
Burley 64	USA	2011
Burley E 531	Serbia	2011
Burley № 1	Bulgaria	2011
Burley 2115	Bulgaria	2011
Burley 1000	Bulgaria	2011
Burley 1317	Bulgaria	2011
Burley 1344	Bulgaria	2011

The resistance is accounted of 16 varieties of tobacco and 24 lines Burley tobacco to ordinary tobacco mosaic of natural infective background and artificial infection with TMV. Observations for spreading diseases to natural infection background respectively in various varieties and lines. lines are conducted 2008-2015, while inoculation is carried out in different years in the period 2008-2014.

Table **2. Lines of varietal group Burley tobacco and years of assessment**

Lines/selection formula	Years of assessment	
	Natural infection background	Artifical infection background
Line 1145 - (Burley 64 x Burley 21)	2011-2015	2014
Line 1231 - [Burley 21 x Burley 21 x Burley 2115)]	2008-2011	2008-2009
Line 1322 - (Banquet 102 x Tennessee 86)	2011-2015	2014
Line 1334 - [(Tennessee 86 x B 21) x B 21 x B2115)]	2011-2015	2014
Line 1349 - Burley 1000 x Burley №1)	2008-2011	2009-2010
Line 1354 - [(Burley 64 x Burley 21) x B 1000]	2008-2011	2008-2009
Line я 1383 - [(B 64 x B 21) x (Tennessee 86 x BNSZ)]	2011-2015	2014
Line я 1390 - (Burley 21 x Burley 2115)	2011-2015	2012-2013

Line 1393 - (Burley 1317 x Burley 21)	2008-2011	2009-2010
Line я 1399 - (Burley 1317 x Tennessee 90)	2008-2011	2008-2009
Line я 1409 - [(B 21x (Banquet 102 x Tennessee 86)]	2008-2011	2009-2010
Line я 1421 - (BM x Burley 1344)	2008-2011	2009-2010
Line 1435 - (Line 1334 x T Tennessee 86)	2008-2011	2008-2009
Line 1467- (Burley 1344 x Burley 1317)	2010-2014	2012-2013
Line 1471A - (Burley 1344 x Burley 1317)	2010-2014	2011-2012
Line 1472 - (Burley 1344 x Tennessee 90)	2010-2014	2012-2013
Line 1473 - (Burley 1317 x Kentucky 907)	2010-2014	2011-2012
Line 1475 -(Burley 1317 x Kentucky 908)	2010-2014	2012-2013
Line 1477 - (Burley 1317 x Coker 46)	2010-2014	2011-2012
Line 1478 - (Burley 1317 x Tennessee 90)	2008-2011	2011-2012
Line 1485 - (Line 1189 x Burley 1317)	2010-2013	2010-2011
Line 1493-(Kentucky 908 x Б 1317)	2010-2014	2011-2012
Line 1488 - (E 531 x Burley 1344)	2010-2013	2010-2011
Line 1500- F8 [Kentucky 908 x (B 21 x Burley 2115)]	2010-2014	2012-2014

For artificial inoculation strain used TMV-TGM. The strain is reproduced in N. tabacum cv. Samsun N'N ', in order to prevent contamination of TMV with ToMV (Tomato mosaic virus) (Stoimenova and Yordanova, 2005). After which the virus is a purified by the method of Gooding and Heber, 1967. The infection is carried out by mechanical means, such as the leaves are dusted with carborundum 600 meshes and rubbed with an inoculum of virus concentration of 5 μg / ml. The preparation of the strain TMV-TGM with a concentration of 1 mg / ml was diluted with 0.1 M sodium-potassium phosphate buffer pH 7.

Testing of plants for resistance to the virus is carried out in the morning hours of the day at a temperature of 18-20 ° C. Plants in phase 4-5 leaf infested by rub in two opposite well-formed sheet, marked on top. The inoculum was applied over the entire surface of the leaf, taking care not to damage the main nerve and tissue below the epidermis. Immediately after inoculation the leaves are washed with water and shade under wet conditions for 24 hours before and after inoculation. The plants are inoculated with TMV once. Symptoms of inoculated leaves are reported to the 3rd, 5th and 10th days after infection, systemic symptoms in young leaves of 10th, 14th and 21st days after infection.

The identification of the ordinary tobacco mosaic on tobacco genotypes tested under natural conditions of infection is carried out by visual inspection of routing areas, based on typical TMV symptoms. For proof of TMV under field conditions is worked with tracer method.

Cucumber Mosaic Virus CMV and TMV tobamoviruses and ToMV cause the occurrence of similar symptoms in infected tobacco plants, which is why bioassay test isolates using key indicator plants. Separation of CMV and tobamoviruses are inoculated with the sample plant N. tabacum cv. Samsun NN, who responds with a hypersensitive reaction to infection tobamoviruses and mosaic - CMV. In some cases, instead of N. tabacum cv. Samsun NN, used Nevrokop 1146 and Nicotiana glutinosa, having the same genes for resistance tobamoviruses. The identification of TMV and ToMV is carried out by infecting plants of N. tabacum cv. Samsun N'N ', which react to ToMV a hypersensitive response and mosaic TMV.

RESULTS AND DISCUSSION

Of the tested by us tobacco varieties resistant to TMV are the majority of genotypes, only 2 of them are susceptible to the virus (Table 3). Corresponding data from literature sources are identical to those obtained by us. Exception is the results for the American Tenneessee 86 variety where literature data, the variety is susceptible to TMV (Miller, 1987)

The used by us a sample of Tennessee 86 variety is resistant to the virus. To resolve the existing conflict is conducted three times testing. In inoculated with TMV leaves of all plants develop necrotic lesions (HR) and no systemic spread of the virus (mosaic), i.e. the population of the variety that testing is resistant to TMV (Yonchev, 2014).

The results of inoculation with TMV of prospective lines of varietal group Burley tobacco are presented in Table 4. The lines sensitive to the virus are Line 1383, Line 1485 and Line 1488. All other 17 lines Burley tobacco possesses resistance virus ordinary tobacco mosaic. Persistent viral parents who are donors of this quality are set out in the relevant column of Table 4.Lines created with the participation of the tested varieties of tobacco are tested for resistance to tobacco mosaic simple and natural infective background. Although the observations of the lines are at least 3 years old, it is evidence of sustainability are not clear due to the low pressure of infective virus. Therefore lines reacted with sensitivity to TMV in artificial contamination of natural infective background showed no signs of ordinary tobacco mosaic.

Table 3. Assessment of varieties of tobacco from the group Burley natural infective background (tobacco mosaic) and artificial infection with TMV.

Сорт	TMV		
	*Natural infection background	Artifical infection background	Literature data
Kentucky 17	0	R	R
Kentucky 907	0	R	R
Kentucky 908	0	R	R
Tennessee 86	0	R	S
Tennessee 90	0	R	R
Coker 46	-	R	-
Banquet 102	0	R	-
Burley NSZ	0	S	-
Burley 21	0	R	R
Burley 64	0	R	R
Burley E 531	-	S	-
Burley № 1	-	R	-
Burley 2115	-	R	-
Burley 1000	0	R	R
Burley 1317	0	R	-
Burley 1344	0	R	-

* Natural infection background - % of infected plants

R - no systemic spread of the virus in inoculated leaf form necrotic spots

S – virus spreads systemically in the plant appear like typical TMV symptoms.

Table 4. Immunological assessment of perspective lines of tobacco group Burley after TMV-infection

Lines	* Background	**Assesment	***Variety
Line 1145	0	R	Burley 64, Burley 21
Line 1231	0	R	Burley 21, Burley 2115
Line 1322	0	R	Banquet 102, Tennesse 86
Line 1334	0	R	Burley 21, Burley 2115, TN 86
Line 1349	0	R	Burley 1000, Burley № 1
Line 1354	0	R	Burley 1000, Burley 21, Burley 64
Line 1383	3.5	S	-
Line 1390	0	R	Burley 1317, Burley 1000
Line 1393	0	R	Burley 1317, Burley 21
Line 1399	0	R	Burley 1317, Burley 21
Line 1409	0	R	Burley 21, Banquet 102, TN 86
Line 1421	0	R	Burley 1344
Line 1435	0	R	Line 1334, Tennesse86
Line 1467	0	R	Line 1390, Kentucky 908
Line 1471A	0	R	Burley 1344, Burley 1317
Line 1472	0	R	Burley 1344, Tennesse 90
Line 1473	0	R	Burley 1317, Kentucky 907
Line 1475	0	R	Burley 1317, Kentucky 908
Line 1477	0	R	Burley 1317, Coker 46
Line 1478	0	R	Burley 1317, Tn 90
Line 1485	0	S	-
Line 1488	0	S	-
Line 1493	0	R	Kentucky 908, Burley 1317
Line 1500	0	R	Ky 908, Burley 21, Burley 2115

* Natural infection background - % of infected plants
**Assessment of resistance after infection with TMV
*** Variety - parental form of which is inherited respective resistance

CONCLUSION

In specific conditions of the experience and the strain composition of the virus, which works immune to TMV of the tested varieties and lines are not established.

Phytopathological assessment of sixteen varieties of Burley tobacco showed that resistant to TMV are the majority of genotypes, only two of them are susceptible to the virus.

Established is inconsistency in our results for the reaction to TMV of Tennessee 86 variety and the data in literature.

Of the tested twenty-four lines Burley tobacco sensitive of TMV are Line 1383, Line 1485 and Line 1488. All other seventeen lines tobacco possesses resistance to TMV

Created is rich selection material Burley tobacco resistant to TMV.

REFERENCES

1. Bozukov H. 2013. Phytopathological assessment of varieties of tobacco. Jubilee International Scientific and Practical Conference “Bulgaria regions” VUARR, pp. 111-112
2. Garcia-Arenal, F. B. A. McDonald. 2003. An analysis of the durability of resistance to plant viruses. Phytopathology, 93, 941-952.
3. Drumeva M. Chinchev B., 2006. Save, use and enrich the gene pool in Virginia tobacco. Bulgarian tobacco 4: 15-17
4. Dyulgerski Y., 2011. Selection and genetic research of Burley tobacco, Thesis, Plovdiv.
5. Lecoq, H., B. Moury, C. Desbiez, A. Palloix, M. Pitrat. 2004. Durable virus resistance in plants through conventional approaches: A challenge. Virus Research, 100, 31-39.
6. Maiss, E., 2004. Recombination and spatial separation of potyviruses in transgenic plants and in mixed infections, The 8th International Symposium on the Biosafety of Genetically Modified Organisms, Montpellier, France, 26-30 pp. 39-41.
7. Miller R., 1987. TN 86: A Burley Tobacco Resistant to TVMV, TEV and PVY. The University of Tennessee Experiment Station Bulletin Number 657.
8. Stoimenova E. and Yordanova A., 2005. Vaccine strain of tomato mosaic virus obtained after serial passages of attenuated strain of tobacco mosaic virus in tolerant to tobamoviruses tomato, J. Culture Collections, 4: 68-77.
9. Stoimenova E., 2009. Genetics of resistance to viral diseases. Agricultural Science, 42: 3-17.
10. Van den Bosch, F., G. Akudibilah, S. Seal, M. Jeger. 2006. Host resistance and the evolutionary response of plant viruses. J. Applied Ecology, 43, 506-516
11. Yonchev J., 2014, Study of the distribution of some viral diseases and the respective resistance in Virginia and Burley tobacco in South Bulgaria, Dissertation.

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Original Scientific paper

HELICOVERPA ARMIGERA ON TOBACCO

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ABSTRACT

Helicoverpa armigera Hubner is an economically important agricultural pest well adapted in tobacco areas. The larvae feed on reproductive organs of tobacco plants causing severe damages on tobacco seed.

The population of H. armigera was visual investigated throughout the summer and beginning of autumn, 2015/2016 in tobacco field in Prilep, with application of the method check of 100 tobacco stalks. The investigations of tobacco stalks attacked by H. armigera larvae are made in three replications in middle/margin plots. Field and laboratory trials were carried out for control of H. armigera, with four insecticides: Chlorcyrin 550 EC (a.i chlorpyrifos 500 g/l + cypermethrin 50 g/l) 1.5 L/ha; Alverde (a.i metaflumizone 240 g/l) 1 L/ha; Affirm (a.i emamectin 9.5 g/kg) 2 kg/ha and Belt 24 WG (a.i flubendiamide 480g/L) 25 g/100L.

Population dynamics of H. armigera shows that the species is present on tobacco from the beginning of July until the end of tobacco vegetation. It was observed that larval densities on tobacco plants were significantly higher at the margins of the fields than in the centre.

Plants should be checked regularly for eggs or young caterpillars. All insecticide applications are really effective when larvae are small. Larvae during L5 and L6 consume about half of their overall diet. They burrow into the seed capsules where they become less accessible to insecticides.

According filed trials and laboratory investigations during 2015/2016, all applied insecticides showed high effectiveness in H. armigera control. The highest average mortality of 100% was caused by Affirm.

Direct damage of the larvae on flowers, seed capsules and top leaves resulted in low tobacco seed yield. Chemical control is still the most reliable and economic way of protecting tobacco crop from H. armigera.

Keywords: tobacco, H. armigera, population, chemical control

HELICOVERPA ARMIGERA НА ТУТУНОТ

Helicoverpa armigera Hubner е економски значаен земјоделски штетен вид, кој е добро прилагоден на тутунот. Ларвите се хранат на репродуктивните органи на тутунот и предизвикуваат сериозни оштетувања на семето од тутунот.

Популацијата на H. armigera беше визуелно проучувана во текот на летото и почетокот на есента, 2015/2016, на тутунот во Прилепско, со примена на методот преглед на 100 тутунски стракови. Набљудувањата на бројот на тутунските растенија нападнати од ларвите од H. armigera се направени во три повторувања, во средината на парцелите и во маргината. Полските и лабораториски испитувања беа спроведени за контрола на H. armigera, со четири инсектициди: Chlorcyrin 550 EC (a.i chlorpyrifos 500 g/l + cypermethrin 50 g/l) 1.5 L/ha; Alverde (a.i metaflumizone 240 g/l) 1 L/ha; Affirm (a.i emamectin 9.5 g/kg) 2 kg/ha и Belt 24 WG (a.i flubendiamide 480g/L) 25 g/100L.

Според популационата динамика на H. armigera, видот е присутен на тутунот од почетокот на јули до крајот на вегетацијата. Може да се констатира дека ларвите се повеќе застапени на маргините на полиња отколку во центарот.

Растенија треба да се проверува редовно за јајца или млади гасеници. Сите инсектициди се ефикасни кога ларвите се мали. Ларви во L5 и L6 ја јадат околу половина од нивната севкупна храна. Тие влегуваат во

семенските чушки и стануваат помалку достапни за инсектицидите.
Според полските и лабораториските испитувања во текот на 2015/2016, сите применети инсектициди покажаа висока ефикасност во контролата на H. armigera. Највисока ефикасност, со морталитет од 100% беше утврдена кај Affirm.
Директната штета на ларвите на цветовите, семенските чушки и врвните листови резултира со низок принос на тутунско семе. Хемиска контрола се уште е сигурен и економичен начин за заштита на тутунот од H armigera.
Клучни зборови: тутун, H. armigera, популациона застапеност, хемиска контрола

INTRODUCTION

H. armigera is an agricultural, polyphagous pest of worldwide significance. This species belongs in order Lepidoptera, suborder Macrolepidoptera, family Noctuidae, subfamily Melicleptrine, genus Helicoverpa, species armigera.
It is an important pest of many crops in many parts of the world and is reported to attack more than 60 plant species belonging to more than 47 families (such as soybean, cotton, sorghum, maize, sunflower, tomato, green pepper and ect.). Also a wide range of wild plant species support larval development [3, 4, 5, 9, 10].
For management of H. armigera greater attention should pay on biological parameters of this pest, including adult movement. This migratory species is highly adaptable and with great capacity for flight [6, 11, 12]. This noctuid specie may easy adapt to seasonal changes and survive in different habitats and went to facultative diapause [15, 16, 17].
Light and feromone traps are used in ecological studies of Helicoverpa armigera. For many years, light traps have been used to monitor Helicoverpa moth populations. Pheromone traps are highly efficient to detect emergence of males [12, 13, 14].
Larvae H. armigera prefer reproductive tobacco tissue. In laboratory conditions (25 °C) eggs hatch in about three days, larvae develop over 19-24 days, pupae over 11-20 days and longevity of adults is about 3 weeks. (7)
Many studies were conducted for investigation of resistance of H. armigera against insecticides [1, 2, 7, 8]. The pest has developed high levels of resistance to conventional insecticides such as synthetic pyrethroids, organophosphates and carbamates.
Resistance factors varied between H. armigera populations and it has moderate to high resistance to cypermethrin and cyfluthrin; a low to moderate resistance to deltamethrin and alpha-cypermethrin; and a comparatively low resistance to bifenthrin, lambda-cyhalothrin and zeta-cypermethrin (11).

MATERIALS AND METHODS

The study was carried out on tobacco plants in Experimental field of Scientific Tobacco Institute-Prilep during 2015/2016. Monitoring of population dynamic of *H. armigera* was performed from July (when plants produce flowers) until the end of September, in a period of 10 days, with application of the method check of 100 tobacco stalks, with Zig-Zag method of pest scouting. The investigations of tobacco stalks attacked by *H. armigera* larvae are made in three replications, in the middle/margin plo ts.
During 2015 and 2016, field experiments were performed to establishing the biological efficacy of some insecticides against *H. armigera*. Diferent active ingredients are compared in experimental design study consisted in random blocks, in 4 repetitions. The following insecticides were applied ac-

cording to the recommended rates: Chlorcyrin 550 EC (a.i chlorpyrifos 500 g/l + cypermethrin 50 g/l) 0.15% (1.5 L/ha); Alverde (a.i metaflumizone 240 g/l) 0.1% (1 L/ha); Affirm (a.i emamectin 9.5 g/kg) 0.2% (2 kg/ha); Belt 24 WG (a.i flubendiamide 480g/L) 0.025% (25 g/100L). The insecticides were applied foliary, with knapsack sprayer, at 200C. The severity of damage induced by 40 larvae per tretmant and the influence of insecticides was recorded after 24 hours, three, five, seven, eleven, fifteen days, after application. Eventual phytotoxic effects were also evaluated.

A laboratory trial was performed on 40 larvae of the tobacco bollworm in petri dishes for each variant. The collected larvae were further nourished and bred according standard laboratory methodology with treated tobacco flower and seed capsules during whole life cycle of the pest.

RESULTS AND DISCUSSION

H. armigera represents a significant challenge to tobacco fileds and it is producing high economic losses of tobacco seed each year. There is no simple solution to Helicoverpa control in tobacco. For management of *H. armigera*, it is required understanding of morphology, lifecycle and biology of the pest. H. armigera overwinter as pupae in the soil. In spring under favorable conditions the adult eclode. Most adults that appear in spring come from populations that survive locally from last year on tobacco fields. Although there may originate from the migratory population of neighboring crops. *H. armigera* is a polycyclic species, and after generation in spring-early summer, on wide range of food sources, *H. armigera* migrate on tobacco plants. Climatic conditions in 2015/2016 were optimal for H. armigera development. Adults are very variable in both size and colour. Females are always darker than the males (Figure 1).

Figure 1. ♀ and ♂ of H. armigera

The females are characterized with high fecundity and they lay large number of eggs on tobacco plants. There is a generally extremely variation in colour of caterpillars: from shades of green, straw-yellow, and pinkish to reddish-brown or even black.

Larvae is develop through six growth stages. Figure 2 shows the larvae of H. armigera collected from one stalk at the same time.

Figure 2. Larvae of H. armigera

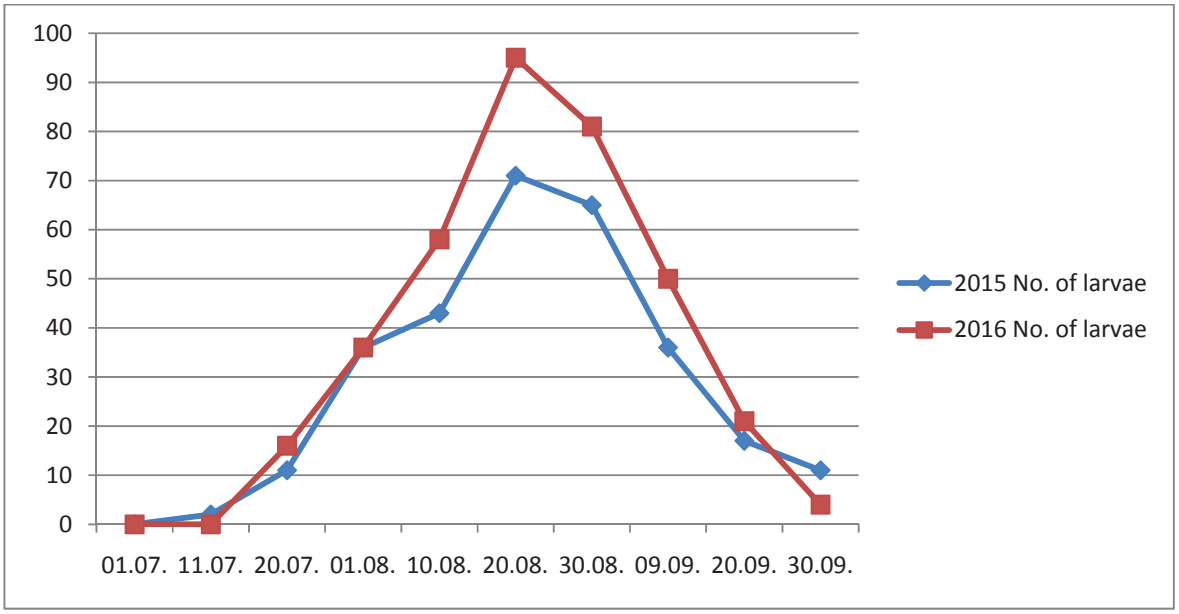
Larvae greedily feed on tobacco, esspecially on reproductive organs of tobacco plants, where they cause serious damages. Flower buds and flowers are damaged, bore holes are visible at the base of flower buds, and they may fall. Larger larvae bore into seed capsules and consume developing seed. We found that in severe infestations larvae may destroy them completely: flower buds, flowers, seed capsules also top leaves (leaving only the main veins) (Figure 3).

Figure 3. Damaged tobacco plants

On completion of growth larva dig a tunnel in the soil. Then the larva build the cocoon (pupal chamber), in which change to the pupal stage (Figure 4).

Figure 4. Pupa of H. armigera

According to our observations the H.armigera is present on tobacco from July (when plants produce flowers) until the end of September, causing severe damages on tobacco seed. The maximum number of larvae was recorded in August, and early September. Larvae of different generations usually overlap. The population is mostly numbered in August and reached the highest level of density on 20 August in 2015/2016 (Graph. 1).



Graph. 1 Population dynamics of *H. armigera* -method check of 100 tobacco stalks

Larval densities on tobacco plants were significantly higher at the margins of the fields than in the centre (Table 1 and 2). In the middle of the plots tobacco plants were mostly in the flowering stage and the percentage of plants infested by larvae of the tobacco bollworm is 22.67% in 2015 and 27.67% in 2016. In the margin parts of the plots, most of the stalks had formed seed capsules and the percentage of plants infested by larvae of the tobacco bollworm is 43.67% in 2015 and 54.33% in 2016.

Table 1- Quantitative representation of the *H. armigera* larvae on tobacco-2015

Replication	No. of tobacco stalks	Place of sampling			
		Plot margins		Middle of the plot	
		No. of larvae	attacked plants %	No. of larvae	attacked plants %
I	100	61	61.00	36	36.00
II	100	33	33.00	21	21.00
III	100	37	37.00	11	11.00
Total	300	131	43.67	68	22.67

Table 2- Quantitative representation of the *H. armigera* larvae on tobacco-2016

Replication	No. of tobacco stalks	Place of sampling			
		Plot margins		Middle of the plot	
		No. of larvae	attacked plants %	No. of larvae	attacked plants %
I	100	46	46.00	24	24.00
II	100	85	85.00	39	39.00
III	100	32	32.00	20	20.00
Total	300	163	54.33	83	27.67

H. armigera has developed resistance to many insecticides, and it is more difficult to control.

IPM is effective component of H. armigera management. Alternative strategies for H. armigera control are biological methods (predators, parasitoids, entomopathgenos) and cultural methods (ploughing in winter/ spring to destruct diapausing pupae, crop rotation, planting of healthy seedlings, use of trap crops, destruction of crop residues, etc).

Tobacco bollworm usually laid on plants which are flowering, or are about to produce flowers.

The importance of scauting of eggs and young larvae of H. armigera suggests that we could provide an early warning of its invasion of an area or crop.

In areas in which are produced certified tobacco seed, Helicoverpa eggs and larvae should be monitored at least twice per week, through July, August and beginning of September .

For management of insecticide resistance we tested different chemical products (Table 3 and 4).

Table 3 -The effectiveness of different insecticides against *H. armigera* larvae-2015

Chemical/rate		No. of larvae	2015 Mortality of larvae/days after treatment					Effect. %	No. of pupa	No. of adult
	Investigation		1	3	5	7	11			
Chlorcyrin 550 EC	Field	40	33	4	-	-	-	92.5	3	1
0.15%	Laboratory	40	18	8	4	3	-	82.5	7	4
Alverde	Field	40	3	8	18	4	1	85.0	6	4
0.1%	Laboratory	40	-	6	15	7	4	80.0	8	4
Affirm	Field	40	-	30	10	-	-	100.0	-	-
0.2%	Laboratory	40	-	21	8	5	3	92.5	3	-
Belt 24 WG	Field	40	-	10	14	6	7	92.5	3	1
0.025%	Laboratory	40	-	6	13	8	5	80.0	8	3

The treatment is recommended to carried out in the evening, because some products lose contact degradation effect due to light or high temperatures. Also the adults are active at twilight and night (noctuid moths).
Insecticidal treatment should be performed after emergence of the caterpillars and before they enter into the seed capsules. Targeting of L1 or L2 is essential but young larvae are difficult to find on tobacco plants. Larvae during L5 and L6 consume about half of their overall diet. They burrow into the floral organs where they become less accessible to contact insecticides, require higher doses to kill and cause direct economic loss.
Older caterpillar has characteristic posture when it is disturbed or exposed on insecticidal tretmant. It lifts its head and curls it under the front of the body, or rolling into a spiral or drops from the plants into the soil. With rational and rotational use of insecticides will get profitable production.
According to the result Affirm was found to be the most effective for the control of H. armigera.
Emamectin benzoate has shown, in two years of testing, a 100% control of H. armigera in field condition.
Keeping all the results in view, it can be conclude that Belt 24 WG, Chlorcyrin 550 EC and Alverde also gave good mortality of H. armigera larvae and can play an important role in managing this insect pest. No effect of phytotoxicity was noticed on tobacco.

Table 4 -The effectiveness of different insecticides against *H. armigera* larvae-2016

Chemical/rate		No. of larvae	2015 Mortality of larvae/days after treatment					Effect. %	No. of pupa	No. of adult
	Investigation		1	3	5	7	11			
Chlorcyrin 550 EC	Field	40	30	3	4	-	-	92.5	3	3
0.15%	Laboratory	40	17	8	4	3	-	80.0	8	7
Alverde	Field	40	-	10	22	4	-	90.0	4	2
0.1%	Laboratory	40	-	8	14	8	4	85.0	6	6
Affirm	Field	40	-	39	1	-	-	100.0	-	-
0.2%	Laboratory	40	-	27	10	1	1	97.5	1	-
Belt 24 WG	Field	40	-	31	8	-	-	97.5	1	-
0.025%	Laboratory	40	-	14	13	7	1	87.5	5	2

For successful management of H. armigera, more attention should be devoted to some basic information such as monitoring efforts, forecasting activities and economic thresholds. We need to study trophic interactions among the tobacco, H. armigera and its natural enemies.

CONCLUSIONS

The tobacco bollworm is one of the major pests of tobacco seed causing both quantitative and qualitative losses.Understanding of the morphology, biology and population dynamics of H. armigera, can be of great help in predicting potentially severe infestations.

Population dynamics of H. armigera shows that the species is present in tobacco biocenosis from the beginning of July until the end of tobacco vegetations, causing severe damages on tobacco seed.

In 2015/2016, the percentage of tobacco stalks attacked by H. armigera ranged from 11.00% to 39.00% in the middle of the plots, where tobacco was in the stage of flowering, and from 32.00% to 85.00% in the margin parts of the plots, where stalks had formed seed capsules.

It‘s control with insecticides, alone or within the context of an IPM programme on tobacco is necessary. To decide whether an insecticide is required, more attention should be devoted to eggs and young larvae. Older larvae are more resistant and burrow into seed capsules and they become less available to be exposed of applied insecticides. Therefore suppression of young larvae (L1 or L2) is essential.

During 2015/2016, all insecticides, in all treated plots have shown high efficacy in control of larvae.W

REFERENCES

1. Cameron PJ, Walker GP, Herman TJB 1995. Development of resistance to fenvalerate in Helicoverpa armigera in New Zealand. New Zealand Journal of Crop & Horticultural Science 23: 429-436.
2. Cameron P.J. ,Walker G.P., 2005 Helicoverpa armigera resistance management strategy. Pesticide Resistance: Prevention and Management. New Zealand Plant Protection Society, 55-60 Inc.Hastings, New Zealand
3. Camprag, D., R. Sekulic, 2002, Vaznije stetocine duvana i njihovo suzbijanje. Biljni lekar, Godina XXX, broj 5.
4. Camprag, D., R. Sekulic, T. Kereši, F. Baca, 2004, Corn earworm (Helicoverpa armigera Hübner) and measures of integrated pest management. Faculty of Agriculture, Novi Sad, YU, 183 pp.
5. Fathipour Y, Naseri B. 2013. Integrated Management of Helicoverpa armigera in Soybean Cropping Systems, book edited by Hany A. El-Shemy, ISBN 978-953-51-0978-5, chapter 9 , p.321-280
6. Fatma, Z., P. H. Pathak, 2011, Food plants of Helicoverpa armigera (Hübner) and extent of parasitism by its parasitoids Trichogramma Chilonis Ishii and Campoletis chlorideae Uchida- a field study International Journal of Entomology, 2 (1): 31-39
7. Krsteska, V., V. Dimeska, P. Stojanoski, 2007, Heliothis armigera Hbn on tobacco and application of some insecticides in its control. Tutun/Tobacco, Vol.57, No 1-2, 42-48, 2007
8. Krsteska V.,Srbinoska M., Stojanoski P., 2014. Helicoverpa armigera –pest control on tobacco. Book of proceedings Challenges in modern production Section 3 , p. 123-129
9. King EG, Coleman RJ, 1989. Potential for biological control of Heliothis species. Annual Review of Entomology, 34:53-75
10. Lammers, J.W., MacLeod A., 2007, Report of a Pest Risk Analysis Helicoverpa armigera

(Hübner, 1808) Plant Protection Service (NL) and Central Science Laboratory (UK) http://www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/documents/helicoverpa.pdf
11. Muhammad Tariq Ch., Muhammad Asghar Malik and Naveed Iqbal. Barani Agri. 2005. Management of Helicoverpa armigera with different insecticides. Pak. J. Agri. sa., Vol. 42(1-2),. Training Institute, Dahgal, Rawalpindi.
12. Vasilev, Lj., 1976, Biology and ecology of the tobacco moth-bollworm Heliothis armigera Hbn. in Macedonia. Ph.D. thesis, Agricultural University, Skopje
13. Vasilev, Lj., 1985, Rezultati i iskustva u suzbijanju Noctiudae na duvanu u Makedoniji u 1985 god. Zbornikradova. Sveska 7, Jugoslovensko savetovanje o primeni pesticida, Opatija;
14. Vasilev, Lj., Lj. Janušeska, P. Stojanoski, V. Dimeska, P. Taškoski, S. Stojkov, 1996, Forecast and integrated battle against the migration Noctuidae of tobacco in the Republic of Macedonia. A project of the Ministry of Science.
15. Zalucki MP, Murray DAH, Gregg PC, Fitt GP, Twine PH, Jones C. Ecology of Helicoverpa armigera (Hübner) and H. punctigera (Wallengren) in the inland of Australia: larval sampling and host plant relationships during winter and spring. Australian Journal of Zoology 1994; 42 329-346.
16. Helicoverpa armigera -http://www.cabi.org/isc/datasheet/26757 (accessed November 2014)
17. Helicoverpa armigera EPPO Standards 2003, Bulletin OEPP/EPPO Bulletin 33, 245–247, PM 7/19
18. Helicoverpa armigera. EPPO/CABI (1997). Quarantine Pests for Europe, 2nd edn, pp. 289–294. CAB International, Wallingford (GB).

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Original Scientific paper

COMPARATIVE INVESTIGATIONS ON THE EFFECTS OF FERTILIZATION AND IRRIGATION OF THE SEMI-ORIENTAL VARIETIES OTLJA O 9-18/2 AND OTLJA-ZLATOVRV

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ABSTRACT

The aim of the research was to estimate the fertilization and irrigation regimes for production of a good quality semi-oriental varieties Otlja O9-18/2 and Otlja-Zlatovrv. Three-year trial was set up in 12 variants with three replications. The trial was bifactorial, with three rates of nitrogen (25, 30 and 45 kg N/ha), constant amount of phosphorus (80 kg/ha) and potassium (100 kg/ha) and two irrigation regimes (45 and 60% FC). The obtained results confirm that fertilization and irrigation have positive impact on the production properties of both varieties. Compared to the control variety, the yield was increased by 86.89% in Otlja O9-18/2 and 89.98% in Otlja- Zlatovrv variety. Fertilization and irrigation have statistically significant impact on reduction of nicotine, proteins, mineral matter and on the increase of soluble sugars. We can conclude that by proper application of agrotechnics we can dictate the yield and quality of semi-oriental tobacco.

Keywords: semi-oriental tobacco, fertilization, irrigation, yield, quality

КОМПАРАТИВНИ ИСПИТУВАЊА НА ЕФЕКТОТ ОД ЃУБРЕЊЕТО И НАВОДНУВАЊЕТО КАЈ ПОЛУОРИЕНТАЛСКИТЕ СОРТИ ОТЉА О 9-18/2 И ОТЉА-ЗЛАТОВРВ

Целта на ова истражување беше да се проучи влијанието на ѓубрењето и наводнувањето врз некои производни својства кај сортите Отља О 9-18/2 и Отља-Златоврв. Тригодишен полски опит беше поставен на делувијално-колувијален почвен тип, со 12 варијанти, во три повторувања. Опитот беше двофакторијален, со три количини на азотно ѓубриво (25,35 и 45 кг/ха), константни количини на фосфор (80 кг/ха) и калиум (100 кг/ха) и два режима на наводнување (45 и 60% од ПВК). Добиените резултати потврдија дека ѓубрењето и наводнувањето имаат позитивно влијание врз производните својства на двете испитувани сорти тутун. Во споредба со контролната варијанта, приносот е зголемен за 89,98% кај Отља 09-18/2 и за 89.98% кај сортата Отља-Златоврв. Ѓубрењето и наводнувањето покажале статистички значајно влијание врз намалувањето на содржината на никотин и белковини и зголемување на растворливите шеќери во тутунот. Можеме да заклучиме дека со правилна примена на агротехничките мерки можеме да го диктираме приносот и квалитетот на полуориенталскиот тутун.

Клучни зборови: полу ориенталски тутун, ѓубрење, наводнување, принос, квалитет

INTRODUCTION

Semi-oriental tobacco is characterized by fine tissue and delicate plate, small basal rib and good factory reproduction. In fabrication it is treated as neutral feedstock with full, sweet to neutral flavor that does not burn and scratches throat while smoking. Therefore, Otlja tobacco is notable additional type of tobacco (Uzunoski 1985). Dry tobacco is used in the manufacture of cigarettes to enhance the taste and charge (Naumoski et al., 1977). Semi-oriental type of tobacco Otlia accounts for 25 % of cigarette mixture. In the Republic of Macedonia, Otlja tobacco has been produced for a long period of time on limited areas. According to Димитриески и сор. (2004), with regard to rational production of this tobacco, its using value in fabrication and the ability to burn without removing the main rib of the leaf (which is not the case with large-leaf tobacco), thus reducing production costs of cigarettes, there is necessity of producing semi-orienatal tobacco types. Spasovski (1957) points out that Otlja tobacco as semi-oriental variety is grown as additional material, its lower, middle and upper middle leaves are first class material which are most prevalent in tobacco harvesting and using proper agronomy factors, irrigation and drying we can get high quality tobacco raw. Patche (1960) studies are based on that tobacco type Otlja should be grown on slope, lightweight, permeable and shrew soils where there are conditions for watering. In the last years there is increased interest from the tobacco companies for this type of tobacco that imposes the need of restarting production in different production regions in our country. Based on the above, our main objective was to perform research on the impact of the fertilization and watering mode for the production of semi-oriental varieties O9-18/2 and O Zlatovrv so we can add our contribution to the prospects for the production of these varieties in Prilep production area.

MATERIAL AND METHODS

Three-year field trials with semi-oriental tobacco variety Otla O9-18/2 and Otlja-Zlatovrv (O Zlatovrv) were performed on the experimental field of Scientific Tobacco Institute – Prilep. The experiment was two factorial with three different nitrogen fertilizers rate (25.30 and 45 kgN/ha), constant amounts of phosphorus (80 kg/ ha), potassium (100 kg/ha) and two irrigation regimes (45% and 60% of water capacity), set up in randomized complete block design in three 3 replications, in the following variations:

1. Ø unfertilized unirrigated control, (Ø)
2. $N_{25}P_{80}K_{100}$ (N_1)
3. $N_{35}P_{80}K_{100}$ (N_2)
4. $N_{45}P_{80}K_{100}$ (N_3)
5. ∅ unfertilized + 45 % of FC (Ø+ W_1)
6. $N_{25}P_{80}K_{100}$ + 45 % of FC (N_1+W_1)
7. $N_{35}P_{80}K_{100}$ + 45 % of FC (N_2+W_1)
8. $N_{45}P_{80}K_{100}$ + 45 % of FC (N_3+ W_1)
9. ∅ unfertilized + 60 % of FC (Ø+ W_2)
10. $N_{25}P_{80}K_{100}$ + 60 % of FC (N_1+ W_2)
11. $N_{35}P_{80}K_{100}$ + 60 % of FC (N_2+ W_2)
12. $N_{45}P_{80}K_{100}$ + 60 % of FC (N_3+ W_2)

Soil preparation was performed with an autumn (30 cm depth) and two spring plowings (8-20 cm depth). Before setting up the experiment, soil tests were done in order to determine the agrochemical and physical properties of the soil. Fertilization was done using inorganic mineral fertilizer NPK 8:22:20, and 27% KAN. 50% of the nitrogen amount was applied in the last powing, before planting together with phoshorus and potassium, the rest 50 % were applied on the first digging. Each elementary plot has 4 rows with 8 plants in a row or a total of 32 plants in the plot. The whole experiment has a total of 1152 plants, with a distance of transplanting 50 x 25 cm. All indispensable agro-technical and plant protection practices were applied during the vegetation period of tobacco. Water quantities for maintaining regimes 45 and 60% of WC were calculated depending on current soil moisture. Harvesting was done manualy in 5 harvests. Tobacco is yellowed and sun cured. After drying, dry tobacco was graded, weighted and qualitative assessment was done according to purchase measures for dried tobacco. Agrochemical parameters of soil and chemical components of tobacco raw are determined by standard methods in accredited laboratories in Scientific Tobacco Institute. The obtained results were statistically processed with ANOVA – LSD test (Filiposki 2011). Tobacco tasting was made by the anonymous comparative tasting method and scoring was done by key for tobacco tasting.

RESULTS AND DISCUSSION

Experiments were carried out on colluvial-alluvial soil; light loamy with slightly acidic pH reaction in water and moderate acid pH (KCl), low amount of organic matter and medium content of soil available phosphorus and potassium (Table 1). Lazarevski et al. (1982) point out that producing semi-oriental and big leaf tobaccos need slightly richer soils with higher clay content to enable the formation of larger organic production.

According to Naumoski et al. (1977) varieties of this type are adapted to mild slope, permeable and shrew soils, were specific quality of Otlja depends on the irrigation.

Table 1 Agrohemical propertis of the soil

Depth (cm)	pH		Humus	mg/100 g soil		Physical clay
	H_2O	KCl	%	P_2O_5	K_2O	%
0 - 30	6.64	5.98	0.81	15.69	13.30	24.5
30 - 60	6.46	5.78	0.65	11.81	12.22	26.8
Classification	low acid	moderately acid	low	medium	medium	light loamy

According to the obtained data (Table 1) it can be seen that the soil is poorer and will not meet the needs of semi-oriental tobacco, so it can be expected that fertilization and irrigation will have a more pronounced effect.

The meteorological conditions during the vegetation period of three years are presented in Table 2.

Table 2 Meteorological data during the vegetation

Month	Year	Average air temperature (^{o}C)			Precipitations mm	Days with precipitations
		Daily	Min	Max		
May	2007	16.9	11.5	22.5	74.3	14
	2008	16.7	9.7	23.0	41.3	8
	2009	15.8	8.7	25.0	55.0	10
June	2007	21.6	15.6	28.0	79.5	11
	2008	19.9	12.3	29.1	10.0	5
	2009	18.5	11.8	27.6	75.0	10
July	2007	25.3	17.1	32.8	5.3	1
	2008	22.3	13.7	31.3	11.0	4
	2009	21.9	13.4	30.6	8.0	3
August	2007	23.7	17.0	30.4	54.2	2
	2008	23.6	14.1	33.3	11.0	2
	2009	21.4	14.0	29.2	43.0	7
September	2007	16.9	10.6	23.5	16.6	5
	2008	15.8	9.7	23.9	110.0	10
	2009	17.1	11.0	25.3	15.0	5
Average/Total (V - IX)	2007	20.9	14.4	27.4	229.9	33
	2008	19.7	11.9	28.1	183.3	29
	2009	18.9	11.8	27.5	196.0	35
Annual average	2007-2009	19.8	12.7	27.7	203.1	32
	1999 -2008	19.9	13.4	26.5	205.7	34

Meteorological data show average day temperature during the vegetation period of 19.8 ^{o}C that it is no different from the ten-year average and is suitable for semi-oriental tobacco growth. According to (Atanasov 1965) optimal temperature for tobacco plant is average day temperature from 22 to 25 ^{o}C, and lower ranges for lack and excess are between 18 and 30 ^{o}C. Average precipitation during the vegetation is 203.1 mm. Year 2007 has highest precipitation rate of 229.9 mm, and lowest precipitation rate is noted in year 2009 with precipitation rate of 196.0 mm. As it can be seen the quantities of the precipitation are uneven and not favorable for high tobacco production with the required quality properties. The changes of climatic factors in recent years including increased temperatures, reduced rainfall and its erratic distribution during the growth phase led to drought in different growth stages of plant and crop yield levels significantly reduced in dry conditions (Salehzadeh et al, 2009). Based on the data on the amount of rainfall and literature data it can be concluded that without irrgation we can not ensure normal growth and cost-effective yield of the tobacco. That is why furhter irrgation was provided, particularly in critical periods when tobacco has the greatest needs for water.

To maintain the level of water in the soil according to established methodology, during 2007 - 2009 four irrigations were done and water supplies were different for the two maintained humidity levels (40% and 60% of WC), depending on the current soil moisture.

The results (Table 3 and Figure 1) clearly show that fertilization and irrigation in particular, and their interaction had a strong impact on increasing the yield of tobacco, compared to the control.

Table 3 Yield of tobacco (kg/ha)

N°	Variant	O 9-18/2						O Zlatovrv					
		2007	2008	2009	$\bar{X}$	Difference Aps	Difference %	2007	2008	2009	$\bar{X}$	Difference Aps.	Difference %
1	**Ø**	**1271**	**1300**	**1294**	**1288**	**-**	**100.00**	**1345**	**1436**	**1322**	**1368**	**-**	**100.00**
2	N_1	1387	1565	1356	1436	+ 148	111.49	1417	1570	1413	1467	+ 99	107.24
3	N_2	1352	1875	1354	1527	+ 239	118.56	1419	1982	1488	1630	+ 262	119.15
4	N_3	1536	2042	1420	1666	+ 378	129.35	1452	2209	1539	1733	+ 365	126.68
5	**Ø + W_1**	**1569**	**1472**	**1417**	**1486**	**+ 198**	**115.37**	**1528**	**1541**	**1430**	**1500**	**+ 132**	**109.65**
6	N_1+ W_1	1853	1904	1692	1816	+ 528	140.99	2019	2141	1809	1990	+ 622	145.47
7	N_2+ W_1	2093	2249	1939	2094	+ 806	162.58	2302	2473	2155	2310	+ 942	168.86
8	N_3 + W_1	2449	2384	2206	2346	+ 1058	182.14	2577	2597	2425	2533	+ 1165	185.16
9	**Ø + W_2**	**1657**	**1474**	**1369**	**1500**	**+ 212**	**116.46**	**1718**	**1587**	**1430**	**1578**	**+ 210**	**115.35**
10	N_1+ W_2	1884	1847	1797	1843	+ 555	143.09	2038	1981	1874	1964	+ 596	143.57
11	N_2+ W_2	2194	2177	2053	2141	+ 853	166.23	2406	2459	2185	2350	+ 982	171.78
12	N_3+ W_2	2458	2446	2397	2434	+1146	188.98	2593	2643	2561	2599	+ 1231	189.99
Average		1808	1894	1691	1798	-	-	1901	2051	1802	1918	-	-
Index		100.0	100.0	100.0	100.0	-	-	105.17	108.29	106.56	113.42	-	-

LSD	2007	2008	2009	2007	2008	2009
0.05	197 kg/ha	186 kg/ha	85 kg/ha	125 kg/ha	141 kg/ha	78 kg/ha
0.01	268 kg/ha	253 kg/ha	116 kg/ha	169 kg/ha	191 kg/ha	106 kg/ha
0.001	360 kg/ha	340 kg/ha	155 kg/ha	257 kg/ha	257 kg/ha	143 kg/ha

At variety O 9-18/2, which is treated as standard variety, increase is from 11.49 % (Variant 2) to 88.98 % (Variant 12). Same trend have variety O Zlatovrv from 7.24% (Variant 2) to 89.99% (Variant 12). According to the results, also it can be noted that the yield is increased with the increasing quantities of nitrogen. Fertilized variants with the highest quantities of nitrogen has increase yield at both varieties O 9-18/2 and O Zlatovrv for 29.35% and 26.68% respectively, compared to the control. Irrigation without fertilization increases yield by about 15%. Lower water factor effects are due to the low fertility of the soil based on the analyses of the soil samples.

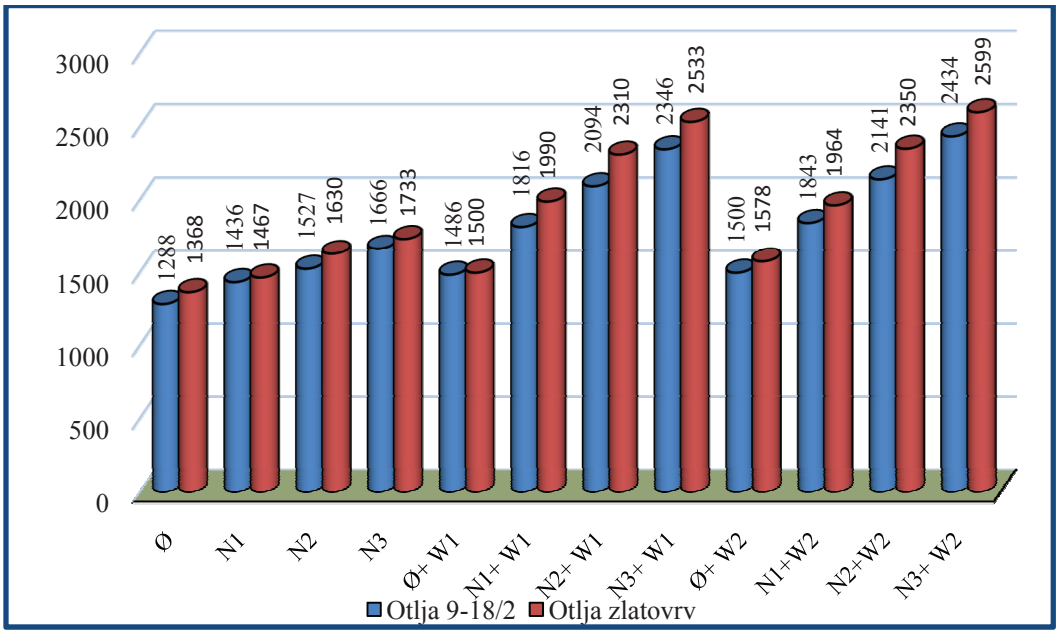

Figure 1. Yield of tobacco (kg/ha)

Interaction level of fertilizing and watering have increased the yield by 40.99 % (variant 7) to 88.98% (variant 12) at variety O 9-18/2 and from 5.17 % to 89.99 % at variety O Zlatovrv. Based on the statistical analysis of the achieved yield by years, in all fertilized and irrigated variants there is statistically significant impact on all three levels of probability, indicating full justification of agro-technical measures applied in the cultivation of tobacco type Otlja.

The table also presents data where is given comparison between the yield in given years of the standard variety O 9-18/2 and variety O Zlatovrv. Based on the data we can see that variety O Zlatovrv has higher yield during the all three years (5.17 -8.29%). The quality of tobacco raw material is expressed by an average purchase price of one kilogram of dry tobacco. Presented data (Table 4) point out that all variants in both studied varieties have higher average price compared to the control. Best results are achieved at variants 7 and 11. Average price at O 9-18/2 is increased by 12.10 %, while O Zlatovrv by 13.57 % (variant 7) and 13.02 % (variant 12). Compared by years, O Zlatovrv has higher average price that in year 2008 is increased by 6.96 %. Therefore we can conclude that variety O Zlatovrv grown with appropriate agronomy measures, have higher quality tobacco raw compared to the standard variety O 9-18/2. This was confirmed and by statistical analyze of the data.

Economical effect as gross income of tobacco on ha, (Table 5) represents the yield and quality of tobacco for each set of variants. Great yield per hectare and quality of raw tobacco (average price per kg of dry tobacco) means greater economic impact and vice versa.

In the examined varieties lowest gross income have controls (46 657 den/ha, 51165 den/ha), and the highestfrom variant 12 (93,471 den/ha, 106960 den/ha). That is gross income of 100.34% at variety O 9-18/2, and 109.05% at variety O Zlatovrv more than unfertilized and unirrigated variants.

Table 4. Average price of tobacco (den/kg)

N⁰	Variant	O 9-18/2					O Златоврв				
		2007	2008	2009	$\bar{X}$	%	2007	2008	2009	$\bar{X}$	%
1	**Ø**	**30.64**	**23.59**	**54.34**	**36.19**	**100.00**	**31.99**	**25.32**	**56.08**	**37.80**	**100.00**
2	N_1	32.59	27.13	53.32	37.68	104.11	32.79	27.66	57.32	39.26	103.86
3	N_2	31.68	27.71	57.03	38.81	107.23	32.53	28.43	57.26	39.41	104.25
4	N_3	30.05	27.21	55.65	37.64	104.00	31.73	27.89	56.08	38.57	102.03
5	**Ø + W_1**	**31.50**	**25.99**	**52.95**	**36.81**	**101.71**	**32.61**	**26.03**	**56.09**	**38.24**	**101.16**
6	N_1+ W_1	33.19	28.42	56.84	39.48	109.09	35.93	30.47	59.24	41.88	110.79
7	N_2+ W_1	34.47	29.40	57.84	40.57	112.10	35.70	32.17	60.91	42.93	113.57
8	N_3 + W_1	33.35	27.94	56.22	39.17	108.23	34.24	32.67	59.05	41.99	111.08
9	**Ø + W_2**	**30.10**	**26.58**	**52.89**	**36.52**	**100.91**	**32.24**	**26.34**	**56.91**	**38.50**	**101.85**
10	N_1+ W_2	30.69	28.12	58.57	39.12	108.09	33.66	31.15	60.17	41.66	110.21
11	N_2+ W_2	32.70	28.98	60.03	40.57	112.10	34.23	32.20	61.74	42.72	113.01
12	N_3+ W_2	31.92	27.95	54.50	38.12	105.33	33.72	31.60	58.55	41.29	109.23
Average		**31.91**	**27.42**	**55.85**	**38.39**	-	**33.45**	**29.33**	**58.28**	**40.35**	-
Index		**100.0**	**100.0**	**100.0**	**100.0**	-	**104.83**	**106.96**	**104.36**	**105.12**	-

LSD	2007	2008	2009	2007	2008	2009
0.05	n.s.	1.26 den/kg	2.86 den/kg	1.65 den/kg	1.43 den/kg	1.64 den/kg
0.01	n.s.	1.72 den/kg	n.s.	2.24 den/kg	1.94 den/kg	2.22 den/kg
0.001	n.s.	n.s.	n.s.	n.s.	2.62 den/kg	2.99 den/kg

Variants fertilized and irrigated with 45% of FC showed a significant increase in gross income compared to the fertilized and unirrigated variants. Compared with variants fertilized and irrigated with 60% of the FC there is no significant increase in gross income. The analysis of the results for this indicator showed statistically significant difference between control variant and fertilized and irrigated variants.

Table 5. Gross income of tobacco (den/ha)

N^{O}	Variant	O 9-18/2					O Zlatovrv				
		2007	2008	2009	$\bar{X}$	%	2007	2008	2009	$\bar{X}$	%
1	**Ø**	**38916**	**30679**	**70377**	**46657**	**100.00**	**42991**	**36398**	**74106**	**51165**	**100.00**
2	N_1	45172	42445	72287	53301	114.24	46457	43419	81013	56963	111.33
3	N_2	42821	51892	77233	57315	122.84	46142	56397	85174	62571	122.29
4	N_3	46224	55546	78963	60244	129.12	46078	61588	86174	64613	126.28
5	**Ø + W_1**	**49260**	**38242**	**75125**	**54209**	**116.18**	**72498**	**40140**	**80226**	**64288**	**125.64**
6	N_1+ W_1	61311	54078	96210	70533	151.17	72498	65234	107200	81644	159.57
7	N_2+ W_1	72238	66149	112135	83507	178.98	82155	79518	131290	97654	190.86
8	N_3 + W_1	81729	66648	124073	90817	19464	88185	82231	143183	104533	204.30
9	**Ø + W_2**	**49837**	**39180**	**72409**	**53809**	**115.32**	**55388**	**41806**	**81396**	**59530**	**116.34**
10	N_1+ W_2	57709	51970	105230	71636	153.53	68892	61714	112787	81131	158.56
11	N_2+ W_2	71516	62753	123287	85852	184.00	83490	79168	134951	99203	193.88
12	N_3+ W_2	81594	68390	130428	93471	200.33	87420	83502	149959	106960	209.04
Average		**58194**	**52331**	**94813**	**68446**	-	**66016**	**60926**	**105622**	**68446**	-
Index		**100.0**	**100.0**	**100.0**	**100.0**	-	**113.44**	**116.42**	**111.40**	**113.26**	-

LSD	2007	2008	2009	2007	2008	2009
0.05	6983 den/ha	6324 den/ha	7292 den/ha	4538 den/ha	4540 den/ha	6326 den/ha
0.01	9491 den/ha	8595 den/ha	9912 den/ha	6168 den/ha	6171 den/ha	8598 den/ha
0.001	12768 den/ha	11562 den/ha	13333 den/ha	8297 den/ha	8301 den/ha	11566den/ha

Chemical composition of tobacco type Otlia are presented in Table 6. Based on the data it can be concluded that the tested agromeasures have a pronounced influence on the important chemical parameters, and thus of their relationship. Increasing the content of nitrogen in the fertilizer increases the content of nicotine (1.6-1.88% and 1.35-1.89%), protein (9.15% -10.24% and 8.22-9.82%) and mineral matter (14.43-15.43 and 12.26-14.20%) respectively in two varieties, without significant change of the content of soluble sugars compared to the control. Irrigation without fertilization adversely affects the contents of the tested parameters. %. In irrigated and fertilized and irrigated varieties we can notice reduction in the content of nicotine, protein and mineral matter and very pronounced increasement in the content of soluble sugars. The content of soluble sugars at variety O 9-18/2 is increased to 24.48 %, and 184.98%. At variety O Zlatovrv is increased to 20.77 or 186.74 % compared to the control. The results of our research are within typical type Otlja (Uzunoski 1985). A certain deviation is observed in soluble sugars that are present in a higher percentage, but these values are close to the research of Богданчески (1981) which found that the content of soluble sugars is from 15.23 to 25.16 %. Lazareski 1977 points out that irrigated tobacco have high carbohydrate content, a lower percentage of nicotine and protein, leafs are soft, with good flavor and low flammability. The chemical composi-

tion of the raw material depends on the type of tobacco, applied agro-technical measures during vegetation, insertion and processing technology (Dimitrieski 2004). From this research it can be concluded that in terms of the same farming practices i.e. production technology chemical composition of the tobacco raw is strongly dependent and by the variety of the same type tobacco.

Table 6. Chemical composition of tobacco (2007-2009)

N⁰	Variant	Nicotine		Protein		Soluble sugars		Mineral matter	
		Otlia 09-18/2	Otlia zlatovrv	Otlia 09-18/2	Otlia zlatovrv	Otlia 09-18/2	Otlia zlatovrv	Otlia 09-18/2	Otlia zlatovrv
1	**Ø**	**1.60**	**1.35**	**9.15**	**8.82**	**8.59**	**11.12**	**14.43**	**12.26**
2	N_1	1.74	1.41	9.54	8.29	8.83	11.91	14.84	12.83
3	N_2	1.87	1.53	10.28	9.70	8.80	11.65	15.30	13.60
4	N_3	1.88	1.89	10.24	9.82	8.84	11.17	15.43	14.20
5	**Ø + W_1**	**0.58**	**0.51**	**7.50**	**7.40**	**22.17**	**19.68**	**11.82**	**10.91**
6	N_1+ W_1	0.71	0.62	8.45	7.68	22.96	17.46	13.83	12.67
7	N_2+ W_1	0.88	0.71	8.01	7.86	22.75	18.22	13.89	13.20
8	N_3 + W_1	0.90	0.87	8.99	8.08	22.14	19.53	13.74	13.31
9	**Ø + W_2**	**0.47**	**0.49**	**6.48**	**7.01**	**23.87**	**20.06**	**14.00**	**12.12**
10	N_1+ W_2	0.51	0.60	6.29	6.45	23.15	20.79	13.27	12.52
11	N_2+ W_2	0.77	0.61	6.79	7.09	22.98	20.34	13.80	12.55
12	N_3+ W_2	0.99	0.82	7.66	7.25	24.48	20.77	13.99	13.67
Average		**1,08**	**0,95**	**8,28**	**7,95**	**18,30**	**16,89**	**14,03**	**12,82**
Index		**100.0**	**88,45**	**100.0**	**96,05**	**100.0**	**92,32**	**100.0**	**91,39**

The properties that tobacco exerts on smoking are called tasting properties (Uzunoski 1985). Tasting is the oldest and the best method for determining the quality of tobacco. In these studies the quality of tobacco from both tested varieties is rated according to the total number of points obtained as a sum of points of irritation properties, taste, aroma, strength, flammability and compactness. Average data is presented on Figure 2.

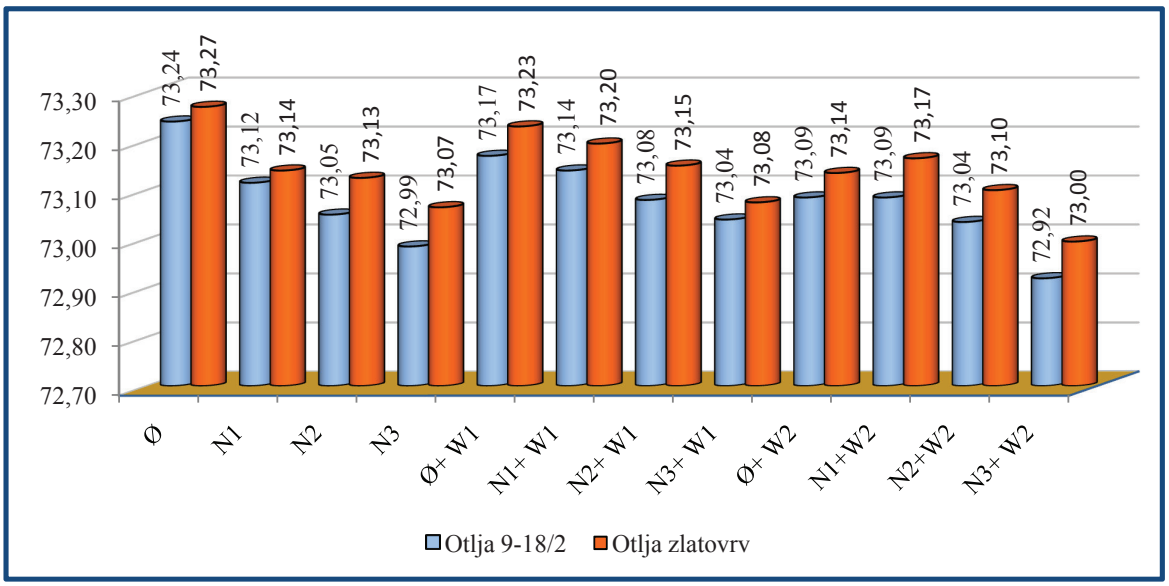

Figure 2. Tasting properties

Based on the assessment of the Tasting Commission, the tested varieties have good tasting properties characteristic of semi-oriental type. At both tasted varieties, best tasting performance are manifested at the control variant. With increasing doses of nitrogen, there was slight decrease in the specific tasting properties of the raw material. From the irrigated and fertilized variants, highest score has variant 6, fertilized with 25 kg nitrogen, with maintained soil humidity of 45% of FWC. Based on the scoring of the Tasting Commission raw tobacco varieties O Zlatovrv has better tasting properties compared to standard variety O 9-18/2.

CONCLUSIONS

Based on the results it can be concluded that fertilizing and irrigation should be mandatory measures in agricultural breeding of semi-oriental variety O 9-18/2 and O Zlatovrv in the Prilep production region. Fertilizng and irrigation influence the increase in the yield of tobacco, the average price and gross income from unit area. Satisfactory yield, better quality and economic viability is achieved with fertilized variants of 35 and 45 kg nitrogen/ha and maintaining soil moisture at 60% of FWC. Maintaining the soil moisture at 60% of the FWC did not influence the quality of the tobacco raw. Fertilizing and irrigation individually, as well as their interactive effect have significant impact on the chemical composition of tobacco. This suggests that with their proper application they can significantly influence the content and interrelationship of important chemical indicators that dictate the quality of the tobacco raw. Based on the scoring of the Tasting Commission best with quality and intensity has the control variant and variant fertilized with 25 kg nitrogen, with maintained soil humidity of 45% of FWC. Tobacco variety O Zlatovrv has better tasting properties compared to standard variety O 9-18/2.

REFERENCES

1. Atanasov D., 1972. Tjutjunoproizvodstvo, Plovdiv.
2. Богданчески М., 1981. Резултати од компаративните испитувања на некои полуориенталски сорти тутун во реонот на Куманово, во 1979 година. Тутун/Tobacco, 1-2, 15-28, Институт за тутун – Прилеп.
3. Димитриески М., Мицеска Г., Ристески И., Кочоска К., 2004. Варијабилноста на хемиските својства на тутунската суровина од полуориенталски тип, отља во зависност од сортата и начинот на одгледување. Јубилејна научна конференција со меѓународно учество „60 година ИТТП“ Пловдив, 153-160.
4. Лазарески Т., 1977. Влијание на наводнувањето врз приносот и поважните хемиски карактеристики на тутунот - сорта Маја. Тутун/Tobacco, 3-4, 155-172, Институт за тутун – Прилеп.
5. Лазарески Т., Пашовски Д., Тодороски П., Филипоски К., 1982. Влијание на ѓубрењето и наводнувањето врз приносот и квалитетот на тутунот тип отља. Тутун/Tobacco, 3-4,

41-51, Институт за тутун – Прилеп.
6. Наумоски К. и сор., 1977. Современо производство на тутунот, Скопје.
7. Salehzadeh , H., Fayyaz Moghaddam A., Bernosi I., Ghiyasi M. and Amini P., 2009. The effect of irrigation regims on yield & chemical quality of oriental Tobacco: research journal Of biological science, 4(5), 632-636.
8. Печијарески Ѓ., Наумоски Ј., Смоквоски М., 1980. Хемиски карактеристики на тутунската суровина од типот прилеп произведена во услови на интервенција со вода. Тутун/Tobacco, 7-8, 41-51, Институт за тутун – Прилеп.
9. Спасовски Б., 1957. Опити со одгледување на тутунот од типот отља во полскиот дел од подреонот на град Битола. Тутун, година VII, 12, 449-453, Институт за тутун Прилеп.
10. Узуноски М., 1985. Производство на тутун, Скопје.
11. Филипоски К. и сор., 1997. Изнаоѓање на економски оправдан најпогоден хранидбен и поливен режим на површините под тутун од хидросистемот "Прилепско поле"-Прилеп, Прилеп.
12. Филипоски К., 2011. Статистички методи во земјоделските истражувања – одбрани поглавја. Институт за тутун – Прилеп.

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Original Scientific paper

AGROECOLOGICAL FEATURES OF TOBACCO PRODUCING REGIONS IN SOUTHERN BULGARIA

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ABSTRACT

Investigations were made on the agri-environmental features of tobacco growing regions in Southern Bulgaria. Soil samples in the vicinity of Gotse Delchev, Kardzhali and Haskovo are the object of study. Soil texture, pH, organic matter content, total nitrogen content, and content of mobile forms of phosphorus, potassium, iron, manganese, copper and zinc were determined in this study.
According to all indices examined, the majority of soils in the researched tobacco producing regions are perfectly suitable for growing oriental tobacco and yield good quality tobacco material, irrespective of the predominantly monocultural planting.
The favorable climate and soil conditions, the experience of several generations of farmers and the established tobacco production traditions are an important prerequisite for the continuation of the production practices and support of the tobacco sector.

Keywords: soil, soil characteristics, soil macro and microelements, oriental tobacco

АГРОЕКОЛОШКИ СВОЈСТВА НА ТУТНОПРОИЗВОДНИТЕ РЕОНИ ВО ЈУЖНА БУГАРИЈА

Вршени се испитувања на агроеколошките својства на тутунопроизводните реони во јужна Бугарија. Анализирани се почвени проби од околината на Гоце Делчев, Карџали и Хасково. Одредувани се структурата на почвата, pH, содржината на органска материја, вкупниот азот и мобилните форми на фосфор, калиум, железо, манган, бакар и цинк.
Според испитуваните показатели, најголем дел од почвите во испитуваните реони е совршено погоден за одгледување на ориенталски тутун и за добивање на висококвалитетен тутунски материјал, и покрај доминантниот монокултурен начин на производство.
Поволните климатски и почвени услови, искуството на неколку генерации тутунопроизводители и воспоставените традиции во одгледувањето на оваа култура се важни предуслови за континуитет на производната пракса и поддршката на тутунскиот сектор.

Клучни зборови: почва, карактеристики на почвата, почвени макро и микроелементи, ориенталски тутун

INTRODUCTION

Oriental tobacco is generally grown in poor and desolate areas with arid climate. Even though tobacco is a highly hygrophilous plant in its homeland, America, in the weather conditions of Bulgaria, characterized by drought in the months of July and August, this former mesophyte has become a xerophyte (Timev et al., 1974). The optimal amount of rainfall during oriental tobacco's growing season (May-September) is 150-200 mm (Donev et al., 1974).

To obtain high quality production from the early ripening oriental tobacco varieties, an average diurnal temperature of at least 17 °C is required during ripening of the leaves, and temperature over 20 °C is considered optimal.

Soils suitable for Oriental tobacco contain 10% to 50% clay, 0.5 to 2.8% organic matter and 0.05 to 0.15% total nitrogen. Soils with 2.5 to 3.8% organic matter and total nitrogen 0.13 to 0.9% are moderately suitable, and can be used in the absence of better conditions (Tanov et al., 1978). Organic matter horizon thickness and erosion rates, too, affect oriental tobacco development. High quality oriental tobacco is obtained on eroded slopes due to the small organic matter horizon thickness. Oriental tobacco yield and quality depend on soil reaction, and good quality tobacco could be obtained over a wide range of pH levels: from 5 to 8.5 (Donev and Zlatev, 1974).

Bulgaria is a traditional producer of oriental tobacco. The tobacco industry continues to have strategic importance for the country's economy in spite of perturbations in recent years. Oriental tobacco production in Bulgaria takes place in established tobacco-growing regions. The boundaries of tobacco-producing regions and those of administrative areas do not coincide: the former cover much broader areas. Each of these areas is characterized by specific climatic conditions, soil, and relief peculiarities, which give rise to a variety of technological qualities of the tobacco, denoting the specific place of origin (varietal subgroup).

The issue of soil fertility preservation in existing extended oriental tobacco monocultures in most tobacco producing areas is particularly relevant (Yancheva et al., 2010, Zapryanova et al., 2014). Therefore, monitoring of the agri-environmental characteristics of soils used for tobacco production is necessary, as could bring about new cultivation technologies for a high quality production that would meet the interests of producers and merchants.

The aim is:

- To study the agri-environmental characteristics of soils from major tobacco-growing regions in southern Bulgaria.
- To assess soil fertility and suitability in areas used for tobacco growing and raw material production.

MATERIAL AND METHODS

Study area

Soils collected from three major tobacco producing regions in southern Bulgaria – Gotse Delchev, Kardzhali and Haskovo regions (Zaprianova, 2006) were subject of this study.

Gotse Delchev region

In this region, areas with altitude of 500-700 m are predominant, followed by areas with 700-1000 m altitude. Soil types are mainly Deluvial and Alluvial; and subtypes

of Cinnamon forest soils eroded to varying degrees are found on the southern erosion forms of the relief. Tobacco production on higher altitudes is done on lands with transitioning soil types, from Cinnamon to Brown forest soils, and occurs in reliefs where temperatures are generally low.

The annual rainfall is 730 to 810 mm with a maximum in December, and minimum in August. During tobacco growth period the amount of rainfall is about 170 mm. The average diurnal temperature during the maturation of the leaves is 20.2 °C.

Kardzhali region

This is the largest tobacco-producing region in Bulgaria. It covers the eastern, middle and western parts of the Rhodopes mountain range, and is located on highlands and hilly terrain. The relief is low-lying in the east and the altitude gradually rises to the west. There are predominantly Cinnamon forest soils, eroded to varying degrees. Common are Alluvial- and Deluvial-meadow soils, Rendzinas, Pseudopodzolic, and Brown forest soils. Vertisols are found in several small areas.

Annual rainfall varies between 700 and 1020 mm. The amount of rainfall during the growing period is from 135 to 160 mm. The average diurnal temperature during tobacco leaves ripening is about 21.8 °C, with a maximum in July: 23.4 °C.

2.3. Haskovo region

The relief of the area is characterized by low, rounded hills and altitude of about 300 m. Settlements and arable land are located between the low ridges of the hills. Soil types in the area are mainly Cinnamon forest, Vertisols and Alluvial meadow.

The climate is continental Mediterranean and the area belongs to the climatic region of the East Rhodopes river valleys. Annual rainfall is about 570 mm with a maximum in December and a minimum in August. The amount of rainfall during the growing period is about 190 mm. The average diurnal temperature during ripening of the leaves is about 22.3 °C.

For the purposes of this agri-environmental study soil samples from the following regions were analyzed:

1. Gotse Delchev region: Gotse Delchev Municipality – the town of Gotse Delchev and villages: Breznitsa, Banichan, Borovo, Kornitsa, Kondovo, Musomishte, Debren, Lazhnitsa, Ablanitsa;
2. Kardzhali region: Kardzhali Municipality – villages: Vishegrad, Beli Plast, Ranilist, Rudina, Gluhar, Yastreb; Kirkovo Municipality – villages: Erovete, Chakalarovo, Dyulishte, Fotinovo;
3. Haskovo region: Haskovo Municipality – the town of Haskovo and villges: Gorno Voyvodino, Maslinovo; Stambolovo Municipality – villages: Stambolovo, Tankovo, Dolno Botevo, Kralevo, Gledka; Ivaylovgrad Municipality – villages: Plevun and Kondovo.

The following parameters were determined:

1. Soil separates - pipette method (by Wigner);
2. pH in aqueous extract - potentiometric (ISO 10390);
3. Organic matter content - in Turin;
4. Total nitrogen content - Kjeldahl;
5. Mobile phosphorus content - by Egner-Rheem;
6. Mobile potassium content - with 2n HCl;
7. Contents of mobile forms of iron, manganese, copper, zinc - 0.005M DTPA + 0.1M TEA, pH 7.3 (ISO 14870).

Elemental contents were determined in accordance with ISO 11047 using atomic absorption spectrophotometer ‘Varian Spek-

trAA 220', Australia, with the following operating wavelengths: Fe – 259.9 nm, Mn – 257.6 nm, Cu - 324.8 nm, Zn – 213.9 nm.

RESULTS AND DISCUSSION

A. Main soil features

Soil texture Figure 1 shows the soil texture in the areas surveyed. In the region of Gotse Delchev the predominant soil type is loamy sand soils (50%), followed by light clayey-sandy soil (40%). In Kardzhali region loamy sand soils are also predominant (30%), however, the soil texture is diverse - from very light to moderately heavy. Most of the soils on which tobacco is grown are eroded to varying degrees. In Haskovo region tobacco is mainly grown on soil with diverse textures: loamy sandy, light and medium clayey-sandy, light loam. Each of them represents 20%. Physical clay content ranges from 16 to 67% (Table 1).

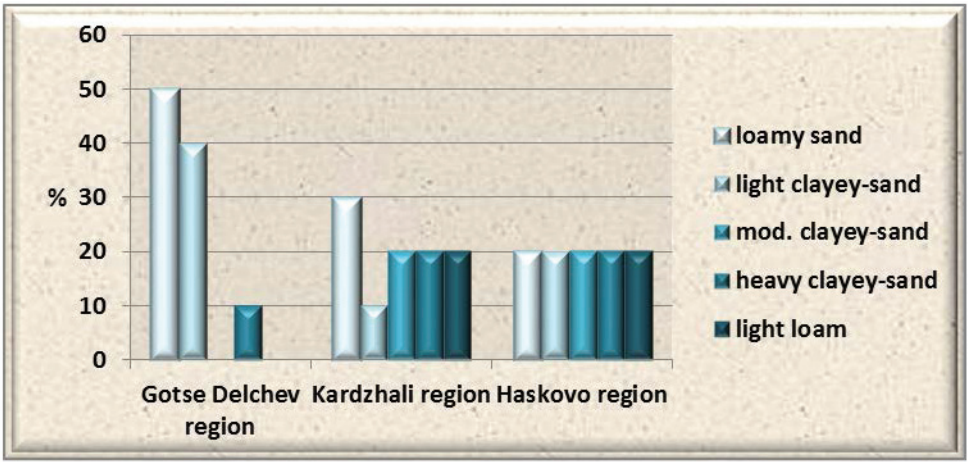

Figure 1. Soil texture

Soil reaction

Growth and development of any tobacco plant depend on soil pH. In all areas surveyed, the presence of soils with acidic, neutral and alkaline reaction to the soil solution was determined.

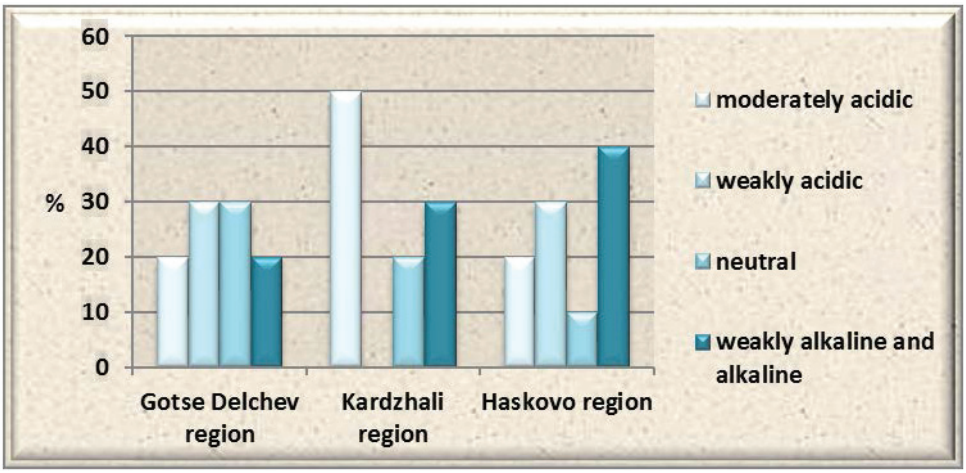

Figure 2. Soil reaction

The data shown in Table 1 shows that soil reaction is in the range between 5,20 to 8,00. In Gotse Delchev region predominant are soils with weakly acidic and neutral reactions. In Kardzhali region, most widespread are soils with a moderately acidic reaction, followed by soils with weakly alkaline and alkaline reactions. In Haskovo region, the majority of the soils sampled had weak alkaline and alkaline reactions (Fig. 2). In general, in terms of pH, all soils analyzed meet the requirements for normal growth and development of tobacco plants.

Organic matter content

According to Artinova's classification (Gyurov and Artinova, 2015), soils from the region of Gotse Delchev, on which tobacco plants are grown, are characterized by low organic matter – this is the case with 100% of the soil samples (Fig. 3). In the region of Kardzhali organic matter content varies from 0.86 to 3.11% (Table 1), and in Haskovo region – from 1 to 5%. In Haskovo predominant are soils with moderate organic matter content. In terms of organic matter content, all soils samples collected in the tobacco producing areas of Gotse Delchev region, and most of the samples collected from Kardzhali region, meet the requirements for oriental tobacco production. Exceptions are a small number of soils samples collected in Haskovo region, which are characterized by high organic matter content.

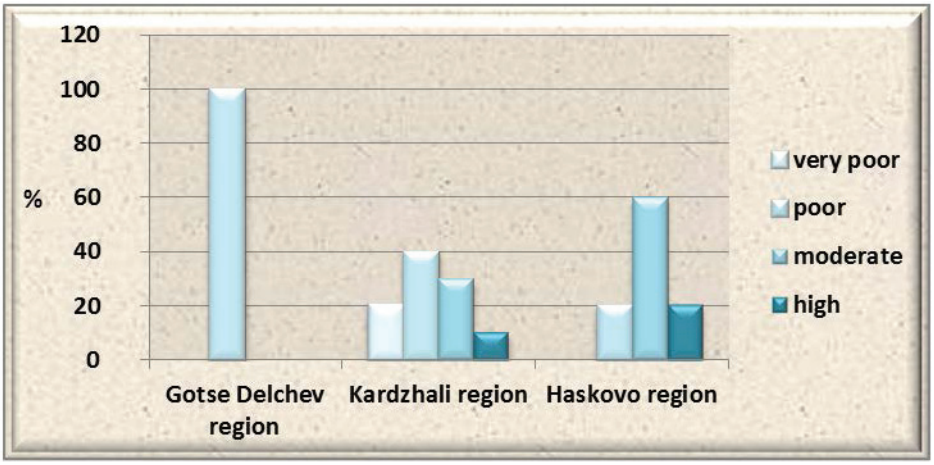

Figure 3. Organic matter content

Table 1. Soil features in the studied areas

Statistical indices	Soil texture	pH	Organic matter
	%		%
Gotse Delchev region			
Mean	21,70	6,30	1,31
St. deviation	8,29	0,55	0,28
Minimum	14,00	5,22	1,08
Maximum	42,10	7,06	1,94
CV, %	38,20	8,70	21,37
Kardzhali region			
Mean	39,58	6,53	1,72
St. deviation	18,84	0,99	0,77
Minimum	16,30	5,43	0,86
Maximum	62,80	7,98	3,11
CV, %	47,60	15,16	44,77

Haskovo region			
Mean	40,24	6,81	2,80
St. deviation	19,85	0,94	1,36
Minimum	16,20	5,70	1,04
Maximum	67,00	8,07	5,00
CV, %	49,33	13,80	48,57

B. Content of macro and trace elements in soils from tobacco producing regions

Total nitrogen availability

Table 2 shows that the availability of total nitrogen in Gotse Delchev region ranges from 0.05% to 0.10%, in Kardzhali region – from 0.05 to 0.19% and in Haskovo region – from 0.07 to 0.28%. In Gotse Delchev region predominant are soils with moderate availability, and in Haskovo region - those with relatively high availability (Fig. 4). It is known that the highest quality of our tobaccos is achieved whenever they are grown on soils with relatively low organic matter and total nitrogen contents.

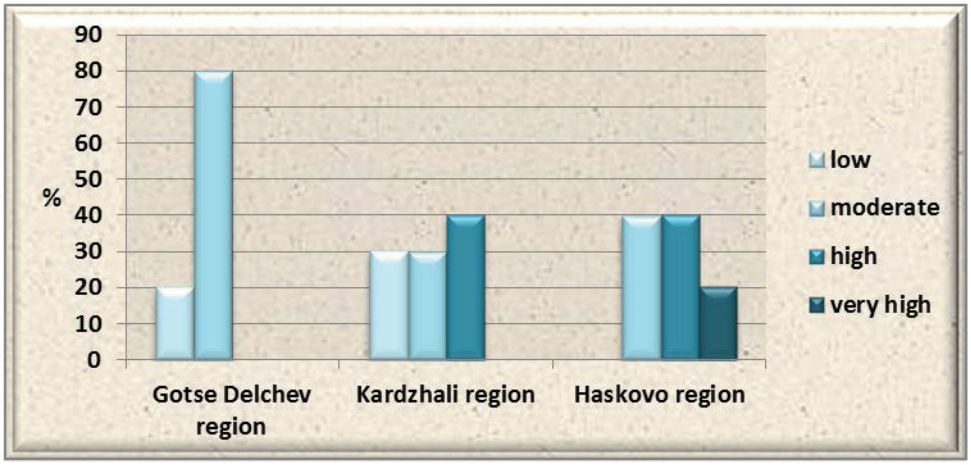

Figure 4. Total nitrogen content

In terms on total nitrogen content, all soil samples collected from the region of Gotse Delchev, and some of the samples from the regions of Kardzhali and Haskovo, are suitable for oriental tobacco production. The unfavourable effects of high total nitrogen content on the tobacco material quality can be overcome with the implementation of appropriate agricultural techniques and farming practices.

Table 2. Macro and microelements content

Statistical indices	total N	P_2O_5	K_2O	Fe	Mn	Cu	Zn
	%	mg/100g		mg/kg			
				Gotse Delchev region			
Mean	0,07	5,77	21,68	25,11	45,30	1,92	1,28
St. deviation	0,01	3,97	25,96	12,06	19,38	1,56	0,512
Minimum	0,06	2,49	9,85	11,70	12,66	0,50	0,69
Maximum	0,10	15,00	95,33	44,82	68,64	6,24	2,40
CV, %	14,29	68,80	119,74	48,03	42,78	81,25	40,00
				Kardzhali region			
Mean	0,10	14,21	33,14	22,54	52,36	1,88	6,21
St. deviation	0,04	19,91	19,65	16,91	35,78	1,19	15,55
Minimum	0,05	0,98	6,15	7,40	7,70	0,36	0,59

Maximum	0,19	62,25	69,80	60,00	130,48	3,74	50,44
CV, %	40,00	140,11	59,29	75,02	68,33	63,30	250,40
Haskovo region							
Mean	0,16	25,44	36,93	20,34	59,73	3,28	2,93
St. deviation	0,071	27,41	23,40	15,09	26,49	2,26	2,29
Minimum	0,07	4,21	13,91	5,54	20,10	0,68	0,80
Maximum	0,28	76,25	78,95	50,84	111,48	7,54	7,73
CV, %	44,38	107,74	63,36	74,19	44,35	68,90	78,16

Mobile phosphorus content

Phosphorus deficiency could cause a number of developmental and growth disorders resulting in their delay, and punctate necrotic spots on the leaves in the case of abundant nitrogen fertilization and possibly with higher content of mobile forms of manganese in the soil.

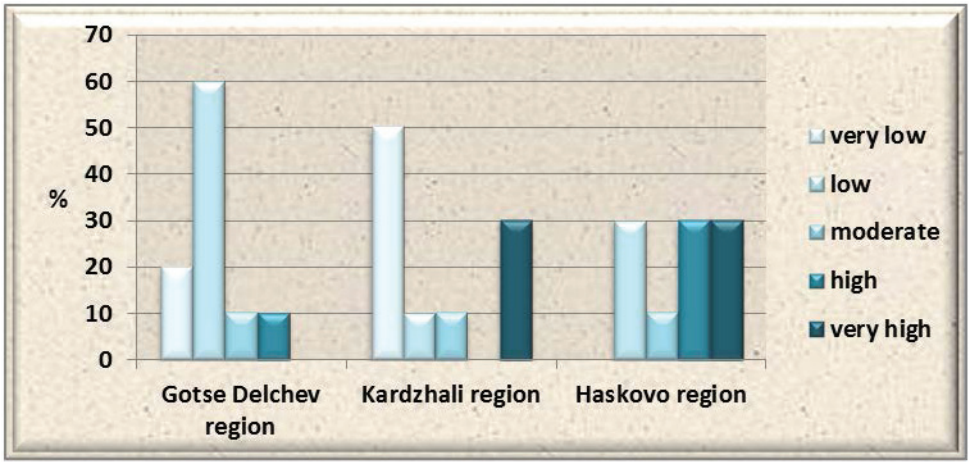

Figure 5. Mobile P availability

In Gotse Delchev region predominant are soils with low availability of mobile P, expressed as P205. They represent 60% of the analyzed soil samples. Very poor levels of P availability were determined 20% of the samples. In Kardzhali region phosphorus availability varies within a very wide range – from 0.98 to 62 mg/100g. The variation coefficient reaches 140% (Table 2). Soils with very poor phosphorus availability represent 50% of all samples, followed by soils with a very good availability – 30 %. In Haskovo region soils with excellent and very good availability represent 60 % of the samples. The observed high values could be explained by manure fertilization.

Availability of mobile forms of potassium

The content of potassium mobile forms in Gotse Delchev region varies greatly – from very low to high, and the variation coefficient reaches 119% (Table 2). This variation is maintained not only between different soil types, but also within the same soil type. A comparison between this region and other two shows that it is characterized by relatively low soil potassium content. Soils with medium potassium availability are observed in 60% of the samples, followed by soils with low availability – 30% (Fig. 6).

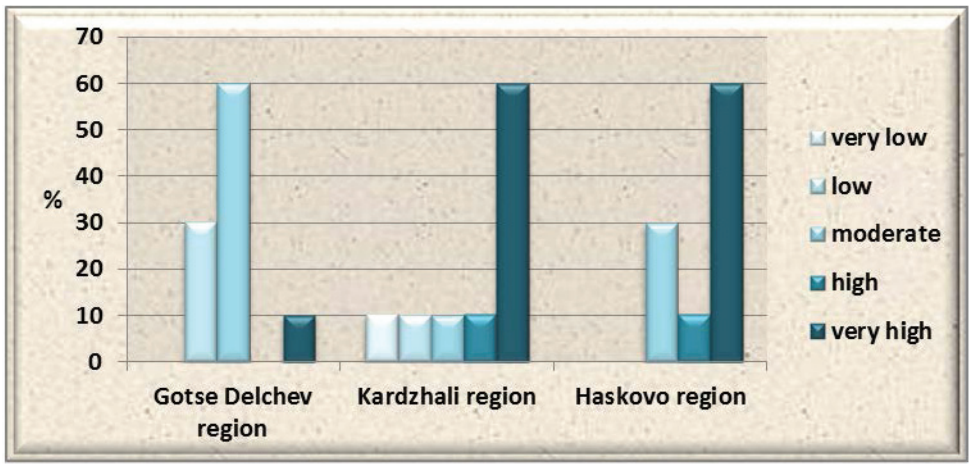

Figure 6. Mobile K availability

Soils collected in Kardzhali and Haskovo regions have a very good level of availability of potassium mobile forms, which predetermines a favorable potassium cycle. In terms of content of mobile forms of potassium, the majority of the soils analyzed are suitable for oriental tobacco growing.

Availability of mobile forms of iron

The content of mobile forms of iron in Gotse Delchev region ranges between 11.72 mg/kg and 45.00 mg/kg; in Kardzhali region – between 7.40 mg/kg and 60 mg/kg; and in Haskovo region – between 5.50 mg/kg and 51 mg/kg (Table 2). According to O'Hallorans et al. (2004), iron deficiency in tobacco occurs at less than 5 mg/kg content of mobile forms of iron in the soil. In all samples analyzed the availability of mobile forms of iron is good.

Availability of mobile forms of manganese

According to the classification of MAFF (Mitsios et al., 2005) the samples collected from all three regions have a very high content of mobile forms of manganese, which predetermines favourable manganese cycle (Fig. 7). It is notable that even if soil reactions are alkaline, the contents of mobile forms of manganese are high. A possible explanation could be the relatively high total content of this element in the soils of Bulgaria. According to Brashnarova (1981), the average content of Mn in Bulgaria is 1200 mg/kg, and the average value for soils in the world is about 545 mg/kg (Kabata-Pendias and Pendias, 1989).

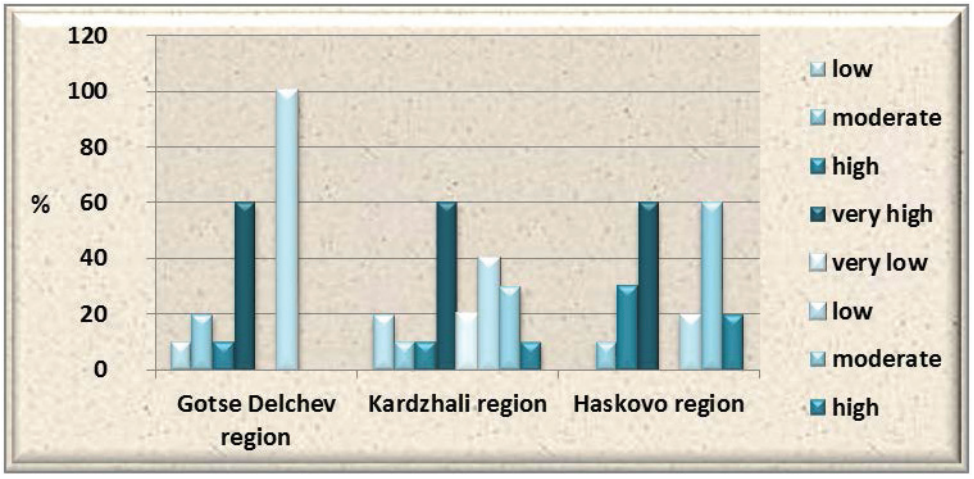

Figure 7. Mobile Mn availability

Availability of mobile forms of copper

The samples collected from tobacco growing areas in Gotse Delchev and Haskovo regions have a level of availability of mobile forms of copper that is considered good (Fig. 8). In Kardzhali region low copper content was determined in 30 % of the samples.

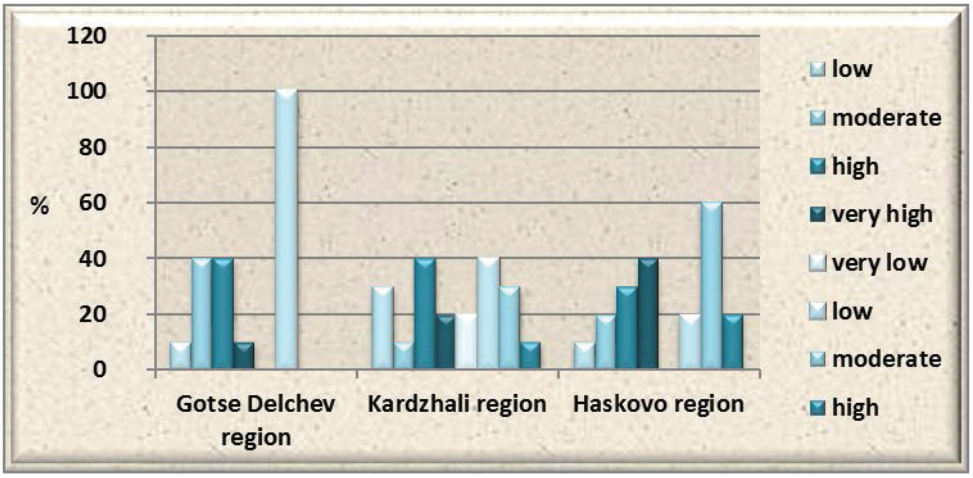

Figure 8. Mobile Cu availability

Availability of mobile forms of zinc

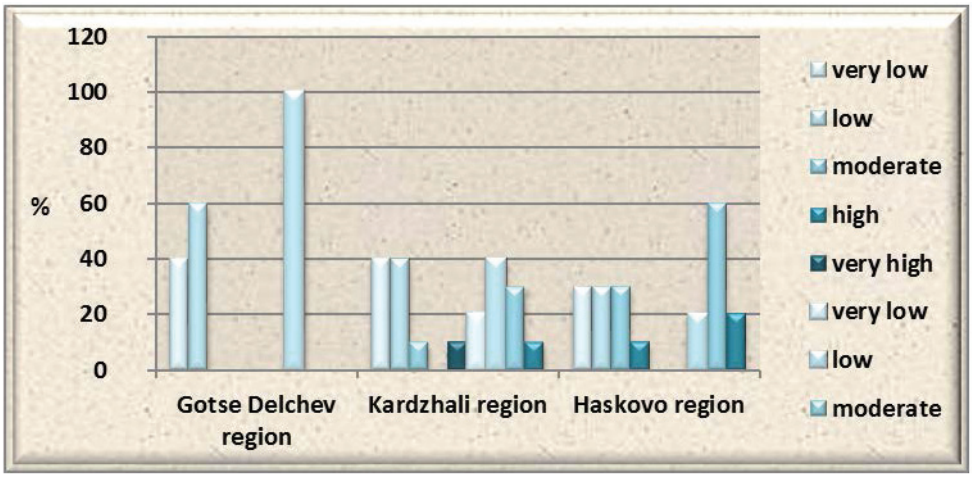

Figure 9. Mobile Zn availability

Soil samples collected in Gotse Delchev region are characterized by low and very low contents of mobile forms of zinc. It is notable that even when soil reactions are acidic, contents of mobile forms of zinc are low. In Kardzhali region contents of mobile forms of zinc vary greatly – from 0.59 to 50 mg/kg. The variation coefficient reaches 250% (Table 2). The highest value was determined in the village of Vishegrad, Kardzhali municipality, located near a lead-zinc plant. In Haskovo region soils with very low, low, and sufficient content represent 30% of all samples analyzed, and those with a high content – 10%.

CONCLUSIONS

Based on the agri-environmental analysis made and the results obtained, the following conclusions are drawn:

In terms of soil texture, soils in Gotse Delchev and Kardzhali regions are very suitable for oriental tobacco production. Some of the soils in Haskovo region also meet the criteria for oriental tobacco growing.

Soil reaction ranges between 5.0 and 8.0 and meets the requirements for normal growth and development of tobacco plants.

According to organic matter contents, soils in the tobacco production areas in Gotse Delchev region and most of the soils in Kardzhali fully meet the requirements for oriental tobacco production. Exceptions are some of the soils in Haskovo region, characterized by high organic matter content. With regard to total nitrogen content, all soil samples from Gotse Delchev region and some of the samples from Kardzhali and Haskovo regions are suitable for oriental tobacco growing. The adverse effects of high organic matter and total nitrogen contents on the tobacco material quality could be overcome with the implementation of appropriate farming practices.

A significant part of the soil samples collected in Gotse Delchev and Kardzhali regions are poor in terms of availability of mobile forms of phosphorus, which requires replenishment with appropriate amounts of phosphorus fertilizers. Soils in Haskovo region are characterized by high phosphorous content.

With regard to potassium contents, most of the soils in Kardzhali and Haskovo regions have an increased availability for this element (high and very high levels of availability) and hence, a favourable potassium cycle. Some of the soils in Gotse Delchev region require potassium fertilization.

The contents of mobile forms of iron and manganese in most of the soils from the three tobacco growing regions sampled were determined to be high to very high, which evidences favorable iron and manganese soil cycles. The availability of mobile forms of copper is moderate to very high, mobile zinc availability is low to moderate.

According to all indicators determined, regardless of the predominantly monocultural planting of oriental tobacco, most of the soils collected in the studied tobacco producing regions are perfectly suitable for the production of oriental tobacco and yield good quality tobacco raw material.

The favorable climate and soil conditions, the experience of several generations of farmers and the established tobacco production traditions are an important prerequisite for the continuation of the production practices and support of the tobacco sector.

REFERENCES

1. Brashnarova A. 1981. Content and distribution of copper, zinc, lead, cobalt, nickel, chromium, manganese, iron and aluminium in the some soils of South Bulgaria; Soil Science and Agrochemistry, 1, 39-47.
2. Gurov D., N. Artinova. 2015. Soil Science, Plovdiv, p. 474.
3. Donev N., G. Zlatev 1974. Tobacco in Bulgaria, Plovdiv, p. 550.
4. Zapryanova P., 2006. Soil-climatic Conditions in the Broadleaf Tobaccos Growing Regions. Ecology and future, 2, 68-76.
5. Zaprjanova P., R. Bozhinova, D. Yancheva, 2014. Nutrients and Heavy metals content in Tobacco soils in the Region of Kardzhali, Jubilee Scientific Conference with Irticipational Participation “70 years Tobacco and Tabacco Products Institute”, 13-14.11.2014.

6. Kabata Pendias A., H. Pendias, 1984. Trace Elements in Soils and Plants, 2nd ed.; CRC Press: Boca Raton, FL, 424.
7. Tanov E., Lukanov K., Miljanchev I., Penchev P., Andonov A., Konarev A. 1978 District-division, concentration and specialisation of tobacco-cultivation and tobacco-processing in Bugaria. Plovdiv, 327.
8. Timev, A., M. Veselinov, K. Atanasov, Ts. Dimitrov. 1974. Oriental tobacco in Bulgaria, Sofia, p. 447.
9. Yancheva D, P. Zaprjanova, R. Bozhinova. 2010. Towards the monitoring of agrochemical characteristics of tobacco soils in the Kardzhali region, Scientific researches, Scientific conference with internatiol participation, v. IV, Part II, 51-57.
10. ISO 10390, 2005. Soil quality – Determination of pH.
11. ISO 14870, 2001. Soil Quality. Extraction of trace elements by buffered DTPA solution.
12. ISO 11047, 1998. Soil quality – Determination of cadmium, chromium, cobalt, copper, lead, manganese, nickel and zinc in aqua regia extracts of soil – Flame and electrothermal atomic absorption spectrometric methods.
13. Mitsios I. K., Golia E.E., Tsadilas C.D. (2005) Heavy metal concentrations in soils and irrigation waters in Thessaly region, central Greece, Communications in Soil Science and Plant Analysis 36, 487-501.
14. O'Hallorans J. M., W. C. Lidemann, R. Steiner., 2004. Iron Characterization in Manure Amented Soils, Communication in Soil Science and Plant analysis, 35, (15-16), 2345-2356.

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Original Scientific paper

INFLUENCE OF CIGARETTE CIRCUMFERENCE ON TAR, NICOTINE AND CARBON MONOXIDE YIELDS

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ABSTRACT

Differences in length and circumference of cigarettes influence smoker behavior and exposure to smoke constituents. Super slim cigarettes (17 mm circumference versus 25 mm circum¬ference of conventional king-size cigarettes), have gained popularity the world in the mistaken belief that smokers will be exposed to less harmful chemicals than traditional brands.

The objective of this study was to determine the effect of circumference on tar, nicotine, and carbon monoxide yields on five commercial brands of super slim and king-size cigarettes, both with tar yield of 1 mg, 4 mg and 7 mg/cig. The content of tar (as NFDPM), nicotine and carbon monoxide in mainstream smoke of cigarettes are measured using ISO standards 4387, 3400, 10362-2, and 8454.

Our results indicated significant reductions of tar yield in 4 and 7 mg super slim cigarettes, compared with the equivalent tar yield king-size products. However, mean tar and nicotine of 1 mg super slim cigarettes and king-size products was not significantly different.

Keywords: super slim, king-size, cigarettes, circumference, tar, nicotine, carbon monoxide

ВЛИЈАНИЕТО НА ОБЕМОТ НА ЦИГАРИТЕ ВРЗ СОДРЖИНАТА НА КАТРАН, НИКОТИН И ЈАГЛЕРОДЕН МОНОКСИД

Разликата во должината и обемот на цигарите влијае на однесувањето на пушачот и изложеноста на штетните состојки од чадот. Super slim цигарите (со обем 17 mm наспроти king-size цигарите со обем 25 mm) имаат стекнато голема популарност во светот поради погрешното верување дека пушачот е помалку изложен на штетните материи споредено со традиционалните брендови.

Целта на ова испитување беше да се утврди ефектот на обемот врз содржината на катран, никотин и јаглероден моноксид кај пет комерцијално достапни марки на super slim и пет king-size цигари со содржина на катран 1 mg, 4 mg и 7 mg/циг.

Содржината на катран (како NFDPM), никотин и јаглероден моноксид во чадот од цигарите е определена со ISO стандардите 4387, 3400, 10362-2 и 8454.

Нашите резултати покажаа значајно намалување на содржината на катран кај super slim цигарите со декларирана содржина од 4 и 7 mg, во споредба со содржината на катран кај king-size производот.

Сепак, просечната содржина на катран и никотин кај super slim цигарите од 1 mg/циг. не се разликуваше значајно споредено со king-size производ.

Клучни зборови: super slim, king-size, цигари, обем, катран, никотин и јаглероден моноксид

INTRODUCTION

In 2015, over 5.5 trillion cigarettes were sold to more than one billion smokers worldwide. In the European Union, research by the European Commission has shown that the market share of reduced circumference cigarettes grew significantly within a declining EU cigarette market from 3.6% in 2006 to 6% in 2012. Today the market share of slim and super slim cigarettes has grown significantly in an overall declining European Union cigarette market (WHO report). In the past ten years, smokers have widely shifted from high-nicotine and high tar cigarettes to lower yield brands. Also, they base their perceptions of risk on length and circumference of cigarettes, believing that longer and slimmer cigarettes are less harmful to smoke than king-size or regular cigarettes.

Slim cigarettes were first brought to market in the 1960s, almost half a century ago and they are intended to the woman. Developing this trend, the leading tobacco companies are launching super slim tobacco products approximately the twenty years later. Slim and super slim cigarettes are widely acknowledged to increase perceptions of stylishness and to generally appeal to women and teenagers. They are promoted via advertising and cigarette packs with prominently displayed tar values (Ford et al. 2014, Kotnowskiet al.2015).

Over many years there have been numerous technological approaches to the reduction of tar, nicotine and carbon monoxide yields in cigarette smoke. Reducing tar levels is typically achieved through cigarette designing, changing features such as filter density, tipping paper length, filter ventilation levels, circumference and tobacco density (O'Connor et al. 2008, Stephens 2007).

Although cigarettes are generally cylindrical they have been made in a variety of lengths and circumferences. Traditional king-size cigarettes have circumferences of 24–25 mm, while "slim" cigarette can have circumferences that range from 22 and 24 mm. The cigarettes with circumference between 19 and 22 mm are referred to as demi-slims and those in the range 16–19 mm are termed "super slim".

Changing the cigarette dimensions may affect the smoke formation and transport processes within the cigarette rod which in turn could affect the quantities and chemical composition of the mainstream (MS) and sidestream (SS) smoke (Norman 1999, Baker 1999).

Slims and super slim cigarettes design has reduced tobacco weight, smaller rod dimension and circumference, a longer and more efficient filter, filter ventilation and high permeability paper. The combination of these design elements results in tar and nicotine yields that are less than regular cigarettes.

The effects of cigarette rod circumference have been well studied (Yamamoto et al. 1984, Yamamoto et al.1985, Muramatsu 2005) on nicotine and other MS components.

Dittrich et al. (2014) described a series of prototype, reduced-toxicant cigarettes which included three 7 mg ISO tar yield cigarettes manufactured at 17, 21 and 24.6 mm circumferences but with the same tobacco blend and packing density. For MS yields of tar, nicotine and carbon monoxide the yields either diminished as circumference decreased or the differences between yields were not significant.

The amount of tobacco varies with the circumference of the cigarette.The tobacco weight used in a cigarette has an important role in determining both the level of smoke emission from a cigarette and product quality. For a fixed length cigarette, tobacco weight can be reduced by decreasing either the tobacco packing density or the circumference.

Reducing circumference has been one of cigarette design parameters that have been investigated as a potential tool for emission reduction.

We are focus mainly on the effects of cigarette circumference to assess the interaction of other physical parameters with tar, nicotine and carbon monoxide (CO) in mainstream smoke.

The objective of this study was to determine the effect of circumference on tar, nicotine, and carbon monoxide yields on five commercial brands of super slim and king-size cigarettes, both with tar yield of 1 mg, 4 mg and 7 mg/cig.

MATERIAL AND METHODS

Samples

We investigated two different type of cigarette: five brands of super slim (SS) and five brands of king–size (KS) cigarettes within the ISO pack tar bands of 1 mg, 4 mg and 7 mg. All cigarettes were filter-tipped and purchased at retail outlets in Republic of Macedonia.

Condition the test samples, prior to physical measurements and mainstream smoke analysis, in a conditioning enclosure in accordance with ISO 3402 (1999) (60 ± 3 % relative humidity and 22 ± 1°C temperature for a 48 h period).

Physical parameters

The pressure drop and filter ventilation measurements were recorded on 20 cigarettes from each pack using the QTM 5 - PD & Ventilation apparatus (Cerulean,Milton Keynes, UK) according ISO 6565.

Calibrated Vernie Caliper (Mitutoyo, Japan) was used to measure the circumference, diameter of cigarette, the length the tobacco rod, filter and tippingand overall cigarette (SRPS E.P2.01:1965).

The cigarette weight measurements were taken on 20 cigarettes from each pack using an analytical balance (Sartorius BP 221 S, Germany) (SRPS E.P2.01:1965).

Collection of mainstream tobacco smoke (MS) and analysis

For mainstream smoke analyses, the cigarettes were smoked under ISO 3308 (2000) by ISO smoking conditions (a35-ml puff of 2 seconds duration taken every 60 secondswith ventilation unobstructed).

The tar (NFDPM) content was determined according to ISO 4387 (2000), nicotine content according to ISO 10315 (2000), and the carbon monoxide by ISO 8454 (1995). Each cigarette sample was prepared for analysis according to ISO 8243 (2006) and evaluation of statistical variation of tar, nicotine, and carbon monoxide yields.

The total particulate matter (TPM) was obtained by calculating the weight difference in the Cambridge filter pads (CFP) before and after the smoking process divided by the number of cigarettes smoked.

The tar content was calculated by subtracting the water and nicotine content from the TPM. Tar, nicotine and carbon monoxide values listed on product packages were presented in Table 1

Table 1.The labeling of tar, nicotine and carbon monoxide on cigarette pack

Cigarette samples	Tar (mg/cig.)	Nicotine (mg/cig.)	Carbon monoxide (mg/cig.)
KS 1 mg	1	0.1	1
KS 1 4 mg	4	0.4	4
KS 2 4 mg	4	0.4	4
KS 1 7 mg	7	0.7	7
KS 2 7 mg	7	0.7	7
SS 1 mg	1	0.1	1
SS 1 4 mg	4	0.4	3
SS 2 4 mg	4	0.4	4
SS 1 7 mg	7	0.6	7
SS 2 7 mg	7	0.6	7

RESULTS AND DISCUSION

This study set out to compare the physical design characteristics and smoke emissions of two commercial available cigarette format, super slim and king-sized.

Although cigarette rods are generally cylindrical in shape they have been made in a variety of overall lengths, filter and tipping paper lengths and circumferences.

There are the major differences in sizes of different styles of European cigarette brands (king – size, slim, super slim and compact size cigarette (nanokings).The cigarette size refers to the length and circumference of the cigarette (Dittrich et al. 2014).

Selected physical characteristics including overall cigarette length, filter and tipping length, circumference, weight, and amount of filter ventilation and pressure drop for the cigarette sample analyzed were measured for each cigarette sample.

Results from measuring the length the tobacco rod, filter and tipping and overall cigarette are presented in Figure 1, 2 and 3.

King-sized cigarettes are typically 84 mm long. In tested samples of king-size cigarettes (KS) the overall length ranges from 83 to 84 mm.

The super slim cigarettes (SS) are visibly longer and slimmer in diameter than king-size cigarettes. Tested super slim cigarettes with the overall length from 96.0 to 96.3 mm are longer than king-size cigarettes (KS).

Tobacco rod or column represents a cylindrical rod containing all the tobacco components of the cigarette blend. The measured length of the tobacco rod of SS cigarettes is higher compared to the KS cigarettes in average 13.02 mm.

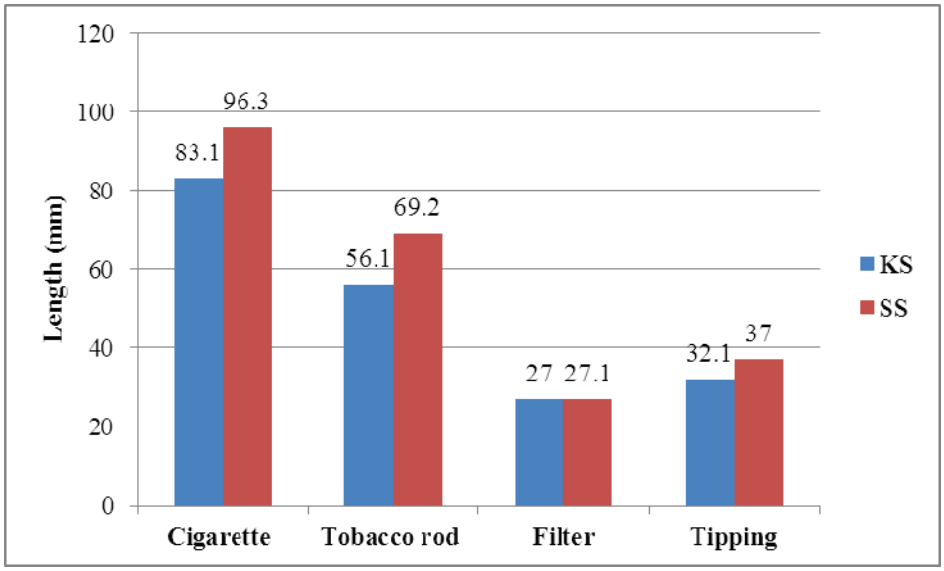

Figure 1. Comparison of the length of KS and SS cigarettes,Tar 1 mg/cig.

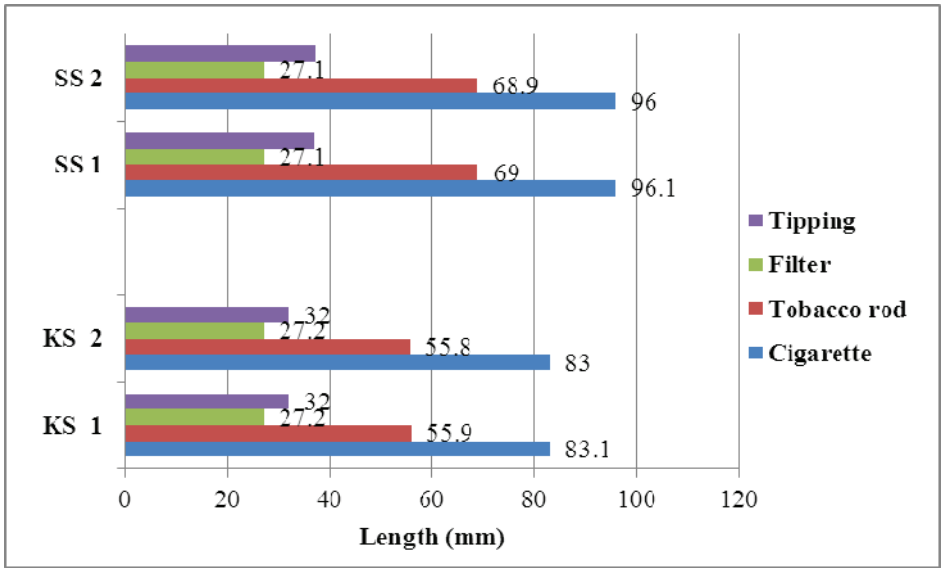

Figure 2.Comparison of the length of KS and SS cigarettes, Tar 4 mg/cig.

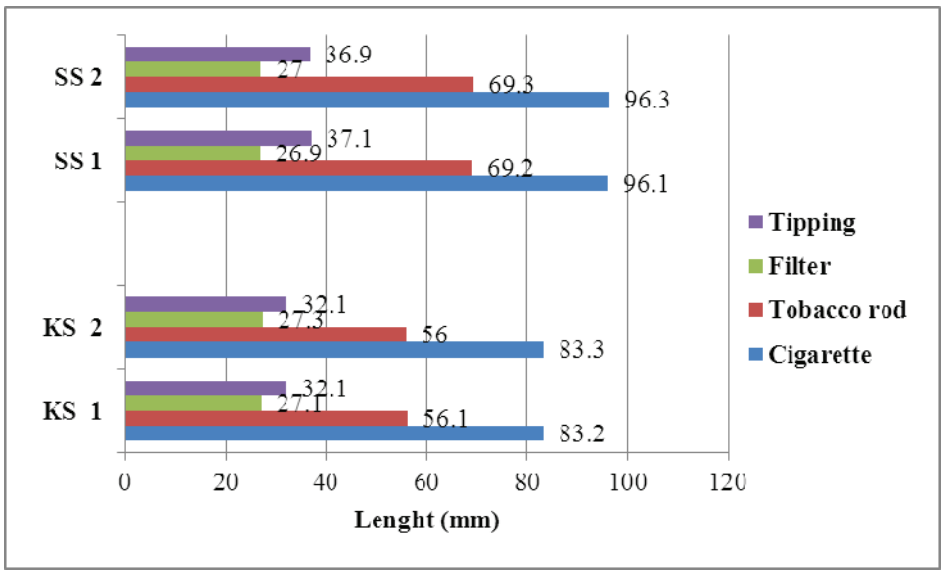

Figure 3.Comparison of the length of KS and SS cigarettes, Tar 7 mg/cig.

"Filter" means filter rod. Regarding the filter length, results shown in both types of products filter length varied slightly (27-27.1 mm). In terms of dimensions, the average length of a cigarette filter is approximately 27.05 mm in both cigarette sizes. The measured length of the tipping paper of SS cigarettes is higher compared to the KS cigarettes in average 5.1 mm.

Parameter circumference of cigarettes is associated with the diameter of the cigarette. But, those are two different physical characteristics of cigarette and filter. The circumference is a perimeter of a circular cross section of cigarette.

The measured circumference in SS cigarettes is visibly lower compared to the KS cigarettes. The circumference of KS cigarettes varies in the range of 24.8 to 25.1 mm. The circumference of all SS samples varies slightly from 16.9 mm to 17.1 mm (Table 2).

Table 2. Comparison of circumference and diameter

Cigarette samples	Circumference (mm)	Diameter (mm)
KS 1 mg	24.7	7.9
KS 1 4 mg	24.9	7.9
KS 2 4 mg	24.8	7.8
KS 1 7 mg	24.9	7.9
KS 2 7 mg	25.1	8.0
SS 1 mg	16.9	5.4
SS 1 4 mg	16.9	5.4
SS 2 4 mg	17.0	5.5
SS 1 7 mg	17.1	5.4
SS 2 7 mg	17.0	5.5

The cigarette diameter impacts the density of tobacco within the product and therefor directly affects various smoking parameters. A varying cigarette diameter during manufacture of cigarette can cause disruption, air pockets in the cigarette, filter drop-outs and various other process problems.

King-size cigarettes measure average 7.9 mm diameter.

Super slims cigarettes measure from 5.4

from to 5.5 mm diameter (Table 2).
Two important properties of ventilated cigarettes that must be controlled are ventilation and cigarette pressure drop.
Table 3 summarizes the measured ventilation and pressure drop of the KS and SS cigarettes. The studied cigarettes covered the wide range of average ventilation from 41.3 to 79.7 %.
The differences in the determined pressure drop in the examined cigarettes were significant. The biggest pressure drop was determined in the SS cigarettes compared to pressure drop measured in KS cigarettes.

Table 3. Physical parameters in KS and SS cigarettes

Cigarette samples	Ventilation (%)	Pressure drop (mmWG)	Tobacco weight (mg)
KS 1 mg	67.1	109.3	549
KS 1 4mg	58.9	92.1	570
KS 2 4 mg	60.1	90.2	592
KS 1 7 mg	43.0	80.3	658
KS 2 7 mg	41.3	81.8	643
SS 1 mg	79.7	90.8	301
SS 1 4 mg	62.0	121.0	315
SS 2 4mg	63.8	124.6	319
SS 1 7 mg	45.5	149.0	359
SS 2 7 mg	47.2	156.3	350

The amount of tobacco varies with the circumference of the cigarette. Super slim cigarettes contain 53-55 % less tobacco than their king-size counterpart (Table 3).
Super slim (SS) cigarettes are considered a less harmful alternative to king-size cigarettes (KS), due to their longer filter and relatively low CO, tar, and nicotine contents.
We examined the influence of circumference on mainstream smoke yields. The impact of altering circumference on the yield of toxicants in MS cigarette smoke was examined using 1 mg, 4 mg and 7 mg/cig. ISO tar KS cigarettes and an equivalent series of 1 mg, 4 mg and 7 mg/cig. ISO tar SS cigarettes (Figure 4, 5, and 6).
For mainstream smoke components we found that in SS cigarettes with smaller circumference, tar and CO yields decrease accordingly. Reducing circumference affects by reducing the total amount of tobacco burnt; this means it will take less time for the tobacco rod to burn and will result in fewer puffs.
Decreasing cigarette circumference increases flow rates, which reduces the amount of time for the smoke to pass and decreases the filtration achieved by the tobacco rod and retention by the filter. That reduce filtration by the tobacco rod or retention by the filter may result in higher levels of MS smoke emissions.
The accuracy of the tar and nicotine values on packets is verified in accordance with ISO standard 8243: confidence intervals around yields required for nicotine and tar are ±20%, and for CO ±25% when sampling at one point in time.

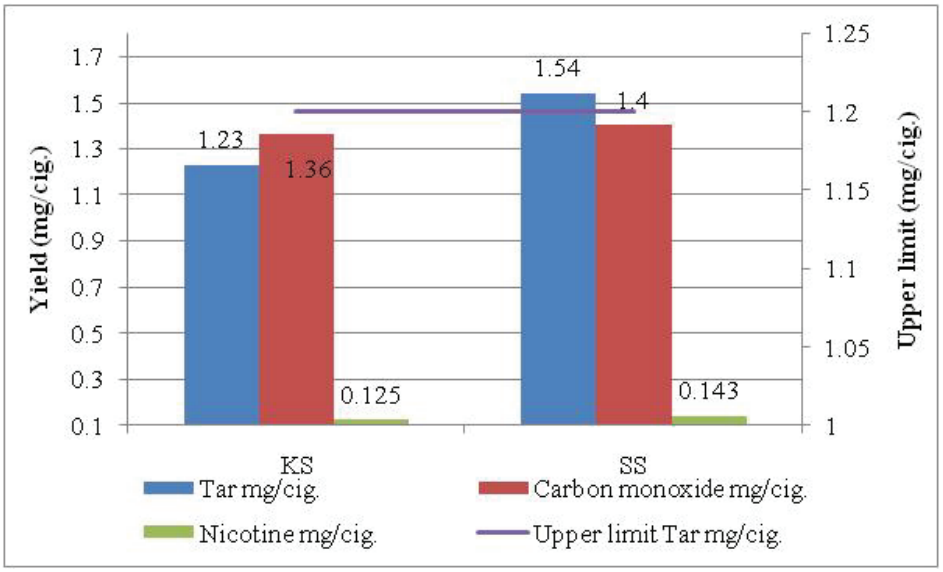

Figure 4.The mean tar, nicotine, and CO smoke yields for KS and SS cigarettes, Tar 1 mg/cig.

Our results indicated reductions of tar yield in 4 and 7 mg super slim cigarettes, compared with the equivalent tar yield king-size products. However, mean tar and nicotine of 1 mg super slim cigarettes and king-size products was not significant different.

Figure 4 shows that mainstream yields in cigarettes increase with increasing cigarettes circumference for tar, nicotine and CO.

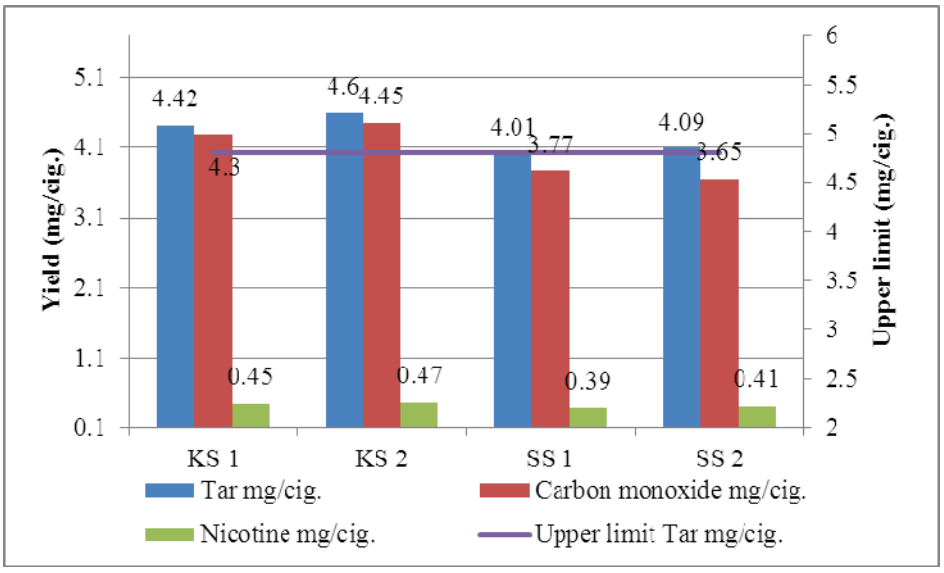

Figure 5.The mean tar, nicotine, and CO smoke yields for KS and SS cigarettes, Tar 4 mg/cig.

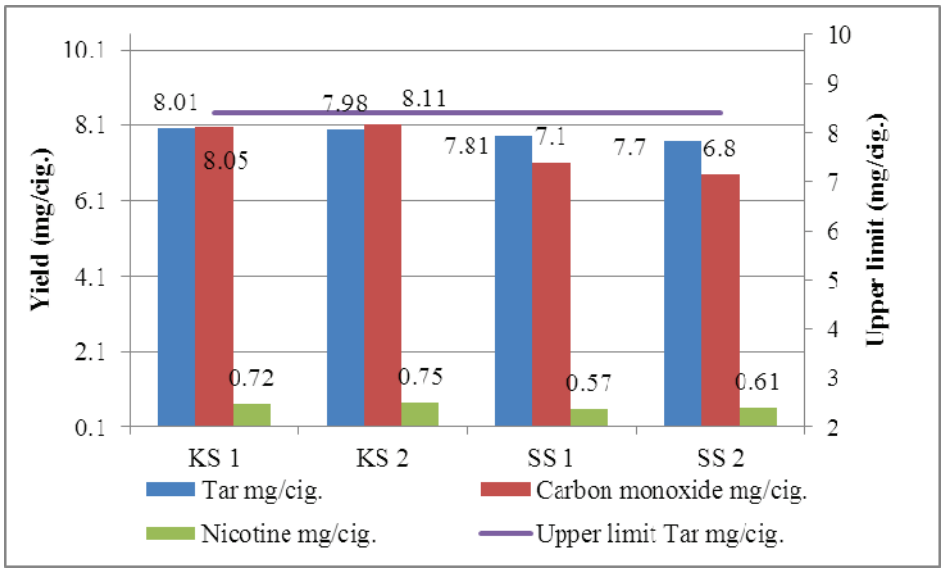

Figure 6.The mean tar, nicotine, and CO smoke yields for KS and SS cigarettes, Tar 4 mg/cig.

Further study measuring smoke constituent are required to help determine the effect of reducing cigarette circumference and other physical parameters on the exposure of smokers to smoke constituents.

CONCLUSIONS

Given the increasing interest in slim circumference and longer length cigarettes, this study examines the effects cigarette circumference on mainstream MS smoke component. Super slim cigarettes may give smokers the impression of reduced yields of smoke because they are longer than king-size cigarettes.

Our results indicated reductions of tar yield measured with standard ISO method in 4 and 7 mg super slim cigarettes, compared with the equivalent tar yield king-size products. However, mean tar and nicotine of 1 mg super slim cigarettes and king-size products was not significantly different.

Super slim cigarettes (SS) are designed to have lower weight of tobacco, and circumference but higher pressure drop and the length of the rod and the whole cigarette. But, the combination of these design elements results in tar and nicotine yields that are not less than king-size cigarettes.

Effectiveness of tobacco weight reduction through decreased circumference on yields of tar, nicotine and CO is diminished.

REFERENCE

1. Baker R.R., 1999. Smoke chemistry. In: Davis DL, Nielsen MT (eds) Tobacco production, chemistry, and technology, Malden, MA: Blackwell Science. 398–439
2. Dittrich D.J., Fiebelkorn R.T,. Bevan M.J. Rushforth D., Murphy J.J., Ashley M., McAdam

K.G., Liu C.,. Proctor A C. J., 2014. Approaches for the design of reduced toxicant emission cigarettes Springer Plus, 3, 374
3. Ford A., Moodie C., Mackintosh A.M., Hastings G., 2014.Adolescent perceptions of cigarette appearance. European Journal of Public Health, 24(3) 464-468
4. ISO 10315 (2000) Cigarettes -- Determination of nicotine in smoke condensates-Gas-chromatographic method International Organization for Standardization, Geneva
5. ISO 3308 (2000) Routine analytical cigarette smoking machine definition and standard conditions. International Organization for Standardization, Geneva
6. ISO 3402 (1999) Tobacco and tobacco products -atmosphere for conditioning and testing. International Organization for Standardization, Geneva
7. ISO 4387 (2000) Cigarettes-determination of total and nicotine free dry particulate matter using a routine analytical smoking machine. International Organization for Standardization, Geneva
8. ISO 8243 (2006) Cigarette-sampling International Organization for Standardization, Geneva
9. ISO 8454 (1995) Cigarettes -- Determination of carbon monoxide in the vapour phase of smoke (NDIR method) International Organization for Standardization, Geneva
10. Kotnowski K., Fong G.T., Gallopel-Morvan K., Islam T., Hammond D., 2015. The Impact of Cigarette Packaging Design Among Young Females in Canada: Findings From a Discrete Choice Experiment Nicotine & Tobacco Research, 1–9
11. Muramatsu M., 2005. An approach to modelling a burning cigaretteBeitr Tab. Int., 21 286–293
12. Norman A., 1999. Cigarette design and materials. In: Davis DL, Nielsen MT (eds) Tobacco production, chemistry, and technology, Malden, MA: Blackwell Science. 353-387
13. O'Connor R. J., Hammond D., McNeill A., 2008. How do different cigarette design features influence the standard tar yields of popular cigarette brands sold in different countries? Tobacco Control, vol. 17, no. 1, i1–i5
14. Stephens W. E., 2007. Dependence of tar, nicotine and carbon monoxide yields on physical parameters: implications for exposure, emissions control and monitoring, Tobacco Control, vol. 16, no. 3, 170–176
15. World Health Organization (WHO). WHO Report on the global tobacco epidemic, 2015: Raising Taxes on Tobacco. Geneva: WHO; 2015. Available from: http://apps.who.int/iris/bitstream/10665/178574/1/9789240694606_eng.pdf?ua=1&ua=1
16. Yamamoto T., Anzai U., Okada T., 1984. Effect of cigarette circumference on weight loss during puff sand total delivery of tar and nicotine.Beitr.Tabakforsch.lnt. 12, 259-269
17. Yamamoto Y., Suga C., Tokura T. T., Okada T. 1985. Effect of Cigarette Circumference on Formation Rates of various Components in Mainstream Smoke. Beitri.ge zurTabak. forschung International.• Volume 13, No. 2, 81-87

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Original Scientific paper

CONDITIONS IN SUPPLY AND DEMAND OF TOBACCO IN THE REPUBLIC OF MACEDONIA

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ABSTRACT

World tobacco production,in spite of the powerful anti-smoking campaign, does not stagnate, but continues to grow in correlation with population growth in the world. Thus, in twenty leading tobacco producing countries, the production ranged from 5.942.748 tons in 2008 to 6.558.000 tons in 2013. It must be noted that nowadays the share of raw tobacco in cigarette content is 0,70-0,80g, while less than 10 years ago it was 1 g, which shows that higher number of cigarettes are smoked, i.e. that the consumption increased. According to our projections, tobacco production in R. Macedonia until 2020 will reach 35000tons, without particular ventures, investments or human resources.

Keywords:supply, demand, tobacco production,a nti-smoking campaign, investment

СОСТОЈБИ ВО ПОНУДАТА И ПОБАРУВАЧКАТА НА ТУТУН ВО РЕПУБЛИКА МАКЕДОНИЈА

Светското производство на тутун иако под голем притисок на антипушачката пропаганда не стегнира,но истото продолжува да расте и е во корелација со порастот на населението во светот.За илустрација, во дваесет водечки земји во производство на тутун кои ја сочинуваат понудата, пропизводството во 2008 година изнесувало 5.942.748 тони, во 2013 година 6.558.000 тони и тоа во услови кога тутунската суровина во цигарата сега изнесува од 0,70-0,80грама, а пред неполни 10 години суровината учествувало со повеќе од 1 грам во цигара.Овој индикатор покажува дека се пушат повеќе парчиња цигари што значи дека е зголемена потрошувачката.Конкретно за Република Македонија согледувањата до 2020година покажуваат дека може да се постигне производство на тутун кое ќе се нуди на пазарот на тутун до 35000тони без посбни вложувања,инвестиции и човечки ресурси.

Клучни зборови: понуда, побарувачка, тутунско производство, анти-пушачка пропаганда, инвестиции

INTRODUCTION

Tobacco production of the Republic of Macedonia was traditionally oriented toward exports on the world market, especially after independence of the country. This trend has been strengthenedafter 2000, and the last five years over 90% of tobacco has beenexported on the world market and the remaining 10% is used in domestic cigarette manufacture.In the above period,almost entireproduction was exported in the foreign marketthe same year it was produced anditwas often higher than the sale of leaf to-

bacco during the year.Thisis big qualitative change in the marketing of processed tobacco, as can be concludedfrom theconstant reduction of fermented tobaccostocks. Today they are at their minimum and are residues fromthe industrial manipulation.Fermented tobacco stocks before 2000 were significant, presenting almostannual production of raw tobacco, which hold a large amount of working capital ofthe companies.Turnover of thecurrent assets was often 1 or below1. Carriers of the sales of fermented tobacco are buyers- processors of raw tobacco. The most dominant are foreign subsidiaries of some multinational companies.Currently, three foreign companies and one domestic account for 80% of sales of Macedonian tobacco in the world market, while the other 20% are smaller companies with only 200-500tons annual sales of tobacco. Tobacco market in Macedonia is controlled by 9-10 companiesand they have an oligopolistic position (oligopolyis a market structure in which a small number of companies has the large majority ofmarket share). Such structurepoints out to a monopoly position in relation to the sellers, i.e.to imperfect competition.Each step of any of these companiesis followed by the other competitive companies and they can even make agreement regarding certain actions, for example, agreement on the criteria for purchase, beginning of purchase, price etc.

TRENDS IN THE REALIZATION OF TOBACCO IN MACEDONIA

In the sphere of supply and demand, raw tobacco market is faced with cyclical, conjuncturalphenomena. These phenomena are rather influenced by cyclical policies in the production of tobacco rawthan by the law of supply and demand. At a global level, these phenomena have a tendency to equalize due to the differences in tobacco production in different parts of the world. Namely,due to the influence of natural conditions,certain areas produce less raw tobacco and other areas produce more.In a long term, tobacco production is equalized and it affects the balance of supply and demand. In the case of more significant deviationof these phenomena, interventions are made from the raw tobacco stocks found in the entities of supply and entities of demand.

The equalization of raw and fermented tobacco productionin the Republic of Macedoniain the period 2011-2015 is presented in Table 1.

Table 1. Production of raw tobacco and fermented tobacco in tons

Year	Raw tobacco	Fermented tobacco
2011	21000	21400
2012	27900	22400
2013	30900	25400
2014	24800	22500
2015	19000	22400
Total	123600	114100
Average	24700	22800

Source: Ministry of Agriculture, Forestry and Water Economy of R. Macedonia

The table shows that the difference of 1900 tons in the production of raw tobacco and fermented tobacco is actually the amount of wastage that occurs during industrial manipulation (percentually it is 9%), which means that the average supply during this period was 22800 tons. The annual production reached 24700 tons and it exceeds the average production of 23000 tons raw tobacco in the period 1991-1995 (source-analysis of Yugotutun- Skopje).

During 2011 – 2015, the average supply reached 22820 tons and the demand was 22520 tons (Table2).

Table 2.Sales of fermented tobacco in the period 2011-2015 (tons)

Year	Fermented tobacco	Tobacco Exports	Difference between supply and demand
2011	21400	21490	90
2012	22400	22460	60
2013	25400	25440	40
2014	22500	22550	50
2015	22400	22600	200
Total	114100	114540	440
Average	22820	22908	88

Source: Ministry of Agriculture, Forestry and Water Economy of R. Macedonia

The sales and exports of fermented tobacco in 2011-2015 was 440tons higher as a result of the sales of tobacco stocks from the previous period. Data based on analytical calculations of tobacco stocks in 2002-2009 are presented in Table 3.

Table 3.Tobacco stocks movement in 2002-2009

Year	Stocks
2002	4897
2003	590
2004	4392
2005	5774
2006	8244
2007	7246
2008	- 3188
2009	7410
Total	35365
Average	4421

During 2011 - 2015, the amount of sold tobacco was 400 tons higher, due to the sale of transitional stocks from previous years which amounted 4421 tons, i.e. that the raw tobacco material which in this period remained for domestic market and the quantities of tobacco stocks were 4421 tons. For illustrate this, it should be mentioned that tobacco stocks from each harvest in the period 1991-1995 measured 18530tons, i.e. 84% of the current crop, as a result of liberalism in the production of varieties that belonged to the same type of tobacco, but had a different quality. Such tobaccos were eliminated by the market itself and they were hold as buffer stocks of the country.

Based on data from Yugotutun-Skopje, tobacco stocks and exports in the period 1991-1995 are presented in Table 4.

Table 4.Movements in production of fermented tobacco and tobacco exports(in tons)

Year	Fermented tobacco	Tobacco stocks	Exports
1991	16526	10274	17000
1992	22997	13046	12000
1993	25964	22105	12000
1994	21143	24634	9000
1995	16152	22595	12000
Average	20556	18530	12400

Source: Analysesof Yugotutun Skopje

In the beginning of transition period (1991-1995), tobacco industry of the Republic of Macedonia had an average production of 20556 tons of fermented tobacco and 18530 tons of stocks stored in warehouses. It represents 39000 tons of fermented tobacco along with the stocks, plus the current harvest in the related year.

The above data point out to apparent qualitative and quantitative change of the situation in the market and trends int obacco market in the period 2011-2015, which is confirmed by the following:

-the average production of fermented tobacco in 2011-2015 was 11% higher than the average production in 1991-1995 (22820:22556, Table 4).

-the average tobacco stocks of 18530 tons in 1991-1995 amounted to 35365 tons in 2002-2009, which is 4421 tons in average and which denotes stocks reduction of 4110 tons.

Tobacco exports in the period 2011-2015 averaged 22520 tons, compared to 12400 tons in 1991-1995, which is an average increase of 10120 tons.

These trends in tobacco market point out to increasing demand of tobacco, covered by reduction of tobacco stocksin order to meet the requirements for tobacco exports. The main factor of this qualitative change and increased sales of tobacco is the improvement of the technological process of fermentation and processing of tobacco. Until 2002, privatized enterprises performed the process of fermentation in the so called FERM plants with multiple series of chambers, but it was long and expensive process (in 1997, according to the plan for processing of tobacco in Tobacco Company - Prilep, only the cost of energy accounted for 14% of the cost of fermented tobacco). The purpose of change of technology is to remove in the most efficient way the threat of catalase (mold) of tobacco, which was taking too much timein the old system of fermentation. The introduction of a new technological system in tobacco fermentation,

so called Redrying system (pasteurization procedure) effectively eliminates the risk of catalase, which significantly shortens the time for tobacco processing. Beside this, where this technological process is applied, tobacco is packed immediately and directly delivered to customers, i.e. cigarette plants. This means that the sales of processed tobacco go continuously throughout the whole year and thus provides higher turnover and saving in binding cash assets, whichcould not be provided by the previous system of fermentation.

CONCLUSION

Investigations onthetrends in sales of tobacco in R. Macedonia lead to the following statements:

1. Demand of tobacco in this period is higher than supply.
2. In addition to technology, the sales of tobaccois significantly affected by the process of globalization of tobacco industry.
3. In case several companies merge into multinational coorporation, rationalization is made in binding the stocks on their way to tobacco factory.
4. It should be emphasized that over 80% of the supply of raw tobacco from Macedonia is concentrated in three foreign daughter-companiesof the multinational companies (Socotab Bitola-45%, Alliance Kavadarci - 27% and Tobacco Company - 12%).
5. Globally, there is a trend of increasing demand for raw tobacco. Thus,oriental tobacco, asthe smallest component in the production of American blend cigarettes, cannotsatisfy the needs of cigarette manufacturers. The analysts dealing with marketing and sale of tobacco raw predict that requirements for oriental tobacco are at least 400.000tons.Realization of this level of production depends on the producers and sellers of oriental tobacco.

REFERENCES

1. Попоски Љ., 2014."Тутунски комбинат-Прилеп, подем и пад", Друштво за Наука и уметност-Прилеп
2. Попоски Љ., 2015."Тутунски атлас", Друштво за наука и уметност-Прилеп
3. Здружение на производители на тутун во лист, Стопанска комора, 2015
4. Податоци од Министерство за земјоделство, водостопанство и шумарство
5. Tobacco Journal Internationa l3/2015
6. FAOSTAT (Food and agriculture organization of the United Nations statistics), 2015/2016

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The publication presents:original scientific papers,review articles, short reports, professional papers and other works related to tobacco science and practice.
Original scientific papers-should contain original scientific research results, previously unpublished. They must be presented in a manner enabling the experiment, i.e. research method, to be repeated and accuracy of the analysis, results and conclusions confirmed.
Review articles-should contain critical surveys of the accomplishments in the fields encompassed in the Journal, papers by an individual researcher or a group of researchers with the purpose to undertake, analyze, evaluate or synthesize previously published information. They should present the latest ideas and theories or new scientific achievements.
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Professional papers-should present useful contributions from the field of an applied science whose problematic is not related to the original research. The aim of these papers is not to present new findings but to use already acquired knowledge and implement it into practice.

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Titles in the text (INTRODUCTION, MATERIAL AND METHODS, RESULTS...) should be centered, boldfaced, written with capital letters, font size 12; Subtitles should be written with initial capital letter, boldfaced, 12-point font size, aligned to the center;
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Arrangement of the paper:

Title-in capital letters, boldfaced, 12-point font size, aligned to the center;
Full name and surname of the first author-capital initial letter, other letters small, font size 12, centered;
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ABSTRACT-at the beginning of the paper, both in English and Macedonian, should not exceed 150 words. It should mention the techniques used without going into methodological detailsand should summarize the most important results. Abstracts should not include citations toreferences.Font size 10, centered.

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INTRODUCTION should provide a brief statement of the subject, comprehensive survey of the relevant lietrature and objectives of the paper;

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RESULTS AND DISCUSSION should be presented in tables, figures, diagrams and photographs, which must accurately describe the findings of the study, ordered sequentially as they appear in the text;

Tables should be numbered with Arabic numeralsaccording to their sequence in the text. The table title should be always above the table centered, in 10 pt font, the text in side of table should be in 10 pt, with one empty row between the title and the table and another one between the table and the text.Tables should be simple and should not duplicate the information given in figures.

Reference to the Table, example: It could be seen from Table 1...., or: The nicotine content in tobacco is 0.98% (Table 4).

Illustrations should be numbered consecutively in Arabic numerals, with centered titles below each of them and with 10 pt.font.

The title of graphical presentation should be in 10 pt font, all graphical presentations, (including graphs, schemes, drawings, photographs etc) should be submitted on CD together with the text. Minimum resolution for images is 200 -300 dots per inch.

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