UDC 633.71 ISSN 0494-3244

ТУТУН

TOBACCO

Vol. 66	Nº 1-6

BULLETIN OF TOBACCO SCIENCE AND PROFESSION

TUTUN TOBACCO	Vol. 66	Nº 1-6	pp. 1-60	PRILEP REPUBLIC OF MACEDONIA	JANUARY JUNE	2016

ISSN 0494-3244

Тутун/Tobacco,Vol. 66, N⁰ 1-6, 3-12, 2016 UDC: 633.71-152.61:631.574(497.7)"2013/2014"

Original Scientific paper

MORPHOLOGICAL STUDIES OF THE LEAVES IN SOME AUTOCHTHONOUS AND COMMERCIAL TOBACCO VARIETIES IN THE REPUBLIC OF MACEDONIA

Ana Korubin – Aleksoska

Scientific Tobacco Institute – Prilep, Kicevski pat bb, Prilep, Republic of Macedonia

e-mail: anakorubin@yahoo.com

ABSTRACT

Investigations were carried out with five aromatic autochthonous oriental tobaccos of the types: Prilep (P 10–3/2 and P 12–2/1), Djebel (Dj № 1) and Yaka (YK 7–4/2 and KY) and five commercial oriental varieties of Prilep tobacco (P–23, P–84, NS–72, P–66–9/7 and P–79–94), to study the number, length, width and area of the middle belt leaves per stalk. The trial was set up in the Experimental field of Tobacco Institute–Prilep in 2013 and 2014, in randomized block design with three replications, using traditional agricultural practices.

The aim of the investigation is to study some autochthonous varieties and new commercial varieties for the stated quantitative traits, and with analysis of variance to estimate the significance of differences betwin varieties and betwin years, which will improve our knowledge on stability of the traits, genotypical homogeneity and progress in selection of oriental tobacco in the Republic of Macedonia.

Differences in leaf the number and size among genotypes in the two-year investigations are highly significant, which is genetic indicator of their mutual differences. The error of the mean value is low, indicating stability and homozygosity of the genotypes. The variety P–66–9/7 is characterized with the highest number of leaves (60) and it has 33 leaves more than YK 7–4/2 and Dj № 1. The largest leaf size was measured in P–79–94 (23,3cm – length, 12,1 cm – width, 179 cm^2 – area). It has 4,8 cm longer, 3,2 cm wider and 74,5 cm^2 larger leaves then YK 7–4/2, which is characterized by the smallest leaves. These data point out to a successful breeding activity of the Tobacco Institute in the selection of oriental aromatic tobaccos. Through evaluation of stability of varieties, the breeder improves its knowledge on their homozygosity and higher security in the choice of parental pairs for implementing selection programs.

Keywords: tobacco (*Nicotiana tabacum L.*), old varieties, commercial varieties, quantitative traits, analysis of variance.

МОРФОЛОШКИ ПРОУЧУВАЊА НА ЛИСТОВИТЕ КАЈ НЕКОИ АВТОХТОНИ И КОМЕРЦИЈАЛНИ СОРТИ ТУТУН ВО РЕПУБЛИКА МАКЕДОНИЈА

Испитувани се пет ароматични автохтони ориенталски тутуни од типовите: прилеп (П 10–3/2 и П 12–2/1), џебел (Џ бр.1) и јака (ЈК 7–4/2 и КЈ) и пет комерцијални ориенталски сорти од типот прилеп (П–23, П–84, НС–72, П–66–9/7 и П–79–94), за бројот на листови по страк и должината, ширината и површината на листовите од средниот појас. Опитот беше поставен на експерименталното поле во Научниот институт

за тутун–Прилеп во 2013 и 2014 година, по случаен блок–систем во три повторувања, со примена на вообичаени агротехнички мерки.
Целта на истражувањата е да се проучат автохтоните сорти и новите комерцијални сорти за наведените квантитативни својства, а со анализа на варијансата да се процени важноста на разликите помеѓу сортите и годините, со што ќе се добие визија за стабилноста на својствата, генотипската хомогеност и напредокот на селекцијата на ориенталски тутуни во Република Македонија.
Разликите во бројот и димензиите на листовите помеѓу генотиповите во двегодишните проучувања се високосигнификантни, што е генетски показател за нивната меѓусебна различност. Грешката на средната вредност за сите податоци е ниска, што е знак за стабилноста и хомозиготноста на генотиповите. Сортата П–66–9/7 се одликува со најголем број листови (60). Таа има 33 листови повеќе од ЈК 7–4/2 и Џ бр.1. Што се однесува до димензиите на листовите предничи П–79–94 (23,3cm – должина, 12,1 cm – ширина, 179 cm^2 – површина). Таа има за 4,8 cm подолги, за 3,2 cm пошироки и за 74,5 cm^2 поголеми листови од ЈК 7–4/2 која има најмали ливчиња. Овие податоци кажуваат за успешната облагородувачка дејност на Институтот за тутун во областа на селекцијата на ориенталски ароматични тутуни. Преку евалуација на стабилноста на сортите, селекционерот добива поголемо сознание за нивната хомозиготност и поголема сигурност во изборот на родителски парови за имплементирање на селекционите програми.
Клучни зборови: тутун (*Nicotiana tabacum L.*), стари сорти, комерцијални сорти, квантитативни својства, анализа на варијанса.

INTRODUCTION

Tobacco is a crop that is grown for its leaf and all breeding activities are directed to increasing its yield and quality. Many authors consider leaf size (length and width) as an important parameter in determination of tobacco quality. During the evaluation, dry oriental tobacco leaves which length exceeds 20 cm are classified as additional tobacco because they have very little or no aroma. Scientific Tobacco Institute - Prilep dates from 1924 and its main activity is the selection of oriental, small-leaf, aromatic tobaccos.

Atanasov (1972) reported that the leaf number in selected varieties is of approximately constant size. According to Uzunoski (1985), leaf number varies depending on agro-ecological conditions and it is important character, since it greatly determines the yield of tobacco. Dimitrieski, Miceska (2011) reported that the newly created variety P-66-9/7 in 2010 accounted for 70-80% of the primary production of tobacco type Prilep. The average leaf number of this tobacco was 52, with an average length of 16-18 cm for the middle and 8 - 10 for the top leaves. Aleksoski (2013) in his three-year investigations on a diallel cross with four parental genotypes of P-84 (breeded for higher yield), received about 52 leaves per stalk and the leaf area of the middle belt was 146 cm^2 in 2007, 138 cm^2 in 2008 and 142 scm^2 in 2009. Korubin-Aleksoska, Aleksoski (2013) in the two-year investigations of six tobacco varieties and their diallel crosses in F1 and F2 generations reported that the variety P-23 showed low variability of number of leaves per stalk, which confirms the homozygosity of the variety i.e. its stability and uniformity. Miceska et al. (2014) in the two-year biometric investigations of autochthonous tobacco varieties in the Republic of Macedonia for the leaf number per stalk reported that the obtained parameters of variability are low, which indicates high genetic homogeneity.

The aim of the study is to investigate and compare the number of leaves per stalk and size of the middle belt leaves in some old domestic varieties and new commercial varieties, in order to get a better knowledge and more successful selection of oriental tobacco in Macedonia.

MATERIAL AND METHODS

Two-year investigations were made on the number of number, length, width and area of the middle belt leaves in five old domestic varieties of the types: Prilep (P 10–3/2 and P 12–2/1), Djebel (Dj № 1) and Yaka (YK 7–4 / 2 and KY – Kishinska Yaka), and five new commercial varieties of the type Prilep: P–23, P–84, NS–72, P–66–9/7 and P–79–94. The traits were measured during tobacco growth in the field. The leaf area (relative area) was calculated by multiplying the length by the width, using the coefficient k = 0,6354 (Gornik, 1973). Each amount represents the arithmetic average for the examined property that is accompanied by error of the mean value (Najceska, 2002).

During tobacco vegetation in field (May - September) in 2013, mean monthly temperature was 19,40C, number of rainy days 34 and total precipitation amount 153 mm. In the same period in 2014 mean monthly temperature was 18,30C, number of rainy days 33 and total precipitation amount 223 mm.

General characteristics of the old domestic tobacco varieties

The cultivation of old tobacco varieties in this region began long ago, during the Ottoman Empire. The centuries–long presence led to their adaptation to the present agro–ecological conditions. Through successive natural selection they have acquired resistance to drought and diseases and can rightly be called autochthonous. Today, the old varieties make a valuable material for breeding activity in the Institute.

Prilep P 10–3/2 – characterized by cup-like habitus, average stalk height 50cm, with 30-36 sessile leaves, dry mass yield averages 1200 kg/ha (Fig.1).

Prilep P 12–2/1 – characterized by cup-like habitus, average stalk height 55cm, with 34-38 sessile leaves, dry mass yield averages 1500 kg/ha (Fig.2).

P 10-3/2 and P12-2/1 are put into production in the 30-ies of the last century; phenotypic and genotypic are very similar; originating from the local tobacco variety Djumaj–bale from Gorna Djumaja – Bulgaria.

Djebel Dj № 1 – released in the first half of the last century; Originated from the local variety Xanthian Yaka grown in the Djebel tobacco producing region in Bulgaria; characterized by a cylindrical habitus, average stalk height 80cm, 26–30 sessile leaves erected toward the stalk, dry mass yield averages 1000 kg/ha (Fig.3).

Yaka YK 7–4/2 – released in mass production in 1932. Originated from Xanthian Yaka originating from Xanthy – Greece; aplant with narrow, spindle shaped–elliptic habitus; average stalk height 100 cm, with 26–32 sessile leaves, dry mass yield averages 1000 kg/ha (Fig.4).

These four domestic varieties were created in the Tobacco Institute–Prilep by Rudolf Gornik (Gornik, 1973). by individual selection.

KY (Kishinska Yaka) – According to some unconfirmed reports, this variety originates from village Kishino in the region of Veles, Republic of Macedonia. According to other surces it was transmitted in the past from Moldova (Uzunoski, 1985). Environmental conditions had a great influence on the morphology and chemistry of this genotype and with multi–decades selection a uniform and stable variety was formed. It is characterized by elongated–elliptic habitus with about 40 sessile leaves (Fig.5).

General characteristics of the new commercial tobacco varieties

The new commercial oriental sun–cured varieties created in the Scientific Tobacco Institute–Prilep present a high quality raw material for the domestic and world market. Due to their pleasant aroma and harmonious chemical composition they enter in the mixtures of the highest–quality cigarette brands.

Prilep P–23 – created by Kostadin Nikoloski and Milan Mitreski, through hybridization and selection in Tobacco Institute – Prilep; recognized by the Ministry of Agriculture, Forestry and Water Management of the Republic of Macedonia in 1995 (Korubin – Aleksoska A., 2004). It has elliptical–conical habitus, average stalk height 65 cm, with about 55 densely arranged leaves, dry mass yield 2000–2500 kg/ha (Fig.6).

Prilep P–84 – created by Kiril Naumovski and Ana Korubin – Aleksoska, through hybridization and selection; recognized in 1988 in former Yugoslavia, as one of the first varieties of the type Prilep. Characterized by cylindrical – elliptical habitus, average stalk height 65 cm, with approximately 40 – 42 sessile leaves, elliptical in shape, dry mass yield 2500–3200 kg/ha (Fig.7).

Prilep NS–72 – created by Dushko Boceski and Simeon Karayankov; recognized in 1984 in former Yugoslavia as one of the first varieties of the type Prilep obtained by crossing (Korubin–Aleksoska A. et al., 2012); characterized by cylindrical–elliptical habitus, average stalk height 75 cm, with approximately 50 sessile leaves, elliptical in shape, dry mass yield 2800–3300 kg/ha (Fig.8).

Prilep P–66–9/7 – created in Tobacco Institute–Prilep by Miroslav Dimitrieski and Gordana Miceska; recognized by the Ministry of Agriculture, Forestry and Water Management of R. Macedonia in 2004 (Korubin–Aleksoska A. et al., 2012); characterized by elliptical–conical habitus, with 54–60 ovate leaves, sessile and evenly distributed on the stem, dry mass yield 3000–3600 kg/ha. It has been the most represented tobacco variety in our country in recent years (Fig.9).

Prilep P–79–94 – created in Tobacco Institute–Prilep by Milan Bogdanceski; recognized by the Ministry of Agriculture, Forestry and Water Management of R. Macedonia in 2001 (Korubin – Aleksoska A., 2004); characterized by cylindrical–elliptical habitus, average stalk height 75 cm, with about 55 sessile leaves densely distributed, especially in the upper part of stem, dry mass yield 2500–3000 kg/ha (Fig.10).

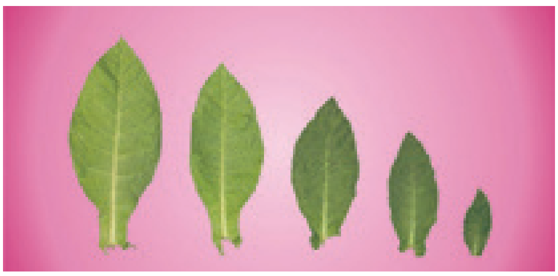


Fig. 1. Prilep P 10–3/2

Fig. 2. Prilep 12-2/1

Fig. 3. Djebel Dj № 1

Fig. 4. Yaka YK 7–4/2

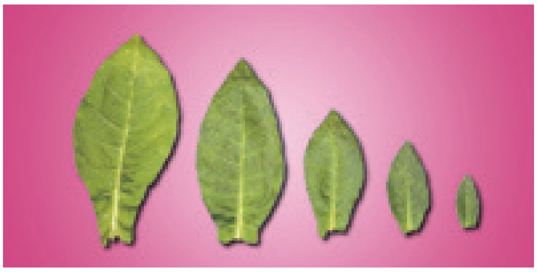


Fig. 5. KY (Kishinskan Yaka)

Fig. 6. Prilep P–23

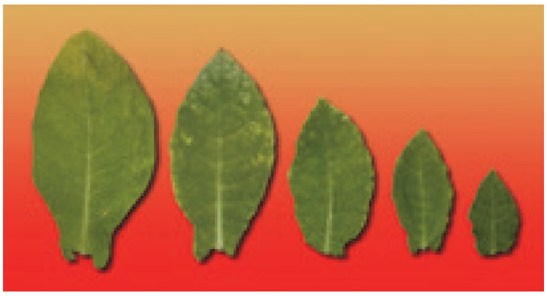


Fig. 7. Prilep P–84

Fig. 8. Prilep NS–72

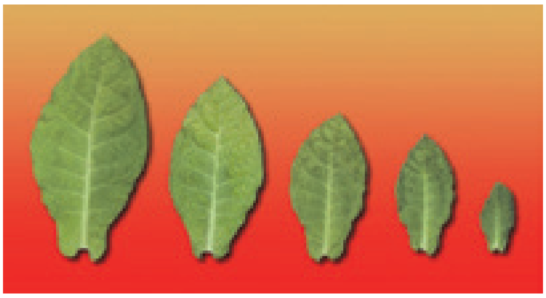


Fig. 9. Prilep P–66–9/7

Fig. 10. Prilep P–79–94

RESULTS AND DISCUSSION

The highest leaf number among the old varieties was found in Kishinska Yaka – KY (36 – 2013, 38 – 2014, i.e. $\bar{x}$ = 37) and the lowest in YK 7–4/2 (26 – 2013, 28 – 2014, i.e. $\bar{x}$ = 27) and Dj № 1 (26 – 2013, 29 – 2014, i.e. $\bar{x}$ = 27.5). Among the new commercial varieties, the highest leaf number was found in P–66–9/7 (58 – 2013, 62 – 2014, i.e. $\bar{x}$ = 60) and the lowest in P–84 (40 – 2013, 42 – 2014, i.e. $\bar{x}$ = 41). The variety P–66–9/7 has 33 leaves more than YK 7–4/2 and Dj № 1 (Table 1).

Analysis of variance for the number of leaves per stalk showed highly significant differences among the varieties. Only in the combination Dj № 1 – YK 7–4/2 the difference was not significant (Table 2). This is an indication of different genotypes. Differences in leaf number per years are small but significant, as a result of the different conditions in the two years of investigation (Table 6).

The highest leaf length among the investigated varieties was measured in P 12-2/1 and P–79–94 ($\bar{x}$ = 23 cm) and the lowest in YK 7–4/2 ($\bar{x}$ = 18 cm). The difference in length of the middle belt leaves between the two varieties is 5 cm (Table 1). The comparison of the middle belt leaf length of the semi–oriental variety Otlja O 9–18/2 – Fig. 11. (32,5 cm – 2013, 33 cm – 2014, i.e. $\bar{x}$ = 32,75 cm), with that of the variety P–79–94 shows that leaves of O 9–18/2 are 9,5 cm longer compared to P–79–94 and 14,3 cm longer compared to YK 7–4/2.

Fig. 11. Отља 9 - 18 / 2

The analysis of variance for the length of the middle belt leaf shows highly significant differences among varieties in 82,2% (2013) and 77,8% of the combinations (2014) and 0,05 significance in 6,7% (2013) and 8,9% (2014). No significance was observed in about 12,2% (P 10-3/2 – KY, 10-3/2 – NS-72, P 12-2/1 – P-79-94, KY – NS-72, P-23 – P-84, P-23 – P-66-9/7 and P-84 – P-66-9/7). The significance of differences in about 87% of the combinations indicates that this trait is varietal characteristic (Table 3).

The highest leaf width among the varieties was measured in P–79–94 (11,9 cm – 2013, 12,3 cm – 2014, i.e. $\bar{x}$ = 12,1 cm), and the lowest width in YK 7–4/2 (8,7 cm – 2013, 9,1 cm – 2014, i.e. $\bar{x}$ = 8,9 cm). The difference in width of the middle belt leaves between these two varieties is 3,2 cm (Table 1). Comparison of width in the middle belt leaves shows that the semi–oriental variety O 9–18/2 (17,5 cm – 2013, 18 cm – 2014, i.e. $\bar{x}$ = 17,75 cm) has 5,65 cm wider leaves than P–79–94 and 8,85 cm wider compared to YK 7–4/2.

The analysis of variance for the width of the middle belt leaves shows highly significant differences among varieties in 88.9% and 82.2% (2013 and 2014 respectively) and 0.05 significance in 4.4% and 8.9% (2013 and 2014, respectively), while in 6.7% (2013) and in 8.9% (2014), i.e. in only five combinations no significance was observed (P 12-2/1 – P-23, P 12-2/1 – P-84, P 12-2/1 – NS-72, Dj № 1 – KY and P-23 – NS-72). The significance of differences in about 93% of the combinations indicates that the investigated trait is varietal characteristic (Table 4).

The largest area of the middle belt leaves was measured in variety P–79–94 (175,9 cm^2 – 2013, 183 cm^2 – 2014, т.е. $\bar{x}$ = 179 cm^2), and the lowest in YK 7–4/2 (101 cm^2 – 2013, 108 cm^2 – 2014, i.e. $\bar{x}$ = 104,5 cm^2). This means that P–79–94 has about 74,5 cm^2 larger leaves than YK 7–4/2 (Table 1). Still, this difference is within the allowed limits for oriental tobaccos. Comparison of the area of middle belt leaves in the semi-oriental variety Otlja O 9–18/2 (361 cm^2 – 2013, 377 cm^2 – 2014, i.e. $\bar{x}$ = 369 cm^2) shows that it has 190 cm^2 larger leaves than P–79–94 and 264 cm^2 larger than YK 7–4/2, which indicates that they are two different categories of tobacco.

The analysis of variance for the area of the middle belt leaves in 2013 shows highly significant differences among varieties in 66,7%, 0,05 significance in 20% and no significance in 13,3% of the combinations, while in 2014 high significance was assessed in 62,2%, 0,05 significance in 20% and no significance in 17,8%. The significance of differences in about 84% of the combinations indicates that the investigated trait is varietal characteristic (Table 5).

The differences in leaf size by years are small but significant and they appear as a result of various meteorological factors during the growing season in the two years of investigation (Table 6).

Table 1. Number and size of the middle belt leaves in autochthonous and commercial tobacco varieties from the Republic of Macedonia

Tobacco varieties	Number of leaves per stalk $\bar{x} \pm s\bar{x}$ 2013	2014	$\bar{x}$	Length of the middle belt leaves (cm) 2013	2014	$\bar{x}$	Width of the middle belt leaves (cm) 2013	2014	$\bar{x}$	Area of the middle belt leaves (cm^2) 2013	2014	$\bar{x}$
Autochthonous varieties												
P 10-3/2	32 0,09	34 0,08	33	22 0,10	22 0,11	22	10,2 0,05	10,5 0,04	10.4	143 1,65	149 1,63	146
P 12-2/1	34 0,10	36 0,11	35	23 0,12	23 0,11	23	10,4 0,07	11,1 0,06	10.8	149 1,83	164 1,70	156.5
Dj № 1	26 0,16	29 0,14	28	19 0,11	20 0,10	20	11,5 0,04	11,8 0,04	11.8	142 1,55	151 1,49	146.5
YK 7-3/2	26 0,17	28 0,15	27	18 0,08	18 0,09	18	8,7 0,08	9,1 0,07	8.9	101 1,59	108 1,52	104.5
KY	36 0,15	38 0,15	37	22 0,12	22 0,11	22	11,6 0,07	11,8 0,05	11.7	161 1,93	166 1,84	163.5
KY	36 0,15	38 0,15	37	22 0,12	22 0,11	22	11,6 0,07	11,8 0,05	11.7	161 1,93	166 1,84	163.5
Commercial tobacco varieties												
P-23	45 0,20	48 0,18	47	20 0,13	22 0,12	21	10,7 0,04	11,1 0,03	10.9	137 1,85	156 1,74	146.5
P-84	40 0,14	42 0,15	41	20 0,10	21 0,09	21	10,5 0,05	10,7 0,03	10.6	136 1,77	145 1,59	140.5
NS-72	43 0,21	45 0,17	44	22 0,17	23 0,15	22	10,8 0,07	10,9 0,06	10.9	149 2,17	158 2,03	153.5

P-66-9/7	58 0,15	62 0,15	60	21 0,12	22 0,14	21	11,2 0,05	11,2 0,04	11.2	149 1,58	153 1,54	151
P-79-94	52 0,14	54 0,15	53	23 0,12	23 0,11	23	11,9 0,06	12,3 0,02	12.1	175 1,69	183 1,62	170.5
$LSD_{0,05}$				0,581				0,4200,				1635,655
$LSD_{0,01}$				1,053				0,756				10,179

Table 2. Significance of differences in number of leaves per stalk between varieties (from the values in Table 1)

Differ.	Signi.	Differ.	Signi.	Differ.	Signi.	Differ.	Signi.	Differ.	Signi.
S1 – S2	-2**	S2 – S3	7.5**	S3 – S5	-9,5**	S4 – S8	-17**	S6 – S8	2,5**
S1 – S3	5,5**	S2 – S4	8**	S3 – S6	-19**	S4 – S9	-33**	S6 – S9	-13,5**
S1 – S4	6**	S2 – S5	-2**	S3 – S7	-13,5**	S4 – S10	-26**	S6 – S10	-6,5**
S1 – S5	-4**	S2 – S6	-11,5**	S3 – S8	-16,5**	S5 – S6	-9,5**	S7 – S8	-3**
S1 – S6	-13,5**	S2 – S7	-6**	S3 – S9	-32,5**	S5 – S7	-4**	S7 – S9	-19**
S1 – S7	-8**	S2 – S8	-9**	S3 – S10	-25,5**	S5 – S8	-7**	S7 – S10	-12**
S1 – S8	-11**	S2 – S9	-25**	S4 – S5	-10**	S5 – S9	-23**	S8 – S9	-16**
S1 – S9	-27**	S2 – S10	-18**	S4 – S6	-19,5**	S5 – S10	-16**	S8 – S10	-9**
S1 – S10	-20**	S3 – S4	0.5	S4 – S7	-14**	S6 – S7	5,5**	S9 – S10	7**

Signi. – * - significance for 5%; ** - significance for 1%

Differ. – differences between varieties

Varieties: S1 = P 10-3/2, S2 = P 12-2/1, S3 = Dj № 1, S4 = YK 7-3/2, S5 = KY, S6 = P-23, S7 = P-84, S8 = NS-72, S9 = P-66-9/7, S10 = P-79-94

Table 3. Significance of differences in length of the middle belt leaves between varieties (from the values in Table 1)

Differ.	Signi.	Differ.	Signi.	Differ.	Signi.	Differ.	Signi.	Differ.	Signi.
S1 – S2	-0,65*	S2 – S3	3,1**	S3 – S5	-2,15**	S4 – S8	-3,9**	S6 – S8	-1,2**
S1 – S3	2,45**	S2 – S4	4,45**	S3 – S6	-1,35**	S4 – S9	-2,75**	S6 – S9	-0,05
S1 – S4	3,8**	S2 – S5	0,95**	S3 – S7	-1,05**	S4 – S10	-4,85**	S6 – S10	-2,15**
S1 – S5	0,3	S2 – S6	1,75**	S3 – S8	-2,55**	S5 – S6	0,8**	S7 – S8	-1,5**
S1 – S6	1,1**	S2 – S7	2,05**	S3 – S9	-1,4**	S5 – S7	1,1**	S7 – S9	-0,35
S1 – S7	1,4**	S2 – S8	0,55*	S3 – S10	-3,5**	S5 – S8	-0,4	S7 – S10	-2,45**
S1 – S8	-0,1	S2 – S9	1,7**	S4 – S5	-3,5**	S5 – S9	0,75*	S8 – S9	1,15**
S1 – S9	1,05**	S2 – S10	-0,4	S4 – S6	-2,7**	S5 – S10	-1,35**	S8 – S10	-0,95**
S1 – S10	-1,05**	S3 – S4	1,35**	S4 – S7	-2,4**	S6 – S7	0,3	S9 – S10	-2,1**

Table 4. Significance of differences in width of the middle belt leaves between varieties (from the values in Table 1)

Differ.	Signi.	Differ.	Signi.	Differ.	Signi.	Differ.	Signi.	Differ.	Signi.
S1 – S2	-0,4**	S2 – S3	-0,9**	S3 – S5	-0,05	S4 – S8	-1,95**	S6 – S8	0,05
S1 – S3	-1,3**	S2 – S4	1,85**	S3 – S6	0,75**	S4 – S9	-2,3**	S6 – S9	-0,3**
S1 – S4	1,45**	S2 – S5	-0,95**	S3 – S7	1,05**	S4 – S10	-3,2**	S6 – S10	-1,2**
S1 – S5	-1,35**	S2 – S6	-0,15	S3 – S8	0,8**	S5 – S6	0,8**	S7 – S8	-0,25**
S1 – S6	-0,55**	S2 – S7	0,15	S3 – S9	0,45**	S5 – S7	1,1**	S7 – S9	-0,6**
S1 – S7	-0,25*	S2 – S8	-0,1	S3 – S10	-0,45**	S5 – S8	0,85	S7 – S10	-1,5**
S1 – S8	-0,5**	S2 – S9	-0,45**	S4 – S5	-2,8**	S5 – S9	0,5**	S8 – S9	-0,35**
S1 – S9	-0,85**	S2 – S10	-1,35**	S4 – S6	-2**	S5 – S10	-0,4**	S8 – S10	-1,25**
S1 – S10	-1,75**	S3 – S4	2,75**	S4 – S7	-1,7**	S6 – S7	0,3**	S9 – S10	-0,9**

Table 5. Significance of differences in area of the middle belt leaves between varieties (from the values in Table 1)

Differ.	Signi.	Differ.	Signi.	Differ.	Signi.	Differ.	Signi.	Differ.	Signi.
S1 – S2	-10.5**	S2 – S3	10*	S3 – S5	-17**	S4 – S8	-49**	S6 – S8	-7*
S1 – S3	-0.5	S2 – S4	52**	S3 – S6	0	S4 – S9	-46.5**	S6 – S9	-4.5
S1 – S4	41.5**	S2 – S5	-7*	S3 – S7	6*	S4 – S10	-66**	S6 – S10	-24**
S1 – S5	-17.5**	S2 – S6	10*	S3 – S8	-7*	S5 – S6	17**	S7 – S8	-13**
S1 – S6	-0.5	S2 – S7	16**	S3 – S9	-4.5	S5 – S7	23**	S7 – S9	-10.5**
S1 – S7	5.5	S2 – S8	3	S3 – S10	-24**	S5 – S8	10*	S7 – S10	-30**
S1 – S8	-7.5*	S2 – S9	5.5	S4 – S5	-59**	S5 – S9	12.5**	S8 – S9	2.5
S1 – S9	-5	S2 – S10	-14**	S4 – S6	-42**	S5 – S10	-7*	S8 – S10	-17**
S1 – S10	-24.5**	S3 – S4	42**	S4 – S7	-36**	S6 – S7	6*	S9 – S10	-19.5**

Table 6. Significance of differences for investigated caracteristics of varieties between years (from the values in Table 1)

Varieties	Differences between years (2013 - 2014)			
	Number of leaves per stalk	Length of the middle belt leaves	Width of the middle belt leaves	Area of the middle belt leaves
Autochthonous tobacco varieties				
1. P 10-3/2	-2**	-0.3	-0.3**	-6*
2. P 12-2/1	-2**	-0.8**	-0.7**	-15**
3. Dj № 1	-3**	-0.8**	-0.3	-9*
4. YK 7-3/2	-2**	-0.5*	-0.4**	-7*
5. KY	-2**	-0.3	-0.2*	-5
Commercial tobacco varieties				
6. P-23	-3**	-1.9**	-0.4**	-19**
7. P-84	-2**	-0.9**	-0.2*	-9*
8. NS-72	-2**	-1.1**	-0.1	-9*
9. P-66-9/7	-4**	-0.6*	0	-4
10. P-79-94	-2**	-0.2	-0.4**	-25**

* - Significance for 5%;

** - significance for 1%

CONCLUSIONS

Two-year investigations of some old domestic oriental varieties (P 10–3/2, P 12–2/1, Djebel Dj № 1, Yaka YK 7–4/2, KY – Kishinska Yaka) and new commercial varieties (P–23 P–84, NS–72, P–66–9/7, P–79–94) showed a small error of the mean value for the traits: number, length, width and area of the middle belt leaves per stalk, which is an indication of correct setting of the experiment, high genetic stability of the traits and homozygosity of the varieties.

The highest leaf number per stalk was obtained in P–66–9/7 (60), which is 33 leaves more than YK 7–4/2 and Dj № 1 – characterized by the lowest number of leaves. The aanalysis of variance shows highly significant differences between varieties and no significant differences between the years, which is indication that these are different genotypes and that the investigated trait is highly heritable and varietal characteristic. The highest leaf length and width was measured in P–79–94 ($\bar{x}$ = 23,3 cm length, $\bar{x}$ = 12,1 cm – width), and the lowest length and width in YK 7–4/2 ($\bar{x}$ = 18,45 cm length, $\bar{x}$ = 8,9 cm - width), showing that the leaves of P–79–94 are 5 cm longer and 3,2 cm wider compared to YK 7–4/2. The significance of 87% for the leaf length and 93% for the width indicates the differences between varieties, suggesting that these traits are varietal characteristics.

The largest area of the middle belt leaves was measured in P–79–94 ($\bar{x}$ = 179 cm^2), and the lowest in YK 7–4/2 ($\bar{x}$ = 104,5 cm^2), which means that P–79–94 has 74,5 cm^2 larger leaves than YK 7–4/2. The significance of differences between the varieties in about 84% of the combinations confirms that the investigated trait is a varietal characteristic.

Compared to the semi–oriental variety Otlja O 9–18/2 it can be concluded that differences in leaf size among the ten genotypes is within allowed limits for oriental aromatic tobaccos.

REFERENCES

1. Aleksoski J., 2013. The mode of inheritance of quantitative characters and heterotic effect in F1 hybrids in a diallel of different tobacco tipes. Тутун/Tobacco,Vol.63, N°1-6, p. 56-62.
2. Atanasov D., 1972. Tobacco production (Тютюнопроизводство). ITTI, Plovdiv.
3. Dimitrieski M., Miceska G., 2011. A new and more productive variety of Prilep tobacco. Тутун/Tobacco, Vol.61, No 1-6, p. 59-62.
4. Gornik R., 1973. Oblagoroduvanje na tutunot. Tutunski kombinat-prilep: 168.
5. Korubin – Aleksoska A., 2004. Tobacco varieties from Tobacco Institute – Prilep. Alfa Print – Skopje: 92.
6. Korubin – Aleksoska A. et al., 2012. Dry tobacco yield in some Prilep tobacco varieties. Proceedings, pp. 78-85. International symposium for agriculture and food. Skopje.
7. Korubin – Aleksoska A., Aleksoski J., (2013). Investigations of the variability of quantitative characters in tobacco varieties and their F1 and F2 hybrids. Тутун / Tobacco, Vol.63, N□ 7-12, p. 20-26.
8. Miceska G., Gveroska B., Dimitrieski M., 2014. Investigation of some quantitative traits in autochthonous tobacco varieties in Republic of Macedonia. Тутун/Tobacco,Vol.64, N□ 1-6, p. 1-4.
9. Najčevska C., 2002. Eksperimentalna statistika primeneta vo zemjodelskite i biološkite istražuvanja. Bona, Skopje: 202.
10. Uzunoski M., 1985. Production of tobacco. Economic Journal, Skopje, pp.543.

ISSN 0494-3244

Тутун/Tobacco,Vol. 66, N⁰ 1-6, 13-21, 2016 UDC: 633.71-152.61(497.2)"2013/2015"

Original Scientific paper

ASSESSMENT OF BIOLOGICAL INDICATORS AND PRODUCTION CHARACTERISTICS OF PERSPECTIVE LINES BURLEY TOBACCO

Yovko Dyulgerski

Tobacco and Tobacco Products Institute (TTPI), Markovo, Bulgaria

(e-mail:yovko_dulg@abv.bg)

ABSTRACT

Examined are the biological and production parameters of seven newly created lines of Burley tobacco. Research results show that with the best biological assessment presents Line 1540, followed by a line 1521. With the shortest and yet most favourable vegetative period differs Line 1531 (74,7 days). With favourable the vegetative period is presented and Line 1521 (76,2 days). All test variants have a shorter vegetative period than the standard variety, Pliska , which is a success in selection work by this indicator. Line 1540 gives the highest yield per hectare (3607 kg/ha) as an average over the period of study and three years of study. From this line gets the highest percentage of first class (45%) and at lower than third class (6%), as average for the period of study and for three years of study. All new created lines strongly outweigh the testimony of the standard variety, both in terms of production and in terms of percentage of classes, which is an indication of the success of the selection work. Line 1540 Line 1521 and Line 1536 are formed as options with the highest production and selection value. In complex of biological indicators and productive characteristics most stands Line 1540 and therefore may being offered for production test and presentation for recognition as a new variety Burley tobacco.

Key words: Burley tobacco, new created lines, biological indicators, production characteristics

ОЦЕНКА НА БИОЛОШКИ И ПРОИЗВОДНИ КАРАКТЕРИСТИКИ НА ПЕРСПЕКТИВНИ БЕРЛЕЈСКИ ЛИНИИ ТУТУН

Истражувањата се за биолошко-производните показатели на седум новосоздадени линии тутун Берлеј. Резултатите од истражувањето покажуваат дека со најдобра биолошка проценка се одликува Линија 1540 и Линија 1521 . Со најкраток и истовремено доста поволен вегетационен период се одликува Линија 1531 (74,7 дена). Со поволен вегетационен период се одликува и Линија 1521 (76,2 дена). Сите испитувани варијанти имаат пократок вегетационен период од стандардната сорта, Плиска, што е поволен показател при избор и работа по овој показател. Линија 1540 дава најголем принос по хектар (3607 кг/ха), како просек за периодот на истражување, така и за трите години на истражување. Од оваа линија се добива највисок процент на првата класа (45%) и низок од трета класа (6%), како просек за периодот на истражување, така и за трите години на истражување. Сите новоселкционирани линии покажуваат голема предност и ја надминуваат стандардната сорта, како во однос на производството, така и во однос на процентот на класите што е индикација за успех на избор и работа. Линија 1540, линија 1521 и линија 1536 се варијанти со највисока биолошко-производна, и селекциона вредност. Според биолошките и производните индикатори најмногу се издвојува Линија 1540 и поради тоа може да се предложи за производство, тестирање и перформанси и за признавање како новата сорта тутун Берлеј.

Клучни зборови: Берлејски тутун, нови линии, биолошки показатели, производни карактеристики

INTRODUCTION

Burley tobacco is indispensable component of American blend cigarettes (Tomov and Minev, 1996; Davis and Nielsen, 1999). Although he held increasingly important part of total tobacco production in Bulgaria, the yield and quality of the produced raw material in the country significantly inferior to that in the traditional producing countries (Bozukov, 2012). The unsatisfactory situation of the tobacco production in country is a serious obstacle for even greater expansion of cultivated areas (Dyulgerski, 2011).

The main reasons for this are old varieties in production (Dimanov and Masheva, 2011). Embedded currently in production varieties Burley tobacco not satisfy the contemporary requirements, neither farmers nor the tobacco industry (Dyulgerski, 2011; Masheva, 2008; Nikolov et al., 2004). Imported Burley tobaccos seriously outperform our tobaccos from this varietal group on most indicators. (Docheva and Stoilova, 2011; Kirkova et all., 2006; Milanova all., 2013; Stoilova and Bojinova, 2007; Nicolova and Drachev, 2006; Popova et all, 2006).

The unsatisfactory situation of the Burley tobacco requires the creation and deployment of new, xigh-yielding and higher quality varieties, which can only be done through selection-research for development and introduction of new higheffective varieties (Dyulgerski, 2011; Yonchev, 2015; Calvert et all, 2000; Dimitrieski et all., 2006; Pearce et all., 2014; Risteski et all, 2012; Snell, 2006).

The purpose of this study is to evaluate the most important biological indicators and productive characteristics of new lines Burley tobacco and the possibilities for their use in selection programs, as well as a possible submission of the best of them for production testing and recognition, new varieties of Burley tobacco.

MATERIAL AND METHODS

For the achievement of defined goal for the period 2013 - 2015 in experimental fields of TTPI - Markovo are tested eight samples of Burley tobacco, namely: Line 1500, Line 1521, Line 1525, Line 1531, Line 1535, Line 1536, Line 1540 and Pliska 2002 variety used to standard in Burley tobacco. Subject of research and analysis are the most important biological and economic parameters in Burley tobacco. Of the biological parameters are researched: plant height, number of leaves; length and width of 13 leaf, respectively, for mid harvesting belt. Estimated is the length of the vegetative period. Dimensions are 120 plants from the option. Of economic indicators are calculated yield per hectare and percentage of first, second and third class.

All the options apply a uniform technology of cultivation. The harvesting of tobacco is performed on whole plants and the air drying is performed in a heating base of TTPI. Field trials are set according to the methodology of Zapryanov and Dimova (1995).

Mathematical treatment of the data is made to the accompanying products SPSS 20. Experimental data are processed by a process of analysis of variance (Anova), a difference between the variant are established by of many ranking test of Dunkan (1995).

RESULTS AND DISCUSSION

1. Biometrical indicators

Not observed significant differences in the height of the options explored in the three years of study. The highest plants are in 2014 and the lowest in 2015 at all options. In 2013 and 2014 the greatest height of plant develops Line 1521 and Line 1540 through 2015.

Average for the period of study with the highest values in terms of the height of the plants presents Line 1521 (169.8 cm) and Line 1540 (168,7 cm). With the smaller height are formed Line 1500 and Line 1531. There are no significant differences between the testimonies of the variants. All present values for height of plants that are optimal for group Burley tobacco (Table 1).

In unison with the the biggest height of most leaves all options developed in 2014, with no significant differences in the number of leaves over the years. And in the three years of researches most leaves gives Line 1540 (32.7 leaves), followed by Line 1521 (Table 1). At least leaves and in the three years of study gives Line 1525 (26.6 leaves).

Most leaves - 33 averages during the reporting period develops Line 1540, followed by a small but proven difference Line 1521. These lines are the only one the variant that give more than 30 leaves. With at least leaves are shape Line 1525 (26.6 leaves) and Line 1500 (27 leaves). Although the testimony of options regarding the number of leaves are not optimal, though they satisfy the requirements of Burley tobacco of this indicator (Table 2).

And in terms of size of the leaves no significant differences in different years of study, both the width and length of the leaves. In this case, most large leaves in all the variants are produced in 2014. The results also are unidirectional - the greatest length and width of the leaves are derived from Line 1540, invariably followed by Line 1521. And in the three years to study the control variety Pliska is characterized by the smallest width of the leaves.

Average for the period of study with the greatest length of the leaves is presented Line 1540 (63.5 cm) followed a slightly different line from Line 1521 (63.1 cm). All others variant given more than 60 cm long of the leaves and satisfy the requirements of Burley tobacco (Table 2).

Table 1. Data from biometric indicators of studied variants by years in the period of study

Variety/Line	Height in cm.	Number of leaves	Length of 13 leaf in cm.	Width of 13 leaf in cm.	Vegetative period in days
			2013		
Pliska	166,7^{ab}	27,7^{ab}	61,5^{cd}	28,8^{e}	82,7^{a}
Line 1500	163^{b}	26,8^{b}	61,3^{d}	30,3^{de}	78,3^{c}
Line 1521	169,5^{a}	29,2^{ab}	63,3^{ab}	33,2^{ab}	76^{e}
Line 1525	165^{ab}	26,3^{b}	61,7^{bcd}	31,3^{cd}	80,8^{b}
Line 1531	163,7^{ab}	27,4^{ab}	61,8^{bcd}	31,8^{bcd}	74,3^{f}
Line 1535	164,3^{ab}	28^{ab}	62,3^{abcd}	32,1^{abc}	77,4^{d}
Line 1536	166,5^{ab}	28,9^{ab}	62,9^{abc}	32,7^{ab}	77^{d}
Line 1540	169,2^{a}	31,6^{a}	63,6^{a}	33,5^{a}	78,5^{c}
$GD_{5\%}$	5,9	4,5	1,4	1,5	0,8

			2014		
Pliska	$168{,}3^{ab}$	$28{,}6^{bcd}$	$61{,}8^{b}$	$29{,}3^{c}$	$81{,}8^{a}$
Line 1500	$166{,}8^{ab}$	$27{,}8^{cd}$	$62{,}2^{bc}$	$30{,}6^{bc}$	$78{,}5^{b}$
Line 1521	$173{,}3^{a}$	$31{,}4^{ab}$	$63{,}4^{ab}$	$33{,}4^{a}$	$75{,}7^{c}$
Line 1525	$165{,}2^{ab}$	$26{,}6^{cd}$	$61{,}4^{b}$	$31{,}5^{ab}$	$80{,}5^{b}$
Line 1531	164^{b}	$28{,}6^{bcd}$	$62{,}5^{abc}$	$32{,}1^{ab}$	74^{d}
Line 1535	$164{,}6^{ab}$	$29{,}2^{bcd}$	$62{,}4^{bc}$	$32{,}3^{ab}$	77^{bc}
Line 1536	$167{,}7^{ab}$	$30{,}6^{abc}$	63^{ab}	$32{,}8^{ab}$	$76{,}5^{c}$
Line 1540	$171{,}5^{ab}$	$32{,}7^{a}$	$63{,}8^{a}$	33,8a	$78{,}3^{b}$
GD5%	7,8	3,3	2,2	2,1	1,6
			2015		
Pliska	$166{,}5^{ab}$	$27{,}3^{bc}$	$61{,}3^{ab}$	$28{,}5^{c}$	$83{,}7^{a}$
Line 1500	$161{,}1^{c}$	$26{,}5^{bc}$	$61{,}4^{ab}$	$30{,}1^{bc}$	$78{,}7^{cd}$
Line 1521	$166{,}7^{ab}$	$30{,}8^{a}$	$62{,}7^{ab}$	$32{,}7^{a}$	77^{d}
Line 1525	$163{,}3^{bc}$	26^{c}	61^{b}	$30{,}7^{abc}$	$81{,}5^{b}$
Line 1531	163^{bc}	$26{,}8^{bc}$	$61{,}6^{ab}$	$31{,}3^{ab}$	$75{,}3^{e}$
Line 1535	$163{,}4^{bc}$	$27{,}8^{bc}$	$62{,}1^{ab}$	$31{,}6^{ab}$	79^{cd}
Line 1536	$165{,}5^{abc}$	$29{,}2^{ab}$	$62{,}5^{ab}$	$32{,}4^{ab}$	$77{,}5^{d}$
Line 1540	$168{,}6^{a}$	$31{,}3^{a}$	$63{,}1^{a}$	$33{,}1^{a}$	$79{,}7^{bc}$
$GD_{5\%}$	4,9	2,7	2,0	2,6	2,1

The greatest width of the leaves is presented Line 1540 (33,5 cm), followed by a margin of line 1521 (33,2 cm). With the narrower width of the leaves are set forth control variety Pliska, which does not fully meet standards in Burley tobacco. The testimony of others the variant in this index satisfy the requirements of group Burley (Table 2).

2. Length of vegetative period

With regard to the length of vegetation period is observed significant difference between different options. All the variants with the longest vegetative period in 2015 and the shortest in 2014, the difference over the years at different options is not significant (Table 1). This shows that all options show stability on this indicator. And in the three years of study with the longest the vegetative period is the standard variety Pliska, and with the shortest Line 1531.

Average for the period of study with the shortest and yet most favourable the vegetative period differs Line 1531 (74,7 days). With favourable vegetative period is presented and Line 1521 (76,2 days). These two lines can be used as a donor for hybridization to shorten the length of the vegetative period in the selection of Burley tobacco. The greatest length of the vegetation period is characterized standard variety Pliska (82,7 days). All test variants have a shorter the vegetation period than the standard variety, which is a success in selection work on this indicator.

Line 1525 (80,9 days) and especially variety Pliska are presented with too long for Burley tobacco vegetative period. For the rest of the variant the length is with values satisfy the selection criteria for Burley tobacco (Table 2).

No is observed significant variation of biological indicators in the experienced variant during the years of the study, which is prerequisite for vegetative and morphology uniformity.

Table 2. Data from biometric indicators of studied variants average for the period of study

Variety/Line	Height in cm.	Number of leaves	Length of 13 leaf in cm.	Width of 13 leaf in cm	Vegetative period in days
Pliska	166,5[abc]	27,9[d]	61,6[de]	28,9[f]	82,7[a]
Line 1500	163,6[c]	27[de]	61,5[e]	30,3[e]	78,5[c]
Line 1521	169,8[a]	30,5[b]	63,1[ab]	33,1[ab]	76,2[e]
Line 1525	164,1[bc]	26,6[e]	61,4[e]	31,2[d]	80,9[b]
Line 1531	163,5[c]	27,6[de]	62[de]	31,7[cd]	74,7[f]
Line 1535	164,5[bc]	28,3[cd]	62,3[cd]	32[c]	77,8[cd]
Line 1536	167,2[ab]	29,6[bc]	62,8[bc]	32,6[b]	77[de]
Line 1540	169,7[a]	31,9[a]	63,5[a]	33,5[a]	78,8[c]
$GD_{5\%}$	5,2	1,4	0,6	0,6	1,3

II. Production characteristics

1. Yield

The greatest yield per hectare in 2013 gives Line 1540 (3591 kg/ha) followed with proven deference by Line 1521 (3443 kg/ha) (Table 3). At lower yield is obtained from the control Pliska variety (2527 kg/ha). In 2014 again the highest yield is obtained from Line 1540 (3775 kg/ha), followed by Line 1521 (3537 kg/ha). At lower yield is obtained from the standard Pliska variety (2724 kg/ha). This year, all study variants give the highest yield, which in unison with the most favorable biometric identifiers received in the same year. And in 2015 the highest yield is obtained from Line 1540 (3162 kg/ha), again followed by Line 1521 (3637). Again the lowest yield, which is obtained from the control Pliska variety (2582). In that year Line 1521 and especially Line 1531 (3162 kg/ha) show significantly lower yield than in the previous two. It can be concluded that with the exception of Line 1531 other tested variants exhibited stability in terms of yield in individual years.

Average for the period of study Line 1540 gives the highest yield per hectare 3607 kg/ha (Table 4). In unproven difference its results superior to those of the next in the ranking in yield per hectare, namely Line 1521 (3462 kg/ha). The yield of Line 1540 exceeds this of standard variety with almost 38%, and the next Line 1521 by 33%. Only those lines receive over 3500 kg per hectare, and therefore can be defined as high-yielding. Favourable extraction and is third in the ranking Line 1536 (3416 kg/ha). The lowest yield presents the standard Pliska 2002 variety (2611 kg/ha) (Table 4).

All new selection lines outperform production standard variety. This is an indication of the success of the selection work on this most important agronomic indicator. Although Line 1525 exceeds 8% yield of the standard variety, its results and those of variety Pliska should be defined as unsatisfactory.

2. Percentage of classes

In terms of percent of classes is observed a strong influence of factors - year (Table 3). Observed are significant differences between the variant during the three years of study, as by all of them are the most favorable in 2014 and the worst in 2015 and during the three years of study. With the highest percentage of first-class is Line 1540, which it is with the lowest percentage of third class. Second and third place with small difference between them in the three years of study are Line 1521 and Line 1336. And during the three years studied with the lowest rate first and highest third class is the standard Pliska variety.

Average for the period of study highest percentage of first class (45 %) is results from Line 1540 (Table 4). As it is presented in the three years of study in the first place on this indicator, it can be concluded that exhibits stability in terms of quality. This is the variant that gives the lowest percentage of third grade (6%). This line can be defined as a relatively high-quality.

In second place but with a big difference from the first is ranks Line 1521 (42 %). With small ranks third Line 1531 (37 %). These two variants also provide less than 10% third class (Table 4).

The standard variety Pliska 2002 gives the lowest percentage of first class from all studied the variant (12 %). Only in him the percentage of third class exceeds that of the first class (Table 3).

Although all variants provide significantly higher rate of first-class than the standard variety, the results of this indicator can be considered satisfactory, since all of them second-class rate exceeds that of the first (Table 4).

All new created lines surpass strong testimony of the standard variety, both in terms of yield and in terms of percentage of classes. This indicates that the selection work in terms of economic indicators is achieved its goals.

Line 1540 Line 1521 and Line 1536 form as options with the highest productive and selection value. In complex biological and economic indicators most stands Line 1540. Because exhibited optimal biometric identifiers favorable length of the vegetation period, high stable yield per hectare and satisfactory percentage of first class, this line deserves to be presented in IASAS for recognition as variety.

Table 3. Production characteristics of studied variants by years in the period of study

Variety/Line	Yield kg/ha	Percentage of standard	Classes in %		
			I	II	III
		2013			
Pliska	2527[e]	100	10	73	17
Line 1500	3323[c]	131	17	69	14
Line 1521	3443[b]	136	42	51	7
Line 1525	2701[d]	107	28	59	13
Line 1531	3342[c]	132	36	55	9
Line 1535	3362[c]	133	30	58	12
Line 1536	3377[c]	134	37	56	7
Line 1540	3591[a]	142	44	51	5
$GD_{5\%}$	63				

		2014			
Pliska	2724^{f}	100	18	70	12
Line 1500	3391^{d}	124	21	67	11
Line 1521	3637^{b}	134	46	48	6
Line 1525	2956^{e}	109	34	55	11
Line 1531	3420^{d}	126	43	50	7
Line 1535	3454^{cd}	129	41	51	8
Line 1536	3515^{c}	127	38	56	6
Line 1540	3775^{a}	139	51	46	3
$GD_{5\%}$	87				
		2015			
Pliska	2582^{f}	100	8	64	19
Line 1500	3130^{d}	121	15	70	15
Line 1521	3307^{b}	128	37	53	10
Line 1525	2787^{e}	108	20	63	17
Line 1531	3162^{cd}	122	31	56	13
Line 1535	3331^{bcd}	129	27	57	16
Line 1536	3357^{bc}	130	34	55	11
Line 1540	3456^{a}	134	39	52	9
$GD_{5\%}$	136				

Table 4. Production characteristics average for the period of study

Variety/Line	Yield kg/ha	Percentage of standard	Classes in %		
			I	II	III
Pliska	2611^{c}	100	12	72	16
Line 1500	3281^{b}	126	18	69	13
Line 1521	3462^{ab}	133	42	50	8
Line 1525	2815^{c}	108	27	59	14
Line 1531	3308^{b}	127	37	53	10
Line 1535	3382^{ab}	130	33	55	12
Line 1536	3416^{ab}	131	36	56	8
Line 1540	3607^{a}	138	45	49	6
$GD_{5\%}$	221				

CONCLUSION

In our study samples of Burley tobacco with the most favourable biological indicators is characterized Line 1540. With very good evaluation is presented and Line 1521.

Average for the period of study with the shortest and yet most favourable the vegetative period differs Line 1531 (74,7 days). With favourable the vegetative period is presented and Line 1521 (76,2 days).

Line 1540 gives the highest yield per hectare (3607 kg/ha) as an average over the period of study and for the three years of study. From this line is prepared highest percentage of first class (45 %), as average for the period of study and for the three years of study.

All new created lines highly superior to the readings of the standard variety in terms of yield and in terms of percentage of the classes. This is an indication of the success of the selection work.

Line 1540 Line 1521 and Line 1536 stand out as the variants with the highest value selection.

The final results show that Line 1540 is ranked first, with complex of biological indicators and production characteristics and should be proposed for production testing and recognition as a new variety.

REFERENCES

1. Bozukov, Ch., 2012. Situation and Prospects of tobacco in Bulgaria. Report-presentation of the First Agricultural Business Forum „Challenges, Opportunities and Prospects for the sector,“ hotel „Hilton“, Sofia, 27.09.2012
2. Davis L., M. Nielsen, 1999, Tobacco: Production, Chemistry and Technology, Blackwell Science, Oxford, UK
3. Dimanov, D., V. Masheva 2011, New varieties of oriental tobaccos of groups Basma, Bulgarian tobacco, number 6, 23-27
4. Dimitrieski M., G. Miceska, I. Risteski, K. Kososka, 2006, Variability of chemical composition in semi-oriental tobacco type otlia depending on the variety and the way of growing, Tobacco, Vol 56, № 5-6, 92-98
5. Docheva M., A. Stoilova, 2011, Gas chromatographic determination of nicotine and water condensate of smoke, Bulgarian tobacco, issue 5, 18-20
6. Dunkan V. 1995. Multiple – range and multiple F – test Biometrics
7. Dyulgerski, Y, 2011, Selection and genetic research of Burley tobacco, Thesis
8. Yovchev Y., 2015, Study of spread of some diseases and viral resistance to them in large leaf tobacco in Southern Bulgaria, Dissertation
9. Kirkova, S., L. Taskova, Y. Kochev M. Kasheva 2006, Examine the possibility of substitution in a blend cigarettes of Burley tobacco imports the same type grown in our cultivation under controlled conditions, USB, HT-VIII, 11-15
10. Masheva, V., 2008, Study on succession of some agro morphological signs in the selection of oriental tobacco, Plant growing sciences, vol. XLV, 2, 107-109
11. Milanova T., S. Kirkova M Srbinoska, P. Alexieva, 2013, Studies on RIP - papers, conference with international participation, HT-SUB, X, 168-172
12. Nikolov E., V. Masheva, Tz. Hristeva 2004, Assessment of value in the selection of varieties of tobacco from East Balkan origins and Tekne. 1. Genetic analysis of the content of nicotine in the raw tobacco Stara Zagora. S.2, CH.2, 250-253
13. Nicolova V., D. Drachev, 2006, Technologikal study on Burley tobacco of Yambol region, Tobacco, 56, 3 – 4, 68 – 72
14. Palmer G., B. Pearce, A. Bailey, 2007, Selecting Burley Tobacco Varieties for Variety Descriptions, 9 – 11
15. Pearce B., Denton P., Bailey A., Miller B., 2014, Selecting Burley tobacco Varieties, Tobacco production guide, Kentucky and Tennessee, 5-8
16. Popova V., Drachev D., V. Nicolova, 2006, Investigation on the burning properties of Burley tobacco grown in different regions of Bulgaria, Tobacco. Vol. 56, № 7 – 8, 159 – 164

17. Risteski I., K. K. Kososka, B. Gveroska, 2012, Results of the investatigation on some bio-morphological characteristks of domestic and introduced varieties of Burley tobacco, Tobacco, vol. 62, № 1-6, 13-21
18. Snell. W., 2006, Estimating the value of Burley Tobacco Quota. Departament of AAgricultural Economics, University of Kentucky, 1-4
19. Stoilova D., R. Bojinova, 2007, Accumulation of nicotine and other nitrogen-containing substances in Burley tobacco, Agricultural University - Plovdiv, Scientific Works, Volume LII, agri 2007.19-22
20. Tomov A., V. Minev, 1996, The role of Burley tobacco for modern cigarette, Bulgarian tobacco, number 2, 24-26
21. Wilkinson C.A., D. R. Peek, T. D. Reed, C. S. Johnson, 2002, Burley Tobacco Variety information for 2002, Publication Number 436-417, p 2
22. Zapryanov H., D. Dimova, 1995, Manual exercise in experimental work with biometrics, Zemizdat

ISSN 0494-3244

Тутун/Tobacco,Vol. 66, No 1-6, 22-29, 2016 UDC : 633.71-153.037:632.95]:631.427.1

Original Scientific paper

THE EFFECT OF PROBIOTICS ON SOIL MICROFLORA IN TOBACCO SEEDLINGS PROTECTION FROM DISEASES

Biljana Gveroska

University St. Kliment Ohridski –Bitola, Scientific Tobacco Institute – Prilep

Republic of Macedonia

e-mail: bgveros@yahoo.com

ABSTRACT

The basic principle of EM technology is to apply and increase the population of effective and useful soil microorganisms which will displace degenerative microorganisms, especially soil pathogens.
There are data on the use of probiotics not only in soil improvement but also in prevention of disease attacks. With regard to this, our objectives were to study the impact of probiotics on soil microflora and, more specifically, to determine the number of each group of microorganisms and their influence on certain processes in the soil, even in the control of certain pathogens, to estimate the effect of application of one probiotic or their combination, as well as the best model of application - when usual cultural practices are applied in order to produce healthy and good quality tobacco seedlings.
Investigations included two probiotics - EmFarma Plus and Ema 5 (probiotic with enriched formula and with rather fungicidal effect), and their combinations, as well as the modes of application.
It can be concluded from investigations that probiotics have a positive effect on the number of certain groups of microorganisms.
The best results in increasing the number of actinomycetes were obtained with EmFarma Plus + Ema 5 (with and without herbicide application). The small number of bacteria is compensated by increased number of actinomycetes. A balanced ratio of bacteria and fungi was recorded, too. The application of Ema 5 also confirmed its effects.
It may be concluded that probiotics with their effect on increasing some groups of microorganisms can prevent the attack of soil pathogens.
Keywords: probiotic, EmFarma Plus, Ema 5, actinomycetes, bacteria, fungi

ЕФЕКТОТ НА ПРОБИОТИЦИТЕ ВРЗ ПОЧВЕНАТА МИКРОФЛОРА ВО ЗАШТИТАТА НА ТУТУНСКИОТ РАСАД ОД БОЛЕСТИ

Основниот принцип ЕМ технологијата е апликација и зголемување на популацијата на ефикасни и корисни микроорганизми во почвата, со што се истиснуваат дегенеративните микроорганизми, особено почвените патогени.
Постојат податоци за примена на пробиотиците не само во насока на подобрување на својствата на почвата, но и спречување на нападот од болести. Затоа, нашата цел беше да се испита влијанието на пробиотиците врз почвената микрофлора. Поточно, утврдувањето на бројноста на одделни групи микоорганизми, што би значело нивно влијание врз одредени процеси во почвата, дури и нападот од патогените.
Исто така, да се утврди ефектот од примената само еден или нивна комбинација, како и најдобриот модел на апликација при примена на вообичаените агротехнички мерки, за да на крајот произведиме здрав и

квалитетен тутунски расад.
Испитувањата се извршени при апликација на пробиотиците EmFarma Plus и Ema 5 (пробиотик со зјакната формула и чие дејство е повеќе фунгицидно), при разни нивни комбинации, како и начини на апликација.
Од испитувањата може да се констатира дека пробиотиците имаат позитивен ефект врз бројноста на одделни групи микроорганизми.
Забележителен резултат во зголемување на бројноста на актиномицетите се забележува кај третманите со EmFarma Plus + Ema 5 (без и со примена на хербицид). Малата бројност на бактериите се компензирана со зголемената на актиномицетите. Се забележува и избалансиран однос на бактериите и габите. Примената на Ema 5 исто така го потврди своето дејство.
Може да се констатира дека пробиотиците со своето дејство врз намножувањето на одделни групи микроорганизми, можат да влијаат врз спречување на нападот од почвените патогени.
Клучни зборови: пробиотик, EmFarma Plus, Ema 5, актиномицети, бактерии, габи

INTRODUCTION

EM (effective microorganisms) technology is one of the main modes of environmental management aimed at establishing a sustainable production.

The basic principle of this technology is the application and increase of the population of effective and beneficial microorganisms in soil which eliminate degenerative microorganisms, especially soil pathogens, thus creating a healthy environment for plants. Effective microorganisms continue to coexist in the rhizosphere and plants grow well, free of pathogens. Therefore, plant growth regulators which exibit no pesticidal activity but instead can promote, inhibit or modify the physiology of plants, are also regulated as biopesticides (BPIA, 2014).

EM farma are bioproducts based on technology of effective microorganisms. Its include four group of microorganisms: lactic acid bacteria, yests, phototrophic bacteria and actinomycetes in carbohydrate medium. Introduction of beneficial microorganisms in any living system will ensure that the healthy microbes dominate the disease-causing populations (ProBioticsPolska, 2015).

There are data on the use of probiotics not only to improve the soil but to prevent the attack from diseases (Higa et al., 1989; Tokeshi et al., 1997; Okorski et al., 2008). The presence of some groups of micro-organisms implies that they have an influence on certain processes in the soil, on conditions for infection provoked by soil pathogens and on development of diseases.

Our main objective was to study the effect of probiotics on some groups of soil microflora, by indirect monitoring of the intensity of damping off disease. Analyses were also made on the effect of biofungicide EMa 5 used alone or in combination with EM farma probiotics, applying all necessary agro-technical measures. Identification of the best model of application of these products will increase the possibility of realization of the basic principle of effective microflora and EM technology will also become applicable from the aspect of seedlings protection from diseases. The ultimate goal is to provide healthy and good quality tobacco seedlings produced by the standards of sustainable tobacco production.

MATERIAL AND METHODS

Trials were set up in tobacco seedbeds and sown with seed of the variety P-66-9/7 at a rate of 6,75 g / 10 m^2.

Treatment of soil in the appropriate variants was carried out the previous day. Variants and treatments are presented in Table 1. Each variant was tested in three replicates and the area of each replicate was 3,33 m^2.

Table 1. Variants and treatments

No.	Variant	Treatment		
1	Check, untreated			
2	Standard treatment (herbicide, funcigide and saltpeter)			
3	EmFarma Plus +Ema 5; herbicide		EmFarma Plus + Ema 5	EmFarma Plus + Ema 5
4	Ema 5; without herbicide	after emergence of seedlings - Ema 5	Ema 5	Ema 5
5	Ema 5; without herbicide		Ema 5	Ema 5
6	EmFarma Plus +Ema 5 after emergence of seedlings; herbicide	after emergence of seedlings EmFarma Plus + Ema 5	EmFarma Plus + Ema 5	EmFarma Plus + Ema 5
7	EmFarma Plus +Ema 5; without herbicide		EmFarma Plus + Ema 5	EmFarma Plus + Ema 5
8	Seed with EmFarma Plus +soil with Ema5; without herbicide		EmFarma Plus + Ema 5	EmFarma Plus + Ema 5
9	Seed with EmFarma Plus +soil with Ema5; herbicide		EmFarma Plus + Ema 5	EmFarma Plus + Ema 5

Treatment of seedlings

EM-farma was applied in a rate of 1000 ml/100m^2, i.e. 30 ml/3,33 m^2.
Ema 5 was applied in a rate of 300 ml/100 m^2, or 10 ml/3,33 m^2.
Tobacco seed (2,25 g/3,33 m^2) was soaked in 3 ml of the prepared solution of EM farma (100 ml/10m^2) and stored 24 hours before sowing.
In variants using herbicide Gamit 4 EC the rate was 0,07 ml/m^2.
The second treatment was after 15 days in all variants (including 4 and 6).
Standard treatment (Variant 2) was applied on the same day, using the fungicides Top M (0,1%) and Ridomil (0,25%) and fertilization with 15 g/m^2 ammonium saltpeter the day before.
Third treatment with preparations and their combinations followed 15 days after the second one.
Monitoring of seedlings and damping off disease was made on daily basis.
Two evaluations of damping off disease were made – the first one prior to the second treatment and the second one - prior to the third treatment.
The percentage of infected area was calculated for the three replicates of each variant and analysis was made on average values.

Microbiological analysis

The number of major groups of microorganisms (bacteria, fungi and actinomycetes) was counted.

Soil samples from all replicates of each variety including the check were collected in sterile paper bags and they were used to make an average sample of 10 g soil.

Applied method of dilution – from the initial 10^{-1} to 10^{-4}. 1ml of each dilution was transferred in 5 petri dishes. For the three groups of microorganisms, the following dilutions were used: 10^{-4} for fungi, 10^{-5} for actinomycetes and 10^{-6} for bacteria.

For each group of organisms, specific nutrient base was used: Capek's agar for fungi, Mesopepton agar for bacteria and Waxmans agar for actinomycetes. The number of colonies was the average of the 5 replicates multiplied by the appropriate dilution..

RESULTS AND DISSCUSION

The late treatment with Ema 5 (variant 4) influenced the number of fungi. Therefore, there is the lowest number of fungi in this variant (Table 2). But also, in its application before sowing without herbicide (variant 5), the presence of this group of microorganisms is less than the other two. These data also confirming the effect of Ema 5 as biofungicide.

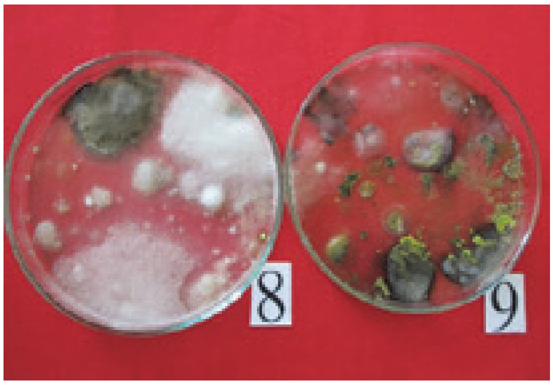
Table 2. Presence of the main groups of microorganisms in the soil

No.	Group of microorganisms / Variant	Fungi ($x 10^4$)	Actinomycetes ($x 10^5$)	Bacteria ($x 10^6$)
1	Check, untreated	5,7	6,0	4,0
2	Standard treatment (herbicide, funcigide and saltpeter)	3,0	1,7	8,3
3	EmFarma Plus +Ema 5; herbicide	3,0	10,0	3,3
4	Ema 5; without herbicide after emergence of seedlings	2,7	6,3	5,0
5	Ema 5; without herbicide	7,3	11,0	13,7
6	EmFarma Plus +Ema 5 after emergence of the seedlings; herbicide	7,3	10,0	13,3
7	EmFarma Plus +Ema 5; without herbicide	12,0	9,6	18,3
8	Seed with EmFarma Plus +soil with Ema5; without herbicide	5,3	7,6	15,3
9	Seed with EmFarma Plus +soil with Ema5; herbicide	9,0	7	20,3

The greatest numbers is determined in the variant 7 (Ph 1), where is the smallest intensity of damping off disease (Graph 1).
Compared to this variant, there is significantly lower number in the variant 3, which indicates effects of herbicide. However, it is same as the amount of fungi in standard treatment, which favors their application in the use of herbicide.
In application of probiotics on the seed at variant 9 there is almost twice higher value than in variant 8 - without herbicide (Ph 2).

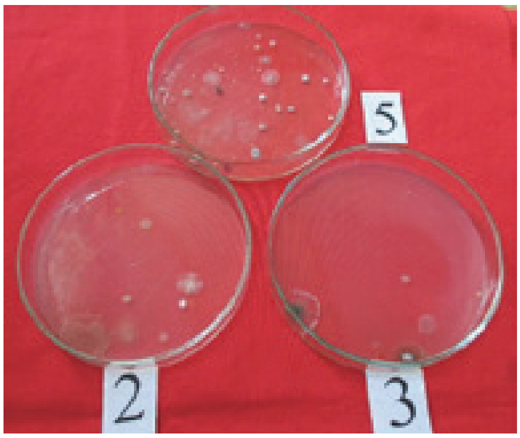
Ph 1. Presence of fungi in Variant 5- Ema 5; without herbicide and 7 - EmFarma Plus + Ema 5; without herbicide

The presence of actinomycetes are the smallest in the standard treatment. In the treatment with probiotics, the largest number (11 x 10^5) is determined in the variant 5- with Ema 5 treatment without the use of herbicide (Ph 3). But also they are represented by 10 and 9.6 x 10^5 in treatments EmFarma Plus + Ema 5 (with and without application of herbicide), which is a remarkable result. The same results was in application of EmFarma Plus + 5 Ema, after emergence of seedlings.
Their number is especially important because actinomycetes are active participants in the decomposition of carbohydrate and albuminous complexes in soil (Консулоска, 1999). Hence, increased numbers of actinomycetes along with numerous enzyme complex means providing more nutrients and destroying of pathogens.

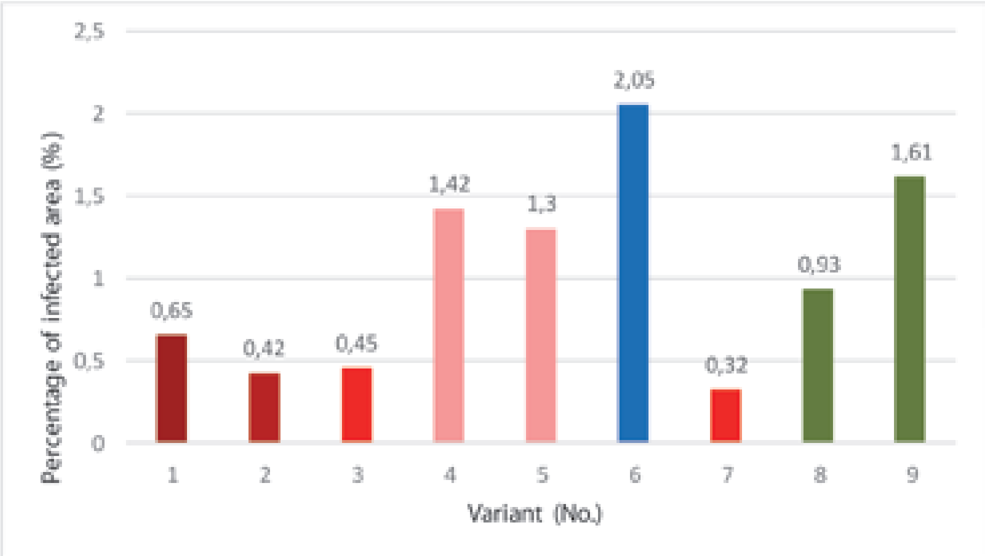


Ph 2. Presence of fungi in variants 8 and 9: seeds with EmFarma Plus + Ema 5; without and with herbicide treatment

Ph 3. Presence of actinomycetes in Variant 5- Ema 5; without herbicide



Ph 4. Presence of bacteria in the variants: 5 - Ema 5 without herbicide, 2- standard treatment and 3- EmFarma Plus + Ema 5; with herbicide



Graph 1. Influence of probiotics on the intensity of damping off disease

Bacteria, as actinomycetes, have the bigest presence in variant 5 (Table 2, Ph 4), followed by variant 9 (20,3 x 10^6). But variant 7 has one of the highest values of the presence of bacteria. This is of great importance for the effect of EM technology on attack of damping off disease. Free living rhizobacteria and endophytic bacteria use some of the same mechanisms to promote plant growth and control phytopathogens (Blomberg, 2001) The small numbers of bacteria in variant 3 is compensated with the number of actinomycetes.

According to the results, variants where the soil is treated with probiotics EmFarma Plus + Ema 5 before sowing, without and with herbicide treatment and two additional treatments, have the smallest intensity of the disease (Graph 1, Ph 5,6).

In these variants, particularly in the application of both probiotics, it is estimated the biggest numbers of fungi (in variant 7) or actinomycetes (in variant 3). At increased presence, their role in the processes influenced by them increased and they have a positive effect on the prevention of attack by pathogens.

The fungi break down highly complex and resistant compounds such as cellulose, starch, gums and lignin (Cinklin, Jr. A. R. 2002, loc cit. Sanko Sangyo Co, 2004). Actinomycetes produce and release in the soil solution antibiotics such as streptomycin, actinomycin and neomycin, and are involved in the decomposition of complex organic compounds such as phospholipids (Cinklin, Jr. A. R. 2002, loc cit. Sanko Sangyo Co, 2004).

The strenghteen formula of EMa 5 certainly contributes to this effect i.e the best results is achieved in their mutual application. Application of these probiotics before sowing as well as aditional treatment unable development and multiplying of beneficial microflora. Therefore, the best result in reducing the damping off disease is achieved when applied in a soil or direct to foliage (Gveroska, 2014a, b).

Higa et al. (1989) emphasize the role of EM on the supression of several destructive soil pathgens, too. Increasing in microbial community – promoters of plant growth are responsible for this effect. The widely recognized mechanisms of biocontrol mediated by plant growth-promoting microorganisms are competition for an ecological niche or a substrate, production of inhibitory allelochemicals and induction of systemic resistance in host plants to a broad spectrum of pathogens (Combant et al., 2005).

According to Okorski et al. (2008), increasing of the microbial diversity of soil improving health and productivity of plants. Sudarma and Suprapta (2011) found that the number of groups of microorganisms like bacteria , fungi and actinomycetes was higher in soil in which there are no symptoms of disease caused by Fusarium, unlike that in which the plants suffer from the disease caused by this pathogen.

Ph 5. Intensity of damping off disease in the check

Ph 6. Seedlings in Variant 3-EmFarma Plus + Ema 5; with herbicide

CONCLUSIONS

- The tested probiotics reduce the intensity of damping off disease.
- Probiotics have a positive effect on the number of some groups of microorganisms and reduce the presence of soil pathogens.
- The lowest intensity of disease was observed in the variants where Ema 5 was applied in combination with EmFarma Plus in the soil before sowing.
- The highest number of fungi was recorded in soil treatment with EmFarma Plus + Ema 5 without herbicide application.
- The highest number of bacteria was recorded by seed treatment with EmFarma Plus + Ema 5, with herbicide application.
- The highest actinomycetes value was obtained during treatment with Ema 5, without herbicide application.
- Significant increase in the number of actinomycetes was recorded in treatments with EmFarma Plus + Ema 5 (with or without herbicide application).
- Increased number of actinomycetes is of major importance because they are active participants in degradation of protein and carbohydrate complexes in the soil.
- Postemergence treatments with biofungicides had no positive effect on microflora or on reducing the intensity of damping off disease.
- The application of bioproducts on the seed before sowing did not give the expected results (with respect to microflora and disease intensity).
- Probiotics with their effect on increasing some groups of microorganisms can prevent the attack of soil pathogens.
- The use of probiotics in the tobacco seedling protection from diseases has good prospects in the sustainable tobacco production.

REFFERENCES

1. Alliance (BPIA), 2014. Advancing Knowlwdge About Biopesticides.
2. www.biopesticideindustryalliance.org op A.Blomberg G,V., Lugtenberg B.J.J. 2001. Molecular basis and of plant growth promotion and biocontrol by rhizobacteria. Curr. Opin. Plant Biol. 4:343-350.
3. Combant S., Duffy B., Nowak J., Clement C., 2005. Use of Plant-Growth –Promoting Bacteria for Biocontrol of Plant Diseases: principles, Mechanisms of Action and Futur Prospects. Applied and Environmental Microbiology, 71 (9): 4951-4959.
4. Gveroska B., 2014 a. Possibilities for application of a biopreparate EMa 5 in tobacco protection from some diseases. Science &Technologies, electronic journal, Vol. IV, Number 6, Stara Zagora, Bulgaria
5. Gveroska B., Miceska G., Dimitrieski M., Korubin-Aleksoska A. 2014 b. Use of Biopreparates in Tobacco Protection – Contribution to Sustainable Agriculture. Turkish Journal of Agriculture and Natural Sciences, Special Issue 2, 2014, pp. 1509-1517.
6. Higa, T. and G.N. Wididana, 1989: Changes in the Soil Microflora Induced by Effective Microorganisms. First International Conference, Kyusei Nature Farming pp 153 – 162, Oct 17-21, 1989.
7. Консулоска Б. 1999. Застапеност на ризосферната микрофлора и нејзиното влијание врз морфолошко-физиолошките карактеристики кај тутунот тип прилеп. Магистерски труд, Универзитет „Св. Кирил и Методиј-Скопје.
8. Okorski A., Olszewski J., Pszczólkowska A., Kulik T., 2008. Effcet of Fungal Infection and the Aplication of the Biological Agent EM 1 on the Rate of Photosynthesis and Transpiration in Pea (Pisum sativum L.) Leaves. Polish Journal of Natural Sciences, Vol. 23 (1): 35-47.
9. ProBioticsPolska, 2015. EmFarma Plus™
10. http://en.probiotics.pl/probio-emy/for-plants-soil/emfarma-plus.html Sanko Sangyo Co, Ltd. 2004. Prospects of EM Technology in Agriculture. http://world.saion-em.co.jp/file_6/file_6-9.pdf
11. Sudarma I.D and Suprapta D.N., 2011. Diversity of soil microorganisms in banana habitats with and without Fusarium wilt symptom. J. ISSAAS, Vol 17, No 1: 147-159.
12. Tokeshi, H., M.J.A. Jorge, A.B. Sanches and D.Y. Harada., 1997: Interaction between microorganisms, soil physical structure and plant diseases. Presented at the 6th EM Technology Conf. Nov. 24-26 1997, Saraburi, Thailand.

ISSN 0494-3244
Тутун/Tobacco,Vol. 66, No 1-6, 30-39, 2016 UDC: 633.71-275.2(497.775)"2011/2013"
Original Scientific paper

PEACH APHID *MYZUS PERSICAE* (SULZER) ON TOBACCO

Krsteska V.

University "St. Kliment Ohridski"- Bitola, Scientific Tobacco Institute – Prilep

Kicevska bb 7500 R. Macedonia

e-mail:vkrsteska@yahoo.com

ABSTRACT

Aphids appear in all tobacco producing regions in Macedonia.
Observations were made on tobacco plants in the region of Prilep during the growing season in 2011-2013, applying the method of 20 tobacco stalks and the method of Davies. Field treatments were carried out for aphid control with: Acetamiprid -0.02%, Imidacloprid -0.03%, 78083 leaf aphids Thiamethoxam -0.02%, Chlorpiriphos -0.15%, Lambda-cyhalothrin -0.025 and Methomyl -0.06%.
The *Myzus persicae* population grew from 79630 in 2011 to 93414 in 2012 and declined to 74440 in 2013, or 247484 on 600 tobacco stalks. According to the method of Davies, 78083 leaf aphids were registered. The maximum incidence of aphids was within 1th and 20th of August.
Applied Neonicotinoides showed excellent effectiveness in aphid control and contact insecticides gave good aphid control. Growers should use insecticides only when necessary. To avoid the possibility of resistance, a change of chemicals during the same growing period is recommended.
Keywords: tobacco, *Myzus persicae*, population dynamics, control

ПРАСКОВАТА ВОШКА MYZUS PERSICAE (SULZER) НА ТУТУНОТ

Лисните вошки се појавуваат во сите тутунопроизводни реони во Македонија.
Набљудувањата беа извршени во регионот на Прилеп, за време на вегетацијата на тутунот во текот на 2011-2013, со апликација на метод на 20 страка тутун и метод на Davies. За контрола на лисните вошки беа поставени полски опити со: Acetamiprid -0.02%, Imidacloprid -0.03%, Thiamethoxam -0.02%, Chlorpiriphos -0.15%, Lambda-cyhalothrin -0.025 и Methomyl -0.06%.
Популацијата на *Myzus persicae* од 79630 во 2011, порасна до 93414 во 2012 и опадна до 74440 во 2013 или вкупно беа утврдени 247484 лисни вошки на 600 тутунски страка. Според методот на Davies беа утврдени вкупно 78083 лисни вошки. Максималната појава на лисните вошки беше од 1 до 20 август.
Аплицираните неоникотиноиди покажаа инзвонредна ефикасност во контрола на вошките, а конатактните инсектициди дадоа добра заштита. Тутунопроизводителите треба да ги применуваат инсектицидите кога е неопходна контролата на лисните вошки. За да се избегне можноста за појава на резистентност, се препорачува смена и ротација на инсектицидите во текот на истата вегетацијата.
Клучни зборови: тутун, *Myzus persicae,* популациона динамика, контрола

INTRODUCTION

The green peach aphid, *M. persicae*, is a highly polyphagous species, colonizing over 500 species of host plants from at least 40 different families (Blackman and Eastop, 2000, cit. Srigiriraju, 2008; Grigorov, 1979). In field conditions of Macedonia, it has a holocyclic life cycle where the sexual phase is completed on a peach and asexual phase occurs on tobacco and other secondary host species (Janusevska, 2001; Krsteska, 2007).

The aphid attack commercial varieties of *Nicotiana tabacum* L. and forms large, dense colonies at the growing points and on the youngest leaves (Helmut and Harrington, 2007).

Aphid diet causes damages on tobacco leaves and reduction of carbohydrates, soluble sugars and glucoses. The aphids may also cause water stress and reduced growth rate of tobacco plant. They deposit honeydew on tobacco leaves resulting in the subsequent growth of a black sooty mold. Contamination of tobacco plant with aphids, or with aphid honeydew, also causes loss and reduces leaf quality. *M. persicae* is vector of several important plant viruses (Todoroski, 1965; Todoroski and Maceljski, 1983; Srigiriraju et al., 2010; Maric and Camprag, 1982).

M. persicae has high potential for reproduction and development. In tobacco biocenosis in the region of Prilep high quantities of this aphid wepe recorded. Due to the unsuitable climate conditions in 2003, the number of its generations on tobacco was reduced to 8, while in 2004 and 2005 there were 10 generations of *M. persicae* (Krsteska, 2007).

Early in the season, aphid infestations are often spotty and if such plants or areas are treated in time, serious damage can be prevented later in the season. In some cases, use of insecticides for other, more damaging insects leads to outbreak of green peach aphid. Inadvertent destruction of beneficial insects is purported to explain this phenomenon, but aphid resistance to some types of insecticide may also be involved (Srigiriraju, 2008). *M. persicae* develop resistance to many insecticides (Harlow, 1990; Kerns et al., 1998; Silva et al., 2012; Eleftherianos et al., 2008).

The main goal of the investigations was to perform analysis of population dynamics of aphids in tobacco fields and their control.

MATERIAL AND METHODS

Investigations were carried out during 2011-2013, on tobacco plants in Prilep. The observations were made with application of the following methods:

a. Method of survey of 20 randomly selected tobacco stalks infested with aphids. Tobacco stalks were sampled from the whole area of the trial at 10-days interval, starting from June 1, up to the end of September. The investigationss were performed on parts of tobacco (leaves, tobacco flowers, seed capsules). 10 checks were made by this method in each of the three years of investigations, i.e. 200 stalks per year, or 600 stalks in total. The investigation included a total of 17603 tobacco leaves (5837 in 2011, 5849 in 2012 and 5917 in 2013).

b. Method of Davies - survey of 100 randomly selected tobacco leaves infested with aphids. Tobacco leaves were sampled from the whole area of the trial at 10-days interval, during tobacco vegetation. 10 checks were made by this method in each of the three years of investigation, i.e. 1000 leaves per year, or 3000 leaves in total.

Field trials were carried out in the Experimental field of Tobacco Institute-Prilep for aphids control with 6 insecticides of different chemical classes.

The treatments included:

1. Acetamiprid (Bubastar 20 SP) -0.02% (neonicotinoid)
2. Imidacloprid (Confidor SL 200) -0.03% (neonicotinoid)
3. Thiamethoxam (Actara) -0.02% (neonicotinoid)
4. Chlorpyriphos (Pyrinex 48 EC) -0.15% (organophosphate)
5. Lambda-cyhalothrin (King) - 0.025% (pyrethroid)
6. Methomyl (Metomyl 90-SP) -0.06% (carbamate)
7. Untreated control
8. The chemicals were applied foliary, with knapsack sprayer, at 20°C. The treatment included 400 tobacco plants in flowering stage. The treatments and the untreated check were set up in a randomized complete block design with four replications. The border rows of the foliar treatments were left untreated.
9. Effectiveness of the applied insecticides was estimated 1, 4, 7, 11, 15 and 21 days after application.
10. Climate conditions are important factors for development of tobacco and aphids. Data presented in Table 1 were obtained from the meteorology station of Scientific Tobacco Institute in Prilep.

Table 1. Climate conditions in the Prilep region

Year	Meteorological factors	Decade	May	Jun	July	August	Septem.	X / ??
2011	**Mean decade air temperature (°C)**	**I**	11,3	18,5	19,4	20,7	21,1	
		II	13,6	18,3	24,4	22,2	21,0	
		III	16,5	18,8	20,00	22,5	16,0	
	Mean monthly air temperature (°C)		**13,9**	**18,5**	**21,3**	**21,9**	**19,6**	**19,1**
	Precipitations (mm)	**I**	20,0	31,0	8,0	9,0	/	
		II	32,0	20,0	/	1,0	23,0	
		III	11,0	/	9,0	1,0	15,0	
	Total precipitations (mm)		**63,0**	**51,0**	**17,0**	**11,0**	**38,0**	**180,0**
2012	**Mean decade air temperature (°C)**	**I**	16,2	19,1	24,2	23,9	20,9	
		II	13,6	21,6	23,7	21,6	17,3	
		III	13,9	22,1	24,8	23,3	18,1	
	Mean monthly air temperature (°C)		**14,6**	**20,9**	**24,3**	**23,0**	**18,8**	**20,3**
		I	6,0	/	/	14,0	/	
		II	56,0	/	/	6,0	23,0	
	Precipitations (mm)	**III**	49,0	20,0	12,0	/	/	
	Total precipitations (mm)		**111,0**	**20,0**	**12,0**	**20,0**	**23,0**	**186,0**

2013	**Mean decade air temperature (°C)**	**I**	17,4	16,0	20,3	24,6	20,0	
		II	16,2	20,6	20,9	23,3	15,6	
		III	15,7	20,4	23,9	20,9	15,5	
	Mean monthly air temperature (°C)		**16,4**	**19,0**	**21,7**	**22,9**	**17,0**	**19,4**
	Precipitations (mm)	**I**	33,0	24,0	11,0	/	/	
		II	11,0	11,0	/	1,0	21,0	
		III	3,0	16,0	/	8,0	14,0	
	Total precipitations (mm)		**47,0**	**51,0**	**11,0**	**9,0**	**35,0**	**153,0**

RESULTS AND DISCUSSIONS

During investigation of the species of Aphididae family, tobacco was attacked only by *Myzus persicae* Sulzer (Photo 1).

The body in apterous aphid is oval and 1.5 to 2.6 mm long. The wingless aphids may occur in light green, dark green, orange or red color (Photo 2). This color morphism in *M. persicae* results from the presence of a series of glycosides in the aphid hemolymph (Blackman, 1974).

Photo 1. Aphids on tobacco

Photo 2. Apterous aphids with several distinctive colors

Nymphs are greenish or yellowish, and they go through four stages during development, and the duration of each stage is in average two days. Redish eyes of young progeny, often easily observable in the abdomen of the mother.

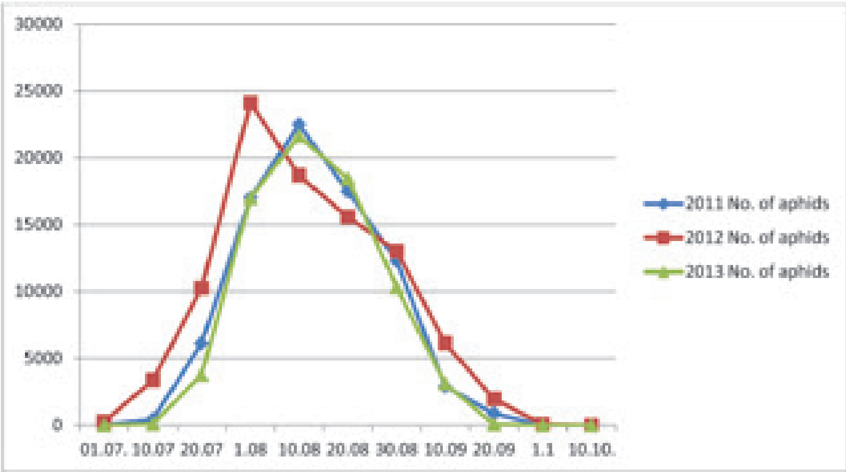
Photo 3. Female, with immatures

As aphid densities increase winged forms are produced to aid dispersal. Alate aphids have a black head and black-redish thorax, and a yellowish green abdomen with a large dark patch dorsally (Photo 3).

Photo 3. Occurrence of alate aphid in aphid colony

During the summer, *M. persicae* developed many parthenogenic generations of apterous aphids on tobacco, which depends primarily on temperature fluctuations and precipitation, as well as the physiological state of the plant.

Following the dynamics of the population of aphids in the Prilep tobacco producing region during 2011-2013, we concluded that the aphids can be observed on tobacco plants from early July to the end of September. Individual samples were found until early October. The most intensive attack of aphids on tobacco occurs from the end of July to mid-August (Graph. 1).



Graph. 1. Dynamics of the population of aphids in tobacco production region in Prilep

Table 2 shows the numerical coverage of leaf aphids in accordance with the Method of 20 stalks. Between 2011 and 2013 a survey of leaf aphids was conducted on 600 stalks containing 17603 tobacco leaves. The population of leaf aphids grew from 79630 in 2011 to 93414 in 2012 and declined to 74440 in 2013 or, in total, 247484 over the years.

Table 2. Quantitative representation of aphids 2011 - 2013
Method: survey of 20 tobacco stalks

Date	N^0 of tobacco leaves 2011	N^0 of aphids 2011	N^0 of tobacco leaves 2012	N^0 of aphids 2012	N^0 of tobacco leaves 2013	N^0 of aphids 2013
01.07	332	17	317	267	329	
10.07	368	451	361	3360	365	98
20.07	521	6121	522	10254	519	3775
01.08	628	17003	586	24098	603	16995
10.08	653	22498	639	18679	673	21648
20.08	710	17491	716	15573	722	18435
01.09	724	12275	764	13023	785	10311
10.09	678	2890	663	6128	681	3107
20.09	615	873	668	1956	647	71
01.10	608	11	613	76	593	-
Total	**5837**	**79630**	**5849**	**93414**	**5917**	**74440**

In the first year of investigation (2011), aphid population was very low at the beginning (17 aphids /sample) and maximum density was achieved in August 10 (22498 aphids/sample). In the peak period, average temperature of 22.2 ^{0}C and precipitations of 9 mm were recorded. The aphid population showed a declining trend from the mid-September, due to gradual decrease in temperature.

In the second year of investigation (2012), the infestation of aphids on tobacco started from the first week of July and the maximum incidence was achieved in the first decade of August (24098 aphids/ sample), at 23.9 ^{0}C mean temperature and 14 mm precipitations. After the second week of September, aphid density declined with the decrease of temperature.

In 2013, aphids were present from the second decade of July to September 20 and maximum population was recorded in August 10 (21648 aphids/sample).

Table 3 gives numerical presentation of leaf aphids according to the Method of 100 tobacco leaves infested with aphids. Over the years, 3000 tobacco leaves were tested and 78083 aphids were recorded. Peak period of aphid population is from early August to mid-August.

Table 3. Quantitative presentation of aphids, 2011-2013
Method: survey of 100 tobacco leaves

Date	N° of aphids		
	2011	2012	2013
01.07	53	54	
10.07	764	811	143
20.07	1231	3262	925
01.08	5083	9143	3206
10.08	8321	8148	8249
20.08	6078	6021	7054
01.09	2174	3067	1702
10.09	136	1189	556
20.09	117	213	210
01.10	52	121	
Total	**24009**	**32029**	**22045**

During tobacco growing season in 2011-2013 there were quantitative differences in aphids occurrence depending on climate conditions. Beside the climate conditions, parasites and predators limit the number of insects per plant. Some cultural practices as weed control, inter-row cultivation and use of recommended nitrogen rates may help delay infestations of aphids or reduce the aphid populations in tobacco. However, insecticides play a major role in aphid control, alone or within the IPM program.

For successful aphid control higher attention should be paid to monitoring. Tobacco leaves should be regularly checked for aphid colonies, but yellow traps and water pan traps can be also used in monitoring of aphid population.

According to our investigations, treatments are recommended if the populations reach economic threshold levels, i.e., when at least 10 of 100 plants are infested with small aphid colonies. Remedial treatments are recommended if the TGPA populations reach economic threshold levels, i.e., when at least five of 50 plants are infested with 50 or more aphids on any one leaf (Semtner, 2007, cit. Srigiriraju, 2008).

Aphids have developed resistance to many insecticides and they are more difficult to control.

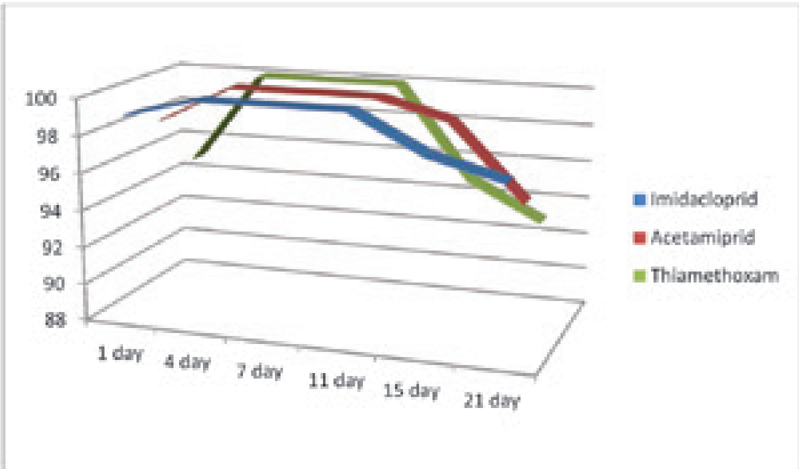
The extensive use of one insecticide during tobacco vegetation creates conditions that favor the fast development of *M. persicae* resistance.

The first report of resistance in this species dates back to 1955 from Anthon, with resistance now reported to most classes of insecticide, including the organophosphates, carbamates, pyrethroids, cyclodienes, and neonicotinoids (Bass et al., 2014).

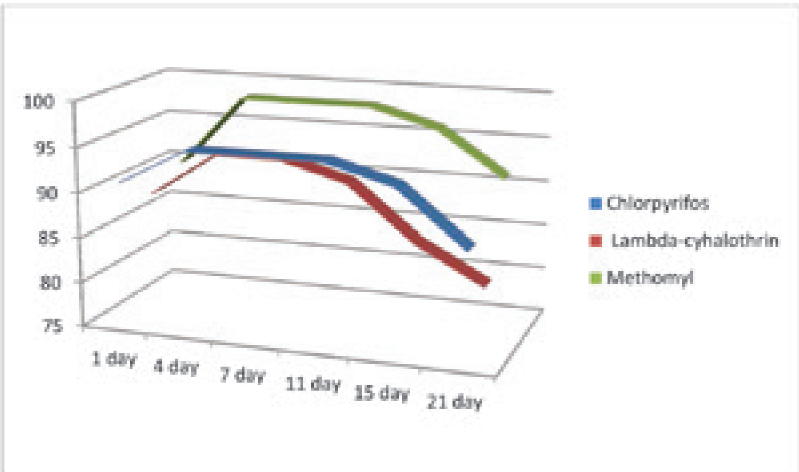
The green peach aphid, *M. persicae*, has developed resistance to at least seventy different synthetic compounds and different insecticide resistance mechanisms have been reported worldwide (Silva et al., 2012). Eleftherianos, 2008, reported strong resistance to pyrethroid insecticides in the peach-potato aphid, *M. persicae.*

Field trials were conducted to evaluate the performance of various chemical classes for aphid control on tobacco, in order growers to rotate insecticides during vegetation (Graph. 2 and 3).

24 hours after application. The efficacy is excellent up to the 21st day. Contact insecticides Chlorpyrifos (organophosphate), Lambda-cyhalothrin (pyrethroid) and Methomyl (carbamate) gave good aphid control, 95 %, 90 % and 98 % respectively. The aphids usually feed on young leaves, flowers and top of the stem. They are often found on the underside of tobacco leaves and are difficult to kill with contact insecticides, therefore careful application of insecticides with knapsack sprayer is necessary.



Graph. 2. Efficacy of Neonicotinoides against leaf aphids



Graph. 3. Efficacy of contact insecticides against leaf aphids

Neonicotinoides: Imidacloprid, Acetamiprid and Thiamtexam provide effective long-lasting protection of aphids, because of their systemic nature. They reached 100 % effectiveness, visible from the first check

The insecticides were also applied in field trial in Chile and, according to Silva et al. 2012, *M. persicae* has been chemically controlled by the application of almost all classes of insecticides, including neonicotinoids, pyrethroids, organophosphates and carbamates.

Aphid control with insecticides on tobacco is necessary. The number of treatments depends on the density and intensity of the attack of leaf aphids. To avoid the possibility of resistance, a change of chemicals during the same growing period is recommended. Growers must apply insecticides only when essential for control, using correct label rates and application procedures and avoiding unnecessary or excessive spraying.

According to Srigiriraju, 2008, the control of *M. pesricae* presents a special challenge to tobacco growers because aphids develop resistance to many insecticides and the facts is that many of the insecticides have been withdrawn from use due to environmental safety or poor performance.

Foster et al. 2007, cit. Srigiriraju, 2008, explain that the extensive use of imidacloprid and other neonicotinoids on tobacco and the reduced availability of insecticides with other modes of action (rotation with neonicotinoids) could create conditions that favor the development of neonicotinoid resistance in *M. persicae.*

Tobacco growers are relying on a few insecticides for control of aphids but the list of insecticides authorized for use on tobacco must be expanded.

CONCLUSIONS

During investigation of fauna species of the Aphididae family it was stated that tobacco was attacked only by *M. persicae*.

From the analyses conducted between 2011 and 20013 to determine the number of leaf aphids in the region of Prilep, it can be concluded that there was variable quantitative coverage.

In 600 tobacco stalks tested the population of leaf aphids grew from 79630 in 2011, to 93414 in 2012 and declined to 74440 in 2013 or, in total, 247484 aphids were counted over the years. According to the method of Davies, in 3000 tobacco leaves a total of 78083 leaf aphids were recorded. In all years, the maximum incidence of aphids was between 1 st and 20 th of August.

For successful management of aphids greater attention should be paid to monitoring. Tobacco leaves should be checked regularly for aphid colonies and treatments are recommended when the populations reach economic threshold levels.

Insecticides play a major role in keeping this pest under control. Development of insecticide resistance in field populations must be avoided.

It can be concluded that *M. persicae* has been successfully controlled by the application of all classes of insecticides applied in field trial. Neonicotinoides showed excellent effectiveness in aphid control and contact insecticides gave good aphid control.

To avoid the possibility of resistance, rotation of chemicals during the same growing season is recommended.

Growers should apply insecticides only when essential for the control, using a correct label rates and application procedures and avoiding unnecessary or excessive spraying.

REFERENCES

1. Bass C., Puinean M. A., Zimmer T. C., Denholm I., Field M. L., Foster P. S., Gutbrod O., Nauen R., Slater R., Williamson S. M.., 2014. The evolution of insecticide resistance in the peach potato aphid, Myzus persicae. Insect Biochemistry and Molecular Biology 51 (2014) 41e51,Grigorov S., 1979. Study on the biology of the peach aphid Myzus persicae Sulz. (Homoptera, Aphididae). Rasten. Nauk, 1979, 15-9/10, p. 184-193.
2. Harlow C. D., Lambert E. P., 1990. Resistante mechanisms in two color forms of the tobacco aphid (Homoptera: Aphididae). J. Econ. Entomol., 83-6, p. 2130-2135, USA.
3. Helmut F. E., Harrington R., 2007. Aphids as Crop Pests. CAB International.
4. Eleftherianos I., Foster S. P., Williamson M. S., Denholm I., 2008. Characterization of the M 918T sodium channel gene mutation associated with strong resistance to pyrethroid insecticides in the peach-potato aphid, *Myzus persicae* (Sulzer). Bulletin of Entomological Research 98: 183–191.
5. Janusevska V., 2001. Predators and parasites on leaf aphid Myzus persicae Sulz on tobacco. Master's work, Faculty of Agricultural Sciences and Food Skopje
6. Kerns D. L, Palumbo J. C., Byrne D. N., 1998. Relative Susceptibility of Red and Green Color Forms of Green Peach Aphid to Insecticides. Southwestern Entomologist Journal p. 17-24.
7. Krsteska V., 2007. Aphidophagous hoverflies (Diptera, Syrphidae) on tobacco in the region of Prilep, doctoral dissertation, Faculty of Agricultural Sciences and Food Skopje
8. Maric A., Camprag D., 1982. Stetocine i bolesti secerne repe. Nolit, Beograd.
9. Silva A. X., Jander G., Samaniego H., Ramsey J. S., Figueroa C. C., 2012. Insecticide Resistance Mechanisms in the Green Peach Aphid Myzus persicae (Hemiptera: Aphididae) I: A Transcriptomic Survey. PLoS ONE 7(6): e36366. doi:10.1371/journal.pone.0036366 Subba Reddy Palli, U. Kentucky, United States of America
10. Srigiriraju L., 2008. Quantification of Insecticide Resistance in the Tobacco-Adapted Form of the Green Peach Aphid, Myzus persicae (Sulzer) (Hemiptera: Aphididae) dissertation Faculty of the Virginia Polytechnic Institute and State University, Virginia
11. Srigiriraju L., Semtner P. J., Anderson T. D., Bloomquist J. R., 2010. Monitoring for MACE resistance in the tobacco-adapted form of the green peach aphid, Myzus persicae (Sulzer) (Hemiptera: Aphididae) in the eastern United States. Crop Protection 29: 197–202.
12. Todorovski B., 1965. Lisna vaš Myzus (Myzodes) persicae Sulz. i rezultati uporednih ogleda njenog suzbijanja različitim pesticidama. Tutun, br. 7-8, Prilep.
13. Todoroski B., Maceljski M., 1983. Myzus (Myzodes) persicae, Priracnik izvestajne i prognozne sluzbe zastite poljoprivrednih kultura. Kolektiv autora, 1983, Beograd.

ISSN 0494-3244

UDC: 633.71-153.037:632.48]:632.952
632.952:[633.71-153.037:632.48

Тутун/Tobacco,Vol. 66, No 1-6, 40-47, 2016

Original Scientific paper

APPLICATION OF SOME NEW FUNGICIDES IN THE CONTROL OF *RHIZOCTONIA SOLANI* KÜHN IN TOBACCO SEEDLINGS

Petre Tashkoski

St "Kliment Ohridski" University - Bitola,
Scientific Tobacco Institute-Prilep, Kicevski pat bb, 7500 Prilep,
R. Macedonia
e-mail: taskoskip@yahoo.com

ABSTRACT

Tobacco plants in seedbeds are often attacked by the soil phytopathogenic fungus *Rhizoctonia solani*, the causing agent of damping off disease. Investigations were made in biological laboratory of the Scientific Tobacco Institute - Prilep with tobacco variety NS72 in order to determine the effectiveness of some newer fungicides in the control of this pathogen. Seedlings were sown in 0,3 m^2 plastic trays in naturally infested soil and in soil inoculated with fungal culture before sowing. The following fungicides were used in investigation: Top M 0.1% (70% thiophanate methyl), Orvego 0,1% (ametoctradin 300 g/l + dimethomorph 225 g/l), Enervin 0,2% (ametoctradin 120 g/kg + metiram 440 g/kg) and Signum 0,1% (boscalid 267 g/kg + pyraklostrobin 67 g/kg). Seedlings were treated twice, by watering with 1 l/m^2 of the solution. The first watering was done in the 4-leaf stage and the second in the stage of rapid growth. The coefficient of fungicide effectiveness was calculated according to Abbott's formula. In seedlings treated with Signum no symptoms of the disease were observed, while in the check variant the infection rate was 75.00 to 100.00%. Due to its high effectiveness, the Signum fungicide can be used in practice, in protection of tobacco seedlings from this pathogen.

Key words: tobacco seedlings, *R. solani*, disease, fungicides, effectiveness

ПРИМЕНА НА НЕКОИ НОВИ ФУНГИЦИДИ ЗА КОНТРОЛА НА *RHIZOCTONIA SOLANI KÜHN* ВО ТУТУНСКИОТ РАСАД

При производството на расад, често во леите се појавува и почвената фитопатогена габа *Rhizoctonia solani*, причинител на болеста сечење. За да се заштити расадот од ова заболување, направивме испитување на понови препарати, со цел да се провери нивната ефикасност во сузбивањето на овој патоген. Испитувањето е изведено во биолошката лабораторија на Научниот институт за тутун-Прилеп на расад од сортата НС72. Расадот е одгледуван во пластични корита со површина од 0,3 m^2 на природно инфицирана почва и почва инокулирана со култура од габата каде инокулумот беше додаден пред сеидба на расадот. За испитување беа користени стандардниот препарат Top M 0,1% (70% thiophanate methyl) и препаратите Orvego 0,1% (ametoctradin 300 g/l+dimethomorph 225 g/l), Enervin 0,2% (ametoctradin 120 g/kg+metiram 440 g/kg) и Signum 0,1% (boscalid 267 g/kg+pyraklostrobin 67 g/kg). Расадот е третиран два пати со полевање со по 1 l раствор на m^2. Првото полевање е направено во фаза вкрстување на расадот, а второто во буен пораст. Коефициентот на ефикасност на фунгицидите е пресметан според формулата на Abbott. Кај третираниот расад со фунгицидот Signum немаше појава на заболување, додека кај контролата заразата изнесуваше од

75,00-100,00%. Заради високата ефикасност што ја покажа овој препарат, истиот може да најде и поширока практична примена во заштитата на тутунскиот расад од овој почвен патоген.
Клучни зборови: тутунски расад, *R. solani,* болест, препарати, ефикасност

INTRODUCTION

Tobacco production is a complex and specific process. Tobacco growth and development, yield and quality depend on the climate and soil conditions and the applied cultural practices. During the production of seedlings for tobacco, vegetable and ornamental plants, the sprouts and young plants can be seriously damaged. The most common cause of this phenomenon, despite *Pythium debaryanum*, is the soil phytopathogenic fungus *Rhizoctonia solani*. The ground part of the stem is covered with water-soaked spots, the tissue decays and the infected plant eventually dies. The disease spreads in larger or smaller patches and it is necessary to apply protective agro-technical measures as an integral part of the production. For prevention of disease outbreak, it is recommended to apply optimum spacing among plants in the seedbeds, moderate irrigation, regular ventilation and chemical protection. In North Carolina - where 95% of tobacco seedlings are grown in greenhouses (hydroponic float trays) - one of the most common diseases is damping off, caused by the fungus *R. solani* (Gutierrez et al., 2001). According to Kenneth et al. (2011), float trays in which tobacco seedlings are grown are ideal place for development of this pathogen.

In our country, too, damping off disease caused by *Rhizoctonia solani* (teleomorph, *Thanatephorus cucumeris*) is economically important disease. This is plant pathogenic fungus with a wide host range and worldwide distribution. It belongs to the class Basidiomycetes - fungi that do not produce asexual (conidia) spores (Annonimus 2016 a,b). It exists in the soil as mycelium and produces sclerotia on plant residues, but occasionally it can also produce sexual spores - basidiospores. The fungus *Thanatephorus cucumeris* (teleomorph stage) was earlier described under the name *Rhizoctonia solan* and it is an anamorph stage (Pejcinovski et al., 2009). According to Gonzalez et al. (2011), the fungi can persist in soil for years as mycelium and they also survive by producing sclerotia, which makes it difficult to control them with conventional fungicides. The most successful results can be achieved by integrated protection, which is a combination of fungicide application and agro-technical measures.

Shew and Ridge (2016) confirmed that fungicides containing active ingredients azoxystrobin and mancozeb show high effectiveness in protection of tobacco seedlings from this pathogen. According to LaMondia (2012), in vitro tests showed that azoxystrobin inhibited mycelium growth. Initially, it reached 98.8% but the results obtained in biological laboratory show that fungicides with this active ingredient can significantly reduce the pathogen infection. Seedlings treated with Quadris based on azoxystrobin have a more developed root system, greener color and lower rate of infection compared to the untreated seedlings. High effectiveness in the control of this pathogen was achieved in the tests made with a.i. thiophanate methyl and with fungicides from the strobilurin group -Quadris 25SC and Stroby WG (Taskoski et al., 2001, 2015 a).

Signum, a new product of the BASF company, showed high effectiveness in the control of gray mold (*Botrytis cinerea*) on strawberries (Hauke, 2004) and lettuce (Callens, 2005). Taskoski (2015) reported high effectiveness of this fungicide in the control of *Pythium debaryanum* in tobacco seedlings.

The aim of this study was to examine the effectiveness of some newer fungicides, including Signum, in protection of tobacco seedlings from the soil phytopathogenic fungus *R. solani*.

MATERIAL AND METHODS

Investigations with tobacco seedlings of NS 72 variety were performed in 2014 and 2015 at the biological laboratory of Tobacco Institute - Prilep in two treatments with three replications. Seedlings were planted in 0,3 m^2 plastic trays on 5.5.2014 and 7.5.2015. In the first trial, they were planted in soil inoculated with pure culture of the fungus, while in the second trial we used naturally infested soil, without additional artificial inoculation. Culture of the fungus grown on nutrient medium potato dextrose agar was used as inoculum in a thermostat at 25^0 C for a period of 10 days. The inoculum for one tray was prepared from mycelial colony in two Petri dishes, blended in 200 ml distilled water and added to the soil before sowing the seedlings. Two treatments with fungicides were made during seedlings growth - in the 4th leaf stage and in the stage of rapid growth. Seedlings were treated with 1 l fungicide solution/m^2 and check variants were poured only with pure water. Four fungicides were included in the investigation. Their active ingredient and concentration are presented in Table 1. Regular agro-technical measures were applied during the cultivation of seedlings.

Table 1. Investigated fungicides

Fungicide	Active ingredient	Concentration %
Orvego	300 g/l ametoctradin + 225 g/l dimethomorph	0,1
Enervin WG	120 g/kg ametoctradin + 440 g/kg metiram	0,2
Signum WG	267 g/kg boscalid + 67 g/kg pyraclostrobin	0,1
Top M 70 WP	70% thiophanate methyl	0,1

Health condition of seedlings was estimated according to the number of infected plants. Two assessments were made during the growing season - the first one ten days after the second treatment and the second - ten days after the first assessment. Spots where seedlings were infected were measured and the empty area and average intensity of disease attack were estimated by processing the obtained data. According to the disease intensity in the second assessment, coefficient of fungicides effectiveness was calculated by the Abbott's formula (1925).

RESULTS AND DISCUSSION

Results of the two-year investigations on fungicide effectiveness in protection of tobacco seedlings from the soil pathogen *R. solani* are presented in tables. In trials performed during 2014 with seedlings planted in inoculated soil, the disease intensity in the check variant ranged from 83.33% in the first assessment to 100.00% in the second (Table 2). In variants treated with fungicides, the disease intensity varied, reaching 50.00 - 65.30% with Orvego 0,1% , 45,00% with Top M 0,1% and 25.00 - 41.20 % with

Enervin 0,2%. Only the seedlings treated with Signum 0,1% applied alone or in combination with other fungicides showed no symptoms of disease.

Table 2. Disease intensity in seedlings grown in soil inoculated with fungal culture of *R. solani* in 2014

Variant	Infected area, %	
	I assessment	II assessment
Check	83,33	100,00
Orvego 0,1%	50,00	65,30
Enervin 0,2%	25,00	41,20
Signum 0,1%	0,00	0,00
Orvego 0,1%+Signum 0,1%	0,00	0,00
Enervin 0,2%+Signum 0,1%	0,00	0,00
Top M 0,1%	45,00	45,00

According to the results, disease intensity of seedlings planted on naturally infested soil in 2014 in the check variant ranged from 80.00% in the first and 90.00% in the second assessment (Table 3). The highest rate of infection in the first assessment was recorded with Orvego 0,1% - 27,40%, Enervin 0,2% - 13,00% and Top M 0,1% - 2,00%. Higher rate of infection was recorded in the second assessment (40.00% with Orvego 0,1%, 30,50% with Enervin 0,2% and 16.40% with Top M 0 1%). In this case again, no symptoms of disease were observed in variants treated with Signum 0,1%.

Table 3. Disease intensity in seedlings grown in naturally infested soil, 2014

Variant	Infected area, %	
	I assessment	II assessment
Check	80,00	90,00
Orvego 0,1%	27,40	40,00
Enervin 0,2%	13,00	30,50
Signum 0,1%	0,00	0,00
Orvego 0,1%+Signum 0,1%	0,00	0,00
Enervin 0,2%+Signum 0,1%	0,00	0,00
Top M 0,1%	2,00	16,40

In 2014, in soil inoculated with fungal culture, the highest fungicide effectiveness (100.00%) was obtained with Signum 0,1% in all three variants (Table 4). The other fungicides tested showed lower effectiveness, reaching 58.80% with Enervin 0,2%, 55,00% with Top M 0,1% and 34.70% with Orvego 0,1%. Similar effectiveness was achieved in seedlings grown in naturally infested soil. The lowest effectiveness was obtained with Orvego 0,1% (55.55%) and Enervin 0,2% (66,11%), and somewhat higher effectiveness (81.77%) was obtained with Top M 0,1%. No symptoms of disease were recorded in seedlings treated with Signum 0,1%.

Table 4. The effectiveness of investigated fungicides in 2014

Variant	Effectiveness, %	
	Seedlings grown in inoculated soil	Seedlings grown in naturally infested soil
Check	-	-
Orvego 0,1%	34,70	55,55
Enervin 0,2%	58,80	66,11
Signum 0,1%	100,00	100,00
Orvego 0,1%+Signum 0,1%	100,00	100,00
Enervin 0,2%+Signum 0,1%	100,00	100,00
Top M 0,1%	55,00	81,77

In 2015, disease intensity was variable. In soil inoculated with pathogenic fungus, the highest intensity was achieved in the check (Fig. 1), reaching 86.00% in the first assessment and 100.00% in the second (Table 5). Lower disease intensity (60.00 - 80.00%) was achieved in the variant with Orvego 0,1% (Fig. 2). With Enervin 0,2% it ranged from 2.70% in the first assessment to 30.00% in the second assessment (Fig. 3). With Top M 0,1% (Fig. 4) disease intensity of 7.50% was recorded only in the second assessment. In this trial, Signum 0,1% applied alone or in combination with other fungicides showed the highest effectiveness in seedlings protection from the pathogen. In seedlings treated with this product, no symptoms of disease were recorded (Fig. 5).

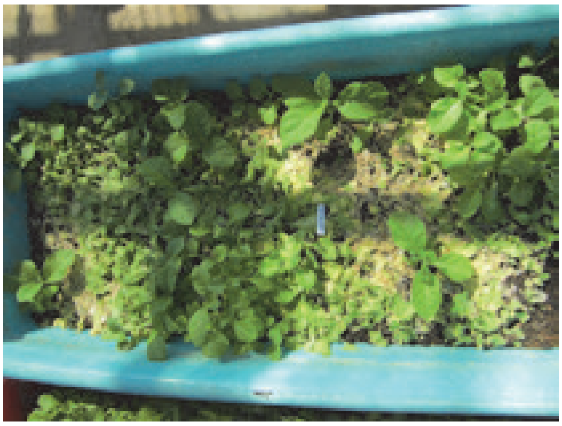


Fig. 1. R. Solani – infected seedlings in the check variant

Fig. 2. Tobacco seedlings treated with Orvego 0,1%

Table 5. Disease intensity in seedlings grown in soil inoculated with fungal culture of *R. solani* in 2015

Variant	Infected area %	
	I assessment	II assessment
Check	86,00	100,00
Orvego 0,1%	60,00	80,00
Enervin 0,2%	2,70	30,00

Signum 0,1%	0,00	0,00
Orvego 0,1%+Signum 0,1%	0,00	0,00
Enervin 0,2%+Signum 0,1%	0,00	0,00
Top M 0,1%	0,00	7,50

Fig. 3. Seedlings treated with Enervin 0,2%

Fig. 4. Seedlings treated with Top M 0,1%

Fig. 5. Seedlings treated with Signum 0,1%

In naturally infested soil, the disease intensity in the check variant ranged from 30.00% in the first assessment to 75.00% in the second (Table 6). Somewhat lower intensity was observed in the variant with Orvego 0,1% (26.00% in the first and 43.40% in the second assessment). The lowest disease intensity of 3.00% in both assessments was recorded with Enervin 0,2%. As in the previous trial, no symptoms of disease were recorded in seedlings treated with Signum 0,1% and Top M 0,1%.

Table 6. Disease intensity in seedlings grown in naturally infested soil, 2015

Variant	Infected area %	
	I assessment	II assessment
Check	30,00	75,00
Orvego 0,1%	26,00	43,40
Enervin 0,2%	3,00	3,00
Signum 0,1%	0,00	0,00

Orvego 0,1%+Signum 0,1%	0,00	0,00
Enervin 0,2%+Signum 0,1%	0,00	0,00
Top M 0,1%	0,00	0,00

According to the results (Table 7), 100.00% effectiveness in protection of tobacco seedlings from this pathogen in 2015 was achieved with Signum 0,1%, applied alone or in combination with other fungicides. In both variants (seedlings grown in soil inoculated with fungal culture and seedlings grown in naturally infested soil), no symptoms of disease were recorded. This fungicide provided maximum effectiveness in protection of tobacco seedlings from the pathogen *Pythium debaryanum,* too (Tashkoski, 2015).

Table 7. The effectiveness of investigated fungicides in 2015

Variant	Effectiveness, %	
	Seedlings grown in inoculated soil	Seedlings grown in naturally infested soil
Check	-	-
Orvego 0,1%	20,00	42,13
Enervin 0,2%	70,00	96,00
Signum 0,1%	100,00	100,00
Orvego 0,1%+Signum 0,1%	100,00	100,00
Enervin 0,2%+Signum 0,1%	100,00	100,00
Top M 0,1%	92,50	100,00

High effectiveness was achieved with Top M 0,1%, reaching 92.50% in seedlings grown in soil inoculated with the pathogen and 100.00% in seedlings grown in naturally infested soil. Effectiveness of 70.00 - 96.00% was achieved with Enervin 0,2% and the lowest effectiveness of 20.00 - 42.13% was obtained with Orvego 0,1%. Enervin is a fungicide which achieved 100% effectiveness in the control of the causing agent of damping off disease Pythium spp. in tomato and pepper (Rusevski et al., 2012) and 98.83% effectiveness against the same pathogen in tobacco seedlings (Tashkoski, 2015).

Due to their high effectiveness, the application of Signum and Enervin fungicides will provide control of the soil pathogen *R. solani* - causing agent of damping off disease on tobacco seedlings.

CONCLUSION

The disease caused by the soil-borne pathogen *R. solani* is a serious problem in production of tobacco seedlings. Severe losses *caused* by *this disease* can be reduced by applying appropriate agro-technical and chemical measures. For this aim, the effectiveness of some newer fungicides was studied during 2014 and 2015.

This effectiveness was achieved at extremely high disease intensity in 2014 which reached 100.00% in the check variant (soil inoculated with culture of the fungus) and 90.00% in the trial with naturally infested soil.

Effectiveness of 55.00% was obtained by application of the standard fungicide Top M 0,1% in soil inoculated with fungal culture and 81.77% in naturally infested soil. The lowest effectiveness (34.70% and 55.55%, respectively) was obtained by the Orvego 0,1% fungicide. In 2015, disease intensity in the check variant was 100.00% in the soil inoculated with fungus and 75.00% in the trial with naturally infested soil.

Application of the above fungicides reduces the infection of tobacco seedlings by this pathogen. The effectiveness achieved with the standard fungicide Top M 0,1% was 92.50% in seedlings grown in inoculated soil and 100.00% in seedlings grown in naturally infested soil. Again, the lowest effectiveness in both variants was obtained with Orvego 0,1% (20,00% and 42.13%, respectively). The effectiveness achieved by Enervin 0,2% was 70,00% in seedlings grown in inoculated soil and 96.00% in seedlings grown in naturally infested soil.

In both years and both trials, the highest effectiveness in protection of tobacco seedlings from *R. solani* was achieved by the fungicide Signum 0,1%, used alone or in combination with other fungicides. Seedlings treated with this fungicide showed no symptoms of disease.

Due to its high effectiveness, it can be recommended for practical application in protection of tobacco seedlings from this soil-borne pathogen.

REFERENCES

1. Abbott W. S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18, 265-267.
2. Annonimous 2016 a. http://www.en.wikipedia.org/wiki/ Rhizoctonia solani.
3. Annonimous 2016 b. http://www.cals.ncsu.edu/course/pp728/ Rhizoctonia.
4. David Shew and Garrett Ridge. 2016. North Carolina State University . Rhizoctonia solani (target spot of tobacco). http://www.wiki.bugwood.org/ Rhizoctonia solani.
5. Gonzalez M., Pujol M., Metraux J.P., Gonzalez-Garcia V., Bolton M.D., Borras-Hidalgo O. 2011. Tobacco leaf spot and root rot caused by Rhizoctonia solani Kühn. Mol Plant Pathology. Apr: 12(3):209-16.
6. Gutierrez W. A., Shew H. D. and Melton T. A. 2001. Rhizoctonia Diseases in Tobacco Greenhouses. College of Agriculture and Life Sciences. Plant pathology extension. North Carolina State University.
7. Kenneth W. Seebold, 2011. Managing Target Spot and Rhizoctonia Damping-Off in the Float System. Kentucky Cooperative Extension Service University of Centucky – college of agriculture, Plant Pathology Fact Sheet, PPFS-AG-T02.
8. La Mondia J. A. 2012. Efficacy of azoxystrobin fungicide against sore shin of shade tobacco caused by Rhizoctonia solani. Tobacco Science 49:1–3.
9. Пејчиновски Ф., Митрев С., 2009. Земјоделска фитопатологија-специјален дел. Универзитет „Гоце Делчев“ Штип.
10. Русевски Р., Кузмановска Б., Банџо К., Ристески М., 2012. Контрола на Pythium spp. кај доматот и пиперката со употреба на нови фунгицидни активни материи. Заштита на растенија, год. XXIII, бр. 24/25, стр. 47-52, Скопје.
11. Ташкоски П., Гвероска Б., Стојков С. 2001. Намалување на појавата сечење кај тутунскиот расад причинета од Rhizoctonia solani Kuhn со примена на одредени фунгициди. Тутун, Tobacoo, Vol. 51, No 3-4, 85-93, Прилеп.
12. Tashkoski P., Krsteska V., Mitreski M., Stojanoski P. 2015 a. The impact of strobilurin fungicides in prevention of damping off disease on tobacco seedlings. 2nd International Symposium for Agriculture and Food - ISAF 2015, 7-9 october, Ohrid, Republic of Macedonia.
13. Tashkoski P. 2015 b. Aplication of the Signum fungicide in control of Pythium debaryanum Hesse on tobacco seedlings. Tobacco, Vol. 65, N0 7-12, pp. 38-46, Prilep.

ISSN 0494-3244
UDC: 633.71(497.7)
Тутун/Tobacco,Vol. 66, No 1-6, 48-52, 2016
Original Scientific paper

STABILIZATION OF TOBACCO PRODUCTION IN R.MACEDONIA

Silvana Pasovska

Un.St.Kliment Ohridski Bitola,Scientific tobacco institute,Prilep Republic of Macedonia

e-mail:Silvana.Pasovska@uklo.edu.mk

ABSTRACT

Tobacco is major agricultural product in many countries in the world. Depending on climate conditions, it is either exceptionally popular or marginally represented. At world level, the annual tobacco production ranges from 6.500.000 to 7.000.000 t. Within this range is the final production of cigarettes which, by acceptance of the American blend, becomes more and more globalized.Trade with raw tobacco in the world is fairly stable, with a balance between supply and demand. The market is traditional and in most cases perfectly defined. Over 95% of tobacco production is spent on cigarette production and the remaining 5% on other tobacco products.
Macedonia has stabilized its tobacco production at approximately 25.000-30.000 tons, with a tendency to improve the quality as much as possible.Macedonia is a country with its own strategy and it will not accept to reduce tobacco production, because replacement of tobacco with another crop will not provide existence to tobacco growers, having in mind the climate conditions and the economic impact. In regions where tobacco is grown, climate and soil conditions are unfavorable for other crops. Regarding the economic impact, production of a new crop will be accepted only if it is profitable replacement for tobacco that will provide existence to growers, which is hardly believable.
Keywords: stabilization, tobacco production, incentives for support and development, productional structure, purchase prices

СЕ СТАБИЛИЗИРА ТУТУНСКОТО ПРОИЗВОДСТВО ВО Р.МАКЕДОНИЈА

Како земјоделска култура,тутунот е значаен за многу земји во светот и во зависност од климатските услови или е изразито масовна култура или е маргинално застапен.На светско ниво,производството на тутун од сите типови се движи од 6.500.000-7.000.000тони годишно и во рамките на ова производство се движи и финалната преработка на цигари,која со прифаќањето на америчкиот бленд,по својот квалитет станува се повеќе интернационална односно глобална.Трговијата со тутунската суровина во светот е доста стабилна,односно постои рамнотежа помеѓу понудата и побарувачката.Пазарот е традиционален и во повеќето случаи перфектно дефиниран.Над 95% од тутунското производство се троши за производство на цигари,додека останатите 5% отпаѓаат на другите тутунски производи.
Македонија го стабилизира производството на тутун. Количините се со тенденција да се задржат на околу 25.000-30.000тони тутун, со што е можен подобар квалитет.Како земја со своја стратегија,Македонија не прифаќа методологија за намалување на производството на тутун која е спротивна на обезбедувањето егзистенција на производителите и во услови кога ќе се замени тутунот со друга култура. При тоа треба да се имаат предвид климатските услови и економскиот ефект. Во регионите каде што се сади тутунот нема климатски услови за други култури бидејќи се тоа суви места. Околу економскиот ефект, се прифаќа само ако нова култура е исплатлива замена, односно им обезбеди егзистенција на производителите, што е малку веројанто.
Клучни зборови: стабилизација,тутунско производство,стимулации за поддршка и развој,производна структура,откупни цени.

INTRODUCTION

Macedonia is traditional producer of oriental tobacco. During the long period of tobacco production it has fluctuated due to various factors, but the most important are weather conditions, which showed the highest impact on quality and quantity of tobacco and according to which we can differentiate weak, average and above average harvests. Other important factors that affect stability of tobacco production are the measures and instruments of economic policy, primarily the prices and incentives.

During the transition period, there were big oscillations in tobacco production compared to the preceding socialist period that were due to the change of socio - political system and the adaptation of tobacco production to the newly created conditions. The production began to stabilize and to approach the quantities achieved in the previous period in 1997, although still with frequent fluctuations. Big and important role in this trend of stabilization played the Government, by introducing special incentives for tobacco production. After 2005, this production ranges between 20,000 and 30,000 tons.

Nowadays, Macedonia has a stable tobacco production compared to neighboring countries, both with regard to quantity and quality.

QUANTITATIVE AND QUALITATIVE CHANGES IN TOBACCO PRODUCTION CONDITIONS IN THE PRE-TRANSITIONAL PERIOD

Macedonian tobacco production in the pre-transitional period consisted of 23 tobacco processing plants and three tobacco companies, organized in the complex organization of associated labor "Yugotutun" – Skopje.

Table 1. Production of raw tobacco in Macedonia in middle-term periods (in tons)

Years	Production
1971 – 1975	29.120
1976 – 1980	30.243
1981 – 1985	29.976

Source: Analysis of the operation of SOZT "Yugotutun" - Skopje

In the period 1981 – 1985, more precisely in 1980, tobacco production engaged 70,000 subcontractors with an average of 0.4 - 0.43 hectares of planted area and 430 kg tobacco per subcontractor. Main tobaccos represented in the production were the oriental types Prilep, Yaka and Dzebel and the semi-oriental type Otlja.. These tobacco types were manufactured in five tobacco producing regions in Macedonia.

Table 2. Structure of tobacco production by types

Type	Tons	%
Prilep	17.345	59
Yaka	6.195	21
Dzebel	1.543	5
Otlja	2.197	8
Others	1.917	7
Total	29.197	100

Source: LJ.Poposki "Uticaj drustveno-ekonomskih i organizaciskih faktora na stanje I razvoj primarne proizvodnje duvana", Beograd 1990

In 1985, the number of registered subcon-

tractors engaged in tobacco production of R. Macedonia was 71,033 and the average yield per subcontractor was 411 kg.
In the above administrative period, prices of raw tobacco were determined by appropriate government authority. There were two types of prices: guarantee prices set by the Government and production prices contracted with purchase companies. No incentives for tobacco existed during this period.

TOBACCO PRODUCTION DURING THE PERIOD OF TRANSITION

After 1991, with acquisition of independence of the Republic of Macedonia, the production of raw tobacco took place in changed socio - economic and political conditions. It was a period of beginning of privatization process, when there was a lack of capital and when the production shifted from public to private foreign and domestic companies. In such conditions, some of the companies ceased the manufacture and purchase of tobacco. Foreign buyers appeared on the raw tobacco market, with only a few domestic companies. Main organizers and buyers are, however, the foreign companies. Macedonia, although small by its area and population, has a solid concentration of tobacco production which, estimated per capita, is higher than that of the surrounding countries. Based on statistics, in 2003 per capita production of raw tobacco in Macedonia was 11.8 kg, in Turkey 2,3kg, in Greece 11,1kg and in Bulgaria 8 kg.

In the last two medium terms, tobacco production in Macedonia ranged as follows:
Table 3.in tons

Year	Production	Subcontractors	
2005 – 2010	**21.726**	**33.785**	
2011 – 2015	**24.773**	**(2014)**	**34.784**

Source: Association of tobacco growers of R. Macedonia 2015

In the period 2006 - 2010, the average number of subcontractors was 33,795, while in 2011-2014 it was 34784 and the average production per subcontractor was 624 kg and 712 kg, respectively.

Table 4. Areas under tobacco in the last medium terms

Years	Hectares
2006 – 2010	16.837
2011 - 2014	16.044

Source: Association of tobacco growers of R. Macedonia 2015

The average production of tobacco in 2006 - 2010 was 643 kg/ha, while in 2011-2014 it mounted to 1,634 kg/ha. The most frequently grown tobacco types were Prilep, Yaka and Basma.

Table 5. Structure of raw tobacco production in 2015godina by types

Tobacco types	kg	%
Prilep	16.684	88,5
Yaka	2.150	11,0
Basma	77	0,5
Total	18.911	100,0

Source: Association of tobacco growers of R. Macedonia 2015

The most dominant variety in the production of raw tobacco is P -66, which accounts for 90% of the total production. This means that it is easily adaptable to most of the productive regions in R. Macedonia. Thus, in 2011-2015 the variety P-66 represented about 78% of the total amount of 24,773 tons purchased tobacco, Yaka - 20%, Basma - 1.7% and other varieties 0, 3%. It is worthy to mention that in 2015 the production of Basma variety was reduced to 77 tons, which is over 90% decrease compared to 730 tons in 2011.

The influence of weather conditions on the quality and quantity of manufactured tobacco affects the structure of purchased classes of raw tobacco.

Table 6. Purchased amount of raw tobacco in tons and share of purchased classes in %

	2013		2014		2015	
Class	Tobacco tons	Tobacco %	Tobacco tons	Tobacco %	Tobacco tons	Tobacco %
I	248	10,0	249	4,3	270	10,0
II	178	57,4	178	28,4	189	65,0
II	136	25,2	136	44,0	136	23,0
IV	53	7,4	55	23,3	55	2,0
Total	615	100,0	618	100,0	650	100,0

Source: A representative part of the purchase of a tobacco purchase company

The data in the table reveal that the 2014 harvest was below the average, the harvest in 2013 was within the average, while in 2015, seen through the quality of purchase classes, the harvest was slightly above the average.

In compliance with the quality of purchased tobacco are the purchase prices by classes.

Table 7. Purchase prices of raw tobacco, denars/kg

Years	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Prices	118	141	167	192	136	165	180	153	117	185

Source: Source: Association of tobacco growers of R. Macedonia

Differences in quality of purchased tobacco expressed through the purchase prices are due to the influence of weather conditions in subsequent years, but subjective influence of the purchase companies must not be excluded.

COMPARATIVE DATA AND INDICATORS OF PRIMARY TOBACCO PRODUCTION IN GRECCE AND BULGARIA

In terms of purchase prices of leaf tobacco, the situation in Macedonia is similar to that in Greece. The average purchase price in Macedonia in 2015, is 4 EUR /kg including the incentives, and in Greece it ranges from 3.8 to 4.5 EUR/kg leaf tobacco. Tobacco production per hectare in Greece is around 2,000 kg, while in Macedonia, in the period 2011-2014, it reached 1,634 kg. The area under tobacco per subcontractor is 1 ha in Greece, 0.5 ha in Macedonia and 0.55 ha in Bulgaria.

The number of subcontractors engaged in tobacco production in Bulgaria in 2015 was 32,000, while in Macedonia 35,000 subcontractors were engaged in the period 2011-2015. The average production of oriental tobacco in Bulgaria for the period 2011 - 2015 was 15,300 tons, while in Macedonia 24,773 tons. The lower production of oriental tobacco in Bulgaria is a due to tha fact that some of the subcontractors are engaged in the production of large-leaf tobaccos Virginia and Burley.

CONCLUSION

After a long time, Macedonia has stabilized its tobacco production both in quality and quantity, which means that it regained the yields of the pre-transitional period.

Tobacco production has increased its productivity and gives higher yields per unit area and per subcontractor, which points out to better utilization of land capacities and available workforce.

The structure of purchased tobacco consists of high quality classes, which is reflected in the pricing policy of purchased tobacco.

The yields and quality of oriental tobacco production in Macedonia is in compliance with those of Bulgaria and Greece, with a strong trend of stabilization.

Big influence on this stabilization has the Government, with its incentives for support and development of tobacco production.

REFERENCES

1. Попоски Љубен,1990. ``Утицај друштвено-економских и организационих фактора на стање и развој примарне производње дувана у СРМ`` Београд,
2. Попоски Љубен,2014. ``Тутунски комбинат – Прилеп, подем и пад``,Друштво за наука и уметност- Прилеп
3. Списание ``Бугарски тутун`` бр.4, Софија 2015
4. Податоци од статистички годишници на СРМ и СФРЈ
5. Здружение на производители на тутун во лист , Стопанска комора, 2015

ISSN 0494-3244

Тутун/Tobacco,Vol. 66, No 1-6, 53-58, 2016 UDC: 633.71-153.1.02:577.115.3}:543.544.3

Original Scientific paper

DETERMINATION OF FATTY ACID COMPOSITION OF SEED OF ORIENTAL TOBACCO

Stefka Kirkova[1] Marija Srbinoska[2] Silvia Ivanova[3]
Anka Georgieva[1]

[1]Tobacco and Tobacco Products Institute –Markovo, Plovdiv, Republic of Bulgaria

[2]Scientific Tobacco Institute-Prilep, Republic of Macedonia

[3]Institute of cryobiology and food technology, Sofia, Republic of Bulgaria

ABSTRACT

The tobacco is grown in many countries in the world and there are areas where the growing tobacco is only economically viable. Fermented tobacco leaf is a commercial product and it is used in the production of cigarettes in the tobacco processing industries. Only a small amount of best quality seeds are collected from fields for next year production, most of them are a by-product of tobacco leaves production.

In our previous studies we found that the seeds of the three tobacco types cultivated in the Balkans, show potential as a nutrient product. In the present study the seeds from oriental tobacco were evaluated as a potential as renewable source of oil.

The aim of the study is revealing the fatty acid composition of seeds of oriental tobacco. For qualitative and quantitative determination of fatty acids in oil from seeds, a GC/FID procedure was performed. The results show that the tobacco seed oil with its unique fatty acid composition may be regarded as special oil suitable for consumption after refining.

Key words: oriental tobacco, seed oil, fatty acid composition, alternative use

ОПРЕДЕЛУВАЊЕ НА СОСТАВОТ НА МАСНИТЕ КИСЕЛИНИ ВО СЕМЕ ОД ОРИЕНТАЛСКИ ТУТУН

Тутунот се одгледува во многу земји во светот, а постојат области каде одгледување на тутун е единствено економски оправдано.Ферментираниот тутун е комерцијален производ кој се користи во производството на цигари во индустријата за преработка на тутун. Само мала количина на семе со најдобар квалитет се собра од полињата за следната производна година, повеќето од семето е нус-производ на производство на тутунски лист.

Во нашите претходни студии,утврдивме дека семето од три типа на тутун кои се одгледуваат на Баканот, покажуваат потенцијал како хранителен материјал. Во ова испитување беше земено семе од ориенталски тутун и беше евалуиран неговиот потенцијал како обновлив извор на масло.

Целта на испитувањето е да се одреди составот на масните киселини во маслото од семе на ориенталски тутун. За квалитативно и квантитативно определување на масни киселини во маслото од семе, беше изведена GC постапка / FID. Резултатите покажуваат дека маслото од семето има состав на масни киселини кој може да се смета погодни за употреба по рафинирањето.

Клучни зборови: ориенталски тутун, масло од семе, маснокиселински цостав, алтернативна употреба

INTRODUCTION

Tobacco is an important industrial crop valued for its leaves. After curing, the leaves are used in manufacturing of different tobacco and tobacco related products. The tobacco industry comprises many companies engaged in the growth, primary tobacco processing, trade, marketing, and distribution of tobacco and tobacco-related products. The international tobacco market is a global, mature, open and cyclical sector. Global because in more than 124 countries in the world is grown different types of tobacco. Mature because growth of production is slow mainly due to strong restrictions WHO anti-smoking campaign. Open because one third of the tobacco produced in the world is exported from the country of origin. And last but not least cyclical due to rapidly changing supply-demand balance and price fluctuations.

A change in European legislation has significantly helped in this regard. Since most subsidized crop, tobacco has become the most negligees. World Health Organization (WHO) policies and measures affected production all types of tobacco, including an Oriental (Health literacy, 1997). The change in the taste of consumers has led to a further reduction share of oriental tobacco in blends. It is known that there are areas where the cultivation of oriental tobacco is the only possible agricultural crop.

Tobacco is excellent source of the phytochemicals, edible proteins, seed oil and organic acids having pharmaceutical and industrial uses.There were reports of studies on its usefulness in pharmaceutical practice. Researchers from the University of South Florida have found successful applicability in degenerative brain diseases, which translates into a gradual reduction of intelligence and memory disorders. Have already been developed preparations that help to delay the symptoms of Alzheimer‘s disease (Health media-Framar, 2012).

Tobacco seed as a secondary product of tobacco leaf production contains oil in a wide range of 36- 41% of the seed weight depending on a number of factors including the variety, growing conditions of tobacco and plantation area (Hutchens,1999.

The focus of researchers has been on the oil content of the tobacco seed which does not contain the alkaloid nicotine (Popov, 1940). The main fatty acids in the oil of tobacco seeds are linoleic, oleic, palmitic and stearic acid (Ashraf-Khorassani at al., 2015).

The oil from the seeds of various types and varieties of tobacco contain various amounts of fatty acids. It has been found that the type of solvent used for extraction of oil does not affect the composition (Srbinoska at al., 2003; Srbinoska at al., 2003a).Ten years ago Prof. Zlatanov and colleagues examined tobacco seeds of “small-leaf” and “large leaf” tobacco regarding their lipid composition and more accurate determination of phospholipid, sterol and tocopherol fraction as biologically active substances in the oil (Zlatanov at all., 2007).

There are studies on their use in animal feed because of its biological value (Rossi et all, 2007). There has been even attempts recovered oil from tobacco seeds to be used in food supplements (Zdremtan and Zdremtan, 2006). There were no reports of research on tobacco seeds as food for the human consumption. In our previous studies we found that the three groups of varieties of tobacco are produced in the Balkans. Their seeds possess superior content of polyunsaturated fatty acids close to the content in poppy and sesame seeds (Kirkova at al., 2015, Kirkova at al., 2016).

However, since the content of particular fatty acids in the seed may vary considerably depending on soil conditions, climate and genetic factors, it would be of interest to analyze seed oil from the oriental tobacco type, which are predominantly cultivated in Balkans. In order to establish the potential of the oriental tobacco seed oil as edible oil the qualitative and quantitative analysis of fatty acids in oil is research subjects of this study.

MATERIALS AND METHODS

It has been studied two harvests tobacco seeds of the same variety oriental tobacco under the same agricultural practice and growing conditions in the Experimental field of Institute of tobacco and tobacco products Markovo, Plovdiv.For the extraction of tobacco seed oil Soxhlet procedure was used (plant material to solvent ratio 1:10 w/v), extraction time 8 h and *n*-hexane as solvent) (Stanisavljevic et al., 2007).
The fatty acid methyl esters (FAMEs) were prepared by boron trifluoride (BF3) method. The FAMEs were then analysed using a gas chromatography (Varian Star 3400 CX) coupled with a FID detector and with a fused silica capillary column-DB-23 (Agilente Technologies, USA) (60 m x 0.25 mm x 0.25 mm).Identification of individual fatty acid methyl ester was achieved by comparison with reference standards. The results are given as the dry weight percentage of total fatty acids.

RESULTS AND DISCUSSION

Results for fatty acid composition of the studied tobacco seeds of oriental tobacco are presented in Figure 1.

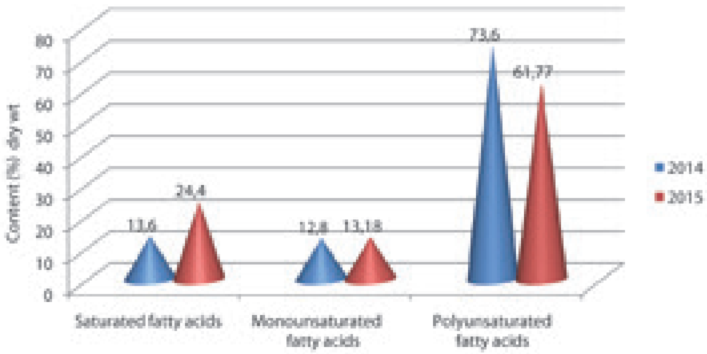


Fig.1. Fatty acid compositionof seeds of oriental tobacco, crop 2014 – 2015 (% dry wt)

The content of fatty acids as well as the ratio between unsaturated and saturated fatty acids is important parameter for determination of nutritional value of certain oil.The tobacco seed oil does is a mixture of saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs). The group of unsaturated fatty acids includes monounsaturated fatty acids (MUFAs) and polyunsaturatedfatty acids (PUFAs).
From the results it appears that the predominant fatty acids present in oil are polyunsaturated fatty acids (PUFAs) with range of 61.77 % to 73.66%on dry weight (Fig.1).

Regarding to the fatty acid composition by groups varies throughout the two years, although variety is the same and tobacco is grown in relatively close agricultural and growing conditions. Content of saturated fatty acids (SFAs) in 2015 have increase compared to polyunsaturated fatty acids (PUFAs). The amounts of monounsaturated fatty acids (MUFAs) are similar (Fig.1).
The results for individual saturated, monounsaturated and polyunsaturated fatty acids contents are presented in Fig. №. 2, 3 and 4.
The results from this study, showed that the percentage of the SFAs ranged from 13.6 % to 90.5% ± 25.41% on dry weight, with the predominant presence of palmitic (C16:0)

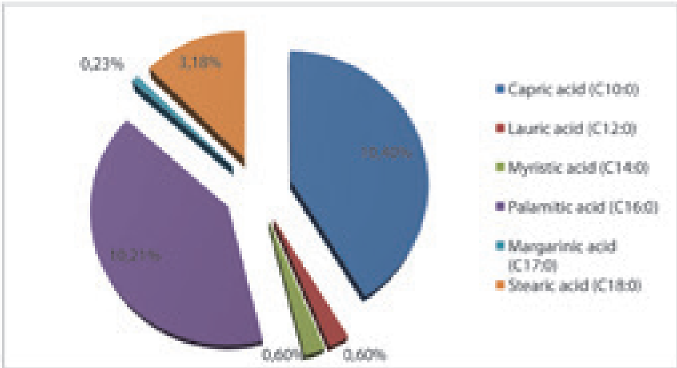


Fig. 2. Content of saturated fatty acids (SFAs) (% dry wt), crop2015

and capric acid (C10:0) (Fig.2).

The fatty acid composition of tobacco seed oil revealed that the oil is rich in unsaturated fatty acids, having linoleic acid, oleic acid and palmitic acid as the most abundant unsaturated and saturated fatty acids respectively. This composition indicates the suitability of the oil as edible oil after refining and also shows that tobacco seed oils follow the usual pattern of seed-fat glyceride structure.

MUFAs refers to present of the major monounsaturated fatty acid in oil, which is oleic acid (C18:1).The content of the MUFAs ranged from 12.8 % to 13.18% on dry weight, with the predominant presence of oleic acid (C18:1)-11.3% and palmitoleic acid (C16:1)-0.26 % on dry weight (Fig.3).

PUFAc refers to the linoleic acid (LA) (C18:2)60.82 % on dry weight, and lower proportion of alpha-linolenic acid (ALA) (C18:3)on 0.64 % dry weight (Fig.4). Among the polyunsaturated fatty acids present in the oil in the largest amount represented linoleic acid 60.82 % dry weight.

From the fatty acid composition, it can be observed that the tobacco seed would be classified as a linoleic oil. γ-Linoleic acid contains ranges from non-detectable to 0.25 (% dry wt).

The data for linoleic are comparable with the data obtained for tobacco seed oils reported by Srbinoska et al.(2003a), and Stanisavljević et al. (2007).

Fatty acid profile of seed oil obtained from Oriental tobacco is presented in Figure 5.

The unsaturated fatty acids are classified into series known as omega, being ω-9 considered nonessential for humans, and the ω-3 and ω−6 as essential fatty acids, because the latter ones cannot be synthesized by mammals; therefore, they are obtained from diet. *Omega-3 (ω3) and omega-6 (ω6) fatty acids are* two different types of PUFAs. Both of

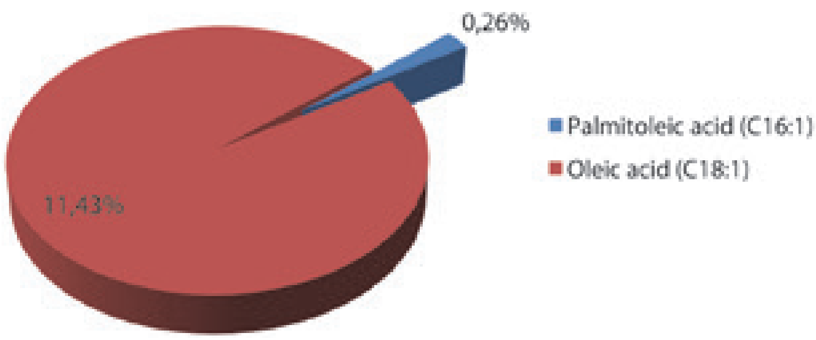


Fig. 3. Content of monounsaturated fatty acids (MUFAs) (% dry wt), crop2015

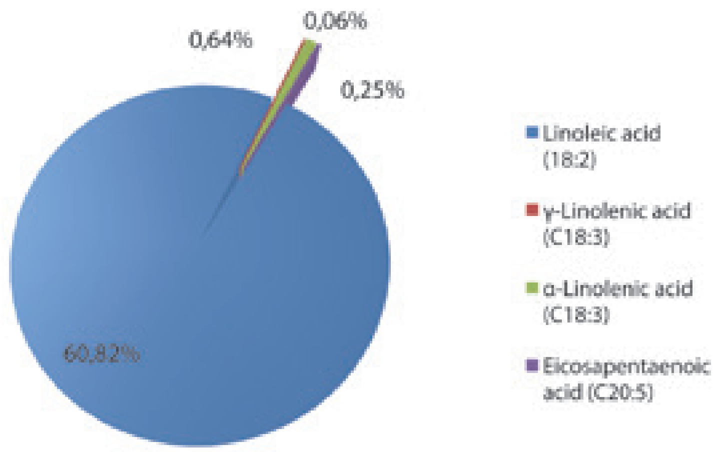


Fig. 4. Content of polyunsaturated fatty acids (PUFAs) (% dry wt), crop2015

themare essential fatty acids required for maintaining healthy skin, regulating cholesterol metabolism, and as a precursor of prostaglandins, hormone-like substances that regulate many body processes.*It was found that if the body synthesizes ω6 absorbs ω6 fatty acids from food. On their ratio have conflicting opinions. Widespread opinion about daily dose is that the ratio of ω3: ω6 be from 1: 1 to 1: 4. Different studies recommend different daily doses, including 2: 3. In the case studied tobacco seed ratio*

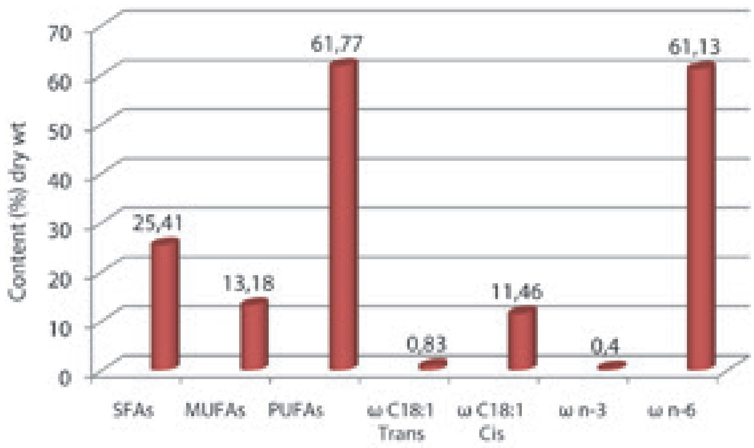


Fig. 5. The fatty acid profile of seed oil from Oriental tobacco

simultaneously takes place and synthesis of omega-3 fatty acids. Otherwise, the body is highly unbalanced in favor of ω6 fatty acids.

CONCLUSIONS

The result showed that the oriental tobacco seed oil contain the highest percentage of long chain mono and polyunsaturated fatty acids: oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3). *Ratio to monounsaturated fatty acids (MUFAs) to polyunsaturated fatty acids (PUFAs) governs the good stability of oil.*

General nutrition quality of oriental tobacco seed oil free from nicotine is close to the safflower oil. The high percentage of unsaturated acids, mainly oleic and linoleic, which has been found comparable to grape seed oil, also suggests potential food use of tobacco seed oil. The quality of the oil would be better if the linoleic acid content is lower.

The chemical characterisation of oriental tobacco seeds has been found important to look at alternative products of the crop i.e. oil and meal and find some uses of these products. The study is also useful for preserving seed purity. Also, the obtained information will make a good contribution to determination of the genotypic and phenotypic variations of tobaccoform different growing regions.

ITERATURE

1. Ashraf-Khorassani M., Coleman W., Dube M., Isaac G., Taylor L., 2015. Synthesis, Purification, and Quantification of Fatty AcidEthyl Esters After trans-Esterification of Large Batches of Tobacco Seed Oil. Beiträge zur Tabakforschung International/ Contributions to Tobacco Research, 26(5):205-213, DOI: 10.1515/cttr-2015-0008
2. From the First to the Last Ash: The History, Economics & Hazards of Tobacco http://healthliteracy.worlded.org/docs/tobacco (10 September, 2016)
3. Health medium - Framar http://media.framar.bg (10 September, 2016)
4. Hutchens T.W (1999). Tobacco Seed in Tobacco Production, Chemistry and Technology, Davis D. L and Nielsen M. T.(edt) Blackwell Science. 66-69
5. Kirkova S., Katsarova S., Zlatanov M., 2015. Preliminary studies on the consumable properties of tobacco seeds, Journal of Global Biosciences, vol.4, 7, 2888-2892
6. Kirkova S., Srbinoska M., Ivanova S., Georgieva A., 2016. Investigation on fatty acid compositions of seeds by type tobacco Virginia, Ecology and Health 2016
7. Popov M., 1940. A comprehensive use of tobacco. Tobacco review, 29-30
8. Rossi L., Selmini G., Cheli F., Fusi E., Fogher C., 2007. Possibile Impiego di Panello di Seme di Tobacco Nell Abimentazione del Suinetto, Large Animal Review, vol.13, 211-215
9. Srbinoska M., Filiposki K, Rafajlovska V.,2003. Fatty Acid Composition of Tobacco Seed Oil and its Potential as Source of Linoleic Acid, 164-167 http://semenarska.rs/UNS-PSU/radovi/2/32%20Fatty%20Acid%20164-167.pdf
10. Srbinoska M., Gercar N., Simonovska B., Najdenova V., Rafajlovska V., 2003. Fatty acids content in oil of the seed of tobacco variety P-23. Tutun/Tobacco. Vol. 53. No.7-8, 225-233
11. Stanisavljević I., Lakičević S., Veličković D., Veljković V., 2007. The extraction of oil from tobacco (Nicotiana tabacum L.) seeds. Chemical Industry& Chemical Engineering Quaterly.13 (1) 41-50.
12. Zdremtan, M., Zdremtan D., 2006.The Tobacco Oil A and Its Qualities. Buletin USAMV-CN, 63:1-4, ISSN 1454-2382
13. Zlatanov M., Angelova M., Antova G., 2007. Lipid Composition of Tobacco Seeds. Journal of Agricultural Science.13:539-544

IN MEMORIAM

Prof. Dr Vera Dimeska

Farewell to a great person.

On 7.12.2015 we lost our dear and respected colleague, Prof. Dr. Vera Dimeska. She was an outstanding expert, scientist and professor to many generations of students from St. Kliment Ohridski Univeristy Bitola - Scientific Tobacco Institute-Prilep.
The news of her death shook all the employees of Tobacco Institute-Prilep. We knew that she fought against a serious disease, but we hoped that she would win yet another victory in her life.
She was a brave and successful woman, fighting with many challenges. Her perseverance, fairness and kindness touched the hearts of many people.
Vera Milan Dimeska was born on 18.11.1943 in Prilep, where she completed the elementary school and high school education. She graduated from Ss. Cyril and Methodius University in Skopje, Faculty of Natural Science and Mathematics – Department of Biology, in 1968. Throughout her education, she was a distinguished student, with high average grades.
She was employed in Tobacco Institute-Prilep in 1969, in the Department of tobacco protection from diseases, pests and weeds, where she worked until her retirement in 2010.
In 1981 she obtained her M. Sci. degree and in 1990 became a doctor of biological sciences from Ss. Cyril and Methodius University in Skopje, Faculty of Natural Science and Mathematics.
She gradually worked her way up from *research associate i*n 1981, senior research associate in 1992 to scientific advisor in 1997. After the years of devoted work in scientific and educational processes, in 1998 Dimeska was appointed full professor at the Postgraduate course in Tobacco Institute-Prilep.
She was a mentor of one master thesis and participated in the preparation of many master and doctoral thesis defended in the Institute.
Prof. Dimeska performed various functions at the Scientific Tobacco Institute-Prilep: head of the Department for tobacco protection from diseases, pests and weeds, member and vice president of the Council of Tobacco Institute, president of the Scientific Council, member of numerous commissions, etc.
She was also a member of the Senate and of the Commission for Education and Science at St. Kliment Ohridski University in Bitola.
Prof.Dr. Dimeska published over 70 papers in national and international journals. As author or co-author of research papers, she participated at many scientific symposia, conferences, congresses and meetings in Macedonia and abroad.
She presented her research work at conferences and congresses of the international tobacco organization CORESTA in New Orleans, Bucharest, Kyoto, Santa Cruz do Sul, Paris, Krakow etc.
She took active part in realization of national and international projects in the field of tobacco.
During her work she received several awards, certificates and plaques.
With her scientific-research activity on weeds, Vera Dimeska gave a great contribution to solving some important problems and with her commitment she contributed to the development of tobacco science. She became a well-known and recognized researcher.
Dr. Vera Dimeska was a good colleague and good friend to all of us, open and honest person. She always had a positive attitude toward solving the working assignments. She remained optimistic until the last moments of her life.
Staff of the Scientific Tobacco Institute Prilep expresses its deepest condolences to her family and gratitude for her contribution to the development of scientific thought and the prosperity of tobacco science. Her death is a great loss to tobacco science and profession in the Republic of Macedonia.
Prof dr Vera Dimeska will always be remembered and will forever remain in our hearts, thoughts and memories.