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STUDY ON INHERITANCE OF THE NUMBER OF LEAVES PER STALK AND DIMENSIONS OF THE LEAVES FROM THE MIDDLE BELT IN TOBACCO VARIETIES FROM DIFERENT TYPES AND THEIR F1 HYBRIDS

Ana Korubin – Alesoska[1], Sime Dojcinov[2]

[1]St. Kliment Ohridski University – Bitola, Scientific Tobacco Institute - Prilep, of Republic of North Macedonia,
[2]Alliance One Macedonia – Kavadarci Republic of North Macedonia
e-mail: anakorubin@yahoo.com

ABSTRACT

Investigations were made with four parent tobacco varieties of different types (Prilep P-23, Prilep P 8-9/80, Floria FL-7 and Samsun S-1) - in the role of mother and MV-1 variety - in the role of father, and their four F1 hybrids for mode of inheritance for morphological properties of the leaves. The crossings were made in 2017, and the experiment with the parent genotypes and their hybrids was set up in 2018, on field trial in Scientific Tobacco Institute – Prilep in a randomized block design with four replications. All appropriate cultural practices were applied during the growing season.
The aim of this work was to study the mode of inheritance of the number of leaves per stalk and the length, width and area of the leaves in the middle belt, in the offspring of the first generation.
The results of the studies indicate the fact that the obtained hybrids did not show a heterotic effect. Intermediate and partial dominance in inheritance is an indicator of good successive selection of individuals in future generations and rapid fixation and stabilization of the studied traits. All crosses (P-23 x MV-1, P 8-9/80 x MV-1, FL-7 x MV-1 and S-1 x MV-1), are rich and interesting selection material.

Key words: tobacco, hybrids, inheritance, morphological traits.

СТУДИЈА ЗА НАЧИНОТ НА НАСЛЕДУВАЊЕ НА БРОЈОТ НА ЛИСТОВИТЕ ПО СТРАК И ДИМЕНЗИИТЕ НА ЛИСТОВИТЕ ОД СРЕДНИОТ ПОЈАС КАЈ ТУТУНСКИ СОРТИ ОД РАЗЛИЧНИ ТИПОВИ И НИВНИТЕ F1 ХИБРИДИ

Трудот опфаќа истражувања на четири мајчински родителски тутунски сорти од различни типови (П-23, П 8-9/80, FL-7 и S-1) и сортата МВ-1 во улога на татко, и нивните четири F1 хибриди, за начинот на наследување на морфолошките својстава на листовите. Вкрстувањата беа направени во 2017 год., а полскиот опит со родителските генотипови и нивните крстоски беше поставен во 2018 год. во Научниот институт за тутун - Прилеп, по рондомизиран блок-систем во четири повторувања. За време на вегетацијата беа применети сите вообичаени агротехнички мерки.
Предмет на овој труд беше да се проучи начинот на наследување на бројот на листовите по страк и должината, ширината и површината на листовите од средниот појас, кај потомството на првата генерација.
Резултатите од проучувањата укажуваат на фактот дека добиените хибриди не покажаа хетеротичен ефект. Интермедијарноста и парцијалната доминантност во наследувањето се показател за добар сукцесивен избор на индивидуи во понатамошните генерации и брзо фиксирање и стабилизирање на проучуваните својства. Сите крстоски (П-23 x МВ-1, П 8-9/80 x МВ-1, FL-7 x МВ-1 и S-1 x МВ-1) претставуваат богат и интересен селекционен материјал.

Клучни зборови: тутун, хибриди, наследност, морфолошки особини.

INTRODUCTION

Tobacco production occupies one of the most important places in the economy of the Republic of North Macedonia. The most part of the tobacco raw material is intended for foreign markets, which shows the importance of this agricultural crop for the state. Our region is very suitable for the production of small-leaf oriental aromatic tobacco which is an integral part in the production of the highest quality cigarette brands.

Having in mind the above, of great importance are the investigations from genetics and tobacco selection. Using the methods of these sciences, the breeders try to create more productive and quality varieties, better in many traits than existing ones.

By introducing new and better varieties in production of tobacco, the economic effect of this crop will increase, which will result in improving the standard of producers, as well as in increasing of the inflow of funds in the country.

The aim of this paper is to study the variability and the mode of inheritance of number of leaves per stalk and dimensions of the leaves from the middle belt of the stalk (traits - directly related to tobacco yield), in diferent types of tobacco varieties, to detect a possible heterotic effect in F1 offspring and to provide basic selection material for further successive breeding activity.

MATERIAL AND METHODS

Five varieties as parental genotypes from the large assortment of Scientific Tobacco Institute – Prilep we chose: Prilep P-23, Prilep P 8-9/80, Floria FL-7, Samsun S-1 and Virginia MV-1. We used the large-leaf flue-cured variety MV-1 as a father, so with its pollen in 2017 we made four F1 hybrids: P-23 x MV-1, P 8-9/80 x MV-1, FL-7 x MV-1 and S-1 x MV-1. They were tested the parent varieties and F1 hybrids by random block-system in four repetitions, in the experimental field at STI–P, in 2018, on a working area of about 291.6 m2 , or on the total area of 655.2 m2 (work area and paths). Large-leaf variety and F1 hybrids were planted at a planting distance of 90 cm (between rows) x 50 cm (between plants in a row), while the oriental varieties and the breeding line with a planting distance of 45 cm (between rows) x 15 cm (between plants in the row). The number of leaves per stalk and the dimensions of the leaves from the middle belt of the stalk (length, width and area), were determined in the full stage of development of the plant, at the beginning of flowering. Mode of inheritance of the components was determined on the basis of test-significance of F1 generation in relation to the average of both parents, according to Borojevic (1981). Intermediate mode of inheritance (i) occurs when the mean value of one trait in the hybrid is equal to the parental average. Partial-dominant mode (pd), has when the mean value of hybrid offspring is approaching to one of the parent varieties. Dominance in inheritance (d), positive or negative, occurs when the mean value of the hybrid coincides with the mean value of one of the parents (+d – when a parent with a higher mean value dominates, -d – when a parent with a lower mean value dominates). Positive heterosis (+h) has a hybrid with a significantly higher value than that of the parent with a higher mean, while negative heterosis (-h) occurs in a hybrid with a significantly lower value than that of a parent with a lower mean value Five varieties as parental genotypes from the large assortment of Scientific Tobacco Institute – Prilep we chose: Prilep P-23, Prilep P 8-9/80, Floria FL-7, Samsun S-1 and Virginia MV-1. We used the large-leaf flue-cured variety MV-1 as a father, so with its pollen in 2017 we made four F1 hybrids: P-23 x MV-1, P 8-9/80 x MV-1, FL-7 x MV-1 and S-1 x MV-1. They were tested the parent varieties and F1 hybrids by random block-system in four repetitions, in the experimental field at STI–P, in 2018, on a working area of about 291.6 m2 , or on the total area of 655.2 m2 (work area and paths). Large-leaf variety and F1 hybrids were planted at a planting distance of 90 cm (between rows) x 50 cm (between plants in a row), while the oriental varieties and the breeding line with a planting distance of 45 cm (between rows) x 15 cm (between plants in the row). The number of leaves per stalk and the dimensions of the leaves from the middle belt of the stalk (length, width and area), were determined in the full stage of development of the plant, at the beginning of flowering. Mode of inheritance of the components was determined on the basis of test-significance of F1 generation in relation to the average of both parents, according to Borojevic (1981). Intermediate mode of inheritance (i) occurs when the mean value of one trait in the hybrid is equal to the parental average. Partial-dominant mode (pd), has when the mean value of hybrid offspring is approaching to one of the parent varieties. Dominance in inheritance (d), positive or negative, occurs when the mean value of the hybrid coincides with the mean value of one of the parents (+d – when a parent with a higher mean value dominates, -d – when a parent with a lower mean value dominates). Positive heterosis (+h) has a hybrid with a significantly higher value than that of the parent with a higher mean, while negative heterosis (-h) occurs in a hybrid with a significantly lower value than that of a parent with a lower mean value.

Parental genotypes:

Prilep P-23 – Authors of the variety are Kosta Nikoloski and Milan Mitreski. The variety belongs to the oriental tobacco type Prilep. It has a strong pleasant aroma. It is characterized by fir-habitus, stem 65 cm high, 45- 50 seated leaves with 20 cm length and 10.5 cm width (Photo 2), and semi-spherical compacted inflorescence with pink flowers (Korubin – Aleksoska, 2004). Belongs to the group of sun-cured tobaccos. The dried leaves have a golden yellow to light orange color, a delicate, elastic and compact leaf plate and a pronounced aroma. The dry leaf mass yield is 2000-2500 kg/ha.

Prilep P 8-9/80 – Creation by Ana Korubin – Aleksoska. The variety belongs to the group of oriental tobacco of the type Prilep. It is characterized by a cylindrical to elongated elliptical habitus, the height of the stalk with the inflorescence is 85 - 90 cm, there are about 45 sitting leaves light green with curly edges. The length of the largest leaf is 20 – 23 cm, and the width 11 - 12.5 cm (Photo 2). The inflorescence is spherical with bright pink flowers. It belongs to the group of sun-cured tobaccos. The dried leaves are golden yellow to orange, with a moderately pronounced mainly rib and a thin weakly expressed secondary leaf veins. The yield of dry leaf mass is 2700-2900 kg/ha.

Floria FL-7 – Breeding linee, creation by Igor Bolsunov. The variety belongs to the group of semi-oriental tobaccos. It has a cylindrical elliptical habitus, the height of the stalk with the inflorescence is 120 - 125 cm, and about 30 sitting laves. The length of the largest leaf is about 30 cm and the width is 17 cm (Photo 3). The leaves have a very delicate leaf plate. The inflorescence is spherical with bright pink flowers. It belongs to the group of sun-cured tobaccos. The dried leaves are golden yellow to orange. This line has genes for resistance to blue mould (Peronospora tabacina Adam.). The yield of dry leaf mass is 2700-2900 kg/ha.

Samsun, S-1 – Belongs to the group of Oriental Turkish tobacco of the type Samsun. The height of the stalk is 80 - 120 cm, it has 28-36 heart-shaped leaves and a short handle, light green with curly edges. The length of the largest leaf is 20 – 23 cm, and the width 11 - 12.5 cm (Photo 4). The inflorescence is spherical with bright pink flowers. It belongs to the group of sun-cured tobaccos. Dried leaves have a yellow-reddish, orange or reddish-copper color, delicate, with a nice and pleasant aroma. The yield of dry mass is 700-1000 kg/ha.

Virginia MV-1 – Dimche Cavkaroski and Mile Uzunoski are the authors of the variety. Belongs to the group of large-leaf tobaccos. It has a conical habitus, tall of the stalk -195 cm, 26-29 sedentary leaves with height of 55 cm and width of 35 cm (Photo 5), and a broom inflorescence with pale pink flowers. There is in fertile and male-sterile form. It belongs to the group of flue-cured tobacco. The dried leaves had a golden yellow color, pleasant taste and aroma. The yield of dry leaf mass is 3500 kg/ha (Korubin – Aleksoska, 2004).

Climatic and soil conditions in the area of investigations

The Earth's entire flora grows, develops and multiplies in favorable climatic and soil conditions and each change affects to the phenotype of the population. So it is with the tobacco plant, environmental factors make changes in a number of quantitative and qualitative traits, but those changes are differently limited, depending on the type of trait and the degree of its heredity. Therefore, in scientific research in terms of selection and genetics, it is necessary to take into account the environmental conditions in which the studies were performed. This is of particular importance in the studies of tha morphological traits. During the tobacco vegetation in 2018, from May to September, the average monthly air temperature was 20,140 °C, average monthly maximum air temperature 25,820 °C, and the average monthly minimum air temperature 13,40 °C. The average monthly relative humidity was 78.8 %. A total of 180.7 mm of precipitation fell during this five-month period. Our research was conducted in the experimental field in the Scientific Tobacco Institute in Prilep on a deluvial (colluvial) soil type, without carbonates, characterized by low humus and total nitrogen content, moderately acidic to neutral reaction, low to extremely low security with easily accessible phosphorus and medium to good potassium supply.

Photo 1. Prilep P-23

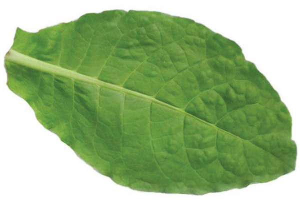

Photo 2. Prilep P 8-9/80

Photo 3. Floria FL-1

Photo 4. Samsun, S-1

Photo 5. Virginia MV-1

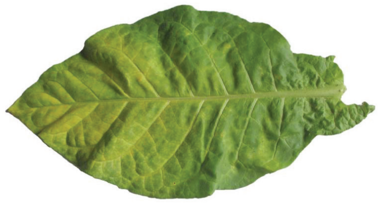

Photo 6. P-23 x MV-1

Photo 7. P 8-9/80 x MV-1

Photo 8. FL-7 x MV-1

Photo 9. S-1 x MV-1

Number of leaves per stalk

The number of leaves in tobacco is an important quantitative trait, due to the direct connection with the increase in yield, which causes great interest among breeders and is always present in programs for creating new varieties and breeding existing ones.

With the smallest number of leaves among the parents is characterized FL-7 (21.6), and with the biggest P 8-9/80 (53.9), while in hybrids the least leaves have P-23 x MV-1 (32), and the most P 8-9/80 x MV-1 (42.2). The standard deviation ranges from 0.9 (MV-1) to 2.5 (P 8-9/80). The coefficient of variability ranges from 3% (S-1 x MB-1) to 6.2% (FL-7). The coefficient of variability of the variants has a value less than 10, which is an indicator of uniformity and stability of the investigated variants.

The mode of inheritance of this trait is intermediate, partially dominant and dominant. There is no heterosis.

Partial dominance in inheritance of leaf number per stalk and absence of heterosis found:

Korubin – Aleksoska (2000), in the crosses of three oriental varieties, Korubin – Aleksoska (2010), in a diallel of three oriental and one semi-oriental variety, Gixhari & Sulovari (2010), in a semi-diallel of eight oriental genotypes. Different way of inheritance and a weak heterotic effect received Aleksoski (2010), in a one-way diallel of three oriental and one Burley variety.

Heterosis with a positive heterotic effect on the trait found: Imtiaz et al. (1997), in a complete diallel of seven Virginia flue-cured genotypes, Butorac et al. (1999), in F_1 offspring of four Burley varieties, Lalitha et al. (2006), in crosses on six lines and six testers, Dimanov & Dyulgerski (2012), at ten crosses of local and introduced Burley varieties (high heterotic effect is detected), Aleksoski et al. (2013), in hybrids of four parent genotypes of tobacco of different types (the heterosis had a weak heterotic effect), Ahmed & Mohammad (2014) in 42 diallel two-way F_1 hybrids of seven flue-cured varieties, Ramachandra et al. (2015), in hybrids obtained from six lines of different types of tobacco and eight testers.

Table 1 shows the mean values, standard deviation, and coefficient of variability for the number of leaves per stalk in parents and F_1 hybrids.

Table 1. Variability and mode of inheritance of number of the leaves per stalk in parents and F_1 hybrids

S.No	Parents and F_1 hybrids		Repetitions				$\bar{x}$ (cm)	σ (±)	CV (%)
			I	II	III	IV			
1.	P-23	P1 (♀)	46.6	45.8	46.3	45.4	46.0	2.4	5.2
2.	P 8-9/80	P1 (♀)	53.6	55	53.6	53.6	53.9	2.5	4.5
3.	FL-7	P1 (♀)	22.2	21.4	21.3	21.6	21.6	1.3	6.2
4.	S-1	P1 (♀)	42.9	41.5	42.6	40.7	41.9	1.3	3.2
5.	MV-1	P2 (♂)	28.1	28.4	28.4	28.3	28.3	0.9	3.5
6.	P-23 x MV-1	F_1	31.9	32.2	32.2	31.8	32.0 ^{pd}	1.2	4
7.	P 8-9/80 x MV-1	F_1	41.9	42.7	42.4	42	42.2 ^{i}	1.5	3.7
8.	FL-7 x MV-1	F_1	39.3	39.3	39.3	38.6	39.1^{+d}	1.4	3.5
9.	S-1 x MV-1	F_1	35.9	36.2	36.3	35.4	35.9 ^{i}	1.1	3
			LSD $_{0.05}$ = 0.68 $_{0.01}$ = 0.92						

Length of leaves from the middle belt of the stalk

The form of the leaves is varietal characteristic, but the dimensions are very variable depending on the environmental factors and the applied agrotechnics.

Among the parent genotypes, the variety P 8-9/80 is with the shortest length of the leaves from the middle belt of the plant (22.7 cm) and with the largest MV-1 (49.7 cm). While, in hybrids with the shortest leaf length is characterized P 8-9/80 x MV-1 (41.6 cm), and with the largest FL-7 x MV-1 (44.3 cm). The standard deviation ranges from 1 (P-23 x MV-1) to 2.5 (S-1 x MV-1) and 2.6 (MV-1). The coefficient of variability ranges from 2.5% (P-23 x MV-1) to 9% (P 8-9/80). The coefficient of variability in hybrids is lower than that in parental genotypes. For all variants the CV-value is less than 10, which means that the tested variants are stable and uniform.

The mode of inheritance of this trait is partially dominant. There is no heterosis.

The partial-dominant way of inheriting of the trait is most common in the studies of: Lee & Chang (1984), in crosses of Korean and Oriental varieties, Gudoy et al. (1987), in hybrids of Burley varieties, Gixhari & Sulovari (2010), in a diallel of eight oriental genotypes, Dyulgerski & Radoukova (2015), in seven Burley-type crosses and seven Virginia-type crosses, and so on.

Heterotic effect in inheriting of the length of the leaves obtained: Lalitha et al. (2006), in 36 F_1s hybrids obtained by crossing of six lines and six testers (the resulting heterosis had a moderate heterotic effect in both directions), Dyulgerski & Dimanov (2012), in ten crosses of local and introduced burley varieties. (for length of 7-8 leaf and 13-14 leaf), Dyulgerski & Dimanov (2013), in six hybrids of flue-cured parental pairs (for length of the leaves from middle belt of the stalk), Dyulgerski & Radoukova (2015), in seven Burley-type crosses and seven Virginia-type crosses, of parental genotypes of local and introduced origin (heterosis had no economic significance), Ramachandra et al. (2015), in crosses on six lines from different types of tobacco and eight testers (hybrids had the longest leaf length: MS PL-5 x Vairam and MS GT-4 x Thangam).

Table 2 shows the mean values, standard deviation and coefficient of variability for the leaf length from the middle belt of the plant in parents and F_1 hybrids.

Table 2. Variability and mode of inheritance of length of the leaves from the middle belt of the stalk in parents and F1 hybrids

S.No	Parents and F_1 hybrids		Repetitions				$\bar{x}$ (cm)	σ (±)	CV (%)
			I	II	III	IV			
1.	P-23	P1 (♀)	24.6	24.2	25.2	24.8	24.7	1.7	7.2
2.	P 8-9/80	P1 (♀)	25	20	22.8	23	22.7	2.0	9.0
3.	FL-7	P1 (♀)	33	33.6	34.1	34.3	33.7	2.0	6.2
4.	S-1	P1 (♀)	26	23.5	26.1	24.5	25.0	1.4	5.0
5.	MV-1	P2 (♂)	50	50.6	49	49.1	49.7	2.6	5.2
6.	P-23 x MV-1	F_1	43	44.1	44.1	41.3	43.1 pd	1.0	2.5
7.	P 8-9/80 x MV-1	F_1	40	41.2	41.4	43.8	41.6 pd	1.4	3.5
8.	FL-7 x MV-1	F_1	43	43.7	44	46.7	44.3 pd	2.1	4.7
9.	S-1 x MV-1	F_1	44	40.5	45.3	46.2	44.0 pd	2.5	5.7

$LSD_{0.05} = 2.11$
$LSD_{0.01} = 2.87$

Width of the leaves from the middle belt of the stalk

The width of the leaves, as well as the length, is a varietal trait on which environmental factors have a great influence.

The variety P 8-9/80 is characterized by the smallest width of the leaves from the middle belt of the plant among the parent genotypes (10,8 cm), and with the largest MV-1 (32.2 cm), while in F_1 hybrids with the smallest leaf width stood out P 8-9/80 x MV-1 (24 cm), and with the largest S-1 x MV-1 (30.1 cm). The standard deviation ranges from 0.6 (P-23 x MV-1), to 2.5 (P 8-9/80). The coefficient of variability ranges from 3% (P-23 x MV-1) to 13.5% (P 8-9/80). The coefficient of variability in hybrids is lower than that in parental genotypes. In all variants CV - value is less than 10 (except in P 8-9/80 where the value is slightly higher than 10), which signals the uniformity of the trait in all investigated variants.

The mode of inheritance of a trait covers all modalities and no heterotic effect is observed.

Partial-dominance and intermediation as the most common way in inheritance of the traits and absence of heterosis found in their research: Fan & Aucock (1974), in crosses of Meryland varieties, Espino & Gil (1980), in diallel in eight varieties of light tobacco, Lee & Chang (1984), in crosses of Korean and Oriental varieties, Legg (1991), in crosses of Burley varieties, Imtiaz & Fida & Ayaz (2012), in a diallel of five flue-cured varieties, Dyulgerski & Dimanov (2013), in six hybrids of flue-cured parent pairs, Dyulgerski & Radoukova (2015), in seven crosses of the Burley type and seven crosses of the Virginia type, creation of parental genotypes of local and introduced origin, etc.

Heterotic effect in inheriting in width of the leaves obtained: Lalitha et al. (2006), in hybrids of six lines and six testers (heterosis had a low to moderate heterotic effect in negative and positive directions). Dyulgerski & Dimanov (2012), in ten crosses of Burley varieties (the best heterotic effect is met by the width of the seventh and eighth leaf, due to which heterosis gains economic importance). Dyulgerski & Dimanov (2013), in six hybrids of flue-cured varieties (for leaf width in the middle belt), Dyulgerski & Radoukova (2015), in seven Burley-type crosses and seven Virginia-type crosses, of varieties of local and introduced origin (heterosis had no economic significance), Ramachandra et al. (2015), in hybrids of six lines of different types of tobacco and eight testers.

Table 3 shows the mean values, the standard deviation, and the coefficient of variability for the leaf width of the middle belt of the plant in parents and F_1 hybrids.

Table 3. Variability and mode of inheritance of width of the leaves from the middle belt of the stalk in parents and F_1 hybrids

S.No.	Parents and F_1 hybrids		Repetitions				$\bar{x}$ (cm)	σ (±)	CV (%)
			I	II	III	IV			
1.	P-23	P1 (♀)	11.4	11.2	12.6	12.8	12	1.9	7.7
2.	P 8-9/80	P1 (♀)	11.2	9.29	11.3	11.2	10.8	1.4	13.5
3.	FL-7	P1 (♀)	20.5	20.7	21.6	22.3	21.3	1.9	9.2
4.	S-1	P1 (♀)	13.4	12.4	14.9	14.9	13.9	0.9	7.0
5.	MV-1	P2 (♂)	31.4	33	32.2	32.3	32.2	1.7	5.5
6.	P-23 x MV-1	F_1	25.8	26.0	25.8	24.6	25.6 ^{pd}	0.7	3.0
7.	P 8-9/80 x MV-1	F_1	24.5	24.5	23.6	23.6	24.0 ^{i}	1.2	4.5
8.	FL-7 x MV-1	F_1	24.6	24.7	24.4	24.5	24.6 ^{pd}	1.0	4.2
9.	S-1 x MV-1	F_1	29.6	29.8	30	30.9	30.1 $^{+d}$	1.1	5.0

$LSD_{0.05} = 1.09$
$LSD_{0.01} = 1.48$

Leaf area of the middle belt of the stalk

The smallest area of the leaves from the middle belt of the stalk in the parental genotypes has the variety P 8-9/80 (156 cm^2), and the biggest MV-1 (1017.2 cm^2), while in F_1 hybrids with the smallest leaf area is characterized P 8-9/80 x MV-1 (635.2 cm^2), and with the biggest S-1 x MV-1 (841.26 cm^2). The standard deviation and the coefficient of variability are not calculated for this trait, because the values are obtained by applying the formula for area, where the mean values of the length and width of the leaves by repetitions are entered, which means that there are no variants needed for these parameters.

The most common way of inheritance in the trait is the intermediate (only in S-1 x MV-1 partial dominance prevails). There is no heterotic effect.

The area of the leaves has been studied by many authors, because the value of this trait correlates with the yield. The most common way of inheritance is the partially dominant and intermediate. Similar results were obtained by: Aleksoski (2010), in a one-way diallel of four parental genotypes of Oriental and Burley origin, Aleksoski et.al. (2013), in a diallel of four parent genotypes of tobacco of different types, Aleksoski (2018), in a diallel of four oriental varieties, Gixhari & Sulovari (2010), in a one-way diallel of eight oriental genotypes, Shah et al. (2017), in 10 hybrids of flue-cured parent varieties, etc.

Positive heterosis in inheriting of leaf area received: Korubin – Aleksoska (2000), in diallel of three oriental and one semi-oriental variety (a positive heterotic effect appeared in two crosses where one parent is the introduced variety Pobeda-2), Lalitha et al. (2006), in hybrids of six line and six testers (the resulting heterotic effect was low to moderate in both directions), Aleksoski (2010), in a one-way diallel of four parental genotypes - three oriental and one Burley (the weak heterotic effect had no economic justification), Gixhari & Sulovari (2010), in a diallel of eight parent oriental genotypes, Aleksoski et.al. (2013), in six diallel crosses of four parent tobacco genotypes of different types, Imtiaz et al. (2014), in 42 bidirectional diallel crosses of seven flue-cured genotypes (a highly significant heterotic effect on the trait has been observed in NC606 x KHG21, due to which there is a possibility to use the hybrid vigor), Ahmed & Mohammad (2014), in 42 two-way F_1 hybrids of seven flue-cured varieties, Shah et al. (2017), in 10 flue-cured exotic hybrids (the studied hybrids showed a significant heterotic effect), Aleksoski (2018), in hybrids of four oriental varieties.

Table 4. Inheritance of the leaf area from the middle belt of the stalk in F_1 hybrids (cm^2)

S.No.	Parents and F_1 hybrids		Repetitions				$\bar{x}$ (cm)
			I	II	III	IV	
1.	P-23	P1 (♀)	177.5	172.3	201.7	201.7	188.3
2.	P 8-9/80	P1 (♀)	178.7	118.0	163.7	163.6	156.0
3.	FL-7	P1 (♀)	429.4	443.0	486.0	486.0	456.6
4.	S-1	P1 (♀)	221.8	185.6	247.1	231.9	221.6
5.	MV-1	P2 (♂)	997.5	1060.9	1002.5	1007.7	1017.2
6.	P-23 x MV-1	F_1	706.2	729.3	722.9	645.5	701.0 ^{i}
7.	P 8-9/80 x MV-1	F_1	622.9	640.3	620.8	656.8	635.2 ^{i}
8.	FL-7 x MV-1	F_1	673.2	687.2	682.1	726.9	692.4 ^{i}
9.	S-1 x MV-1	F_1	826.4	768.1	863.5	907.0	841.3 ^{pd}
			$LSD_{0.05} = 44.9$ $LSD_{0.01} = 61.1$				

CONCLUSIONS

From our investigations on parental genotypes and their F_1 hybrids, as well as the mode of inheritance on the number of leaves per stalk and the dimensions of the leaves from the middle belt, we have got the following conclusions:

- The varieties that are the subject of these investigations differ significantly from each other and are characterized by a high degree of stability and uniformity, as a result of their homozygosity.
- Inheritance of the number of leaves per plant is intermediate, partially dominant and dominant. In the hybrid FL-7 x MV-1 the parent with a larger number of leaves is dominant.
- In inheriting of the length of the leaves from the middle belt of the stalk is found only the partially-dominant way, in both directions of dominance.
- In inheriting of the width of the leaves from the middle belt of the plant covers all modalities (intermediate, partially-dominant and dominant). In the hybrid S-1 x MV-1 the parent with broad leaves is predominant.
- The leaf area of the middle belt of the stalk is inherited intermediate, with the exception of S-1 x MV-1 where partial-dominance occurs.
- There is no occurrence of a heterotic effect in the F_1 population in all examined morphological traits.
- With these investigations we obtained F_1 hybrid offspring, with which we provided material for further selection activity.
- The results obtained with these studies represent useful achievements in the field of genetics and tobacco selection, and they have primary importance for science and practice in the process of creating new superior varieties.

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COMPARATIVE TESTING TRIAL OF ORIENTAL TOBACCO VARIETIES FOR SUSTAINABLE TOBACCO PRODUCTION STRUCTURE IN ALBANIA

Paqsor Cano[1], Vlash Tirana[2], Belul Gixhari[3], Agim Rama[4]

[1]National Tobacco and Tobacco Agency, Tirana, Albania
[2]National Seed and Seedling Institute, Tirana, Albania
[3]Plant Genetic Resources Institute-Agricultural University of Tirana, Albania
[4]Koplik I Siperm, Malsi e Madhe, Shkoder, Albania

e-mail: paqocane@yahoo.comk

ABSTRACT

A comparative field trial of four oriental tobacco varieties (Katerini, Izmir, Prilep 669 and Prilep 1237) was carried out during 2019 to assess bio-morphological and economic quantitative characteristics. The comparison trial was conducted in Koplik of Malsi e Madhe Municipality, situated in the Northern areas of Albania. Experimental design used was randomized block design in four replications. Katerini variety was used as a control. The aim of our investigation was to identify possible new varieties of tobacco to be included in the structure of tobacco production in Albania.
ANOVA analysis and comparisons of means for all pairs using Tukey-Kramer test show the significant differences between and within tobacco varieties at the $P_{0.05}$ and $P_{0.01}$ levels of the probability. Principal Components Analysis on correlations identified the percentage of variation accounted for by three principal components was 86.91%, and the proportion of the total variance accounted for by each factor.

The field comparison trial and factorial analysis permitted the identification of the most important bio-morphological traits with potential for sustainable the future tobacco breeding programs, and found the Pr669 tobacco variety as more appropriate to be included in Albanian tobacco structure.

Key words: tobacco varieties, morphological traits, yield

КОМПАРАТИВЕН ОПИТ ЗА ИСПИТУВАЊЕ НА ОРИЕНТАЛСКИ СОРТИ ТУТУН ЗА ОДРЖЛИВО ТУТУНОПРОИЗВОДСТВО ВО АЛБАНИЈА

Во текот на 2019 година беше поставен компаративен полски опит со четири ориентални сорти тутун (Katerini, Izmir, Prilep 669 и Prilep 1237) за да се проценат био-морфолошките и економските квантитативни карактеристики. Компаративниот опит беше лоциран во Koplik, општина Malësi e Madhe, во северните области на Албанија. Опитот беше поставен по случаен блок–систем во четири повторувања. Како контрола се користеше сортата Katerini. Целта на нашето истражување беше да се идентификуваат можните нови сорти тутун што треба да бидат вклучени во структурата на производството на тутун во Албанија.

ANOVA анализата и споредбите на средните вредности од сите парови со користење на Tukey-Kramer тестот покажаа сигнификантни разлики помеѓу и во рамките на сортите тутун на нивоата на веројатност од $P_{0.05}$ и $P_{0.01}$. Анализата на главните компоненти за корелациите утврди дека процентот на варијабилноста кај трите главни компоненти е 86,91%, а делот на вкупната варијанса е резултат на секој фактор.

Полскиот компаративен опит и анализата на факторите овозможија идентификација на најважните био-морфолошки карактеристики со потенцијал за одржливост во идните програми за облагородување на тутунот, и утврдија дека сортата Pr669 може да се вклучи во албанскиот тутунопроизводен сортимент.

Клучни зборови: сорти тутун, морфолошки особини, принос.

INTRODUCTION

Tobacco conquered the world for the pleasure it gives during smoking and also for the high revenues it brings to the farmers and countries in which it is grown. Introduced in Albania early in XVIII century it has been valued for its economic and social importance. Tobacco production, in Albania, start increasing and takes a very important place after 2nd World War and especially during the centralized economy due both for economic and social reasons, reaching more than 20.200 tons in 1985 (Gixhari et al., 2003).

During the centralized economy all activities and processes related to the cultivation and production of tobacco crops were regulated by law and tobacco producers economies were allowed to use only certified seed materials. For more than a half century, tobacco varieties grown in Albania were of oriental and semi-oriental origin, mainly of the types: Samsun, Bashibagli, Basma and Semi-oriental, diversified in the four principal tobacco regions. Decentralization of economy after 1990 years put down (in collapse) Albanian Tobacco Industry and tobacco growing areas and selected structure varieties were decreased from year to year.

In the latest decades the structure of tobacco cultivation is based especially on the varieties introduced by abroad, and has abandoned the local tobacco varieties used for several decades because of their high adaptability to the local conditions. Actually the structure of tobacco production in Albania consists of oriental tobacco types of Katerini, presented with some varieties which account for about 95% of total tobacco production. This variety, cultivated for more than three decades, due to the Greek tobacco companies that manage tobacco raw material in Albania, is going to be well adapted to different climate conditions of Albania. In the recent years, new tobacco varieties as Prilep, Yaka and Djebel, have been introduced and included in the field trials with purposes to identify any variety appropriate for the tobacco structure of production.

The objective of the study was to investigate the morphological characteristics and biological properties of four oriental varieties grow in different conditions of their habitat range. The aim of our investigation was to identify possible new varieties of tobacco to be included in the structure of tobacco production in Albania.

MATERIALS AND METHODS

The field experiment was carried out during 2019 with four genotypes of the varietal group as Prilep 669 (Pr669) and Prilep 1237 (Pr237) and oriental group as Katerini (Kat) and Izmir (Izm3). A comparative study was conducted in Koplik i Siperm (Malsi e Madhe Municipality of Shkodra District) situated in the Northern areas of Albania. Experimental design used was randomized block design in four replications. Katerini variety, which has been the most cultivated variety in the other regions of Albania for three decades, was used as a control. The area of the experimental elementary plot in total was 30 m^2. Comparative trial was displayed in rotation with wheat and farming cultural practices of oriental tobacco were used. Field transplantation was realized in the end of April.

The following characters are reported: Plant height (PH), Number of leaves (LN), Leaf characters as Leaf Length (LL) and Leaf Width (LW); Stem characters as: Base stem diameter (Bst), Middle stem diameter (Mst), Apex stem diameter (Ast) and mean steam diameter (St.mean); Internode characteristics as: Base internode length (Bint), Middle internode length (Mint), Apex internode length (Aint) and mean internode length (Int.mean); Yield of each six harvesting hands (g) as: Y1Hs, Y2Hs, Y3Hs, Y4Hs, Y5Hs, Y6Hs and the total tobacco Yield for all six hands (Y1-6); Plant number at the first harvesting (PnHs1), Plant number of the latest harvesting (PnHs6) and Mean plant number for all six harvesting hands (PnHs1-6), and Length of the vegetation period from planting to budding (bud) and from planting to flowering, in days; and Economic indicator - Yield of dried tobacco per unit (Y/ha^{-1}) were analyzed.

Cultural practices: Sowing date and growing conditions as the distance between plants in a row and between rows, fertilizer application, number of plants established, plant protection, harvest date etc. were the same for each genotype and consistent with established farming practices of the area and with the variety used.

Statistical Analysis: The differences between tobacco varieties for the mean values of the bio-morphological quantitative characters observed and evaluated were carried out using ANOVA analysis, and a means comparisons analysis for all pairs using Tukey-Kramer HSD (Tukey, 1953) was carried out. Identification of the most important bio-morphological characters that influence highly on the total variation, was realized using Principal Components Analysis (PCA) on correlations.

All statistics data for bio-morphological characters were calculated employing the SAS JMP Statistical Discovery (2012).

RESULTS AND DISCUSSION

Analysis of bio-morphological quantitative characters:

ANOVA analysis and comparisons of means for all pairs using Tukey-Kramer test ($q = 2.96880$, $\alpha = 0.05$) show the presence of significant differences between and within tobacco varieties at the $P_{0.05}$ and $P_{0.01}$ levels of the probability (Table 1).

Significant variation was observed for Plant height (PH), Number of leaves (LN), Stem characters as: Base stem diameter (Bst), Middle stem diameter (Mst), Apex stem diameter (Ast) and mean steam diameter (St. mean); Internode characteristics as: Base

Table 1. Comparisons of means for all pairs using Tukey-Kramer test for the most important traits

Plant traits		Kat.	Izm3	Pr237	Pr669
Plant height	PH	91.650000 b	101.25000 a	70.275000 d	77.250000 c
Number of leafs	LN	26.925000 b	23.525000 b	36.550000 a	38.225000 a
Leaf length	LL	23.050000 a	22.050000 a	21.500000 a	22.500000 a
Leaf width	LW	13.700000 a	11.250000 a	11.100000 a	10.800000 a
Apex stem diameter (mean)	St.mean	1.2075000 a	1.0025000 b	1.0375000 b	0.9750000 b
Internode length (mean)	Int.mean	2.2100000 b	2.3750000 a	1.9775000 c	1.6775000 d
Yield of 1st harvesting hand	Y1Hs	341.00000 a	278.50000 b	361.50000 a	383.00000 a
Yield of 2nd harvesting hand	Y2Hs	532.00000 b	492.25000 b	687.00000 a	725.50000 a
Yield of 3rd harvesting hand	Y3Hs	642.00000 bc	582.25000 c	701.50000 ab	754.00000 a
Yield of 4th harvesting hand	Y4Hs	658.00000 a	634.50000 ab	584.75000 b	670.50000 a
Yield of 5th harvesting hand	Y5Hs	411.50000 a	424.75000 a	429.25000 a	439.75000 a
Yield of 6x harvesting hand	Y6Hs	177.50000 ab	181.00000 ab	138.25000 b	192.25000 a
Yield for all six hands	Y1-6	2762.0000 bc	2548.0000 c	2947.5000 ab	3165.0000 a
Yield of dried tobacco per unit	Y/ha^{-1}	276.20000 bc	254.80000 c	294.75000 ab	316.50000 a

internode length (Bint), Middle internode length (Mint), Apex internode length (Aint) and mean internode length (Int.mean), and yield harvesting hands, except the of 5th harvesting hand, and yield of dried tobacco per unit (Y/ha^{-1}). Most of these quantitative traits were significant at the probability $0.0001 < P_{0.05}$. Meanwhile the other characters (not in table 1) were not significant in means comparisons analysis for all pairs using Tukey-Kramer HSD test.

ANOVA analysis and comparisons of means using Tukey-Kramer test identified the most important quantitative traits with potential for sustainable tobacco breeding programs, and found the Pr669 tobacco as a potential variety to be considered for the enlargement of Albanian tobacco structure.

Principal Components Analysis (PCA) on correlations identified the total variance of the principal components (PC) and the proportion of the variances explained by each factor. All quantitative variables contribute to 100% of total variation.

Comparing the eigenvalues for each factor (Table 2) using the minimum eigenvalue criterion (Kaiser, 1960), only three principal components were retained for further analysis. All 25 quantitative variables contribute in the total source of variation 100% of variance. The percentage of variation accounted for by three PC was 86.91%. The percentages of total variation accounted for by each of the three principal components were 62.7%, 24.5% and 9.0% respectively (Table 2). The two first PCs explain 87.2% of the original variation, which is acceptable to identify the influence of the most important characters that influence on the total variation among four tobacco varieties included in the field comparison trial.

Table 2. Eigenvalues matrix of principal components for 4 tobacco varieties x 25 morphological traits

Principal Components/factor analysis			
PC No.	**Eigenvalue**	**Percent variance**	**Cumulative Percent**
1	8.7743	62.674	62.674
2	3.4349	24.535	87.209
3	1.7908	9.012	96.221

Relationships among the bio-morphological characters and tobacco varieties

In the present study where the first two PCs explain 87.2% of the original variation, the maximum information from morphological quantitative data was received using ordination methods (PCA and principal coordinate's analysis) (Jolliffe, 2002.).

Two-dimensional scaling for relationships among tobacco varieties and quantitative morphological traits that accounts for the larger proportion of the total variance in PC1, PC2 and PC3 revealed by PCA show that the contribution of each tobacco variety and of each quantitative morphological trait on the total of variation is not equal. (Table 3, Figure 1).

Table 3. Matrix of vectors of three PC for 4 tobacco varieties x most important agro-morphological traits

Morphological quantitative characters		ANOVA		Eigenvectors		
		F Ratio	Prob > F	PC1	PC2	PC3
Plant height	PH	145.1987	<.0001*	**-0.30621**	0.02275	**0.31306**
Number of leafs	LN	70.7605	<.0001*	**0.33509**	-0.02324	-0.08498
Leaf length	LL	0.4580	0.7166	-0.08176	**0.46958**	**0.32048**
Leaf width	LW	3.2969	0.0578	-0.17506	**0.44250**	-0.18077
Apex stem diameter (mean)	St.mean	38.1403	<.0001*	-0.13256	**0.48465**	-0.14759
Internode length (mean)	Int.mean	945.6774	<.0001*	-0.32899	-0.08183	-0.12347
Yield of 1^{st} harvesting hand	Y1Hs	17.0782	0.0001*	**0.31021**	0.20189	-0.09352
Yield of 2^{nd} harvesting hand	Y2Hs	14.7546	0.0002*	**0.33595**	-0.04269	-0.04388
Yield of 3^{rd} harvesting hand	Y3Hs	15.3044	0.0002*	**0.33318**	0.08548	0.02210
Yield of 4^{th} harvesting hand	Y4Hs	8.3116	0.0029*	0.23378	**0.38904**	0.01825
Yield of 5^{th} harvesting hand	Y5Hs	1.0014	0.4256	0.18652	**-0.30393**	**0.45911**
Yield of 6^{x} harvesting hand	Y6Hs	3.4920	0.0499*	-0.04600	0.16840	**0.70261**
Yield for all six hands	Y1-6	24.7748	<.0001*	**0.33101**	0.09777	0.05697
Yield of dried tobacco per unit	Y/ha^{-1}	24.7748	<.0001*	**0.33105**	0.09754	0.05636

In bold all eigenvectors > **0.30**

Seven quantitative traits show higher contribution on the PC1 variance that account for 62.7% of total variation. For PC1 the morphological quantitative traits as LN, Y1Hs, Y2Hs, Y3Hs, Y1-6, Y/ha^{-1} and PH (with eigenvectors > 0.30) were the quantitative traits (variables) with larger values and more significant weighting on the PC1 variance. The influence of PH character was found negative in this study. These traits can be used successfully as morphological quantitative marker traits for bio-morphological characterization of tobacco varieties and in tobacco breeding programs. A well understanding of the most important morphological characters can facilitate identification of any individual tobacco variety and selection of desirable traits (genes), increasing the information in tobacco database useful for the sustainable of tobacco structure production.

Variation in PC2 (24.5% of total variance) was mainly the result of differences between quantitative traits as LL, LW, St.mean, Y4Hs and Y5Hs (with eigenvectors > 0.30). The influence of Y5Hs character was found with negative influence on the PC2 variation.

In PC3 there were PH, LL, Y5Hs and Y6Hs characters that account for only 9% of the total of variation. LL trait accounts for PC3 the variance that was not account for in PC2 (Table 3, Figure 1).

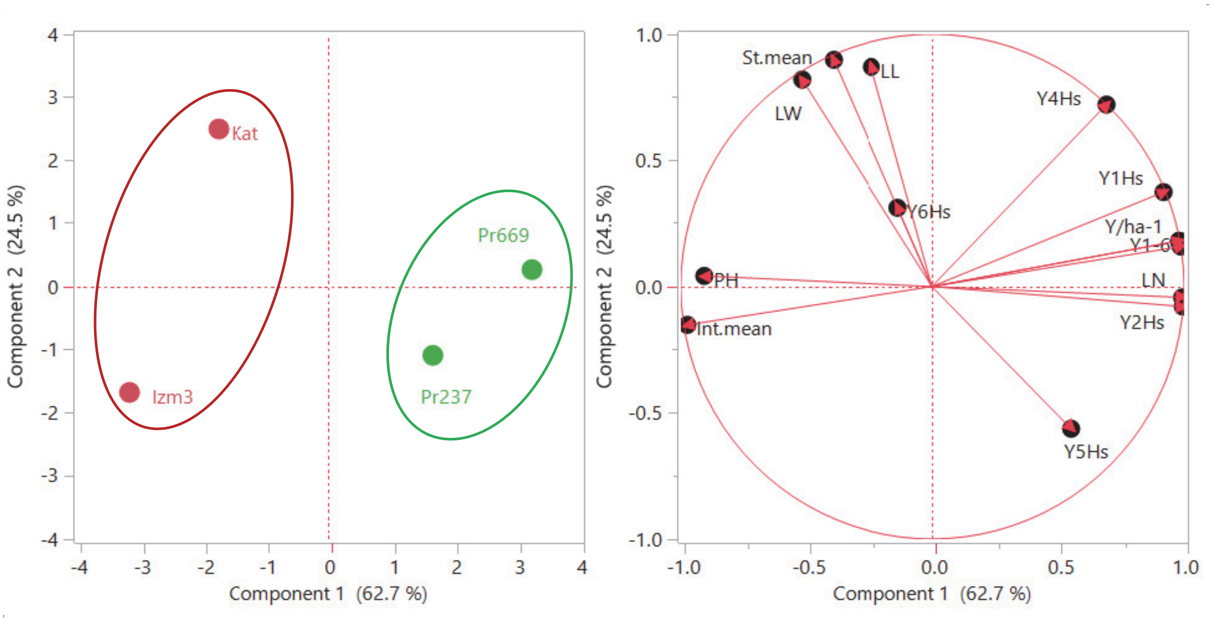

Fig 1. Two-dimensional relationships between the most important tobacco morphological quantitative traits revealed by PCo analyses.

Genetic similarity/dissimilarity evaluated by combination of quantitative morphological traits using cluster analysis (Ward) method show the presence of similarity and distances between tobacco varieties included in field comparison trial. In our study variation among Kat and Izm3 tobacco varieties with distance equal to 5.142713428 (Kat leader and Izm3 joiner) was found higher than the variation between two other tobacco varieties (Pr669 and Pr237). These two latest tobacco varieties with distance equal to 3.620152790 (Pr237 leader and Pr669 joiner) were more similar between those.

The high variation found for the group of tobacco varieties as Izm3 and Kat could be explained by the agro-climatic conditions of trial site (Northern Albania) not very suitable for this tobacco varieties of different origin. The presence of some heterogeneity between these tobacco varieties is due to not very appropriate manner or technology of seed production.

Meanwhile the high similarity found for the most morphological and yield characters, among commercial oriental varieties of Prilep tobacco (Pr237 and Pr669) (Korubin et al., 2017), could be explained be their high homogeneity and genetic stability (Korubin et al., 2014), and perhaps by the appropriate technology of seed production. These tobacco varieties were grown under about the similar agro-climatic conditions to their site of creation.

Two economic characteristics (Y1-6 and Y/ha^{-1}) of tobacco varieties Pr669 and Pr237, highly significant (<.0001*) and with most weight on the PC1 variance, explain the base of yield stability during the most harvesting hands and the higher yield production per unit at the end of the season (trial). In this comparison varietal study Pr669 variety was found more appropriate to be maintain in consideration for possible inclusion in Albanian tobacco structure.

Similar results or results with small differences for these two varieties are reported by Korubin (2004), Korubin et al., (2014; 2017), Dimitrieski et al., (2011), Pelivanoska (2009), Pesevski et al., (2011), Terill et al., (1985).

CONCLUSION

The field comparison trial and factorial analysis permitted the evaluation of variability between four tobacco varieties and identification of the most important agro-morphological traits with potential for sustainable the future tobacco breeding programs.

The traits with more significant weighting on PC1 variance (LN, Y1Hs, Y2Hs, Y3Hs, Y1-6 and Y/ha^{-1}) and on PC2 variance (LL, LW, St.mean and **Y4H**s) can be utilized successfully as morphological markers for the field assessment and comparison of the tobacco varieties, and in tobacco breeding programs.

The comparison field variety trial found the Pr669 tobacco variety as more appropriate to be included in Albanian tobacco structure

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CHEMICAL CHARACTERISTICS OF VARIETIES AND LINES OF THE BURLEY TYPE IN THE 2018 HARVEST

Karolina Kochoska[1], Marija Srbinoska[1], Romina Kabranova[2]

[1]St. Kliment Ohridski University – Bitola, Scientific Tobacco Institute - Prilep, of Republic of North Macedonia
[2]Ss. Cyril and Methodius University in Skopje: Faculty of Agricultural Sciences and Food, Republic of North Macedonia
e-mail: karolina_kocoska@yahoo.com

ABSTRACT

One of the important characteristics of the tobacco raw material is the determination of the quality and the use value of the tobacco leaves, through the chemical composition. These studies cover and analyse: the content of nicotine, proteins, total nitrogen, soluble sugars and ash.

A total of 6 Burley varieties and lines were taken for the study, which included 4 foreign recognized varieties (Enchu Ø, Banket A_1, Habana - 92, B-D-1) and two lines in CMS form created in the Scientific Tobacco Institute - Prilep (B-110/15 CMS F_1 and B-199/11 CMS F_1), which were placed in 4 iterations according to the Random block system method.

The goal of our research was to contribute by introducing the basic chemical properties of newly recognized Burley-type lines created at the Scientific Tobacco Institute – Prilep. According to the obtained data, the newly obtained lines B-210/15 CMS F_1 and B-199/11 CMS F_1, are characterized by typical chemical characteristics for the Burley tobacco type.

Key words: tobacco, chemical composition, nicotine, proteins, sugars.

ХЕМИСКИ КАРАКТЕРИСТИКИ НА СОРТИ И ЛИНИИ ОД ТИПОТ БЕРЛЕЈ ВО РЕКОЛТА 2018

Една од важните карактеристики на тутунската суровина е и одредувањето на квалитетот и употребната вредност на тутунските листови, преку хемискиот состав. Во овие испитувања се опфатени и анализирани: содржината на никотин, белковини, вкупниот азот, растворливи шеќери и пепел.

За испитување беа земени 6 берлејски сорти и линии, каде беа вклучени 4 странски признати сорти (Enchu Ø, Banket A_1, Habana – 92, Б-Д -1) и две линии во ЦМС форма создадени во НИТ – Прилеп (Б-110/15 ЦМС F_1 и Б-199/11 ЦМС F_1), кои беа поставени во 4 повторувања по методот на случаен блок систем. Целта на нашите истражувања беше да се запознаеме со основните хемиски својства од новодобиени линии од типот берлеј кои се создадени во Научниот институт за тутун – Прилеп. Според добиените податоци новодобиените лини Б-210/15 ЦМС F_1 и Б-199/11 ЦМС F_1, се одликуваат со типични хемиски карактеристики за берлејски тип тутун.

Клучни зборови: тутун, хемиски состав, никотин, белковини, шеќери.

INTRODUCTION

Tobacco belongs to the group of strategic crops in the agricultural economy in the Republic of North Macedonia. The cultivation of tobacco is conducted on an area between 12.000 and 15.000 ha with an annual production of 20 to 25 million kilograms of tobacco raw material with good quality. From the aspect of the typical representation, over 95 % from this fields belong to aromatic oriental tobacco type Prilep and Yaka, while the large ones (Virginia and Burley) almost cannot be seen in the fields. By the end of the 90 s of the last century these types were grown here, which reduced the tobacco raw material import. But they were necessary for the production of the most popular "american blend" cigarettes (Mitreski 2018). In this cigarette, (Richard, 2009) first promoted the Camel cigarette, whose composition included Virginia, Burley, and Oriental tobacco. A few years later, in 1916 the ATS (American Tobacco Company) launched the new product Lucky Strike which used toasted Burley leaves for the first time. Since then, the best-selling type of cigarettes in the world are the American blend cigarettes In American blend cigarettes, Burley raw material participates with different percentages, its representation depends on the quality group of cigarettes and the specific demands and needs of a certain market. The average presence of this raw material in technological recipes ranges between 25 and 30 %. Burley tobacco is dried indoors under shade and is an integral part of blend cigarettes, but it is also consumed as pipe tobacco and chewing tobacco. Light, spongy tissue with high ability to absorb liquids is characteristic of Burley tobacco. It has high nicotine and protein content and only trace amount of sugar, as a result of long drying. This tobacco has a sharp taste (pungent ammonia) and is used in production of American blend-cigarettes, with up to 30 % share (Stanković, 2002, Georgiev, 2002, Radojčić, 2011). The leaves are relatively tender and thin. Burley tobacco can absorb and retain liquids up to 25 % of its own weight, which is much higher percentage compared to Virginia tobacco (7 % - 8 %) (Alić-Dzemidzić et al., 1999, Stanković, 2002; Georgiev, 2002). They contain only trace amounts of carbohydrates and the sugar content in some varieties of this tobacco in the world is 2 % - 6 %. Nicotine content ranges from 1.5 % to 3 %. Burley leaves are characterized by excellent combustion, high filling capacity and good absorption capacity that improve the taste of blend cigarettes. Science has a role in the creation of attractive varieties that will meet the requirements of the tobacco producer (quality and quantity) but also by selecting new varieties that are more resistant to diseases, as well as finding simpler ways of growing and drying tobacco. The cultivation of the Burley type in Republic of North Macedonia started in the 60's of the last century, on small areas intended for experimental purposes. Since then until present day, the Department of Genetics, Selection and Seed Control together with the Departments of Agrotechnics, tobacco protection Technology and chemistry, have been working on creating new varieties of the Burley type and finding appropriate agrotechnical measures in order to obtain a raw material with satisfactory use value. In the past few years, several varieties of the Burley type have been created at the Scientific Tobacco Institute - Prilep, which according to many characteristics do not lag behind the results obtained for Burley varieties from famous production regions in the world. The development of chemistry as a scientific discipline and research methods are extremely significant in agriculture and these are factors that accurately determine the quality of consumer products, including tobacco.

The obtained results regarding the chemical properties of the tobacco raw material, would enable to get a clear picture about the raw material of our Burley tobacco varieties, i.e. what producers and fabrication could expect if the production of this type of tobacco is restarted.

MATERIAL AND METHODS

The experiments were conducted during 2018 in the Scientific Tobacco Institute - Prilep on alluvial-colluvial type of soil that was previously prepared and fertilized with 300 kg/ha in the ratio NPK 8:22:20. The experiment included 6 varieties and lines from the domestic and foreign assortment in fertile and CMS form, as follows: Enchu Ø, Banket A_1, Habana - 92, B-D-1 and two male-sterile cross-hybrid lines at the Scientific Tobacco Institute – Prilep B-110/15 CMS F_1 and B-199/11 CMS F_1, which were set in 4 iterations, according to the method of random block system. The transplanting density was 90×50 cm on 6.6.2018. During the vegetation, the tobacco was dug twice, and it was nourished once with 3-4 g/stem of 26 % CAN (calcium ammonium nitrate). The leaves were harvested manually at the time of their technological maturity in 5-6 harvests. During the growing season, several additional irrigations were made. Tobacco leaves were then stringed, matured and cured in special barns for Burley tobacco (air-curing). The qualitative assessment of cured tobacco was made according to the Rules for unique measurement of quality of Burley tobacco leaf. Dry leaves from the middle belt were taken for examination, from each variety and line which was previously subjected to fermentation. The analyses of the chemical properties of tobacco were performed in the accredited Laboratory for the quality control of tobacco and tobacco product, in the Department of tobacco chemistry, chemical smoke, pesticide residues and biochemistry, according to internationally recognized methods.

RESULTS AND DISCUSSION

The tobacco leaves have a complex chemical composition whereby the content of each component separately as well as their mutual ratio depend on the inherited properties of the plant, the position of the leaf (the insertion), the agricultural and environmental factors and the drying technology. The degustational characteristics of the raw material also largely depend on the ratio of the chemical components. These studies cover and analyse: the content of nicotine, proteins, total nitrogen, soluble sugars and ash. Uzunoski (1985), stated that the chemical composition to a certain extent is a typical and varietal characteristic and is highly variable depending on the conditions of cultivation during field tobacco vegetation, tillage and other factors. Baylov (1965) points out that the quality of tobacco depends not only on the contained of chemical components, but also on their interconnection, as well as on their relationship, changes resulting from the conditions of cultivation and the method of drying.

Nicotine content

Nicotine is created in the root system. which with the development of the tobacco plant is synthesized in the leaves, the stem and the leaf veins. The nicotine content is highest in the leaf tissue and is least present in the nervature (main rib and secondary veins) and its content grows from the lower to the upper harvests. The nicotine content is an indicator that determines the use value of the leaves. The nicotine content is greatly influenced by: the type and variety, the soil type, the applied agricultural technique (fertilization and irrigation), as well as the drying technology.

Table 1, shows data about the nicotine content of the middle belt insertions of the examined varieties, whereby these insertions in the assessment are representatives of the upper classes, which is not the case with oriental tobacco, where the upper insertions are representatives of the upper classes (upper belt).

The control variety Enchu is characterized by the lowest nicotine content (1.65 %), while the highest nicotine content is present in the line B-210/15 CMS F_1 with 2.74 %, whose relative difference compared to the control variety is higher by 66.06 %.

In the other examined varieties and lines, the nicotine content ranges from 2.09 % in the Habana variety - 92, to 2.42 % in the line B-199/11 CMS F_1.

According to Hristoski (2013), the nicotine content in the Burley tobaccos with various origins is highest in the raw material of the insertion B, originating from Sri Lanka (2.89 %) and Serbia (2.72 %) while in the tested varieties of the study, the average nicotine content is highest in the insertion T, up to 2.70 % in the Pelagonec variety.

Table 1. Nicotine content %

Variety	Average %	Differences		Rank
		Absolute	Relative	
Enchu Ø	1.65	/	100.00	6
B-D-1	2.13	+0.48	129.09	4
Banket A_1	2.14	+0.49	129.69	3
Habana - 92	2.09	+0.44	126.67	5
B-210/15 CMS F_1	2.74	+1.09	166.06	1
B-199/11 CMS F_1	2.42	+0.80	146.67	2

Zebasil (2007), in Burley tobacco types grown in Zimbabwe, points out that the nicotine content varies, depending on the degree of maturity of the leaves. During the yellowing of the leaves, the content of nicotine and sugars is higher than the content of proteins. Smokvoski et al. (1993**)** found that the nicotine content is higher (3.15 %) in whole stalk harvest compared to harvest by insertions, i.e. dried in rows under polyethylene (1.94 %). In the variant shade-drying in a row the nicotine content was 2.25 % and in the variant shade-drying of full stalk harvest, the nicotine content was 2.22 %. Pelivanoska (1999), from the three-year studies (1996/1998) of the Burley variety Chulinec in the Prilep region, concluded that the nicotine content ranges from 0.63 % (control variety + 50 % of the field water capacity) to 2.60 % (fertilized and non-irrigated). Pelivanoska and Trajkoski (2001) found that in Burley tobacco, fertiliized with higher amount of nitrogen the nicotine content is higher than in non-irrigated and non fertilized varieties, as well as fertilized variants with lower amounts of nitrogen and lower field water capacity (FWC). Pelivanoska and Trajkoski (2004), in Burley tobacco varieties grown in the Polog region, in fertilized and irrigated variants, found that the nicotine content ranged from 1.17% (variant - irrigated with 75% of the FWC) to 2.05 % (control varant). Risteski (2006), came to the conclusion that the nicotine content in all examined varieties of the Burley type, grown in the Prilep region in 1999/2001, in the variant of whole stalk harvesting and drying, expressed in relative numbers, increases from 0.44 % to 61.32 % in comparison with the control variety - Chulinec.

Total nitrogen content

The total nitrogen content negatively affects the tobacco quality by reducing the use value of the raw material, Nicotin content depends on several factors such as the tobacco type, the soil and climate conditions, the fertilization, the irrigation, the leaf location, etc. The lowest total nitrogen content is present in the lower insertions and the highest content is present in the upper insertions. In Table 2 the content of total nitrogen ranges from 4.29 % in the line B-210/15 CMS F_1 and has the lowest value, up to 4.90 % in the variety Banket A_1, with the highest content of total nitrogen. The newly obtained lines are characterized by a lower content of total nitrogen compared to the other examined varieties, B-199/11 CMS F_1 (4.37 %) and B-210/15 CMS F_1 (4.29 %) total nitrogen in insertions of the middle belt of the plant. According to the literature data, the content of total nitrogen does not deviate within large limits.

Table 2. Total nitrogen content %

Variety	Average %	Differences		Rank
		Absolute	Relative	
Enchu Ø	4.61	/	100.00	4
B-D-1	4.79	+0.18	103.90	2
Banket A_1	4.90	+0.29	106.29	1
Habana - 92	4.66	+0.05	101.08	3
B-210/15 CMS F_1	4.29	-0.32	93.06	6
B-199/11 CMS F_1	4.37	-0.24	94.79	5

Uzunoski (1985), concluded that when examining several varieties of the Burley type, the total nitrogen content ranged from 3.04 % in the variety B-21 to 3.36 % in the variety Chulinec.

Leffingwell (1976), when examining the change in the chemical composition of tobacco leaves at different stages of drying, came to the conclusion that the total nitrogen content in the Burley tobacco types ranges from 2 to 6 %.

Pelivanoska and Trajkoski (2004), in the Burley raw material grown in the Polog region (1997/1999) in different variants of fertilization and irrigation, found that the average content of total nitrogen ranges from 2.92 % (75 % available water capacity) to 3.81 % (control variety-fertilized).

Hristoski (2013) indicates that the average content of total nitrogen in all varieties in the test increased from the lower to the upper insertions. In the control variety, the average content of total nitrogen ranges from 2.69 % in the insertion X to 4.02 % in the upper insertion T. The average content of total nitrogen in the variety B-2/93 is within the range of 2.82 % (X) to 3.89 % (B) while in the Pelagonec variety it is within the range of 2.69 % in the insertion X to 3.31 % in the insertion T.

Protein content

The protein substances in the tobacco leaf are an important component in the chemical composition of the raw material. Degustational characteristtics of the tobacco leaf directly affect the protein content. From the past experience in the fabrication when determining the quality and use value of the Burley raw material, the best grades are obtained in the tobacco whose protein content is not higher than 11 % nor lower than 6 %. The protein content does not in itself determine the quality and use value of the raw material, rather this is the function of the ratio nicotine : soluble sugars : protein. The protein content in the test can be seen from the data indicated in Table 3, whereby the protein content is lowest in the control variety Enchu (8.95 %), and it is highest, but still within the indicated limits, in the variety Habana - 92 (11.00 %). The newly obtained lines are also characterized by a protein content within the limits which are a characteristic of the Burley raw material, in the line B-210/15 CMS F_1 with 9.85 % up to 10.41 % in the line B-199/11 CMS F_1.

Table 3. Protein content %

Variety	Average	Differences		Rank
		Absolute	Relative	
Enchu Ø	8.95	/	100.00	6
B-D-1	10.92	+1.97	122.01	2
Banket A_1	9.27	+0.32	103.58	5
Habana - 92	11.00	+2.05	122.91	1
B-210/15 CMS F_1	9.85	+0.90	110.06	4
B-199/11 CMS F_1	10.41	+1.46	116.31	3

The obtained results for the studied varieties are within the expectations and are correlated with the quoted literature data and the results of the Burley raw material.

Uzunoski (1985), quoting Shmuk, points out that in quality tobacco leaves of the Burley type, the protein content should not be higher than 9 %. The optimal limits that determine the quality of the raw material range from 5 to 10 %.

Boceski (1984), while studying the chemical composition of the Burley type, concluded that the protein content progressively increases from the lower to the upper insertions, i.e. from 8.54 % in the first insertion to 11.74 % in the eighth insertion.

Pelivanoska (1999), when examining the chemical composition of the Burley variety Chulinec in the Prilep region (1996/1998) in different variants of fertilization and irrigation, concluded that the protein content ranged from 6.82 % (control variety + 70 % of the FWC) to 11.44 % (fertilized and non-irrigated).

Risteski (2008), from the three-year examinations (1999/2001) in six varieties of the Burley type (Prilep region), came to the conclusion that the average protein content ranges from 7.35 % in the variant of whole stalk harvest to 9.54% in the variant picked-cured.

Risteski (2006), while growing six varieties of the Burley type in the Prilep production region in the period 1999/2001, concluded that the average protein content is within the range of 7.35 % in the variant whole stalk harvest in the variety C-104 up to 9.54 % in the variety Chulinec in the variant picked-cured.

Hristoski (2013) indicates that from the data it can be seen that the average protein content by insertions in the control variety ranges from 8.69 % in insertion X to 13.50 % in insertion T. In the variety B-2/93 in insertion X it is 9.91 % and in insertion T it is 12.84 %. In the Pelagonec variety at the insertion X, the average protein content is 8.32 % and in insertion T it is 11.18 %.

Soluble sugar content

One of the most active chemical components in the tobacco leaf that occurs in the initial processes of photosynthesis are soluble sugars. The Burley type is characterized by a low sugar content. In American blend cigarettes, the Burley raw material, due to its spongy structure, is used as a cigarette filler to increase the volume, i.e. as a flavour corrector by adding various chemicals of natural origin that aim to improve the taste of the cigarette. Table 4 shows data about the content of soluble sugars in the dry leaves of the middle belt of the plants. From the data it can be seen that the average content of soluble sugars in all varieties ranges from 0.68 % in the control variety Banket A_1, to 1.95 % in the variety Habana - 92 which has 105.26 % higher relative difference than the control variety. For other varieties and lines, the average soluble sugar content ranges from 0.95 % in the control variety Enchu and the line B-210/15 CMS F_1, to 1.15 % in the line B-199/11 CMS F_1.

Table 4. Soluble sugar content %

Variety	Average %	Differences		Rank
		Absolute	Relative	
Enchu Ø	0.95	/	100.00	4
B-D-1	1.01	+0.06	106.32	3
Banket A_1	0.68	-0.27	71.58	5
Habana - 92	1.95	+1.00	205.26	1
B-210/15 CMS F_1	0.95	0	100.00	4
B-199/11 CMS F_1	1.15	+0.20	121.05	2

Popović et al. (2000), while examining the qualitative properties of several characteristics in Burley CMS hybrids, concluded that the average content of soluble sugars from three-year studies ranges from 0.69 % to 1.23 %.

Pelivanoska and Trajkoski (2004), while growing Burley tobacco, in the period from 1997 to 1999 in the Polog production region, found that the average content of soluble sugars ranges from 1.48 % in the fertilized-non-irrigated variant to 2.84 % in the non-fertilized-irrigated variant with 60 % FWC.

Risteski (2006), in his three-year studies of six varieties of the Burley type grown in the Prilep region, came to the conclusion that the average content of soluble sugars is within the range of 0.77 % in the variant of whole stalk harvest in the variety B-96/85 to 1.39 % in the Podravac variety.

Risteski (2008), from the studies (1999/2001) of six Burley tobacco varieties in the Prilep region, came to the conclusion that the average content of soluble sugars ranges from 0.61 % in the variant of whole stalk harvest to 1.39 % in the Podravac variety.

Hristoski (2013). In the control variety B-21 the lowest content of soluble sugars (0.89%) was recorded in the insertion X and the highest content (1.83 %) was found in the insertion B from the upper middle belt. In the variety B-2/93 the lowest content (1.27%) is in the insertion X and the highest and almost identical content (1.82 % and 1.84 %) was in the insertions of the middle lower belt (C) and the middle upper (B) belt. Unlike these varieties, in the Pelagonec variety, the average content is the lowest (0.81 %) in the insertion X and the highest (1.44 %) in the insertion T of the upper harvest belt.

Content of mineral matter

The participation of mineral matter is conditioned by the genetic potential of the selected variety, the measures of the agricultural technique, the soil type and the position of the leaves (insertions), etc. Of the entire chemical composition of the tobacco leaves, mineral matter take up a significant part of the dry matter.

In terms of the plant physiology, mineral matter under the action of light and other factors that participate in the assimilation of organic matter, produce new organic matter that is used for building the leaf tissue.

In Burley type tobaccos, the content of mineral matter is an indicator of the quality of combustion of the dry tobacco leaves (combustion, compactness and ash color). In the combustion process, mineral matter have the role of catalysts that regulate the combustion process i.e. the smoldering process and prevent the appearance of flame.

Table 5. Content of mineral matter %

Variety	Average %	Differences		Rank
		Absolute	Relative	
Enchu Ø	21.96	/	100.00	1
B-D-1	18.69	-3.27	85.11	3
Banket A_1	20.41	-1.55	92.94	2
Habana – 92	17.97	-3.99	81,83	5
B-210/15 CMS F_1	18.05	-3,91	82.19	4
B-199/11 CMS F_1	17.87	-4.09	81.38	6

Table 5 shows data about the movement of the content of mineral matter in the studied varieties and lines. The line B-199/11 CMS F_1 (17.87%) has the lowest average content of minerals, while the control variety Enchu (21.96 %) has the highest content of mineral matter. From the tests it can be said that the average content of mineral matter substances ranges from 17.97 % in the variety Habana - 92, to 20.41 % in the variety Banket A_1.

According to (Staniša, 1967), chlorides, sulphates and phosphates have little contribution in the improvement of the combustion process, especially chlorides which create a protective layer around the particles disabling access of oxygen and this completely prevents the oxidation process. The presence of chlorides in tobacco causes difficulty in inhalation and extinguishes the cigarette. Of all metal salts, potassium salts stimulate combustion and calcium and magnesium salts contribute to the production of white ash. Risteski (2006), in the period 1999/2001 in the Prilep region, while studying the characteristics of six varieties of the Burley type, found that the average content of mineral substances ranges from 17.44 % to 19.11 %.

Gjuzelev (1983) points out that leaves of the Burley type are characterized by a high content of mineral substances, which ranges from 18 % -24 %.

Pelivanoska and Trajkoski (2004), in their three-year research (1997/1999) in Polog, on Burley tobacco varieties of tobacco, concluded that the average content of mineral matter ranges from 13.80 % in the non-fertilized - irrigated variant with 60% available water capacity to 15.28 % in the control variant.

Hristoski (2013). He indicates that in insertion X for the Pelagonec variety the average content of mineral substances is 29.55 % and in the raw material originating from India their content amounts to 31.44%. In the variety B-2/93 the average content is the lowest (17.73 %) in the insertion B and an inconsiderable increase (17.85 %) was recorded in the insertion T.

CONCLUSIONS

From the obtained data we can draw the following conclusions:

Nicotine as an important chemical component in the tobacco leaf, ranges from (1.65 %) in the control variety Enchu to 2.74 % in the line B-210/15 CMS F_1.

The total nitrogen content ranges from 4.29 % in the line B-210/15 CMS F_1 and has the lowest value, up to 4.90 % in the variety Banket A_1 which has the highest content of total nitrogen.

The newly acquired lines are also characterized by protein content within the limits of the Burley raw material, in the line B-210/15 CMS F_1 with 9.85 % up to 10.41 % in the line B-199/11 CMS F_1.

The average content of soluble sugars in all varieties ranges from 0.68 % in the control variety Banket A_1, to 1.95 % in the variety Habana - 92.

The line B-199/11 CMS F_1 (17.87 %) is characterized by the lowest average content of mineral substances, and the control variety Enchu (21.96 %) is characterized by the highest content of mineral substances.

According to the obtained data, the newly obtained lines B-210/15 CMS F_1 and B-199/11 CMS F_1, are characterized by typical chemical characteristics for the Burley tobacco type. According to the chemical properties, the newly recognized lines B-210/15 CMS F1 and B-199/11 CMS F1 do not lag behind the foreign Berley varieties, so it is expected that these lines will find their place in production.

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CHECKING THE EFFICIENCY OF THE HERBICIDE GAMIT 4 EC IN TOBACCO SEEDLING

Petre Tashkoski, Petre Stojanoski

St. Kliment Ohridski University – Bitola, Scientific Tobacco Institute - Prilep, of Republic of North Macedonia
e-mail: taskoskip@yahoo.com

ABSTRACT

Weeds have direct effect upon reduction of yield and quality of tobacco, hindering the good performance of other work operations. Indirectly, being a host to many insect vectors of various diseases, they are associated with infections of tobacco. Clomazone is a herbicide with wide application in weed management and our goal was to determine its efficiency in the control of weeds in tobacco seedlings. Experiments were conducted through in seedbeds of the Scientific Tobacco Institute-Prilep. Tests were made with napropamide-based preparations Ohinol 50-S in a rate of 2 kg/ha and Devrinol 45F in a rate of 3 l/ha, used prior to transplanting. Gamit 4EC (clomazone) rates were 0.7; 0.8; 0.9 and 1 l/ha used after transplanting. Sampling and assessment of weed were performed by the method of 1 m^2 squares. The effectiveness of herbicides in control of weeds was evaluated by counting weed populations in plots treated with herbicide and those in the untreated check. The highest efficiency was obtained with the herbicide Gamit - 95.97% at 1 l/ha, 93,76% at 0,9 l/ha and 91.94% at 0,8 l/ha. Herbicides based on napropamide gave poor efficiency.

Key words: tobacco, weed, herbicides, clomazone, napropamide.

ПРОВЕРУВАЊЕ НА ЕФИКАСНОСТА НА ХЕРБИЦИДОТ GAMIT 4 EC КАЈ ТУТУНСКИОТ РАСАД

Плевелите може директно да влијаат врз намалувањето на приносот и квалитетот на тутунот, и да го попречуваат квалитетното извршување на останатите работни операции, а индиректно учествуваат во заразата на тутунот од некои болести бидејќи истите се домаќини на голем број инсекти вектори на разни заболувања. Бидејќи хербицидот clomazone најдува се поширока примена за сузбивање на плевелите кај утунот, нашата цел беше да ја провериме неговата ефикасност во сузбивање на плевелите кај тутунскиот расад. Испитувањата беа спроведени во леи на површина од Научниот институт за тутун-Прилеп. Беа испитувани препарати на база napropamid, Ohinol 50-S со доза 2 kg/ha и Devrinol 45F со доза 3 l/ha употребени пред сеидба. Хербицидот Gamit 4EC (clomazone) беше употребен во доза од 0,7; 0,8; 0,9 и 1 l/ha по сеидба. Собирањето на плевелите и оценувањето на заплевеленоста е вршено по методот на квадрати од 1 m^2. Ефикасноста на хербицидите во уништувањето на плевелите се оценува врз база на заплевеленоста кај третираните парцелки со хербицид и контролата. Највисока ефикасност беше постигната со хербицидот Gamit итоа 95,97% со доза 1 l/ha, 93,76% со доза 0,9 l/ha и 91,94% со доза 0,8 l/ha. Хербицидите на база napropamid покажаа слаба ефикасност.

Клучни зборови: тутун, плевели, хербициди, clomazone, napropamid.

INTRODUCTION

Weeds have direct impact on the reduction of yield and quality of tobacco, hindering the good performance of other work operations. Indirectly, they are associated with infection of tobacco plants, being a host to many insect vectors of various diseases. In many countries in the world, including R. Macedonia, a number of manufacturers apply manual weeding of planted tobacco, removing the weeds by manual pulling or by hoeing. In modern tobacco production, however, chemical protection is the main practice in control of weeds. Although the number of herbicides for weed control in tobacco is much lower than for other crops, they still provide good protection from weeds.

The world's most used herbicides in tobacco are clomazone, metolachlor, napropamid, pebulate, pendimethalin, sethoxydim, trifluralin and sulfentrazone (William A Bailey, 2013; Chapman Koga and Rutendo Nyabadza, 2016). Clomazone is a selective herbicide with contact and residual effect, used for control of annual broadleaf and grass leaf weeds in oilseed rape, cotton, tobacco, soybeans, rice and sugar cane and vegetables like peas, beans, carrots and potatoes (Annonimus 2017). This herbicide shows effective control on tobacco weeds Digitaria spp., Panicum spp., Setaria spp., *Chenopodim album*, *Ambrosia artemisiifolia* et al., incorporated in a rate of 0,84-1,1 kg/ha (William A Bailey, 2013, Andy Bailey et al., 2014). It can be applied within 7 days after planting, since tobacco is tolerant.

Clomazone is a herbicide registered for use in 1986 in the United States (April Van Scoy and Ronald S. Tjeerdema, 2007). It was produced by the FMC company under the trade name Command and Cerano 5M and used for control of annual broad leaf and grass leaf weeds in soy, tobacco and other crops. Command 480EK (clomazone) is a relatively new herbicide with various modes of action and is intended to control weeds in a number of vegetables and in tobacco (Annonimus, 2017b). The herbicide is absorbed from the soil through plant roots or through vegetative mass, if applied post transplanting. As a result, the plant develops chlorosis, i.e. bleaching of the green parts, but is not dangerous to tobacco. Skott et. al (1994) made laboratory analysis and estimations on the susceptibility of tobacco, tomatoes and peppers against clomazone in cotyledons stage. Big differences were found in the content of carotenoids between tobacco and tomatoes treated with clomazone. The chlorosis occurs in treated weeds as a result of inhibition of chlorophyll and carotenoids synthesis that protects chlorophyll from sunlight (Annonimus 2017; Chapman Koga and Rutendo Nyabadza, 2016; William A Bailey, 2013). Due to the lack of chlorophyll, plants die (Annonimus, 2017b). In our tests we used the herbicide Gamit 4EC (clomazone), known under the trade name Gamit or Clon 480 EC (Annonimus, 2017c, 2017d).

The aim of this research was to receive a more detailed information on the effect of herbicide use upon tobacco seedling, the results of which will be very useful for tobacco production.

MATERIAL AND METHODS

Trials were carried out throughout in the field of the Scientific Tobacco Institute-Prilep. Seedlings of the variety P66-9/7 were transplanted in 5 m^2 plot, in polyethylene-covered seedbeds. Herbicides were applied using knapsack sprayer. The trial was performed with standard preparations Ohinol 50-S and Devrinol 45F and the herbicide Gamit 4EC. Data on herbicide treatment, active ingredient and rate of use are presented in Table 1. The following variants were investigated in the trial: Ohinol 2 kg/ha prior to transplanting, Devrinol 3 l/ha prior to transplanting and Gamit 4EC 0,7 l/ha, 0,8 l/ha, 0,9 l/ha and 1 l/ha post transplanting. During the cultivation of seedlings, traditional agro-technical measures were applied.

Table 1. Herbicide application in the trial

Herbicide	Active ingredient	Rate
Gamit 4EC	Clomazone 480 g/l	0,2-1 l/ha
Ohinol 50-S	Napropamid 50%	2 kg/ha
Devrinol 45F	Napropamid 450 g/l	3 l/ha

Determination of herbicide effectiveness was based on weed counting in the treated plots and in the check. Two methods were used for evaluation: visual and quadrat square method (Костов, 1994). Assessment of weed population in this trial was done by the method of quadrats, which consists of collecting and counting the weed plants per unit area (usually 1 m^2). Weeds are collected from each variant separately, during their early stage of growth. When weed population is larger, smaller quadrats are used, but the number of weeds is reduced to 1 m^2. Based on the average results from the three replications, the coefficient of herbicide efficiency was calculated by equation (Костов, 1994):

$$Ce = Wc – Wt/Wc \times 100,$$

where:
Ce= coefficient of efficiency
Wc = number of weeds per 1m^2 in the check plot
Wt = number of weeds per 1m^2 in the treated plots

RESULTS AND DISCUSSION

Results of investigations on weed control in tobacco seedlings in 2015 are presented in Table 2, showing the total number of weeds and their weight by species for each preparation. Both with application of Devrinol and Ohinol, the most abundant weed species in number and weight was the purslane - *Portulaca oleracea* (205-245 plants/m^2, 64,77 - 136,19 g), due to the poor efficiency of the herbicides against this weed. In variants treated with Gamit, the percentage of this weed was minimal, i.e. the herbicide showed high efficiency in its control. In the check variant, 268 plants of this weed were counted, with a weight of 159,87 g. Although insignificant in number, the most common weed species in almost all variants were *Hyoscyamus niger, Chenopodium album, Chenopodium pumilio* and *Amaranthus retroflexus*. The most common among panicum grass weeds was *Echinochloa crus galli* (25 plants/m^2 in the variant treated with Ohinol and 14 plants/m^2 in the check). In the check variant, the number of weeds was 303 plants/m^2, weighing 167,37 g. The lowest number of weeds was counted in variants treated with Gamit - from 20 plants/m^2 in variant treated with 1 l/ha to 45 plants/m^2 in variant treated with 0,8 l/ha, weighing from 1,51 g to 8,79 g. In variants treated with napropamide-based herbicides, total number of weeds per m^2 was 266 plants/m^2 with Devrinol and 288 plants/m^2 with Ohinol, weighing 76,83 g - 143,59 g.

According to the results presented in the table, weed control efficiency of Gamit in tobacco seedlings ranged from 85,14% at 0,8 l/ha to 93.40% at 1l/ha. With regard to weed weight, the efficiency of Gamit ranged from 94.75% at 0,8 l/ha to 99.10% at 1 l/ha. Herbicides with a.i. napropamide show low effectiveness because of the higher presence of *Portulaca oleracea* weed.

Investigations made in 2016 showed approximately the same results as in 2015 (Table 3). The most abundant weed species among variants treated with napropamide was the purslane (*Portulaca oleracea*) - 251 plants/m^2 in variants treated with Ohinol and 335 plants/m^2 in those treated with Devrinol. The green mass weight was 214,57g with Devrinol and 217,05 g with Ohinol. In all variants treated with Gamit, no samples of this weed were found and in the check variant 293 plants/m^2 were counted, with a weight of 242,25 g. Again, a smaller number of the most common broadleaf weeds and grass weeds were counted in tobacco seedlings. The number of weeds ranged from 347 plants/m^2 in variants treated with Ohinol to 507 plants/m^2 in those treated with Devrinol, and in the check variant 571 plants/m^2 were counted. Despite the high number of weeds, big difference was observed in weed weight between the check variant and the treated plots. The weight of the weeds in untreated plot was 723,48 g and in treated variants it ranged from 393,57 g with Ohinol to 504,75 g with Devrinol. In variants treated with Gamit the lowest number of weeds was counted with Gamit 1 l/ha - 23 plants/m^2 and the highest with Gamit 0,7 l/ha - 63 plants/m^2. These data show that the most efficient weed control in tobacco seedlings of 95.97% was obtained with Gamit 1 l/ha and with regard to green mass weight the efficiency was 99.33 %. In other variants treated with Gamit the efficiency ranged from 88.96% to 91.94%. The lowest efficiency was obtained with Devrinol - 11,20% and Ohinol - 39,22%. High efficiency of clomazone was reported in investigations of Kenneth C. Flower (2001), i.e. lower rates of the herbicide reduced the weed vegetation in Virginia tobacco by 75% and the efficiency of higher rates was 98%. William A. Bailey (2013) reported that the application of sulfentrazone in combination with clomazone gave the most effective results in the control of weeds in dark tobacco. Among the herbicides based on sulfentrazone, pendimethalin, napropamide and clomazone (Comand 3ME herbicide), the combination with clomazone was the most effective in the control of weeds in tobacco (Andy Bailey et al., 2014).

The results of investigations carried out in 2017 are presented in Table 4. As in previous years, the most abundant weed species was *Portulaca oleracea*. In the check variety, 212 plants/m^2 of this weed were counted, in variants treated with napropamide 106 - 140 plants/m^2 and the lowest number was observed in variants treated with Gamit: 10 plants/m^2 at a rate of 1 l/ha and 30 plants/m^2 at a rate 0,7 l/ha. The population of other weeds varied, but both the broadleaf and grass leaf weed species were represented. The total population of weeds in seedbeds was 465 plants/m^2 in the check, 165 - 220 plants/m^2 in variants treated with napropamide and 19 - 66 plants/m^2 in variants treated with Gamit. Again, the highest efficiency of 95,91% was achieved with Gamit 1 l/ha and 93.76% with Gamit 0,9 l/ha. Poorer performance was obtained with herbicides Ohinol (52,68%) and Devrinol (63,50%), due to the high population of *Portulaca oleracea*. Some of the effects can be seen in the figures attached (Fig. 1-6).

Fig. 1. Check

Fig. 2. Seedlings treated with Gamit 0,8 l/ha

Fig. 3. Seedlings treated with Gamit 0,9 l/ha

Fig. 4. Seedlings treated with Gamit 1 l/ha

Fig. 5. Seedlings treated with Ohinol 2 kg/ha

Fig. 6. Seedlings treated with Devrinol 3 l/ha

Table 2. Effect of herbicides on weed control efficiency in tobacco seedlings – 2015

Herbicide	Ohinol 2кg/ha PreT		Devrinol 3 l/ha PreT		Gamit 0,7 l /ha PostT		Gamit 0,8 l /ha PostT		Gamit 0,9 l /ha PostT		Gamit 1 l /ha PostT		Check	
Weed species	**No./m²**	**Mass- g**	**No./m²**	**Mass- g**	**No./m²**	**Mass- g**	**No./m²**	**Mass- g**	No./m²	Mass- g	No./m²	Mass- g	No./m²	Mass- g
Portulaca oleracea	245	136,19	205	64,77	6	0,70	8	0,97	6	0,33	1	0,06	268	159,87
Hyoscyamus niger	4	0,29	3	0,36	2	0,02	3	0,12	1	0,17	2	00,5	1	0,25
Chenopodium album	7	1,27	-	-	2	0,20	8	0,53	-	-	-	-	10	0,87
Chenopodium pumilio	1	0,22	46	10,02	19	1,05	15	2,66	12	0,69	13	0,25	7	2,33
Amaranthus retroflexus	2	0,74	2	0,34	10	1,41	3	3,26	1	0,03	4	1,15	2	1,14
Xanthium strumarium	2	0,29	-	-	-	-	2	0,16	-	-	-	-	-	-
Digitaria sanguinalis	-	-	-	-	-	-	6	1,09	-	-	-	-	-	-
Echinochloa crus galli	25	4,03	8	0,82	2	0,13	-	-	5	1,13	-	-	14	2,62
Capsella bursa pastoris	-	-	2	0,52	-	-	-	-	-	-	-	-	1	0,29
Convolvulus arvensis	2	0,56	-	-	1	0,07	-	-	-	-	-	-	-	-
Total No.of weeds/ m² and mass in g	288	143,59	266	76,83	42	3,58	45	8,79	25	2,35	20	1,51	303	167,37
Efficiency-% / No. of weeds	4,95		12,21		86,14		85,14		91,75		93,40		-	
Efficiency-% / green mass	14,21		54,09		97,86		94,75		98,59		99,10		-	

Table 3. Effect of herbicides on weed control efficiency in tobacco seedlings – 2016

Herbicide	Ohinol 2кg/ha PreT		Devrinol 3 l/ha PreT		Gamit 0,7 l /ha PostT		Gamit 0,8 l /ha PostT		Gamit 0,9 l /ha PostT		Gamit 1 l /ha PostT		Check	
Weed species	No./m^2	Mass- g	No./m^2	Mass- g	No./m^2	Mass- g	No./m^2	Mass- g	No./m^2	Mass- g	No./m^2	Mass- g	No./m^2	Mass- g
Portulaca oleracea	251	217,05	335	214,57	-	-	-	-	-	-	-	-	293	242,25
Solanum nigrum	15	26,62	6	10,42	-	-	-	-	-	-	-	-	33	47,09
Hyoscyamus niger	19	66,67	19	101,59	-	-	-	-	-	-	-	-	3	5,90
Chenopodium album	25	17,71	36	23,16	-	-	-	-	-	-	-	-	51	99,82
Chenopodium pumilio	3	1,75	47	33,06	60	30,70	43	13,60	58	23,75	23	4,80	80	79,06
Amaranthus retroflexus	16	21,93	44	71,55	-	-	-	-	-	-	-	-	24	39,93
Capsella bursa pastoris	3	22,77	9	34,72	-	-	-	-	-	-	-	-	13	38,31
Convolvulus arvensis	2	2,43	2	1,75	-	-	-	-	-	-	-	-	-	-
Polygonum aviculare	5	3,57	4	2,21	-	-	-	-	-	-	-	-	-	-
Echinochloa crus galli	7	6,90	5	11,72	3	1,37	-	-	1	0,22	-	-	55	146,72
Digitaria sanguinalis	1	6,17	-	-	-	-	3	0,89	-	-	-	-	9	9,76
Total No.of weeds/m^2 and mass in g	347	393,57	507	504,75	63	32,07	46	14,49	59	23,97	23	4,80	571	723,48
Efficiency-% / No. of weeds	39,22		11,20		88,96		91,94		89,66		95,97		-	
Efficiency-% / green mass	45,60		30,23		95,56		97,99		96,68		99,33		-	

Table 4. Effect of herbicides on weed control efficiency in tobacco seedlings – 2017

Herbicide	Ohinol		Devrinol		Gamit		Gamit		Gamit		Gamit		Check	
	2кg/ha		3 l/ha		0,7 l /ha		0,8 l /ha		0,9 l /ha		1 l /ha			
	PreT		PreT		PostT		PostT		PostT		PostT			
Weed species	**No./m²**	**Mass- g**	**No./m²**	**Mass- g**	**No./m²**	**Mass- g**	**No./m²**	**Mass- g**	**No./m²**	**Mass- g**	**No./m²**	**Mass- g**	**No./m²**	**Mass- g**
Portulaca oleracea	140	489,15	106	420,00	30	28,50	28	27,60	18	20,20	10	15,00	212	450,00
Hyoscyamus niger	30	125,50	18	38,25	6	4,00	-	-	-	-	-	-	8	10,15
Chenopodium album	-	-	24	14,48	14	2,35	6	1,00	6	0,50	4	0,40	128	132,00
Chenopodium pumilio	20	24,40	5	8,55	2	3,6	2	5,00	2	2,00	4	2,80	55	92,00
Amaranthus retroflexus	-	-	-	-	6	7,6	6	2,00	4	0,45	4	0,40	30	38,00
Xanthium strumarium	4	6,80	-	-	-	-	-	-	-	-	-	-	2	8,00
Digitaria sanguinalis	14	2,35	12	44,82	-	-	-	-	-	-	-	-	14	12,50
Echinochloa crus galli	12	8,45	-	-	8	18,00	-	-	1	0,46	1	0,42	16	32,00
Total No.of weeds/ m² and mass in g	220	656,65	165	526,10	66	64,05	40	30,60	29	21,61	19	16,22	465	774,65
Efficiency-% / No. of weeds	52,68		63,50		85,80		91,39		93,76		95,91		-	
Efficiency-% / green mass	15,23		32,08		91,73		96,04		97,21		97,90		-	

CONCLUSION

Weeds are strong competitors to tobacco for food and space and they can negatively affect its yield and quality. The poor choice of herbicides available for use in tobacco and the emergence of the active ingredient clomazone on the market incited us to investigate the efficiency of the herbicide Gamit 4EC (clomazone) in tobacco seedlings.

Investigations were performed in conditions of high abundance of weeds in tobacco seedbeds, the number of weeds in untreated plot (check) ranging from 303 to 571 plants/m^2.

The tests with clomazone conducted through gave very good results. The highest efficiency in all three years was achieved with Gamit 1 l/ha (93.40%, 95.97% and 95,91% respectively). Almost the same results were obtained with Gamit 0,8 l/ha and Gamit 0,9 l/ha. At a rate of 0,8 l/ha, weed control efficiency ranged 85.14% - 91.94% and at a rate of 0,9 l/ha it ranged 89.66% - 93.76%. At the lowest rate of 0,7 l/ha, 85.80% to 88.96% efficiency was recorded. The napropamide herbicides (Ohinol 50-S and Devrinol 45F) gave poor results in weed control. The best efficiency was recorded in 2017 - 52.68% with Ohinol and 63.50% with Devrinol. Because of the high efficiency of the herbicide Gamit, despite temporary chlorosis that appears in tobacco seedlings, it will find wider application in the control of weeds both in seedlings and in transplanted tobacco.

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AUTOMATIC IDENTIFICATION OF *TRIALEURODES VAPORARIORUM* AND *BEMISIA TABACI*

Martin Tashkoski[1], Vesna Krsteska[2]

St. Kliment Ohridski University – Bitola, Scientific Tobacco Institute - Prilep, of Republic of North Macedonia, Kicevska bb 7500 Prilep, R. Macedonia

e-mail: [1]tashkoskim@yahoo.com
[2]vkrsteska@yahoo.com

ABSTRACT

The computers era and the digital revolution has made positive changes in modern life, including entertainment, communication and science. Digital images are just one example of this revolution. Smartphones allow us to take pictures and to modify them applying different filter. Digital image processing is used in digital cameras, to improve image quality, applying various filters that display the image in different ways, extracting information from medical or microscopic images. Image processing often pairs with machine learning and together are powerful method for images classification. New algorithms for image processing, their analysis and obtaining specific information are constantly being developed.

In this paper we present a method for microscopic images classification of two larvae of insects belonging to the family **Aleyrodidae**, subfamily **Aleyrodoidea** or known as whiteflies. Both whiteflies (lat. *Bemisia tabaci* and *Trialeurodes vaporariorum*) feed on the juices of the plant and can easily adapt on different plants. They are very similar and can be distinguished only in a certain stage of their development (pupae stage). This paper presents the process of taking the images, their preprocessing and processing, the creation of a descriptor that best describes the two insect larvae, the method for cleaning noise and the background of the images, and the results obtained from several different classification tests in Weka and SVM light.

Key words: tobacco, whiteflies, identification, distinction, processing, images

АВТОМАТСКА ИДЕНТИФИКАЦИЈА НА TRIALEURODES VAPORARIORUM И BEMISIA TABACI

Компјутерската ера и дигиталната револуција направија позитивни измени во модерниот живот, вклучувајќи ја забавата, комуникацијата и науката. Дигиталните слики се само еден пример од оваа револуција. Паметните телефони ни овозможуваат фотографирање и дигитална измена на фотографиите. Процесирањето дигитални слики се користи во дигиталните камери и фотоапарати, за подобрување на квалитетот на сликата, различни филтри кои ја прикажуваат сликата на различен начин, добивање на некои информации од медицински и микроскопски слики. Процесирањето слики и машинското учење заедно претставуваат моќна метода за класификација на слики. Постојано се развиваат нови алгоритми за процесирање слики, нивна анализа и добивање на конкретни информации.

Во овој труд опишуваме метода за класификација на микроскопски слики добиени од ларвите на два вида инсекти кои припаѓаат во фамилијата **Aleyrodidae**, подфамилија **Aleyrodoidea** или познати како белокрилки. Двете белокрилки (лат. *Bemisia tabaci* и *Trialeurodes vaporariorum*) се хранат со сокот од растението и може да се прилагодат на различни растенија. Тие се многу слични и може да се разликуваат само во одредена фаза од нивниот развој (фаза кукла). Во трудот се презентирани добивањето на сликите, нивно претпроцесирање и процесирање, креирање на дескриптор кој на најдобар можен начин ги опишува двете ларви од инсектите, метода за чистење на сликите од шумот и позадината, како и резултати добиени од повеќе различни тестови од класификација во Weka и SVM light.

Клучни зборови: тутун, белокрилки, идентификација, разликување, процесирање, слики.

INTRODUCTION

One of the most important pests in greenhouse production are two species of moths, the greenhouse or flower moth (*Trialeurodes vaporariorum* Westwood 1856) and the tobacco moth (*Bemisia tabaci* Gennadius 1889). They are especially harmful for the production process in a protected area (greenhouses), where there are suitable conditions for their reproduction. In the plantations in a protected area, these whiteflies are present throughout the entire vegetation, from seedling to harvest, which makes it difficult to control them. Thus Britvec (1966) and Bedeković (1974), (cit. Sekulić, 1974), report a strong attack by these harmful insects, not only attacking horticultural crops and flowers in greenhouses, but also the plants in the open field. The whiteflies are polyphagous insects that, in addition to cultivated plant species, also attack other plants species that are commonly found around protected areas and are a constant source of infection.

According to Šimala et al., (2016), the species *T. vaporariorum* originates from Central and South America from where it is spread all over the world, and attacks a large number of cultivated and wild plant species in the open and in protected areas. It is most harmful to horticultural crops and ornamental plants in a protected area. Over 859 plant species from 121 families are registered as its hosts. According to this author, the species B. tabaci originates from India and Pakistan from where it has spread to all continents. Until recently, this shield bearer moth was known as a pest on areas with cotton, tobacco and other field crops in tropical and subtropical areas. Attacks more than 600 different plant species. B. tabaci is considered a more dangerous species because it is a vector of a number of destructive viruses on the horticultural crops.

In Croatia according to Žanić et al. (2019), the greenhouse moth *T. vaporariorum*, is important for many plant species, from the family Cucurbitaceae, especially cucumbers, and in addition to this pest of special importance is the pest *B. tabaci*, which is very similar to the pest T. vaporariorum, and often occur together in mixed populations.

The adults of *T. vaporariorum* and *B. tabaci* are very similar and resemble small white butterflies, and the larvae of these two moths are morphologically very similar, making it difficult to determine them based on their appearance.

Shield bearer moths are small insects about 2 mm in size the adults and 0.2 to 1 mm in larval stage (Šimala et al., 2016). Plant damage is caused by adult insects and larvae found on the back of the leaf. They suck the juices from the leaves and the attacked plants lose their green color, necrosis appears on the leaves and they dry out. The damage caused by plant viruses is also enormous, because the tobacco shield moth *B. tabaci* also appears as a vector of viral diseases. Damage is also caused by the excreted honeydew on the leaves and fruits, which is a good basis for the development of the fungus, due to which the fruits lose their quality and market price.

According to Žanić et al. (2019), the *T. vaporariorum* imago has two pairs of wings covered with white waxy dust. At rest, its wings are arranged to form a triangular shape. The body size of the imago exceeds 1 mm, and the female lays light yellow elliptical eggs about 0.2 mm long on the back of the leaf. The eggs hatch into larvae that have four developmental stages. The pest has 10-12 generations per year that overlap, so the plants are at the same time all the developmental stages that make it difficult to control them.

Due to the large investments in the greenhouse production, and also for obtaining higher yields and better products quality, it is necessary to apply appropriate protection. Insecticides are commonly used to

protect against these pests, but due to their high degree of resistance to insecticides, their control is difficult.
These pests were often present in the biological laboratory of the Scientific Institute of Tobacco-Prilep where tobacco was grown for experimental purposes, where part of the plant material was used for this study.

Because the two species of moths *T. vaporariorum* and *B. tabaci* are very similar and at first glance it is not possible to distinguish which species they are, the aim of this research was to apply the method of automatic identification of these harmful insect species which would facilitate the work of scientists in their determination.

MATERIAL AND METHODS

Tobacco leaves (*Nicotiana tabacum*) grown in the biological laboratory of the Scientific Institute for Tobacco-Prilep were used as material for this study, and which were attacked by shield bearer moths. In the phytopathological and entomological laboratory of the institute, the larvae of these pests were observed under a microscope. A number of microscopic images were taken and used during further examination to determine the shield bearer moths.
Important macroscopic morphological characteristics for distinguishing the species *T. vaporariorum* and *B. tabaci* listed by Šimala et al., (2016) are the following:

T. vaporariorum has a light yellow body, the wings are dormant, rounded at the front and back, placed on the body in the shape of a triangle and fly in different directions (Photo 1). While the body of *B. tabaci* is dark yellow, the wings are dormant at the front, pointed, elliptical, raised from the body in the form of a sloping roof and flying in a straight line (Photo 2, Malais et al., 2003). Females lay eggs on the back of the leaf individually or in groups, often in a semicircle or circle (Photo 3, Malais et al., 2003) from which larvae hatch (Photo 4, Malais et al., 2003). A female can lay 50 to 300 eggs in her lifetime.

Photo 1. *Trialeurodes vaporariorum* – imago (Tashkoski M. 2014)

Photo 2. *Bemisia tabaci* – imago (Malais, 2003)

Photo 3. *Bemisia tabaci* – eggs (Malais, 2003)

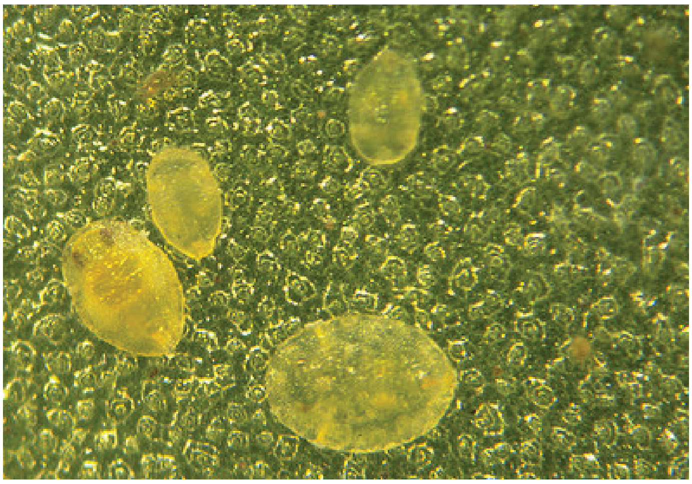

Photo 4. *Bemisia tabaci* – larvae (Malais, 2003)

Although the whiteflies look similar, they have differences in each stage of their development. In entomology, the problem can be that these whiteflies can physically change their look depending on the plant they feed themselves with, their environment and its temperature. According Malumphy (2004) the most accurate indicator for distinction is based almost entirely on their fourth larval stage or "puparial stage", and we can see it under microscope.

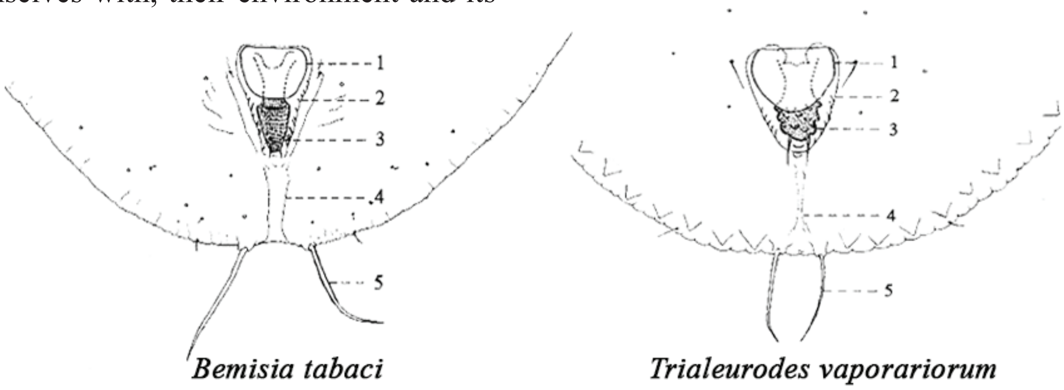

Photo 5. *Bemisia tabac*i and *Trialeurodes vaporariorum* - closer look to "vasiform orifice"

On Photo 5 (Malumphy, 2004), we can see the "vasiform orifice" part of the two whiteflies that is considered in this paper as a main identification for distinction. The shape of the operculum (1) for both pupas is similar, but the lingula (3) of *Bemisia tabaci* is longer. Also the vasiform orifice (2) of *B. tabaci* is thinner than the one of *Trialeurodes vaporariorum*. Shape of the lingula and vasiform orifice in the last (pupal) stage is the best indicator for recognizing the whitefly type. There are also differences in the caudal furrow (4) and caudal seta (5), but those facts are not used in this paper, because they are not clearly visible on the images that were processed.

The images

The number of the images are crucial part of developing algorithm based on machine learning and image processing. The images of *B. tabaci* were found on the Internet, and for the *T. vaporariourum* the images were taken in the Scientific tobacco institute - Prilep. There are several steps of taking these images: preparation of the biological samples, awareness of the mechanical damage of the samples, type and quality of the microscope. The most difficult problem was that each larva was covered with dust which appeared as a noise at the image. At Photo 6 there are microscopic images with different zoom levels of the larva. The image (1) is larva captured with binocular, the image (2) is the larva before removing the dust, and the image (5) is the "vasiform orifice" part needed for the algorithm.

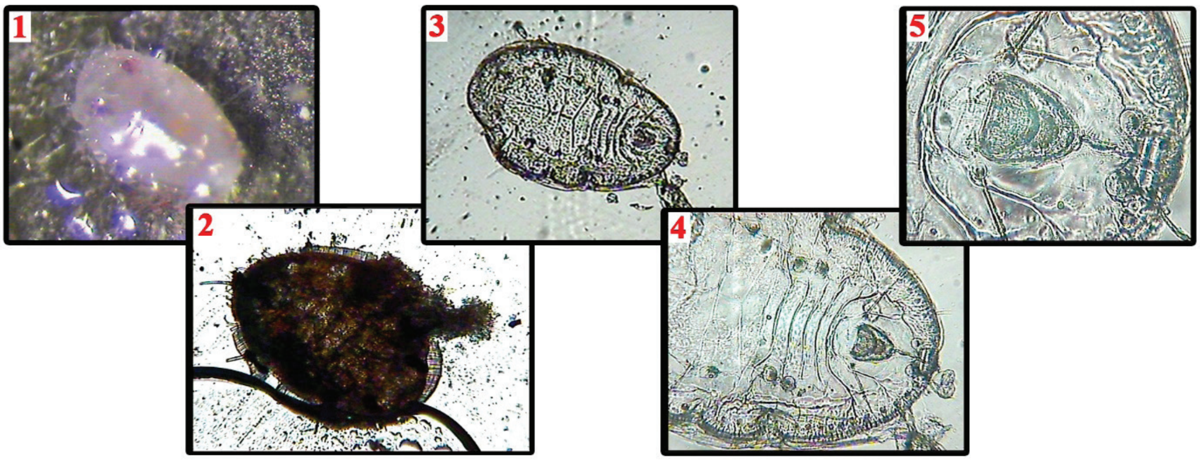

Photo 6. Microscopic images of larva of *Trialeurodes vaporariorum*

Image Pre-processing

In order to create the images database used for learning the algorithm, the images were pre-processed and cropped in order to get clear image of the "vasiform orifice" part. Photo 7 presents images example of the "vasiform orifice" of both whiteflies with the same size. These images were used as input to our algorithm for image processing and automatically removing the background. The database with cleared images was separate in 2 parts, one for training the algorithm and one for testing.

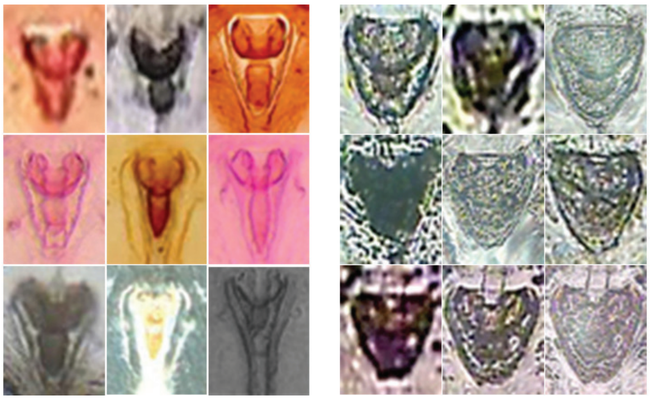

Photo 7. Vasiform orifice of *B. tabaci* (left) and *T. vaporariorum* (right)

This algorithm for clearing the background of the images was developed in the software Wolfram Mathematica. The basic steps (1-6) of the algorithm applied on all of the images are shown in Photo 8:

1. Importing the cropped images with the "vasiform orifice" (it is not relevant which images are processed first);
   1.1. Converting each of the images to "grayscale" image and producing 3 vector pairs for each image and classifying the pixels according to their color intensity (black, grey and white);
2. Applying bilateral filter to the images (in order to reduce the noise);
3. Morphological image processing (in order to reduce the noise);
4. Converting each of the images to binary image;

5. Deleting the isolated small groups of black pixels (in order to get clear white background).
6. The final step is rejecting all images that cannot be cleared.

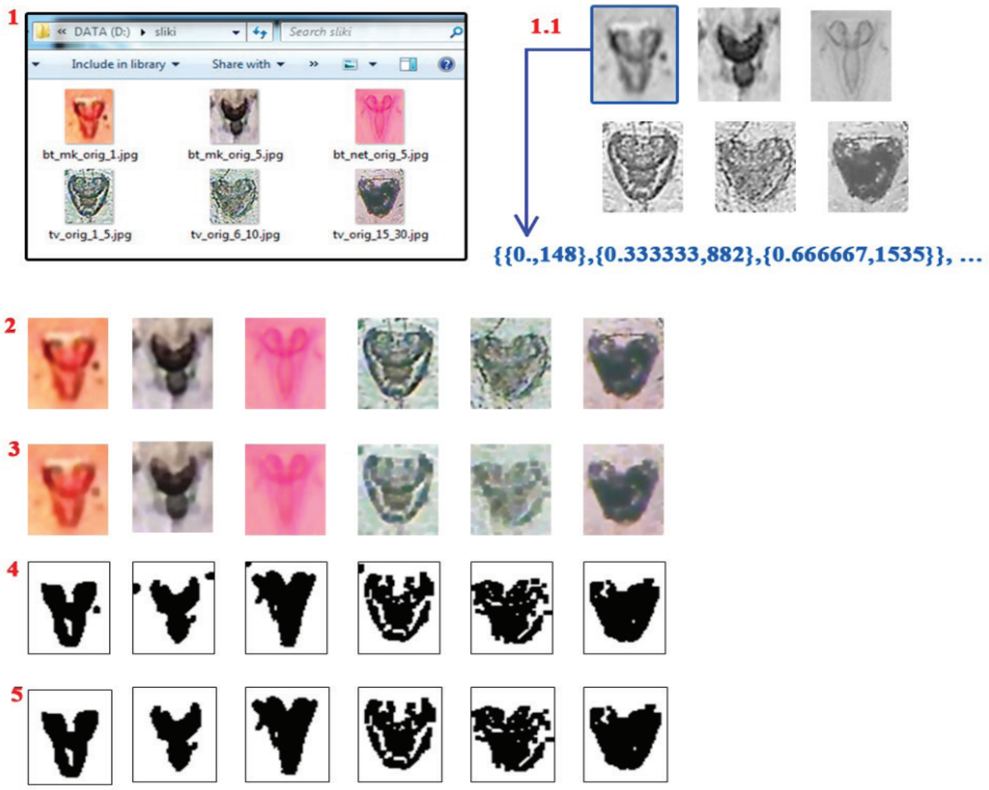

Photo 8. The basic steps in the algorithm for removing the background

In step 1, we import the images with the "vasiform orifice" part. In the step 1.1 the images are converted to "grayscale" style. For each grayscale image, we form three-dimensional vector of pairs, in order to find the parameters that characterize the image the best way according their color intensity. Because the images were converted to "grayscale" style in Step 2, the pixels values variy in scale of 0 to 1 (0 for black and 1 for white). First pair represents the number of pixels that has values 0 – 0.33, the second pair – number of pixels with values 0.33 – 0.67, and the third pair – number of pixels with values 0.67 – 1. This information important in the following steps for adjusting the appropriate filters for image cleaning.

The second step is about applying bilateral filter on each of the images, as it is shown at Photo 8. The bilateral filter according to Tomasi et al. (1998) is a non – linear, edge – preserving and noise – reducing filter for images. The intensity value at each pixel in an image is replaced by a weighted average of intensity values from the nearby pixels. This weight can be based on a Gaussian distribution.

In third step we made some morphological image processing. We used the method of closing as the basic rule for removing noise. Closing removes small holes and it tends to enlarge the boundaries of bright regions in the image and shrink background color holes in such regions.

The fourth step is converting each of the images to binary images, and after that in the step 5 we use function for deleting the small isolated groups of black pixels.

The crucial part in this algorithm is to select just the images with best quality for the training algorithm for distinction. In the final step, the algorithm automatically reject all the images that are not clear enough and can obtain bad results in the training algorithm. In the photo 9 is presented the output of the algorithm with the rejected images. From total of 346 images, 326 were accepted (saved in folder and used as inputs for creating the descriptor - the descriptor represents some parameters that describes "vasiform orifice" of the two whiteflies in a different way) and 20 were rejected. The method shown on Photo 10 counts the pixels at the borders (up, down, right and left). If there are more black pixels than a half of the border length, these images are rejected for the classification process as undistinguishable.

```
> Vkupno: 346 clenovi.

> 326 Prifateni clenovi.

> 20 Otfrleni clenovi:
{bt_art_1.jpg, bt_art_2.jpg, bt_art_4.jpg, bt_mk_orig_10.jpg, bt_mk_orig_2.jpg,
 bt_mk_orig_8.jpg, bt_mk_orig_9.jpg, bt_net_orig_1.jpg, bt_net_orig_2.jpg, bt_net_orig_4.jpg,
 tv_net_orig_12.jpg, tv_orig_12_17.jpg, tv_orig_21_22.jpg, tv_orig_2_1.jpg, tv_orig_2_2.jpg,
 tv_orig_8_7.jpg, tv_orig_9_12.jpg, tv_stoe_orig_21.jpg, tv_stoe_orig_22.jpg, tv_stoe_orig_6.jpg}
```

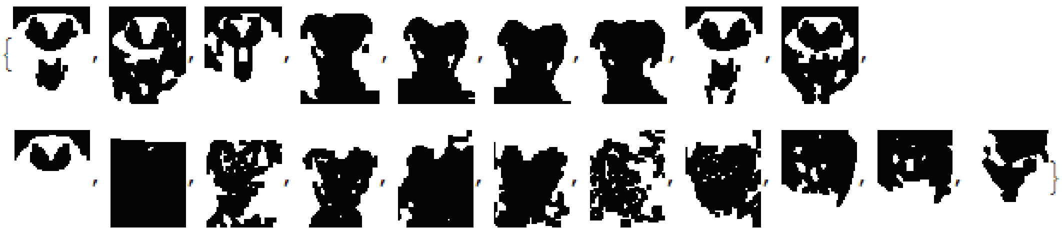

Photo 9. The output of the algorithm for clearing the background of the images (at the bottom are samples of the rejected images)

```
proverkaCrniPix[sl_] :=
  Module[{crniU = 0, crniD = 0, crniL = 0, crniR = 0, crniLD = 0, crniRD = 0, i = 0, podatoci = ImageData[sl], indikator = 0},
   For[i = 1, i ≤ 45, i++, If[podatoci[[1]][[i]] == 0, crniU++]];
   For[i = 1, i ≤ 45, i++, If[podatoci[[57]][[i]] == 0, crniD++]];
   For[i = 1, i ≤ 57, i++, If[podatoci[[i]][[1]] == 0, crniL++]];
   For[i = 1, i ≤ 57, i++, If[podatoci[[i]][[45]] == 0, crniR++]];

   For[i = 47, i ≤ 57, i++, If[podatoci[[i]][[1]] == 0, crniLD++]];
   For[i = 47, i ≤ 57, i++, If[podatoci[[i]][[45]] == 0, crniRD++]];
   If[crniU ≥ 35 || crniD ≥ 30 || crniL ≥ 45 || crniR ≥ 45 || (crniLD != 0 && crniRD != 0), indikator = 1];
   indikator
  ];
```

Photo 10. The function for rejecting the uncleaned images in the algorithm for image processing in Wolfram Mathematica

Algorithm for distinction

For the algorithm for distinction of images of these two whiteflies, we used the following technologies:

Visual Studio with C# for creating the descriptor – some parameters that describes the "vasiform orifice" part in best way.

Weka and SVM light - two software for machine learning

After various tested parameters for descriptor, in this paper as descriptor we used measurements of the "vasiform orifice" part: five widths (distributed at the same distance), height, and ratio height / last width. For example, the parameters of the descrip-

tor for the both whiteflies:
Measurements of “vasiform orifice” *Bemisia tabaci*: 19, 33, 25, 16, 6, 48, 8
Measurements of “vasiform orifice” *Trialeurodes vaporariorum*: 16, 43, 41, 34, 10, 53, 5.3

These parameters were collected automatically from the cleared images and were used in the machine learning software for training and distinction as text files with format understandable for this software.

RESULTS AND DISCUSSION

The previous described algorithm provided total 326 images, 39 of *Bemisia tabaci* and 287 of *Trialeurodes vaporariorum*. In this paper we present the results for distinction of these two whiteflies organized in two groups with 10 tests and used different classifiers in Weka and SVM light (Joachims, 2002). For the first group we maintain even ratio in the test folder (we use 10 instances of both classes for testing), and in the second group we maintain even ratio in the training folder (we use 30 instances of both classes for training).
For the first group of tests we have produced 10 tests, and for each we have taken 10 instances of *B. tabaci* and 10 instances of *T. vaporariorum* for testing of the total set with 326 instances. For every test we performed classifications in Weka (for different classifiers) and in SVM light (for different kernels).
According the average best results (Photo 11) in Weka for *B. tabaci*, were obtained with the classifiers Bayes.BayesNet (**78%** correctly recognized), and best results for correctly recognizing *T. vaporariorum* were obtained with the classifier trees.J48 **89%**.
According to the average best results (Photo 11) in SVM light for correctly recognizing *B. tabaci* were obtained with the RBF kernel (for gamma=0.001) **70%**, and best results for correct recognition of *T. vaporariorum* were obtained with all of the kernels except the RBF kernel (for gamma=0.001) **85%**.

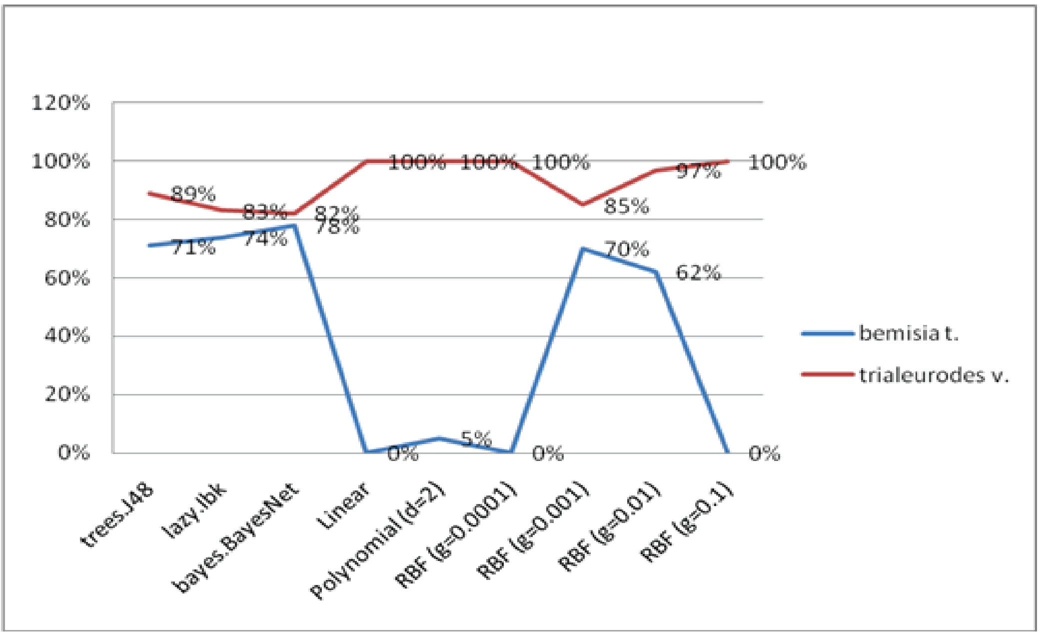

Photo 11. Average of the results in Weka and SVM light with even ratio of the both classes in the testing set

For the second group of tests we have produced 10 tests, and for each we have taken 30 instances of *B. tabaci* and 30 instances of *T. vaporariorum* for training. For every test we performed classifications in Weka (for different classifiers) and in SVM light (for different kernels). For the test set we used 9 instances of *B. tabaci* and 257 instances of *T. vaporariorum*. The average results are presented in Photo 12.

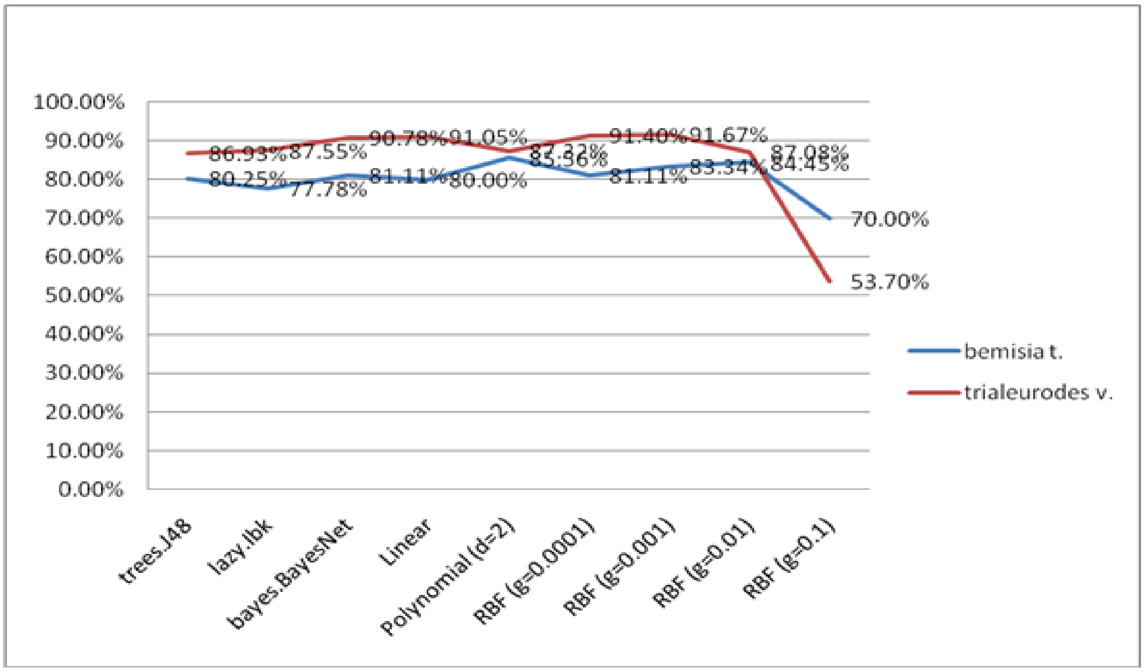

Photo 12. Average of the results in Weka and SVM light with even ratio of the both classes in the training set

CONCLUSION

In this paper, we proposed an algorithm for automatically removing background of the images of *B. tabaci* and *T. vaporariorum* in order to develop an automatic system for distinction. We tested with several classifiers, using the images that we obtained with this method and we presented the results. According the new results, we can conclude that this algorithm has proved as a good solution for automatically cleaning the images. According these tests, we can conclude that these types of problems, where we can make distinction by images is possible if we have big set of sample images. The future work is integrating all of these algorithms into one user-friendly software easy for using and encouraging all scientist to collect images of their work in order to be used in similar manner. We also believe that the database of the whitefly *T. vaporariorum* we created will be useful to the other researchers.

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Review article

POLLUTION IMPACTS OF CIGARETTES BUTTS-A REVIEW

Marija Srbinoska[1], Stefka Kirkova[2], Vesna Radojičić[3], Nermina Đulančić[4]

[1]St. Kliment Ohridski University – Bitola, Scientific Tobacco Institute - Prilep, of Republic of North Macedonia
[2]Tobacco and Tobacco Products Institute, Plovdiv-Markovo, Republic of Bulgaria
[3]University of Belgrade, Faculty of Agriculture, Belgrade, Republic of Serbia
[4]University of Sarajevo, Faculty of Agriculture and Food Science, Bosnia and Herzegovina
e-mail: *srbinoska.marija2014@gmail.com*

ABSTRACT

Annually trillions of cigarettes are smoked worldwide and cigarette butts are the major littered itemin terms of number of pieces released directly into the environment. It is mainly cigarette filter made of cellulose acetate, and unburned tobacco filler. A significant amount of tar containing thousands of organic and inorganic ingredients is trapped in the filter.
Cigarette butts are a significant environmental concern as the chemicals and heavy metals can leach into the soil or water and pose threat to animals and plants, from there they can enter into the food chain as well.
Study attempts the questions have been raised concerning the contaminants that are released from cigarette butts and its impact on the environment, legislation and responsibilities towards environment. Also it attempts to determine the role of the consumers, cigarette companies, authorities and their responsibility for diminishing of pollution. Proposed approaches would have benefit to the more effective protection of the environment.

Key words: Environment; cigarette butts; chemicals; smokers

ВЛИЈАНИЕ НА ОПУШОЦИТЕ ОД ЦИГАРИ ВРЗ ЗАГАДУВАЊЕТО – ПРЕГЛЕД

На годишно ниво се пушат трилиони цигари ширум светот, а отпушоците од цигари се главен извор на отпад според бројот на парчиња одложени директно во околината. Главно се работи за филтерот од цигарите кој е направен од целулоза ацетат и неизгорен тутун. Значителна количина катран кој што содржи илјадници органски и неоргански состојки е заробен во филтерот.
Опушоците од цигари претставуваат значителна еколошки проблем грижа бидејќи хемикалиите и тешките метали можат да преминат во почвата или водата и да претставуваат закана за животните и растенијата, од таму може да влезат и во синџирот на исхрана.
Студија се обидува да покрене прашања во врска со загадувачите што се ослободуваат од отпушоци од цигари и нивното влијание врз животната средина, законодавството и одговорностите кон животната средина.
Истотака, се обидува да ја одреди улогата на потрошувачите, производителите, властите и нивната одговорност за намалување на загадувањето. Предложените приоди ќе придонесат за поефикасна заштита на животната средина.

Клучни зборови:Животна средина; отпушоци од цигари; хемикалии; пушачи

INTRODUCTION

Globally, smoking prevalence — the percentage of the population that smokes has decreased, but the number of cigarette smokers worldwide has increased due to population growth. With 5.8 trillion cigarettes consumed worldwide in 2014, and nine trillion expected by 2025, the global environmental burden of cigarette waste is significant (Eriksen et al. 2015).

Whatever their direct health impact on or benefit to smokers, cigarette filters pose a serious litter and toxic waste disposal problem (Novotny et al. 2009, Novotny and Slaughter 2014).*Discarded* cigarette filter, but or litter is the single most collected item in urban and environmental trash cleanups. An estimated 4.5 trillion of the annual 6.3 trillion cigarettes sold do not end up in a dustbin or ashtray, but are deposited somewhere into the urban and environment area as post-consumption waste. Assuming that each filter weighs 170 milligrams, the weight of all butts discarded annually is about 175 200 tonnes (Novotny et al. 2015). Like other forms of waste, cigarette butts also contribute to greater landfill demands. In addition, when they are littered, they lead to increased costs of municipalities' waste disposal (Barnes 2011).

Many smokers who do not properly dispose of their butts, lighting material, and packaging do not consider their behavior littering (Rath et al.2012). Studies have consistently found that a great majority of smokers litter cigarettes after smoking 76.7% reported by Patel et al. (2013), and 84% by Wilson et al. (2014). The problem has increased in recent years with government legislation for smoking restrictions in public buildings and restaurants forcing smokers outside, where butts are often littered.

Filters are designed to capture various less desirable components of mainstream smoke, with each cigarette brand using different designs (Petraru et al. 2013). The quantity and proportion of chemicals retained in cigarette smokers can be influenced by the tobacco blend, design and selectivity of the filter, and the consumer's smoking habits. The type of filter material will influence the initial chemicals present in the cigarette butt that can potentially be emitted and leached. These chemicals are sourced from agricultural treatments of tobacco plants, uptake from contaminated soils, additives used in the manufacturing process, the attached cellulose acetate filter, and combustion products generated in the process of smoking cigarettes (Novotny et al. 2009).

It has been observed that in recent decades-the cigarette companies have been concerned that aesthetic and environmental concerns related to cigarette butts could contribute to the growing social unacceptability of smoking and regulation that companies responsible for butt disposal (Smith and McDaniel 2011).

Based on the information from the available literaturethere are several strategies to reduce or eliminate the public health and environmental effects of cigarette butt waste have focused on two separate methods: 1) making cigarette butt waste less toxic and persistent, 2) consumer education and responsibility to reducing the number of discarded cigarettes butts and 3) extended cigarette companies responsibility.

Butt litter as an environmental and public health hazard is a relatively new field of study, but recent research and findings provided chemistry-based evidence for the slow degradability of cigarette filters and the harmful presence of their chemicals in the environment.

This study reviews reports on the toxicity of cigarette butts and recommends several approaches to mitigation of this environmental problem and diminished of pollution.

DISCUSSION

With up to two-thirds of every smoked cigarette discarded onto the ground, between 340 and 680 million kilograms of waste tobacco product litters the world each year (WHO, 2017). Cigarette smoke is a complex mixture of gases and submicron-size particulate matter (Harris 1996, Fowles and Dybing 2003, Norman 1999). Cigarette tar, technically the material deposited on a filter when the smoke is passed through, is used as a catch-all term for the particulate components of cigarette smoke, except for alkaloid compounds such as nicotine. Tar is comprised of organic and inorganic compounds, many of which are very toxic (Norman 1999, Eldridge et al. 2015). The increasing popularity of bans on indoor smoking leads to people often smoking outdoors and discard cigarette butts. The discarded cigarette filter may retain many of these potential pollutants that may be leached into the environment and transferred to aquatic organisms, some in the human food chain.

It is very important to increase public awareness about the toxicity and other environmental impacts of discarded cigarette butts.

In areas with substantial amounts of butts, environmental pollutants may arise as nicotine and other chemicals, heavy metals, additives, pesticide residues are leached from the filters and remnant tobacco.

Evidence on environmental pollution due cigarette butts

Cellulose acetate is used in the majority of commercial filter tips for conventional and roll-your own cigarettes. It is synthesized by acetylating cellulose with acetic anhydride in the presence of a catalyst. These fibers, each about 20 μ in diameter, are treated with titanium dioxide and over 12,000 of them are packed tightly together, using triacetin as a binding agent, to create a single filter (Puls et al. 2011, Harris 2011).

Most cigarette filters are surrounded by two layers of paper and rayon wrapping, the porosity of which acts to control the airflow through the filter. Cigarette paper also has many chemicals, including glues to hold the paper together and alkali metal salts of organic acids such as sodium acetate to keep the cigarette burning while smoking (Norman 1999).

Although researchers have confirmed cellulose acetate to be photodegradable UV wavelengths less than 280 nm, there is much debate as to whether it is biodegradable.Exposure to the sun will eventually break the filter down but the source material becomes diluted in water or soil. The titanium dioxide used to whiten filter fibers also functions as a photo oxidation catalyst (Puls et al. 20110.

Initially, scientists also did not consider cellulose acetate to be biodegradable based on early studies that were done with organisms that solely degrade cellulose (Novotny et al. 2009).

But later studies revealed that cellulose acetate is technically biodegradable (Puls et al. 2011, Edgar 2001). Depending on the environmental conditions, filters can resist degradation and only completely disappear after 10 to 15 years.

Many patents exist for enhancing filters' degradations, from increasing the filter's surface area, adding a decomposition accelerating agent to filter fibers to changing the design of cellulose acetate fibre. Some companies have developed biodegradable and compostable cigarette filters using natural fibers like hemp, cotton, and food-grade starch. These alternative filters are intended to help reduce environmental pollution from cigarette butts. Biodegradable and compostable cigarette filters still contain chemical residues from contact with tobacco smoke, and these toxic elements will continue to be released to the environment through combustion or contact with water, or as residue when the filter decomposes (Novotny et al. 2015).

It is not only the synthetic cellulose acetate filter that makes cigarettes an environmental problem. Discarded cigarette butts contain the chemicals, nicotine, pesticides, heavy metals and carcinogens found in cigarettes, and can contaminate the environment and water sources. These chemicals are sourced from agricultural treatments of tobacco plants, uptake from contaminated soils, additives instilled in the manufacturing process, and combustion products generated in the course of smoking cigarettes.

Bonanomi et al. (2015) reported 30–35% mass decomposition of cigarette butts in controlled laboratory conditions took 720 days in grassland soil. The decomposition in sand dune soil was found to be slower.

Green et al. 2014 pointed the littered cigarette butts as source of nicotine in urban waters. The same study also found that the cumulative nicotine release from fifteen consecutive rainfall events with 1.4 mm of precipitation for each event was 3.8 mg of nicotine per g butt, of which 47 % was released during the first event.

Heavy metals from soil, pesticides, insecticides, herbicides, and fungicides are present in tobacco products (Micevska et al.2006).

Moerman and Potts (2011) determined the concentration of Al, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sr, Ti, and Zn from cigarette butts in aqueous solution, including assessment of pH effects and soaking time on metal concentration leached. This research suggests that cigarette butts are potential sources of heavy metal environmental contamination and have the potential to cause acute and chronic harm to various organisms.

Moriwaki et al. (2009)] found that arsenic, nicotine, PAHs, and heavy metals such as cadmium and lead are released into the environment as part of roadside tobacco waste including butts.

Studies show that the chemicals within cigarettes, such as arsenic, nicotine, lead and ethyl phenol, could leach into salt and fresh water and be acutely toxic to aquatic micro-organisms and fish. Marine animals may be especially harmed by nicotine, ethylphenol, and other organic compounds in cigarettes (Micevska et al.2006, Kadir and Sarani 2015).

Researchers examined the toxic effects of smoked cigarette butts (smoked filter and tobacco), smoked cigarette filters (no tobacco), and unsmoked cigarette filters (no tobacco) on to saltwater and fresh water test fish (Slaughter et al.2011). Wright et al. (2015) studied the impacts of smoked cigarette filter toxicants and microfibres on the polychaete worm, a widespread inhabitant of coastal sediments.

Factors affecting selective filtration of mainstream smoke include the material properties and adsorptive capacity of the filters, the volatility and chemical properties of the smoke constituents, and the affinities that the filters and smoke constituents have for each other. The cigarette filters can selective filtered the mainstream smoke, but they do not solve the problem of emission of some chemicals into the environment (Dittrich et al. 2014). Using effective filters the emission rates from cigarette butts into air may be minimal for some heavy chemicals (e.g., metals, tobacco-specific nitrosamines), but may be significant for more volatile chemicals (e.g., nicotine, pyridine, benzene.

There are several potential mechanisms whereby recent smoking could affect responses to air pollutants. Although in most instances the mechanism is inherently reasonable, often direct experimental proof is lacking. The airborne emissions of cigarette butts may be influenced by the cigarette brand, filter material, butt length, environmental temperature, airflow around the cigarette, number of puffs during smoking, and selectivity of the filters.

There a few recent studies about the types of airborne chemicals detected on cigarette filters (Yu et al. 2013, Ji et al.2015). Only some compounds from cigarette smoke that are retained on the discarded cigarette butt are likely to emit into the air. Some compounds can be too volatile to get fully retained in the cigarette filter, and others can get retained but are very unlikely to be emitted from cigarette butts because they are not volatile enough. Petraru et al. (2013) examined the ability of 29 different cigarette filters to retain chemicals, showing that lower molecular weight chemicals breakthrough filters faster.

Compounds with moderate volatility in cigarette smoke like nicotine and PAHs that do get retained in the cigarette butt can potentially be emitted from a cigarette butt after it is disposed, depending on the environmental conditions (temperature, relative humidity, UV intensity, etc.) to which it was exposed.

Consumer education and responsibility

Cigarette butt waste is the last socially acceptable form of littering in what has become an increasingly health and environmental problem. Many smokers who do not properly dispose of their butts, lighting material, and packaging do not consider their behavior littering (Rath et al.2012, Smith and McDaniel 2011, Smith 2011). The problem has increased in recent years with government legislation for smoking restrictions in public buildings and restaurants forcing smokers outside, where butts are often littered.

There are several grass roots organizations and websites addressing the issue of cigarette butt waste. These focus primarily on consumer education and responsibility to dispose of butts properly. However, it is an accepted notion in health behavior science that human behavior changes only slowly if at all unless there are costs, benefits, and social norms to support these changes. Cigarette warning labels informing smokers about the risks of smoking with evidence for the effectiveness of cigarette package warning labels (Hammond et al. 2006).

So, additional package labels and public information about the toxicity of discarded butts may be considered. These would include specific instructions for the safe disposal of the butts and brief information about why this disposal is important. These labels would contribute to public information about butt toxicitywhen they are littered in environment.

Extended responsibility for companies producers of cigarettes

There is increasing awareness of the potential environmental implications of the global scale of discarded cigarette butts, and the World Health Organization's Framework Convention on Tobacco Control provides relevant policy direction. Article 18 Protection of the environment and health of persons could potentially be applied to support prohibition of single-use filters; litigation and economic interventions aimed at recovery of costs of industry misconduct and environmental damages; and to 'innovate, improve and enforce new and existing environmental regulations and agreements' that apply to all stages of tobacco production and post-consumption waste (Harris 1996, Novotny et al. 2015).

Schneider *et al*. (2011) analyze tobacco product litter as an economic issue, with costs of cleanup borne by communities instead of the tobacco companies. Barnes et al. (Rath et al. 2012) describes some important regulatory and environmental principles that should underlie efforts to mitigate cigarette butt waste, including the Precautionary Principle—which states that environmental harm does not have to be proved to justify preventing potential exposures—and Extended Producer Responsibility—which asserts that those who produce a toxic waste product should be held accountable for its cleanup (Curtis et al. 2014, Curtis et al. 2016). As a result of new legislation concerning marketing restrictions and plain packaging, filters are one of the few components left for companies to utilize to differentiate themselves and communicate to the consumer using different sizes, shapes and colors.

The tobacco industry developed program to reduce cigarette litter aims to lower the stigma of smoking, prevent lawmakers from using cigarette butt as a reason to establish further smoking bans, and remove the industry's financial responsibility towards cleaning up cigarette litter (Smith 2011).

Because existing anti-littering laws have not changed smokers' littering habits, Novotny and Slaughter et al. (2011) ask for new environmental interventions and partnerships between tobacco control and environmental groups. They propose litigation to hold the tobacco industry responsible for clean-up costs associated with cigarettes, advocating the use of labels on cigarette packages about the toxicity of discarded butts, and a deposit-return scheme. Other options include requesting the industry to pay an advanced recycling fee or to take back all discarded tobacco waste products.

Several options are available to reduce the environmental impact of cigarette butt waste, including developing biodegradable filters, increasing fines and penalties for littering butts, monetary deposits on filters, increasing availability of butt receptacles, and expanded public education (Curtis et al.2014, Curtis et al.2016). It may even be possible to ban the sale of filtered cigarettes altogether on the basis of their adverse environmental impact.

CONCLUSION

The environment pollutants generated by the cigarette arise from the chemical process of combustion of tobacco and paper, leachates from cigarette filter and remnant tobacco to *cellulose acetate.*

It is found that thestrategies to reduce or eliminate the environmental effects of cigarette butt waste have focused on two approaches: 1) making cigarette butt waste less toxic and persistent, and 2) reducing the number of cigarettes discarded.

The first approach more directly addresses cigarettes as the source of waste. The tobacco industry should enhance the biodegradability of filters, decreasing overall toxicity of cigarettes, avoid introduction of harmful chemicals other than those that are really necessary to maintain the current manufacturing technology.

Authorities need to develop the strategy which includes anti-butts messages on all packaging and advertisements, adding additional package labels and public information about the toxicity of discarded butts, and placement and maintenance of outdoor ashtrays in areas where smokers gather. In addition it is important that smokers' littering behavior be modified to decrease this source of pollution.

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Original scientific paper

TOBACCO PRODUCTION FOCUSED ON THE RESPONDENTS - PRODUCERS AND NON - PRODUCERS OF TOBACCO

Silvana Pashovska

"St. Kliment Ohridski University – Bitola, Scientific Tobacco Institute - Prilep, of Republic of North Macedonia
e-mail: silvana.pasoska@uklo.edu.mk

ABSTRACT

Tobacco production in Republic of North Macedonia is completely oriented to the oriental aromatic types.
The question about replacing the tobacco with another crop that would provide the same or even higher level of income is often subject to analysis and discussion in the scientific circles in the field of agriculture and among the people who are practically involved in this area. The answer is that the possibility of such a replacement is limited because of the lack of alternative employment and due to the unsuitable natural conditions for having another type of agricultural production.
The use of the x^2 - test confirms the basic hypothesis of the research, which is that the prospective production of tobacco will depend on the determination and will of tobacco producers.

Key words: tobacco production, strategy, prospect, world trends, economic indicators

ПОГЛЕДИ ЗА ПРОИЗВОДСТВОТО НА ТУТУН СО ФОКУС НА ИСПИТАНИЦИ - ПРОИЗВОДИТЕЛИ И НЕПРОИЗВОДИТЕЛИ НА ТУТУН

Производството на тутун во Република Северна Македонија е целосно ориентирано кон ориенталните ароматични типови.
Прашањето за замена на тутунот со друга култура што би обезбедило исто или уште поголемо ниво на приход, честопати е предмет на анализа и дискусија во научните кругови од областа на земјоделството и кај луѓето кои се практично вклучени во оваа област. Одговорот е дека можноста за ваква замена е ограничена поради недостаток на алтернативно вработување и поради несоодветни природни услови за да се има друг вид земјоделско производство.
Употребата на x^2 - тестот ја потврдува основната хипотеза на истражувањето, а тоа е дека потенцијалното производство на тутун ќе зависи од определбата и волјата на производителите на тутун.

Клучни зборови: производство на тутун, стратегија, перспектива, светски трендови, економски индикатори

INTRODUCTION

Tobacco production and its social and economic significance are a source of existence, engagement and income of a great part of the population, as well as income of the country made by export. In the past decade, the World Health Organization made efforts to reduce the tobacco-planted surfaces via the Framework Convention on Tobacco Control, but it is successful only in the developed member states of the EU, while in the other parts of the world the situation is not such. There, the production maintains a stable level. This Framework Convention is directed towards solving some important global problems such as: illegal trade with tobacco and tobacco products, control of harmful ingredients in cigarettes and smoke of tobacco, retail and wholesale and international trade.

The Republic of Macedonia does not foresee measures for the reduction of tobacco production because of the sensibility and because of the social and economic aspects; therefore this issue is to be solved in the future, after the potential entrance of Macedonia in the EU when the tobacco production plans shall be harmonized with the EU rules (Poposki, 2015)

Tobacco production in Macedonia in the past few years is about 25.000 tons per year the realistic increase of which greater human resources are needed that in the following period are rather uncertain (aging of the population and youth migration in cities and abroad). There will always be threats such as the different events on the foreign market, the competition coming from the neighbouring countries producing oriental tobaccos (Turkey, Greece and Bulgaria) and from other countries from the Far East (FAOSTAT 2019/2020). The expansion of some new tobacco products that do not depend a lot on the production of tobacco in the field, such as the so called electronic cigarettes and similar products also pose a real threat.

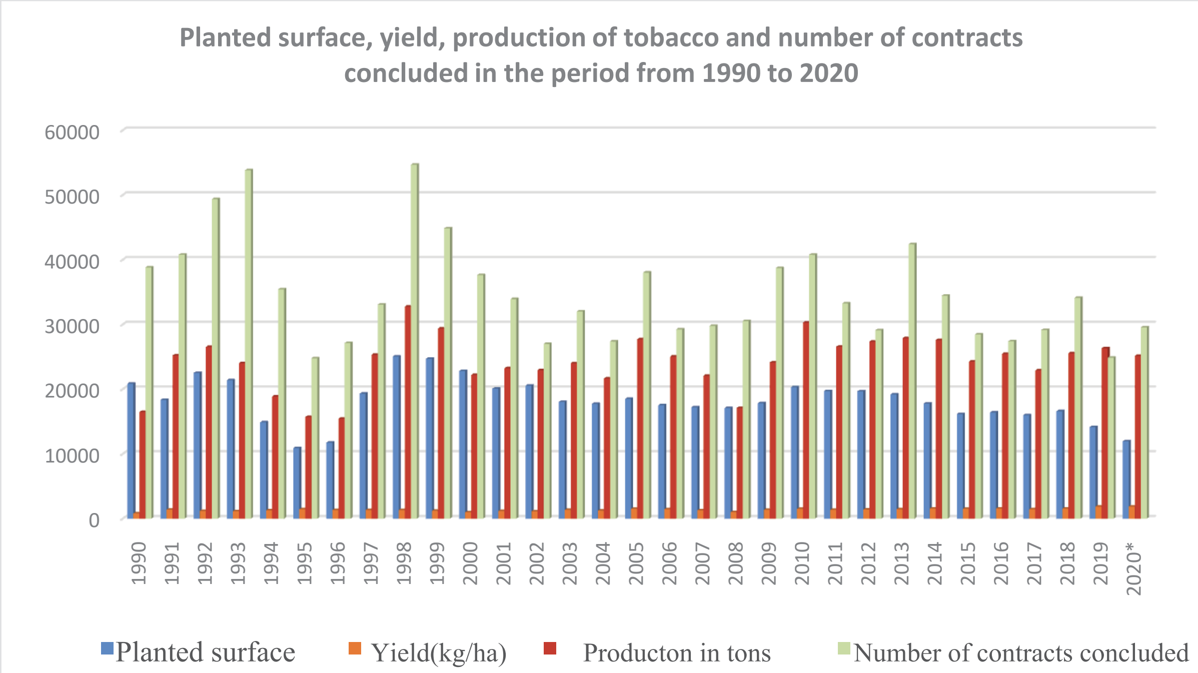
RESULTS AND DISCUSSION

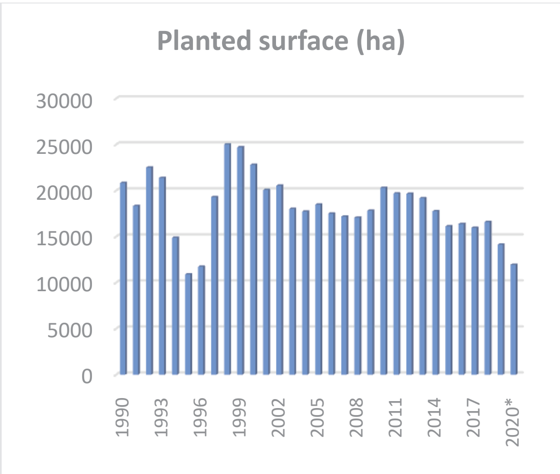
The production of tobacco is has a very important role in the economy of North Macedonia both from economic and form social point of view. The types of tobacco that are being cultivated are of oriental type, mainly the type Prilep, Jaka and Basmak. The average of the tobacco produced in the Republic of North Macedonia in the period from 1990 to 2020 is 24.115 tons (Table 1), with an average yield of 1.861 kg/ha. At first sight it seems that it is an insignificant part of the foreign trade relations of the country, but one can understand its real value when we take into consideration that the total export in 2019 was 22.493 tons of oriental tobacco. The importance of the tobacco for the Macedonian economy is even more obvious when compared with the total export of agricultural products in 2015 in which its share was more than 22%. All activities and processes related to the production of this plant are regulated with the new Law on tobacco, tobacco products and similar products (“Official Gazette of the Republic of North Macedonia” no. 98/19 and 27/20). In our country, as is the case everywhere, the tobacco production varies in separate years. In the past year Macedonia is placed at the 30th place regarding the amount of produced tobacco (30th in 2014, 30th in 2015 and 28th in 2016). According to the data of the State Statistical Office and of the Ministry of Agriculture, Economy and Water Economy, the production, state with the planted surface in hectares and the yield (kg/ha) in the period from 1990 to 2020, as well as the production and the concluded contracts is as stated below:

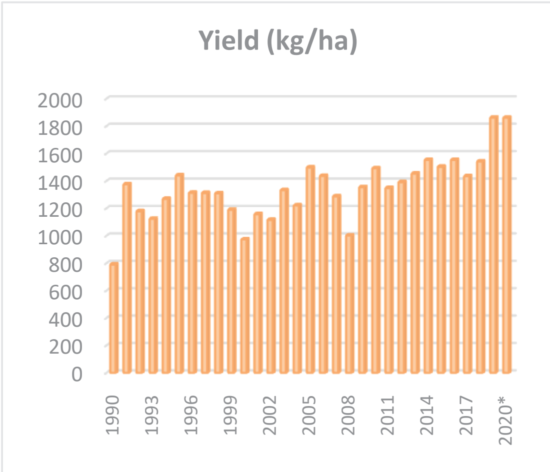
Table 1. Planted surface, yield, production of tobacco and number of concluded contracts in the period from 1990 to 2020

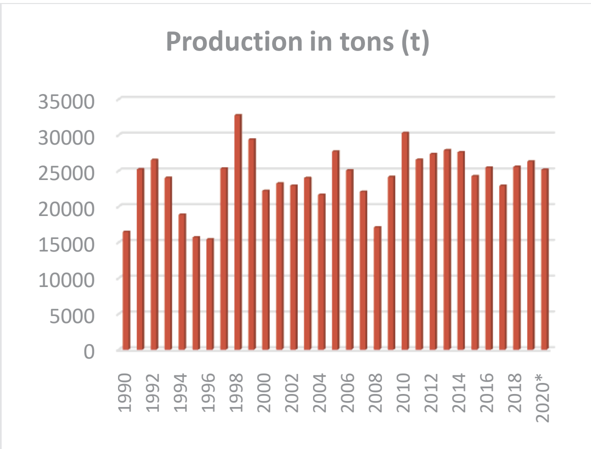
No.	Year	Planted surface	Yield (kg/ha)	Production in tons (t)	Number of oncluded contracts
1	1990	20825	790	16452	38809
2	1991	18324	1375	25195	40750
3	1992	22497	1178	26502	49348
4	1993	21373	1123	24002	53809
5	1994	14864	1269	18862	35416
6	1995	10891	1440	15683	24752
7	1996	11738	1313	15412	27110
8	1997	19290	1312	25308	33050
9	1998	25016	1309	32746	54661
10	1999	24700	1189	29368	44822
11	2000	22790	973	22175	37617
12	2001	20067	1157	23217	33906
13	2002	20530	1116	22911	26971
14	2003	18008	1332	23986	32000
15	2004	17715	1221	21630	27343
16	2005	18485	1498	27691	38000
17	2006	17507	1436	25036	29230
18	2007	17183	1287	22056	29771
19	2008	17064	1001	17087	30519
20	2009	17809	1354	24122	38710
21	2010	20300	1492	30280	40743
22	2011	19693	1348	26537	33234
23	2012	19656	1391	27333	29090
24	2013	19178	1453	27859	42367
25	2014	17758	1553	27578	34445
26	2015	16128	1503	24237	28454
27	2016	16379	1553	25443	27380
28	2017	15961	1434	22885	29132
29	2018	16582	1541	25547	34104
30	2019	14127	1861	26295	24854
Total		552.438	39.802	723.435	1.050.397
Average		18.415	1.327	24.115	35.013
31	2020*	11936	1861	25131	29531

* The information about the purchased amounts in 2020 are not definitive, the factual data are still waited for, the data of the amounts of tobacco bought by the Ministry of Agriculture, Forestry and Water Economy are used in this table









The data shown in the tables and on the charts give a certain global image of the movement of tobacco production in our country in the period of three decades. Regarding the planted surface it can be seen from the data that tobacco production in our country is stable even though there are some oscillations in given years and it varies within the limit of 18.415 hectares. In the past years a reduction is perceive and the limit is about 14.127 ha. As far as the production is concerned it also has certain oscillations and is about 24.115 tons. Changes are seen in the number of contracts concluded that has decreased compared to the first years and now, the average of the analysed period is 35.013 concluded contracts. In the past year this number is relatively stable. The last component- the yield per hectare has grown significantly, i.e. from 790 kg/ha in 1990 it increased to 1327 kg/ha. This shows that tobacco production follows the global trends since there is an increase of the yield as well, so our country is gradually getting closer to the global average of 1800 kg/ha (Ministry of Agriculture, Forestry and Water of R.N.Masedonia).

If the Republic of North Macedonia does not become member of the EU, then, having into consideration the current policy of direct payments of the primary tobacco production, the production would maintain the current level with a possibility of constant increase. The amount of 30.000 tons tobacco can be reached without any significant investments because we have land, human resources, experience and tradition at our disposal. It can be even stated that the amount of bought tobacco of about 30.000 tons in the past has been reached on several occasions. To have higher production greater human resources are needed which in the following period cannot be said with certainty that would be easy to achieve because of the demographic movements (aging of the population and migration of young people in the cities and abroad).

Having into consideration what was stated above, every national economy must commit itself to building a coherent and comprehensive vision for the development of the agriculture and for rural development, or, more precisely, for creating a good strategy for the development of agriculture in every segment, including as well the production of tobacco. This means that the primary focus should be put on institutional development, on the new access to organizational and managerial work and on the technical and technological changes and innovations. Technological development and innovations continue to be important for the development of agriculture and rural development, but they must be supported by the development of suitable structures for the management of that sector. In other words, it is necessary to design, implement and constantly review the set measures of the economic and agricultural policy which are essential for the support of the investments in agriculture such as:

- Maintaining healthy and stable macroeconomic and trade policies that support investments in agriculture;
-Improvement of the functioning of the input markets for production factors (labour, agricultural land and capital) and outputs on local, national and international level;
-Strengthening and improvement of the qualities of human capital via proper education and access to productive resources;
-Establishing strong institutional environment that improves the access to markets provides dissemination of information defines standards and provides suitable legal and regulative framework;
-Providing research services that develop productive technologies;
-Improving the marketing, the transport and communication infrastructure for the support of timely access of agricultural producers to seasonal and long-term capital and production inputs, as well as provision of strong support via the price policy of agricultural products and
-Protection of the natural resources and of the environment.

Starting from what was previously stated; we can state that the agricultural policy is an essential part of the economic policy of every country that reflects the existing economic system, the achieved degree of social and economic development, of the disposability of production factors, of the existing development strategies and external factors (global economic flows, tendencies and development).

The strategy for the development of agriculture and agriculture policy is a system of legal, economic, political, social, technical and other character that is undertaken by the competent governmental bodies and institutions in order to develop the agriculture in all of its elements. The objectives set in the strategy determine the methods, instruments and mechanisms for achieving the objectives of the agricultural development in short, medium and long term. Within the clearly defined strategy, the priorities for the development and the activities holders must be sorted out in order for them to be successfully realized. It is understandable for the strategy adopted to be subject to changes, to be modified and to be adjusted to the needs with the aim of successfully achieving the planned objectives and tasks in the development of agriculture. The success of the agricultural policy and strategy depends on the complexity and on the consistency of the measures that have been planned and their proper implementation.

Because of its specificities it is necessary for the agricultural to be specially treated in the economic policy. It should contain instruments and mechanisms of active assistance for strengthening the agricultural sector, its modernization and suitable protection that will enable timely and quality procurement of the domestic producers and production for export.

Well-made strategy for development and promotion of the agriculture is an instrument that determines the role and place of agriculture in the society- the economic development of every country (Gilligan, Wilson, 2012) . Therefore, the choice of measures and methods to achieve the objective set in the agricultural policy; its proper realization and consistency are of utmost importance in the development regardless whether they are direct or indirect, short-term or long-term. It can be concluded that the agricultural strategy and policy, as system of measures of the country, is an important part of the overall economic policy and as such it is a fundamental factor for permanent development and for increasing the social and individual wellbeing in every national economy.

EMPIRICAL RESEARCH

With the objective to learn the opinions for further production of tobacco in the Republic of North Macedonia a survey was conducted within two groups of respondents:

1. *Non-producers of tobacco,* i.e. inactive producers and
2. *Producers of tobacco,* i.e. active producers of tobacco

The survey, as a method of research, was conducted via questionnaires (provided at the end of the text) including 70 persons in the first group and 93 persons in the second group.

To have a more comprehensive analysis of the data obtained from the empirical research, a basic hypothesis was previously formed which reads as follows: **the future production of tobacco will depend on the determination and will of the tobacco producers.**

The natural, social and global preferences in this field would be undoubtedly respected.

As a support of the general hypothesis the following additional hypotheses were imposed:

1. More extensive positive thinking of the respondents (producers and non-producers of tobacco) about the production of tobacco in order to obtain more com-

prehensive analysis of the data obtained from the empirical research, it has been previously formed

2. Planning of tobacco production by tobacco producers and beyond.
3. Thoughts about the possibilities to develop tobacco production in a family (and more extensive) business

The results of the research, which are given below, give a possibility to comment the feasibility or non-feasibility of the basic hypothesis that has been set and that reads as follows: ***the future production of tobacco will depend on the determination and will of the tobacco producers.***

Initially, the results obtained from the questionnaires have been processed, summarized and then they were presented in the table and in individual graphs.

The x^2 test and the contingency coefficient were used in the processing and presentation of data with the objective to determine the relation between the variables that are in the interest of this research[1]. The x^2 test and the contingency coefficient were used in the processing and presentation of data with the objective to determine the relation between the variables that are in the interest of this research. In this empirical research the frequencies are distributed in two columns and three rows which results in 2 degrees of freedom. The table value of x^2 for 2 degrees of freedom and significance threshold of 0,05 is 5,991 (p - level of probability). The summary of the processed results obtained from the conducted survey are given below.

1 More information on the x^2 – test can be found in the text of prof. Trajche Miceski, (2010) Health statistics, UGD- Faculty of Economics, Shtip, page 154-191

Table 2. Summarized review of the answers of non-producers-inactive producers and producers of tobacco- active producers of tobacco

Questions	*Offered answers*	*Individual answers*			
		Non-producers, inactive producers of tobacco		**Producers, active producers of tobacco**	
		Value	*%*	*Value*	*%*
1. **Do you think that in the future period tobacco will be produced in our country?**	Yes	**39**	**56%**	**63**	**68%**
	No	**9**	**13%**	**9**	**10%**
	No answer	**22**	**31%**	**21**	**23%**
Total		**70**	**100%**	**93**	**100%**
					The calculated x^2-test is = 3,070 C = 0,123
2. Do you plan to produce tobacco?	Yes	9	13%	63	**68%**
	No	39	56%	13	**14%**
	I might	22	31%	17	**18%**
Total		**70**	**100%**	**93**	**100%**
					The calculated x^2-test is = 65,846 C = 0,498
3. Do you think that there are possibilities to develop production of tobacco into a family (or more extensive) business?	Yes	36	51%	**59**	**63%**
	No	9	**13%**	**22**	**24%**
	No answer	25	**36%**	**12**	**13%**
Total		70	**100%**	**93**	**100%**

The calculated x^2-test is = 15,153
C = 0,265

The first question dedicated to both groups (non-producers- inactive producers and producers of tobacco- active producers of tobacco) was: *1. Do you think that in the future period tobacco will be produced in our country?*

Table 3. The answers and the processed data of the respondents

Questions	Offered answers	Individual answers			
		Non-producers, inactive producers of tobacco		Producers, active producers of tobacco	
		Value	%	Value	%
1. Do you think that in the future period tobacco will be produced in our country?	Yes	39	56%	63	68%
	No	9	13%	9	10%
	No answer	22	31%	21	23%
Total		70	100%	93	100%
					The calculated x^2-test is = 3,070 C = 0,123

It can be seen from the table 3 that this question referring to the future of production, i.e. whether the respondents think that tobacco will be produced in the future in our country the first group of respondents, i.e. non-producers- inactive producers of tobacco- responded in the following manner: 56% gave positive answer, 13% gave negative answer and 31% gave no answer, while the second group of respondents- active producers of tobacco responded in the following manner: a high percentage of 68% see future of the tobacco production in Macedonia, 10% gave negative answer and 23% gave no answer. It can be clearly seen that more than 50% of the two groups of respondents- non-producers of tobacco, i.e. inactive producers and producers of tobacco, i.e. active producers of tobacco for a long time- stated that they think that tobacco will continue to be produced in the Republic of North Macedonia. Consequently, the calculated value of the x^2 test is 3,070 which is under the tabular value (5, 991) which shows almost identical answers in the two different groups of respondents. It is certain that these statements are due to the tradition and the practice in this field. This shows that the main hypothesis (the future production of tobacco will depend on the determination and will of the tobacco producers) has been confirmed, as has been the additional hypothesis related to this question and whether a positive answer will be given when it comes to the production of tobacco in our country.

The second question was also dedicated to the two groups (non-producers- inactive producers of tobacco and producers- active producers of tobacco) and it reads as follows: 2. *Do you plan to produce tobacco?*

Table 4. The answers of the respondents

Questions	Offered answers	Individual answers			
		Non-producers, inactive producers of tobacco		Producers, active producers of tobacco	
		Value	%	Value	%
2. Do you plan to produce tobacco?	Yes	9	13%	63	68%
	No	39	56%	13	14%
	I might	22	31%	17	18%
Total		70	100%	93	100%
					The calculated x^2-test is = 65,846 C = 0,498

It can be seen in the table 4 that this question refers to whether the respondents plan to produce tobacco for which the first group of respondents, i.e. the inactive tobacco producers answered in the following manner: 13% gave positive answer, 56% gave negative answer while 31% gave no answer, while a high 68% of the respondents of the second group, i.e. tobacco producers, stated that they plan to produce tobacco, 14% do not plan to produce tobacco and 23% gave no answer.

It can be seen from the second question that both groups of respondents- non-producers of tobacco, i.e. inactive producers and producers of tobacco- active tobacco producers for a long period of time, have given different answers, i.e. more than 68% of the tobacco producers think that they will continue producing tobacco. Conversely, only 13% of the non-producers of tobacco think that there is a possibility for them to continue producing tobacco.

The calculated value of the x^2 test is 65,846 and it falls behind the tabular value (5,991) which shows a difference, i.e. opposite answers given by the two groups of respondents. These statements are a result of their activity until now. There is not joint confirmation of the main hypothesis nor of the additional hypothesis for the readiness for tobacco production on behalf of the two groups. This means that only active tobacco producers plan to continue producing tobacco.

The third question dedicated to the two groups (non-producers- inactive tobacco producers and producers of tobacco- active tobacco producers) reads as follows: Do you think that there are possibilities to develop production of tobacco into a family (or more extensive) business?

Table 5. The answers of the respondents

Questions	Offered answers	Individual answers			
		Non-producers, inactive producers of tobacco		Producers, active producers of tobacco	
		Value	%	Value	%
3. Do you think that there are possibilities to develop production of tobacco into a family (or more extensive) business?	Yes	36	51%	59	63%
	No	9	13%	22	24%
	No answer	25	36%	12	13%
Total		70	100%	93	100%
					The calculated x^2-test is = 15,153 C = 0,265

From the table above (table 5) it can be seen that this question referring to the possibility to develop tobacco production as a family business was answered positively by 51% of the first group of respondents, i.e. non-producers- inactive tobacco producers, while 13% of them gave negative answer and 36% gave no answer, while a high 63% of the second group of respondents, i.e. tobacco producers- active tobacco producers see possibility for the tobacco production to be developed as a more extensive family business, while 24% of them gave negative answer and 13% gave no answer.

This third question shows that the two groups of respondents- non-producers- inactive tobacco producers and tobacco producers- active tobacco producers, have similar, but not fully identical answers, i.e. over 63% of the respondents falling in the tobacco producers group think that there are possibilities to develop the tobacco production into family (or more extensive) business. Furthermore, 51% of the respondents who are not tobacco producers, i.e. inactive tobacco producers think that there are possibilities to develop tobacco production into a family (or more extensive) business.

It is certain that the answers are a result of the traditional tobacco production that has been present for so long and the perceptions about the possibilities for developing a family business.

The calculated value of the x^2 test is 15,153 and it is beyond the tabular value (5,991) which shows that the answers of the two groups of respondents are not identical. These statements are due to their activity until now. There is not joint confirmation of the main hypothesis nor is of the additional hypothesis referring to the opinions about the possibilities for the development of tobacco production into family (or more extensive) business.

Additionally, the calculated values of the x^2 test show that there is a need of training for everyone who wants to work in or to have a business related to tobacco production in which they see their future existence and a possibility to make profit.

CONLCUSION

The analysis of tobacco production through the amount of produced tobacco, the planted surface, number of contracts concluded with tobacco producers and the yield for a period of thirty years, i.e. from 1990 to 2020, as well as the empirical research that has been conducted point to the fact that in the Republic of North Macedonia there are conditions for development and prosperity of tobacco production that, first and foremost, depends on the determination and will of the tobacco producers.

In this context it is important to point out that in this case the state and agricultural policy implemented by providing assistance and stimulation for the tobacco producers, via organizing trainings and educational workshops for raising the education level in this field, via creating conditions for turning small tobacco producers into a more extensive family business, via providing satisfactory prices for the purchased tobacco, via offering support to the associations of tobacco producers and via timely delivery of information for all events, changes and legal acts in this area are of great importance. . This kind of activity and support provided by the state will give inactive tobacco producers or those who have not ever worked in the field of tobacco production a stimulation to start thinking about directing their work towards this production that offers a lot of benefits for the producers. The survey that has been conducted shows that the greatest part of the people, who did not work in the field of tobacco production, does not see their future in this field which creates room to stimulate this part of the population to be interested in tobacco production by offering simulative measures and providing better working conditions.

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QUESTIONNARE for NON-PRODUCERS OF TOBACCO

Dear all,

This questionnaire was prepared for scientific and research objectives. It is anonymous and gives you an opportunity to freely and realistically express your opinions.
We would like to kindly ask you when answering the questions to circle one of the provided answers.

1. Do you think that in the future period tobacco will be produced in our country?
а) Yes
б) No
в) No answer

2. Do you plan to produce tobacco?
а) Yes
б) No
в) I might.

3. Do you think that there are possibilities to develop production of tobacco into a family (or more extensive) business?
а) Yes
б) No
в) No answer

Thank you for your cooperation

QUESTIONNARE for PRODUCERS OF TOBACCO

Dear all,

This questionnaire was prepared for scientific and research objectives. It is anonymous and gives you an opportunity to freely and realistically express your opinions.
We would like to kindly ask you when answering the questions to circle one of the provided answers.

1. Do you think that in the future period tobacco will be produced in our country?
а) Yes
б) No
в) No answer

2. Do you plan to produce tobacco?
а) Yes
б) No
в) I might

3. Do you think that there are possibilities to develop production of tobacco into a family (or more extensive) business?
а) Yes
б) No
в) No answer

Thank you for your cooperation

INSTRUCTIONS TO AUTHORS

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Since the publication is of an international character, all manuscripts should be submitted in English. Authors whose native language is not English should have their papers checked by research workers from the related fields who have good proficiency in the English language. All manuscripts must be proofread prior to submission. Language and style of the manuscripts are responsibility of the author.

The publication presents: original scientific papers, review articles, short reports, professional papers and other works related to tobacco science and practice.

Original scientific papers - should contain original scientific research results, previously unpublished. It must be presented in a manner enabling the experiment, i.e. research method, to be repeated and accuracy of the analysis, results and conclusions confirmed.

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Manuscripts should follow the format INTRODUCTION, MATERIAL AND METHODS, RESULTS AND DISCUSSION AND CONCLUSION, for experimental research where events are presented in chronological order.

Titles in the text (INTRODUCTION, MATERIAL AND METHODS, RESULTS…) should be centered, boldfaced, written with capital letters, font size 12;

Subtitles should be written with initial capital letter, boldfaced, 12-point font size, aligned to the center;

Arrangement of the paper:

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Full name and surname of the authors - capital initial letter, other letters small, font size 12, centered; Name of the institution - for multiple authors from different institutions, each author's surname should be followed by identifying superscript number associated with the appropriate institution.

Address of the institution - full postal address of the institution, as well as the e-mail of the corresponding author; italic, centered.

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Key words - up to 7 essential words, in English and Macedonian

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

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

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

All graphical presentations (including graphs, schemes, drawings, photographs etc) should be submitted on CD together with the text and saved as separate files (graphs should be prepared as Excel files - XLS extension, and schemes, drawings and images should be submitted as .JPG or .TIF files). Minimum resolution for images is 200 - 300 dots per inch.

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Russel E. W., 1973. Soil conditions and plant growth. 10th ed., Longman, London.
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Tobacco Institute 7500 Prilep Kicevska bb
Republic of Macedonia
E-mail: tobacco_institute_prilep@yahoo.com

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