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DIALLEL CROSSES TRIAL – THE BASIS FOR DETECTION OF RESISTANCE TO DISEASES IN TOBACCO

Ana Korubin–Aleksoska[1], Zlatko Arsov[2], Gordana Miceska[1], Biljana Gveroska[1], Jane Aleksoski, Žarko Bebić

[1]Scientific tobacco institute-Prilep, Kicevska bb, Prilep, University of St. Kliment Ohridski, Bitola, Republic of Macedonia (Contact: anakorubin@yahoo.com)
[2]Faculty of Agricultural Sciences and Food, University of Ss. Cyril and Methodius, Bul. Aleksandar Makedonski bb, Skopje, Republic of Macedonia

ABSTRACT

Investigations were made with ten varieties of tobacco types : Prilep (P- 23 , P- 76 , P- 66-9/7 , P-84), Yaka (YK 10-7/1), Djebel (Xanthe, XDj-M), Basmak (MB-3), Samsun (SM-1), Sirdili (SM-LL) and Virginia (MV-1) and their 45 diallel crosses for resistance to diseases, with an emphasis on black shank - *Phytophthora parasitica var. nicotianae*. The trial with parental genotypes and their hybrids was set up in 2011, 2012 and 2013 at the Experimental field of the Scientific Tobacco Institute - Prilep in randomized block design with three replications. Traditional agricultural practices were applied during the growing season. The resistance/susceptibility degree was estimated according to a scale recommended by FAO.

The aim of this paper is the detection of resistance to black shank and creation of resistant lines, using diallel analysis to obtain a knowledge on the genetics of this disease.

The highest resistance to the disease was recorded in YK 10-7/1 and SM-LL, while the varieties MV-1 and P-76 showed to be the most susceptible. The highest resistance in the diallel was recorded in the crosses where one of the parents was YK 10-7/1, which indicates a possession of dominant gene of resistance. In the process of breeding, the method of Back-cross hybridization was used in order to increase the varieties resistance to the black shank disease.

Keywords: tobacco (*Nicotiana tabacum L.*), diallel crosses, Back-cross hybridization, resistance, black shank (*Phytophthora parasitica var. nicotianae*).

ОПИТ СО ДИЈАЛЕЛНИ КРСТОСКИ – БАЗА ЗА ОТКРИВАЊЕ ОТПОРНОСТ НА БОЛЕСТИ КАЈ ТУТУНОТ

Испитувани се десет сорти од типовите: Прилеп (П-23, П-76, П-66-9/7, П-84), Јака (ЈК 10-7/1), Џебел (Ксанти XDj-M), Басмак (МБ-3), Самсун (SM-1), Sirdili (SM-LL) и Вирџинија (МВ-1) и нивните 45 дијалелни крстоски за отпорност на болестите на тутунот со посебен акцент на црнилката - *Phytophthora parasitica var. nicotianae*. Опитот со родителските генотипови и нивните хибриди беше поставен на опитното поле при Научниот институт за тутун – Прилеп по случаен блок-систем во три повторувања во 2011, 2012 и 2013 година. Во текот на вегетацијата беа применети вообичаени агротехнички мерки. За проценката на степенот на резистентност односно сензибилност користевме скала пропишана од ФАО.

Целта на овој труд е откривање на отпорност кон црнилката, како и добивање на отпорни линии, а со дијалелната анализа и добивање сознанија за генетиката на болеста.

Прворангирани сорти отпорни на болеста се ЈК 10-7/1 и SM-LL, додека најголема осетливост покажаа МВ-1 и П-76. Највисока резистентност во дијалелот покажаа крстоските каде еден од родителите е ЈК 10-

7/1 што укажува поседување на доминантен ген за отпорност. За облагородување на сортите во насока на зголемување на отпорноста кон црнилката го користевме методот Повратно вкрстување (ВС).

Клучни зборови: тутун (*Nicotiana tabacum L.*), дијалелни крстоски, повратно вкрстување, отпорност, црнилка (*Phytophthora parasitica var. nicotianae*).

INTRODUCTION

Tobacco, like all other crops, is attacked by many diseases, parasites and pests. The lack of their control can lead to reduction in yield and quality and even to destruction of the entire crop. Nowadays there is a range of products for successful treatment of many diseases and control of pests, parasites and weeds. The most important law in nature, however, is the law of survival - all organisms tend to stay alive. This is performed through occurrence of new races of the pathogen - the causing agent of the disease or new individuals immune to the products for protection on one side and though creation of new resistant crops on the other.

Beside tobacco, which is dangerous to smokers health, the residues of the pesticides further increase the risk of diseases that threaten and destroy smokers life. Therefore, chemical products should be used carefully and properly as a precaution in the production and release of resistant varieties (Dimitrieski et al., 2012).

The purpose of this paper is to detect the resistance to economically important diseases in a trial with tobacco varieties and diallel crosses and to create new resistant lines. The same scheme can be applied in many other crops for various diseases.

Diallel crossing is applied in selection primarily for creation of hybrids and varieties with better yield and quality than the existing ones (Korubin-Aleksoska, 2003), but one replication in the trial with parents and F1 hybrids can be set up on infected area or infestation can be made with pathogen of the disease, which will help to detect resistance among some parents and their hybrids. Diallel crossing provides maximum number of combinations to be made for each parent, by which accurate information on the inheritance of resistance can be obtained.

MATERIAL AND METHODS

Investigation material included 10 varieties representing different types of tobacco: Prilep (P-23 , P-76 , P-66-9/7, P-84), Yaka (YK 10-7/1), Djebel (Xanthe, XDj-M), Basmak (MB-3), Samsun (SM-1), Sirdili (SM-LL) and Virginia (MV-1). In July and August 2010 were made diallel crossing and obtained seed from 45 F1 hybrids (J. Aleksoski). The trial with parental genotypes and their F1 hybrids was set up at an area of 1471,5 m² in the Experimental field of Scientific Tobacco Institute - Prilep, using a randomized block design with three replications. The oriental parents and their hybrids were arranged in three rows per replication, with 34 plants in a row (spacing: 15 cm x 45 cm). The large-leaf parent and its hybrids were arranged in four rows per replication, the parent with nine plants (spacing : 60 cm x 90 cm) and hybrids with 15 plants in a row (spacing: 35 cm x 90 cm). The third replication was set up in a plot infected with black shank disease. Infestation was done with pathogens of powdery mildew (*Erysiphe cichoracearum*), blue mold (*Peronospora tabacina*) and wildfire (*Pseudomonas tabaci*). The results presented in this paper, however, are focused only on black shank disease - *Phytophthora parasitica var. nicotianae*.

For assessment of the resistance/ susceptibility degree of plants we used

a scale recommended by FAO: 0 - no information, 1 – immune, 2 - highly resistant, 3 to 4 – resistant, 4 to 6 – semi susceptible, 7 – moderately susceptible, 8 – susceptible and 9 - highly susceptible. This scale can be changed depending on the disease and crop and it is quite applicable in breeding, because each of its variants is adapted and internationally accepted.

Parental genotypes

(Order - according one-way diallel)

Samsun SM-1 – sun-cured, oriental, aromatic tobacco, brought in Tobacco Institute – Prilep from Turkey. The plant has cylindrical-elliptic habitus, with average stalk height of 85 cm and 25-30 sessile leaves (16 cm x 9 cm). Floral bud is semispherical, with light pink flowers. Cured leaves are gentle and elastic, golden yellow and orange in color, characterized by intensive and specific aroma. Dry mass yield ranges 1000 kg/ha (Fig. 1).

Virginia MV-1 (authors: D. Cavkaroski, M. Uzunoski – 1987) - variety of the type Virginia (flue-cured, large-leaf tobacco). The plant has conical (haystach-shaped) habitus, with average stalk height of 195 cm and 26-29 sessile leaves (55 cm x 35 cm). Floral bud is brushing, loose, cup-shaped, with pale pink flowers. Found both in male-sterile and fertile form (Korubin-Aleksoska, 2004). The middle belt dry leaves are golden-yellow in color. They are characterized by good elasticity, water retention and filling capacity, pleasant taste and aroma. Dry mass yield ranges 2500-3500 kg/ha (Fig. 2).

Yaka YK 10-7/1 (author: A. Korubin – Aleksoska – 2010) – variety of the type Yaka (sun-cured, oriental tobacco).The plant has cylindrical habitus, with average stalk height of 105 cm and 50-60 sessile leaves (17,5 cm x 9 cm). Floral bud is semispherical, with pale pink flowers. Cured leaves are with golden yellow color, characterized by pleasant sweetish taste and intensive specific aroma. Dry mass yield ranges 2500 kg/ha (Fig. 3).

Prilep P-23 (authors: K. Nikoloski, M. Mitreski, 2001) – variety of the type Prilep (sun-cured, oriental tobacco). The plant has a conical (fir tree-shaped) habitus, with average stalk height of 65 cm and 45-50 sessile leaves (20 cm x 10,5 cm). Floral buds are relatively small, dense and semispherical, with pale pink flowers. Cured leaves are golden yellow and the upper ones are light orange, elastic, rich in substance, with poorly defined nervation. They are characterized by an intense and specific aroma (Korubin-Aleksoska, 2004). Dry mass yield ranges 2000-2500 kg/ha (Fig. 4).

Prilep P-76 (authors: D. Cavkaroski et al. - 1987) – variety of the type Prilep (sun-cured, oriental tobacco).The plant has elliptic-conical habitus, with average stalk height of 90 cm and 59 sessile leaves (23 cm x 11,5 cm). Floral bud is dense and semispherical, with white to pale pink flowers. Cured lower leaves are yellow, middle leaves are orange and the upper ones reddish orange, characterized by specific aroma (Korubin-Aleksoska, 2004). Growth period from planting to flowering is 85-95 days (late maturing variety). Dry mass yield ranges 3500-4000 kg/ha (Fig. 5).

Basmak – MB-3 (authors: group of breeders from Tobacco Institute – Prilep and Faculty of Agricultural Sciences and Food - 2010) – variety of the type Basmak (sun-cured, oriental, aromatic tobacco). The plant has cylindrical habitus, with average stalk height of 70 cm and 35-45 sessile leaves (19 cm x 9,7 cm). Floral bud is semispherical, with light pink flowers. Cured lower leaves are yellow-orange and the upper ones red-orange in color. They are characterized by intensive specific aroma. Dry mass yield

ranges 2000-2500 kg/ha (Fig. 6).

Prilep P-66-9/7 (authors: M. Dimitrieski, G. Miceska, A. Siskoski – 2001) – variety of the type Prilep (sun-cured, oriental tobacco).The plant has elliptic habitus, with average stalk height of 80 cm and 45-55 sessile leaves (18 cm x 9 cm). Floral bud is dense and semispherical, with pale pink flowers. Cured lower leaves are yellow and the upper ones are reddish to orange, characterized by intensive specific aroma. Dry mass yield ranges 3000-3500 kg/ha (Fig. 7).

Sirdili, SM-LL – oriental variety of tobacco. It has a cup-like habitus, with average stalk height of 55 cm and 33 elongated sessile leaves (27,5 cm x 6 cm). Floral bud is semi-spherical and sessile in apical leaves, with white-pink flowers. Cured leaves are characterized by pleasant specific aroma. Dry mass yield ranges 1200-1500 kg/ha (Fig. 8).

Xanthe-Djebel, XDJ-M - variety of the type Djebel (sun-cured, oriental, aromatic tobacco). The plant has elliptic habitus, with average stalk height of 65 cm and 17 sessile leaves (17 cm x 8,4 cm), with oval shape and slightly curved tip. Floral bud is loose, with pale pink flowers. Growth period from planting to flowering is 40-45 days (early maturing variety). Cured leaves are golden yellow to light red in color. They are characterized by pleasant specific aroma. Dry mass yield ranges 500-700 kg/ha (Fig. 9).

Prilep P-84 (authors: K. Naumovski, A. Korubin – Aleksoska - 1988) - variety of the type Prilep (sun-cured, oriental tobacco). The plant has cylindrical to oblong-elliptic habitus, with average stalk height 65 cm; 38-42 sessile leaves (20 cm x 10 cm). Floral bud is medium large, semispherical, with pale pink flowers. Cured lower leaves are yellow, middle leaves are orange and the upper ones are red orange in color. They are characterized by specific aroma. Dry mass yield ranges 2500-3200 kg/ha (Fig. 10).

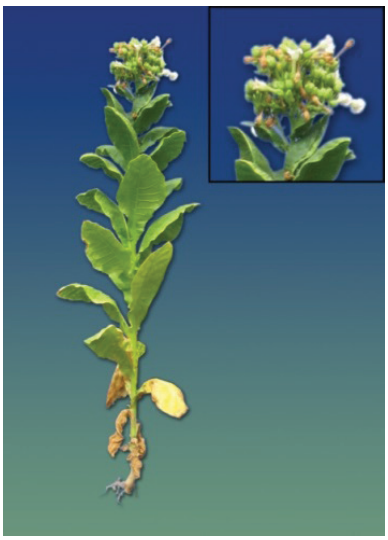

Fig. 1. Samsun, SM-1

Fig. 2. Virginia MV-1

Fig. 3. Yaka JK 10-7/1

Fig. 4. Prilep P-23

Fig. 5. Prilep P-76

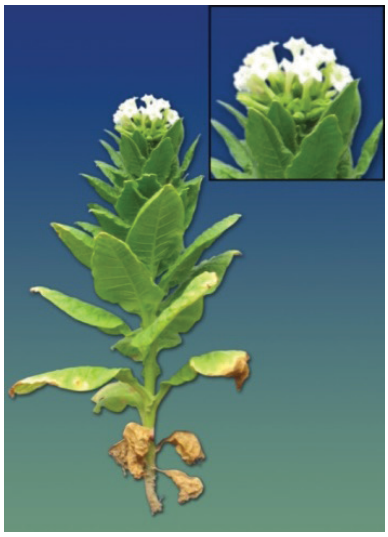

Fig. 6. Basmak MB-3

Fig. 7. Prilep P-66-9/7

Fig. 8. Sirdili, SM-LL

Fig.9. Xanthe-Djebel, XDJ-M

Fig. 10. Prilep P-84

Diallel crosses

(Obtained from J. Aleksoski)

SM-1 x MV-1, SM-1 x YK 10-7/1, SM-1 x P-23, SM-1 x P-76, SM-1 x MB-3, SM-1 x P-66-9/7, SM-1 x SM-LL, SM-1 x XDJ-M, SM-1 x P-84, MV-1 x YK 10-7/1, MV-1 x P-23, MV-1 x P-76, MV-1 x MB-3, MV-1 x P-66-9/7, MV-1 x SM-LL, MV-1 x XDJ-M, MV-1 x P-84, YK 10-7/1 x P-23, YK 10-7/1 x P-76, YK 10-7/1 x MB-3, YK 10-7/1 x P-66-9/7, YK 10-7/1 x SM-LL, YK 10-7/1 x XDJ-M, YK 10-7/1 x P-84, P-23 x P-76, P-23 x MB-3, P-23 x P-66-9/7, P-23 x SM-LL, P-23 x XDJ-M, P-23 x P-84, P-76 x MB-3, P-76 x P-66-9/7, P-76 x SM-LL, P-76 x XDJ-M, P-76 x P-84, MB-3 x P-66-9/7, MB-3 x SM-LL, MB-3 x XDJ-M, MB-3 x P-84, P-66-9/7 x SM-LL, P-66-9/7 x XDJ-M, P-66-9/7 x P-84, SM-LL x XDJ-M, SM-LL x P-84, XDJ-M x P-84

RESULTS AND DISCUSSION

Black shank is a very serious tobacco disease, first identified in 1893 on the islands Java and Sumatra (Fig.11). In the United States it occurred in 1915 on large-leaf tobacco. Later it was observed in some countries of Africa, South America and Europe. In Bulgaria it was first observed in 1928, in Greece in 1975, in Montenegro in 1982 and in Macedonia in 1983 (A. Korubin-Aleksoska, 1989).

Inoculation

A replication of the diallel trial with parental genotypes and F1 hybrids was set up in previously infected soil and additional inoculation with fungus culture was applied by irrigation of the stalk base (Sanches - Monge 1974, Bonnet 1985). This method seems to be the most acceptable because it is cheap, fast and reliable. The inoculum was prepared from stalks of diseased plants (a mixture of the pathogen and optimum amount of water).

Table 1. Assessment of the resistance level in parental genotypes

Parental genotypes	Percentual representation of the disease	Grade (FAO scale)	Rank
1. Samsun, SM-1	48, 5	4 - 6	6
2. Virginia, MV-1	99,5	9	10
3. Yaka, YK 10-7/1	0	0	1
4. Prilep, P-23	72,5	7	8
5. Prilep, P -76	85.3	9	9
6. Basmak, MB-3	59,7	4 - 6	7
7. Prilep, P-66-9/7	19,2	1	3
8. Sirdili, SM-LL	11,7	1	2
9. Xanthe-Djebel, XDJ-M	35,5	3 - 4	4
10. Prilep, P -84	46.8	4 - 6	5

Table 2. Assessment of the resistance level in diallel F1 crosses

A				B			
F1 crosses	Disease (%)	Grade (FAO)	Rank	F1 crosses	Disease (%)	Grade (FAO)	Rank
1. SM-1 x MV-1	64,9	4 - 6	35	24. YK 10-7/1 x P-84	4,9	0	9
2. SM-1 x YK 10-7/1	1	0	2	25. P-23 x P-76	86,5	8	44
3. SM-1 x P-23	61,5	4 - 6	33	26. P-23 x MB-3	70,5	7	37
4. SM-1 x P-76	72,6	7	39	27. P-23 x P-66-9/7	23,3	2 - 4	21
5. SM-1 x MB-3	78,8	8	42	28. P-23 x SM-LL	9,5	0	14
6. SM-1 x P-66-9/7	22,5	2 - 4	20	29. P-23 x XDJ-M	69,8	7	36
7. SM-1 x SM-LL	8,7	0	12	30. P-23 x P-84	57,5	4 - 6	31
8. SM-1 x XDJ-M	40,1	2 - 4	25	31. P-76 x MB-3	73,4	7	40
9. SM-1 x P-84	45.3	4 - 6	27	32. P-76 x P-66-9/7	25,2	2 - 4	23
10. MV-1 x YK 10-7/1	1,4	0	3	33. P-76 x SM-LL	5,9	0	10
11. MV-1 x P-23	79,2	8	43	34. P-76 x XDJ-M	64,3	4 - 6	34
12. MV-1 x P-76	89,5	9	45	35. P-76 x P-84	60,7	4 - 6	32
13. MV-1 x MB-3	71,3	7	38	36. MB-3 x P-66-9/7	25,5	2 - 4	24
14. MV-1 x P-66-9/7	24	2 - 4	22	37. MB-3 x SM-LL	12	1	15
15. MV-1 x SM-LL	9	0	13	38. MB-3 x XDJ-M	48,3	4 - 6	28
16. MV-1 x XDJ-M	57	4 - 6	30	39. MB-3 x P-84	50,5	4 - 6	29
17. MV-1 x P-84	75,5	7	41	40. P-66-9/7 x SM-LL	6,5	0	11
17. YK 10-7/1 x P-23	2	0	6	41. P-66-9/7 x XDJ-M	16,5	1	19
19. YK 10-7/1 x P-76	0,5	0	1	42. P-66-9/7 x P-84	12,4	1	16
20. YK 10-7/1 x MB-3	3,5	0	8	43. SM-LL x XDJ-M	15,2	1	18
21. YK 10-7/1 x P-66-9/7	1,5	0	4	44. SM-LL x P-84	12,8	1	17
22. YK 10-7/1 x SM-LL	2,5	0	7	45. XDJ-M x P-84	41,5	2 - 4	26
23. YK 10-7/1 x XDJ-M	1,9	0	5				

Fig. 11. Phytophthora parasitica Dast. var. nicotianae Breda de Haan - Black shank (crnilka)

Gene – for – gene relationship

The results in Table 4 indicate vertical resistance to Phytophthora parasitica var. Nicotianae in YK 10-7/1. This type of resistance may be determined by a single gene – monogene or several genes - oligogenes with a strong effect, so called major genes. This situation is present when the pathogen does not contain virulence genes. The disease occurs when the pathogen contains additional virulence genes and the plant does not have resistance genes. This is defined as gene - for - gene relationship, which results in specific resistance to certain races of the pathogen (Korubin - Aleksoska, 1989). This situation was defined by Flor (1971), and it denotes that for each pair of resistance or susceptibility specific genes in the host there is a corresponding pair of virulence or avirulence specific genes inside the pathogen.

Pedigree of tobacco variety Yaka YK 10-7/1

The Yaka variety YK 10-7/1 was created by crossing of Yaka YV 125/3 and the Djebelian variety Pobeda 2 (authors: M. Palakarcheva and D. Bajlov). Pobeda 2 is originating from the wild species *Nicotiana debneyi* and a variety of Basma tobacco. *Nicotiana debneyi* is a wild species originating from Australia that blooms throughout the growing season. It brings resistance to many diseases, one of which is the black shank.

Breeding for obtaining the resistance to black shank disease

The highest resistance in the diallel trial was observed in YK 10-7/1 and the lowest resistance, i.e. the highest susceptibility to black shank was observed in MV-1.

In order to increase the MV-1 resistance to black shank the Back-cross hybridization method was used (E. Sanchez-Monge, 1974). Cultivar YK 10-7/1 (resistant to the

pathogen) was used as a mother and MV-1 (susceptible to the pathogen) as a father. After seven successive back-crossings with MV-1 and one self-fertilization of heterozygous individuals (Aa), the susceptible ones (aa) were eliminated, the heterozygous (Aa) were avoided and selection was made with the homozygous resistant (AA) individuals, which phenotypically resemble MV-1 and carry dominant genes for resistance to the disease (Fig. 12).

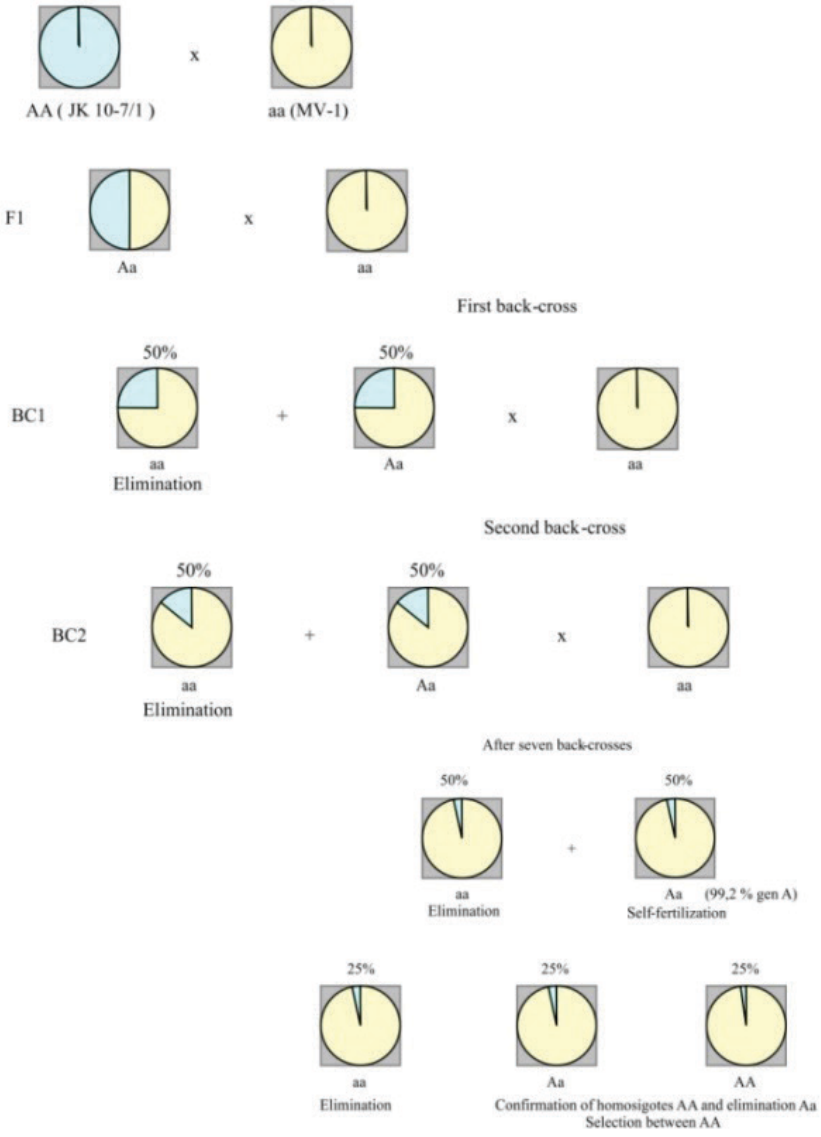

Fig. 12. Developing a resistance to Black shank disease (Phytophthora parasitica var. nicotianae) in tobacco by the use of Back-cross hybridization (E. Sanchez-Monge, 1974).

CONCLUSION

Based on the results of our investigations on detection and selection of disease-resistant tobacco cultivars and F1 hybrids from their diallel crosses, the following conclusions can be drawn:

- A trial with parents and diallel F1 crosses is used for obtaining hybrids

and creation of new superior cultivars; it also offers a possibility for detection of resistant genotypes. The diallel consists of maximum number of combinations which can be obtained among parental genotypes and the diallel analysis will give us the knowledge on the mode of inheritance of characters investigated.

- Among parental genotypes, the first ranked cultivar with resistance to *Phytophthora parasitica var. nicotianae* was YK 10-7/1, and it was followed by Sirdili SM-LL. The highest susceptibility to the disease was recorded in Virginia MV-1 (large-leaf) and in P-76 (oriental).
- The highest resistance to *P. parasitica* in the diallel was recorded in the crosses where one of the parents was YK 10-7/1, indicating a possession of dominant genome for resistance.
- The method of back-cross hybridization was applied to increase the cultivars resistance to black shank. Cultivar YK 10-7/1 (resistant to the pathogen) was used as a mother and MV-1 (susceptible to the pathogen) as a father. After seven successive back-crossings with MV-1 and one self-fertilization of heterozygous individuals (Aa), the susceptible ones (aa) were eliminated, the heterozygous (Aa) were avoided and selection was made with the homozygous resistant (AA) individuals, which phenotypically resemble MV-1 and carry dominant genes for resistance to the disease.
- The scheme for obtaining resistance to *Phytophthora parasitica var. nicotianae* can be applied in many other crops for various diseases, when it reffers to ”vertical (specific) resistance”.

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Original Scientific paper

HYBRIDOLOGICAL ANALYSIS OF THE INHERITANCE OF CHEMICAL COMPOSITION IN VIRGINIA TOBACCO (*NICOTIANA TABACUM L.*) CROSSES

Yovko Dyulgerski

Institute of Tobacco and Tobacco Products (ITTP), Markovo, Bulgaria
(e-mail: yovko_dulg@abv.bg)

ABSTRACT

Investigations were made on the degree of dominance, heterosis and inheritability of chemical composition in Virginia tobacco plants. For that purpose, P_1, P_2, F_1 and F_2 populations of six crosses of introduced Virginia tobaccos were studied. It was found that the inheritance of nicotine and sugar contents is overdominat, incompletely dominant or additive, and that of total nitrogen and proteins was overdominant or incompletely dominant. The direction of inheritance is toward the parent with higher levels of investigated trait. Only the inheritance of total nitrogen content is both from the parent with lower values and from those with higher values. There are high values for heritability coefficient of nicotine, suggesting that genetic factor is a crucial determinating factor for this trait. Therefore, the selection of this trait is effective in early generations. The contents of sugar, total nitrogen and protein showed low values of heritability coefficient, suggesting higher efficiency of selection in later generations.

Keywords: Virginia tobacco, heritability, inheritance, nicotine, sugars, total nitrogen, proteins.

АПСТРАКТ

Истражувани се степенот на доминантност, хетерозисот и наследувањето на хемискиот состав кај некои сорти тутун од типот вирџиниј а. За таа цел, проучувани се P_1, P_2, F_1 и F_2 генерациите кај шест крстоски од интродуцирани вирџиниски сорти. Утврдено е дека во наследувањето на содржината на никотин и шеќери се јавува супердоминантност, парцијална доминантност и адитивност, а во наследувањето на вкупниот азот и протеините преовладуваат супердоминантноста и парцијалната доминантност. Наследувањето е во насока на родителот со повисок степен на истражуваното својство. Само вкупната содржина на азот се наследува и од родителите со пониски вредности и од оние со повисоки вредности. Постојат високи вредности за коефициентот на наследување на никотинот, што укажува на тоа дека генетскиот фактор е клучен одредувачки фактор за ова својство. Затоа, селекцијата на својството е ефикасна во почетните генерации. Коефициентот на наследувањена содржината на шеќери, вкупен азот и протеини има ниски вредности, што укажува на поголема ефикасност на селекцијата во подоцнежните генерации.

Клучни зборови: вирџиниски тутун, херитабилност, наследување, никотин, шеќери, вкупен азот, протеини.

INTRODUCTION

The chemical composition of tobacco is a major quality trait (Davis and Nielsen, 1999; Dimitrieski et al., 2006; Tso, 1988). The most important chemical indicators in Virginia tobacco are nicotine, total nitrogen, sugars and proteins (Kirkova, 2005; Stoilov

et al., 2002). The most important of these is the role of nicotine (Stoilov et al., 2002; Nikolov et al., 2004).

The use of genetic analysis of these indicators will improve the efficiency of the selection process. There are few studies in this field worldwide (Lukrapov, 1958; Matzinger and Wernsman, 1968; Vandenberg 1970; Povilaitis 1971; Korubin-Aleksoska, 2001; Dagnon and Dimanov, 2007). They reveal that in F_1 hybrids inheritance of nicotine is most often negative, as the main type is overdominant and intermediate with a negative sign (Stankev and Trancheva, 1989). Overdominant positive inheritance was observed less often (Manolov, 1979, Nikolov et al., 2004). The literature also refers to additive inheritance of nicotine. In sugars, additive type of inheritance is the most common (Nikolov et al., 2004; Bing-Guang et al., 2005).

The purpose of this study is to apply hybridological analysis to determine the character and extent of gene interactions, inheritance and the number of genes that differ in initial parental forms, inheritability coefficient and the expressions of heterosis and transgression regarding the chemical composition of Virginia tobacco in terms of their use in the selection of this type of tobacco.

MATERIAL AND METHODS

Investigations included P_1, P_2, F_1 and F_2 populations of six crosses, along with the introduced Virginia tobacco varieties: Hybrid 714 (K 730 x K 254), Hybrid 715 (K 730 x K 358), Hybrid 719 (RG 8 x K 358), Hybrid 725 (K340 x K 358), Hybrid 726 (K 358 x NC 729) and Hybrid 727 (K 358 x K 254). The trial was carried out in the Experimental field of the ITTP in Markovo in the period 2007-2011.

The contents of nicotine, sugars, total nitrogen and proteins were estimated using the arithmetic mean ($\bar{x}$), error of the arithmetic mean ($S\bar{x}$), degree of dominance (d/a) using Mather's formula (Mather,1985), occurrence of heterosis (HP) according to Omarov (1975). The method of Sobolev (1976) was used for estimation of: occurrence of transgression (Tn), number of genes by which parental forms differ (N), heritability coefficient (h^2) and coefficient of genotypes selection efficiency in phenotypic expression of the trait (Pp).

RESULTS AND DISCUSSION

Inheritance of nicotine content is overdominant, incompletely dominant and additively dominant depending on the cross (Table 1). The direction of inheritance is toward the parent with higher values. The number of genes determining the expression of the trait in all crosses varies within narrow limits - from 3 to 5.

Heterosis occurrence is variable and depends on the cross. Strong positive heterosis was observed in Hybrid 715 (K 730 x K 358) and Hybrid 726 (K 358 x NC 729), where its values reached 23-24%. Relatively high values were observed in Hybrid 719 (RG 8 x 358 K). Hybrid 727 (K 358 x K 254) has a weak presence of negative heterosis. Coefficients of transgression depend on the manifestations of heterosis and show that from the available homozygous genotypes of the decaying generations in Hybrids 715 and 726, plants can be selected which will exceed the nicotine rate of the parents by 0.8%.

Medium to high heritability coefficients were found, especially in Hybrid 719 (RG 8 x K 358). The most important role in

determining this trait has the genotype and the role of environment is weaker. In this case, the selection of the desired trait can start in earlier generations.

Table 1.Data on the inheritance of nicotine content

Parent/Cross/Index	P_1	P_2	F_1	F_2	d/a	HP	Тн	N	h^2	Pp
Hybrid 714 (K 730 x K 254)	2,6	2,2	2,7	2,4	1,5	103,8	-0,02	3,23	0,56	0,471
Hybrid 715 (K 730 x K 358)	2,6	2,5	3,2	3,0	13	123,1	0,78	4,64	0,66	0,588
Hybrid 719 (RG 8 x K 358)	2,1	2,5	2,8	2,8	0,3	112	0,34	3,60	0,75	0,683
Hybrid 725 (K340 x K 358)	2,4	2,5	2,6	2,4	0,1	104	0,01	4,49	0,47	0,451
Hybrid 726 (K 358 x NC 729)	2,5	1,9	3,1	2,9	0,3	124	0,83	3,51	0,52	0,570
Hybrid 727 (K 358 x K 254)	2,5	2,2	2,4	2,8	0,33	96	-0,07	4,84	0,60	0,532

The inheritance of sugar content is monogenic-overdominat or incompletely dominant, while in Hybrid 726 (K 358 x NC 729) it is additive (Table 2). The direction of inheritance is always toward the parent with higher values and in this case it is favorable. Significant heterosis effects were observed in hybrids 715 and 727 and especially in Hybrid 725 (K340 x K 358). The coefficients of transgression show that from the available homozygous genotypes of the decaying generations in Hybrids 715 and 725, plants can be selected which will exceed the percentage of sugars in the parents by over 1%.

Relatively low values of the heritability coefficient were observed, especially in Hybrid 719 (RG 8 x 358 K), where it was less than 30%. In determination of this trait, environment has a more important role. In this case, the selection of sugars may start in later generations.

Table 2. Data on the inheritance of sugars content

Parent/Cross/Index	P_1	P_2	F_1	F_2	d/a	HP	Тн	N	h^2	Pp
Hybrid 714 (K 730 x K 254)	14,6	15,4	15,6	15,3	0,2	101,3	0,061	1,32	0,350	0,413
Hybrid 715 (K 730 x K 358)	14,6	15,8	17,3	17,1	1,5	109,5	1,053	1,52	0,323	0,382
Hybrid 719 (RG 8 x K 358)	16,4	15,8	16,8	16,5	2,3	102,4	0,037	1,28	0,294	0,467
Hybrid 725 (K340 x K 358)	14,8	15,8	18,1	17,9	2,8	114,6	1,343	1,19	0,402	0,655
Hybrid 726 (K 358 x NC 729)	15,8	17,2	17,3	17,2	0	100,6	0,003	1,25	0,417	0,530
Hybrid 727 (K 358 x K 254)	15,8	15,4	16,8	16,9	6	106,3	0,887	1,13	0,345	0,428

Total nitrogen content is most often inherited with incomplete dominance, only in Hybrid 726 (K 358 x NC 729) it is overdominant. The direction of inheritance is toward the parent with higher nitrogen content, except for Hybrid 725 (K340 x K 358), which is dominated by the parent with lower values. In this case, more factors are responsible for determination of the investigated trait - from 6 to 12.

Significant heterotic effect was observed in all crosses and in Hybrid 725 (K 340 x K 358) it was with a negative sign. It achieved very high values (over 35%) in Hybrid 726 (K 358 x NC 729). Heterotic effect can be used in the selection of Virginia tobacco both to increase and to reduce the nitrogen content. The coefficient of transgression depends on occurrence of heterosis and it also has significant values.

Lower values were recorded for the coefficient of heritability in all crosses. In

this case, the coefficient of efficiency of the selection shows that it may start in later generations.

Table 3. Data on the inheritance of total nitrogen content

Parent/Cross/Index	P_1	P_2	F_1	F_2	d/a	HP	Тн	N	h^2	Pp
Hybrid 714 (K 730 x K 254)	1,4	1,9	2,2	1,9	0,3	115,8	0,661	8,17	0,272	0,365
Hybrid 715 (K 730 x K 358)	1,4	1,7	2,1	2,2	0,4	123,5	0,783	6,38	0,369	0,470
Hybrid 719 (RG 8 x K 358)	1,6	1,7	2,0	1,8	0,3	117,6	0,650	10,62	0,285	0,288
Hybrid 725 (K340 x K 358)	2,0	1,7	1,8	1,7	-0,33	90	-0,57	7,48	0,290	0,423
Hybrid 726 (K 358 x NC 729)	1,7	1,6	2,3	2,2	13	135,3	1,021	9,10	0,402	0,481
Hybrid 727 (K 358 x K 254)	1,7	1,9	2,3	1,9	0,4	121,1	0,771	11,64	0,342	0,396

Inheritance of protein content is overdominant or incompletely dominant, with preponderance of the former. The direction of inheritance is always toward the parent with higher values (Table 4). No variation was observed in the number of genes determining the sign - they are 2 or 3. In all crosses significant heterotic effect was observed and for hybrids 714 and 727 it was more than 20%. Heterosis can be successfully used to increase the protein content in Virginia tobacco. Coefficients of transgression were also significant in all crosses and show that, depending on the cross, selected generations can exceed the protein content of the parents by 0.5 to 1.2%.

The values of heritability coefficient in all crosses were insignificant. In this case, the influence of environment in phenotypic expression of the trait is very high. As with total nitrogen content, the effect of the selection will occur in later generations.

Table 4. Data on the inheritance of proteins content

Parent/Cross/Index	P_1	P_2	F_1	F_2	d/a	HP	Тн	N	H^2	Pp
Hybrid 714 (K 730 x K 254)	5,2	5,4	6,5	6,2	1,1	120,4	0,929	2,23	0,181	0,351
Hybrid 715 (K 730 x K 358)	5,2	6,1	6,7	6,6	0,75	109,8	0,677	2,35	0,242	0,326
Hybrid 719 (RG 8 x K 358)	6,2	6,1	6,9	6,7	15	111,3	0,684	2,77	0,196	0,347
Hybrid 725 (K340 x K 358)	5,1	6,1	6,6	6,5	2	108,2	0,514	2,42	0,156	0,297
Hybrid 726 (K 358 x NC 729)	6,1	6,3	7,2	7,2	0,9	114,3	0,812	3,06	0,153	0,380
Hybrid 727 (K 358 x K 254)	6,1	5,4	7,4	7,0	4,71	121,3	1,236	2,24	0,268	0,322

CONCLUSIONS

1. The inheritance of nicotine and sugar contents was overdominant, incompletely dominant or additive, and that of total nitrogen and proteins was overdominant or incompletely dominant. The direction of the inheritance of nicotine, sugars and protein is toward the parent with higher value, and that of total nitrogen goes both toward the parent with higher and to the one with lower value.
2. The number of genes influencing the expression of the investigated traits by which parental forms are distinguished is small and varies negligibly.
3. Manifestations of heterosis and transgression in significant values were found in all chemical indices.
4. Medium to high heritability coefficients were found for the content of nicotine and

low to negligible values for sugars, total nitrogen and proteins. The efficiency of selection in the content of nicotine will be higher in earlier generations, and for sugars, total nitrogen and proteins in later generations.

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PRODUCTIONAL CHARACTERISTICS OF SOME ORIENTAL VARIETIES OF BASMAK TOBACCO

Karolina Kočoska

St. Kliment Ohridski University - Bitola - Scientific Tobacco institute - Prilep,
Kicevski pat bb, 7500 Prilep
e-mail: karolina_kocoska@yahoo.com

ABSTRACT

Basmak tobacco is included in Macedonian production of oriental tobacco. This type is distinguished by its high quality raw material, typical for the oriental tobaccos, and is highly demanded in world market. Field trials were conducted in Scientific Tobacco Institute - Prilep from 2009 to 2011 with YK 7 - 4/2 as a check (ø) and three Basmak varietiesMK-1, MB-2 and MB-3. The highest values for dry tobacco yield per stalk and per unit area were recorded in MB-3 variety (11.60 g/stalk and 2140 kg/ha). The Basmak varieties showed higher average purchase price and yield per hectare compared to the check. They also achieved higher gross income, the highest value of which was recorded in MB-3 variety (336 075 denars/ha).
The aim of the paper is to give a complete view on productional characteristics of investigated Basmak varieties and to enable their easier implementation in mass production of this tobacco type.

Keywords: tobacco, variety, Basmak, yield, gross income

ПРОИЗВОДНИ СВОЈСТВА НА НЕКОИ ОРИЕНТАЛСКИ СОРТИ ОД ТИПОТ БАСМАК

Во вкупното произботство на ориентalски тип тутун во Македонија регистрирано е и производство на типот басмак. Суровината од овој тип тутун е со висок квалитет, карактеристична за ароматичен ориентalски тип тутун, кој како таков е доста баран на странскиот пазар. При Научниот институт за тутун – Прилеп од 2009 до 2011 година беше поставен опит каде беа вкучени 4 сорти, и тоа: ЈК 7 - 4/2 како контрола (ø), и басменските сорти МК – 1, МБ – 2 и МБ – 3. Приносот на сув тутун по страк и единица површина највисок е кај сортата МБ-3 (11,60 g/страк и 2140 kg/ha). Повисоката просечна откупна цена и принос по хектар кај басменските сорти во споредба со контролата покажаа и повисок бруто паричен приход, кој е највисок кај сортата МБ-3 (336 075 ден./ha).
Целта на овој труд беше да се даде комплетна слика за производните својства на испитуваните басменски сорти, а со тоа да се овозможи нивна полесна имплементација во производство на овој тип тутун.

Клучни зборови: тутун, сорта, басмак, приноси, бруто паричен приход.

INTRODUCTION

Yield and quality of the obtained raw from Basmak tobacco meet the criteria and quality standards of many manufacturers and tobacco purchase companies from these

areas. The taste of smokers is changing and so are the requirements of manufacturers for particular components of oriental tobacco raw used in fabrication of blend cigarettes. Therefore, each year the foreign buyers are offered different types and varieties to meet the requirements in terms of aroma, taste and other tobacco characteristics. Basma is well known and highly appreciated oriental tobacco, primarily grown in Greece and Turkey. However, after the dramatic decline in production of this tobacco in these two countries (especially in Greece), tobacco companies see a possibility to shift a part of this production in the Republic of Macedonia, in areas with favorable soil and climate conditions. In order to make this production more attractive to manufacturers, the purchase price for this tobacco is somewhat higher compared to other oriental tobaccos. The fact that there are practically no problems with exports of Basma tobacco raw is additional motif for production of newly created varieties of this type that will be interesting for farmers, processors, manufacturers, wholesalers etc. The most similar to Basmak tobacco by its morphological characteristics is the check variety YK 7-4/2.

MATERIAL AND METHODS

Investigations were carried out in 2009, 2010 and 2011 with the standard variety YK 7-4/2 as a check (ø) and three Basma varieties (MK-1, MB-2 and MB-3).

The seedling was produced in traditional way at the field of Scientific Tobacco Institute - Prilep, in cold beds covered with polyethylene. The trials were performed with 5 g seed/10 m^2, applying all necessary cultural practices and protective measures. After one autumn and two spring ploughings of soil, the trial was set up in randomized blocks with 5 replicates, at 45 × 12 cm planting density on previously prepared soil. The area of the main plot was 9 m^2 and the useful plot area was 6.16 m^2. The number of rows in each plot was 5 (3 were used for harvest and 2 as protective shield). The number of plants in a row was 42 (38 stalks for harvest and 4 protective). Harvesting and stringing of leaves were performed manually in 7 primings in the stage of technical maturity, followed by sun-curing on horizontal frames. Qualitative assessment of cured tobacco after ironing was done according to the "Criteria for qualitative and quantitative assessment of raw tobacco leaf" (Official Gazette of the Republic of Macedonia, February 12, 2007). Corrected yield per stalk and hectare, the average price for 1 kg dry tobacco and gross income were statistically processed by analysis of variance and tested with LSD method (Najceska, 2002).

RESULTS AND DISKUSSION

Dry tobacco yield per stalk and per unit area

Dry tobacco yield per stalk depends on genetic potential of the variety, soil and climate conditions and applied agricultural practices. The yield per hectare is closely related to the yield per stalk and, along with quality, it is a visual indicator for assessment of economic value of the variety.

Results of the three year-investigations (Table 1, Figure 1) reveal that the yield ranges from 7.01 g/stalk in YK 7-4/2 (∅) in 2011 to 11.98 g/stalk in MB-3 variety in 2010. Compared to the check, highly significant difference of 1 % was observed in MB-3 (2009, 2010 and 2011), MK-1

and MB-2 (2010 and 2011). In 2009, no significant difference was observed in variety MB-2, whereas MK-1 showed significant difference at 5 % level.

The average yield in the investigation period ranged from 7.77 g/stalk in YK 7-4/2 (∅) to 11.60 g/stalk in MB-3 and, expressed in percentage, it was 50.08 % higher compared to the check. Bogdanceski et al., (1991) reported a yield of 9.8 g/stalk or 1959 kg/ha. Data for the yield per hectare (Table 2, Figure 2) reveal the lowest value in the check YK 7-4/2 (1294 kg/ha) in 2011, and the highest in MB- 3 variety (2210 kg/ha) in 2010. High significant difference of 1 % compared to the check was recorded in varieties MB-3 in 2009, 2010 and 2011 and in MK-1 and MB-2 in 2010 and 2011. In 2009, significant difference of 5 % was recorded in MK-1 variety, and the variety MB-2 showed no such difference. The average yield per hectare for the three years of investigation ranged from 1433 kg/ha in YK 7-4/2 (∅) to 2 140 kg/ha in MB-3, which is 49.34 % higher than the check. Basma varieties are characterized by lower yields. (Nuneski, 2008), reported that the yield in variety Izmir Basma (Turkey) ranges from 600 to 1000 kg/ha. Dimov, (2011) stated that the yield of Djebel Basma , Djebel Basma 12 and Djebel Basma 13 varied from 1600 to 1900 kg/ha, depending on the agroclimatic conditions and applied cultural practices. Comparison between yields (g/stalk and kg/ha) obtained in our investigation and the available literature data reveals that the region of Prilep has suitable soil and climate conditions for production of Basma tobacco.

Table 1. Tobacco yield in g/stalk

Variety	Year	Yield, g/stalk	Difference		Average, 2009-2011	Difference 2009-2011		Rank
			Absolute	Relative		Absolute	Relative	
	2009	8.07	/	100.00				
YK 7-4/2 ∅	2010	8.22	/	100.00	7.77	/	100.00	4
	2011	7.01	/	100.00				
	2009	9.99^{+}	+ 1.92	123.69				
MK-1	2010	10.11^{++}	+ 1.89	122.99	9.55	+ 1.78	122.84	2
	2011	8.54^{++}	+ 1.53	121.83				
	2009	9.06	+ 0.99	112.35		+ 1.35		
MB-2	2010	9.39^{++}	+ 1.17	114.23	9.12		117.99	3
	2011	8.93^{++}	+ 1.93	127.39				
	2009	11.10^{++}	+ 3.03	137.45				
MB-3	2010	11.98^{++}	+ 3.76	145.74	11.60	+ 3.83	150.08	1
	2011	11.71^{++}	+ 4.71	167.05				

2009 yield, LSD 5% = 1,76 + 1% = 2,48 ++ | 2010 yield, LSD 5% = 0,55 + 1% = 0,78 ++ | 2011 yield, LSD 5% = 0,71 + 1% = 1,00 ++

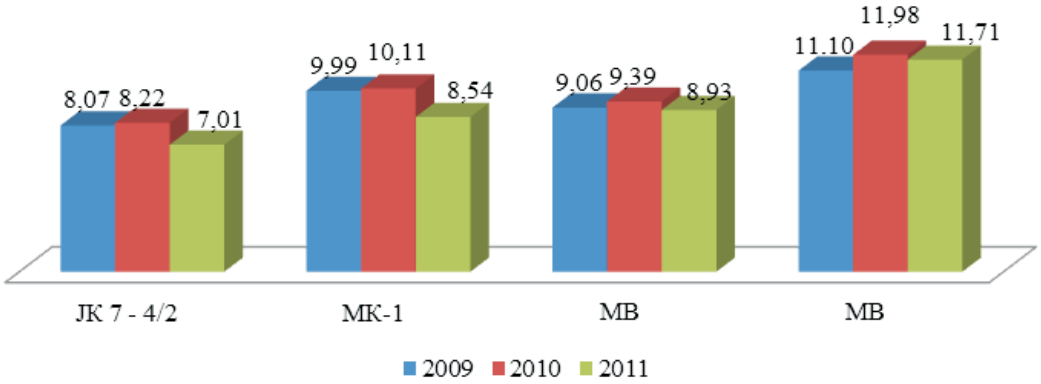

Figure 1 – Dry tobacco yield, g/stalk

Table 2. Dry tobacco yield, kg/ha

Variety	Year	Yield, g/ stalk	Difference		Average, 2009-2011	Difference 2009-2011		Rank
			Absolute	Relative		Absolute	Relative	
	2009	1490	/	100.00				
ЈК 7-4/2 ∅	2010	1515	/	100.00	1433	/	100.00	4
	2011	1294	/	100.00				
	2009	1843⁺	+ 352.40	123.69				
МК-1	2010	1864⁺⁺	+ 349.00	123.04	1761	+328	122.89	2
	2011	1576⁺⁺	+ 282.00	121.79				
	2009	1674	+ 183.20	112.35				
МВ-2	2010	1732⁺⁺	+ 217.00	114.32	1684	+251	117.51	3
	2011	1647⁺⁺	+ 353.00	127.28				
	2009	2048⁺⁺	+ 557.20	137.45				
МВ-3	2010	2210⁺⁺	+ 695.00	145.87	2140	+707	149.34	1
	2011	2161⁺⁺	+ 867.00	167.00				
2009 yield, LSD 5% = 325.31 ⁺ 1% = 457.23 ⁺⁺			2010 yield, LSD 5% = 100.54 ⁺ 1% = 141.31 ⁺⁺			2011 yield, LSD 5% = 130.77 ⁺ 1 % = 183.81 ⁺⁺		

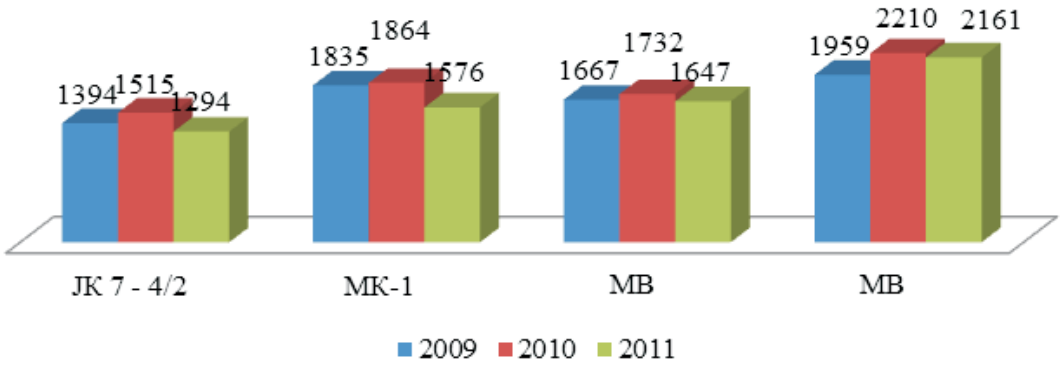

Figure 2 - Dry tobacco yield, kg/ha

Average purchase price

The average purchase price is one of the important indicators of tobacco quality, expressed in monetary value for purchased kg of tobacco. Principal role in formation of this category have the grades of tobacco and their percentage.

According to the data from Table 3 and Figure 3, the lowest average price of 100.03 denars/kg was recorded in YK 7-4/2 (∅) in 2011 and the highest - 178.42 denars/kg - in Basma variety MK-1 in 2010, which is 76.69 % higher than the check. Compared to YK 7-4/2, highly significant difference of 1 % was estimated in the newly created Basma varieties MK-1, MB-2 and MB-3 during the three years of investigation.

Average price of the varieties included in the trial ranged from 107.40 denars/kg in YK 7-4/2 to 168.45 denars/kg in MK-1, which relative difference is 56.84 % higher compared to tyhe check. The average price in other varieties ranges from 157.06 to 163.54 denars/kg in MB-3 and MB-2, respectively.

According to the results, the investigated Basma varieties have a high percentage of higher graded - tobacco raw compared to the check variety Yaka, as a result of which they achieve higher price per kg purchased tobacco.

Bogdanceski et al.,(1997) reported that average price of the standard variety YK 7-4/2 in the region of Strumica was 38.04 denars/kg. The average price of the newly created Basmak varieties is higher, due to the improved quality.

Table 3. Average price, denars/kg

Variety	Year	Average price, denars/kg	Difference		Average price, 2009-2011	Difference, 2009-2011		Rank
			Absolute	Relative		Absolute	Relative	
	2009	121.19	/	100.00				
ЈК 7-4/2 ∅	2010	100.98	/	100.00	107.40	/	100.00	4
	2011	100.03	/	100.00				
	2009	168.47^{++}	+ 47.28	139.01				
МК-1	2010	178.42^{++}	+ 77.44	176.69	168.45	+ 61.05	156.84	1
	2011	158.45^{++}	+ 58.42	158.40				
	2009	166.42^{++}	+ 45.23	137.32				
МБ-2	2010	170.90^{++}	+ 69.92	169.24	163.54	+ 54.16	152.27	2
	2011	153.30^{++}	+ 53.27	152.84				
	2009	164.12^{++}	+ 42.93	135.42				
МБ-3	2010	157.15^{++}	+ 56.17	155.62	157.07	+ 49.67	146.25	3
	2011	149.93^{++}	+ 49.90	149.88				

2009 yield, LSD 5% = LSD 5% = 9.07 $^{+}$ 1% = 12.75 $^{++}$ 2010 yield, LSD 5% = 11.99 $^{+}$ 1% = 16.85 $^{++}$ 2011 yield, LSD 5% = 10.95 $^{+}$ 1% = 15.39 $^{++}$

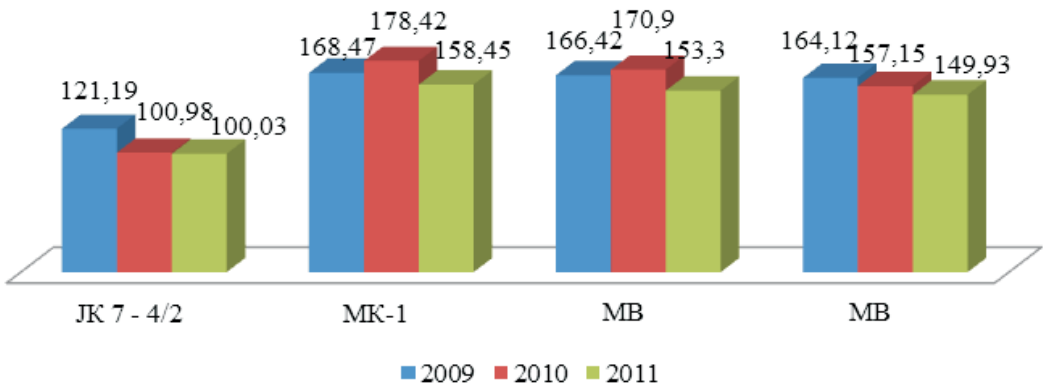

Figure 3 – Average price, denars/kg

Gross income per unit area

Gross income per unit area actually synthetisizes the results for yield and quality of tobacco, expressed through the percentage of high grades and average price (denars/ha).According to the results presented in Table 4 and Figure 4, the gross income of varieties included in the trial varies from 129.598 denars/ha in the check YK 7-4/2 (2011) to 347.119 denars/ha in the variety MB-3 (2010). In relation to average price, it varies from 151.618 denars/ha in YK 7-4/2 to 336.075 denars/ha in MB-3, which is 121.66 % higher compared to the check. It should be noted that key factor in the formation of gross income of the variety MB-3 was the high yield per hectare. MK-1 and MB-2 varieties had higher yields compared to the check, achieving 81.83 % and 96.30% higher gross income, respectively. According to (Bogdanceski et al.,1997), the gross income of Yaka tobacco in the Strumica region ranged from 64,619 to 106,484 denars/ha in varieties YK 7-4/2 (ø) and Yaka 23, respectively. It is interstting to remark that all varieties included in the three years-investigation achieved high significant difference of 1 % compared to the check.

Table 4. Gross income, denars/ha

Variety	Year	Gross income, denars/ha	Difference		Average	Difference		Rank
			Absolute	Relative		Absolute	Relative	
YK 7-4/2 ∅	2009	181 121	/	100.00	151 618	/	100.00	4
	2010	153 134	/	100.00				
	2011	129 598	/	100.00				
MK-1	2009	310 703^{++}	+ 129 582	171.55	297 627	146 009	196.30	2
	2010	332 504^{++}	+ 179 370	217.13				
	2011	249 674^{++}	+ 120 076	192.65				
MB-2	2009	278 499^{++}	+ 97 378	180.35	275 683	124 067	181.83	3
	2010	296 096^{++}	+ 142 962	193.36				
	2011	252 454^{++}	+ 122 856	194.80				
MB-3	2009	336 992^{++}	+ 155 581	213.21	336 075	184 557	221.66	1
	2010	347 119^{++}	+ 193 984	226.68				
	2011	324 115^{++}	+ 194 517	253.95				

2009 yield, LSD 5% = 64 210 $^{+}$ 1% = 90 248 $^{++}$ 2010 yield, LSD 5% = 25 328 $^{+}$ 1% = 35 599 $^{++}$ 2011 yield, LSD 5% = 30 414 $^{+}$ 1% = 42 748 $^{++}$

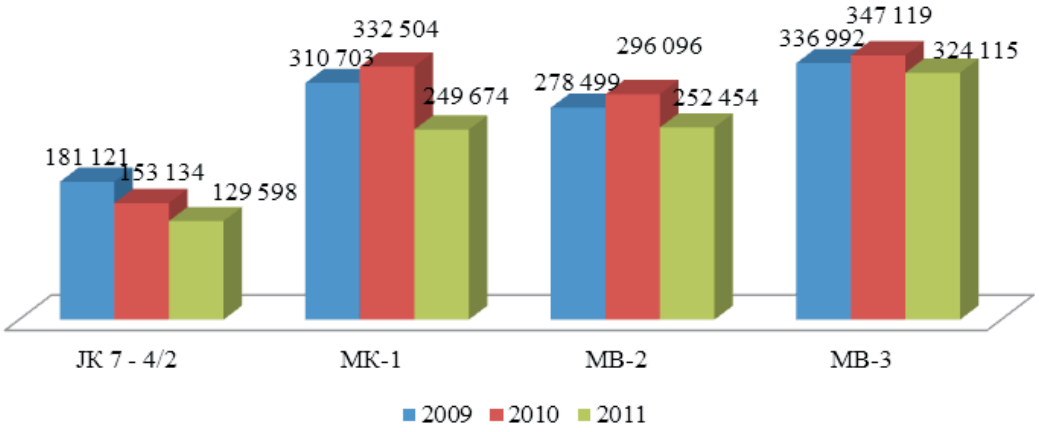

Figure 4 - Gross cash income, denars/ha

CONCLUSIONS

Based on results of the three year-investigations, the following conclusions can be made:

- Dry tobacco yield was the lowest in the check variety YK 7-4/2, amounting 7.77 g/ stalk and 1433 kg/ha, while Basma variety MB-3 had the highest yield of 11.60 g/stalk and 2140 kg/ha. Expressed in percentage, MB-3 achieved 50.80 % higher yield per stalk and 49.34 % higher yield per hectare compared to the check.

Compared to the check dry tobacco yield was the lowest in the check, highly significant difference of 1 % was observed in MB-3 (2009, 2010 and 2011), MK-1 and MB-2 (2010 and 2011). In 2009, no significant difference was observed in variety MB-2, whereas MK-1 showed significant difference at 5 % level.

-The lowest purchase price of tobacco was recorded in the check YK 7-4/2 (107.40 denars/kg). Basma varieties achieved higher purchase price (157.07 denars/kg in MB-3 to 168.45 denars/kg in MK-1). Compared to YK 7-4/2, highly significant difference of 1 % was estimated in the newly created Basma varieties MK-1, MB-2 and MB-3 during the three years of investigation.

- Gross income was the lowest in YK 7-4/2 (151.618 denars/ha) and the highest values were achieved in MB-3 variety (336.075 denars/ha), which is 221.66 % higher than the check variety.

It is interesting to remark that all varieties included in the three years-investigation achieved high significant difference of 1 % compared to the check.

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EVALUATION OF SOME *TRICHODERMA* ISOLATES FOR BIOCONTROL EFFECT ON *RHIZOCTONIA SOLANI*

Biljana Gveroska

Scientific Tobacco Institute-Prilep, Kicevski pat bb,
Republic of Macedonia
e-mail: gveroska@t-home.mk

ABSTRACT

Tobacco production is affected by the need to reduce the use of pesticides due to strict standards in recent years. Therefore, all methods and means to control the harmful agents with minimal environmental impact and economic consequences are included.

Biological control is a modern, environmentally friendly approach in plant protection, which is easily incorporated into the system of Integrated Pest Management. In plant pathology, the term biocontrol usually is concerning to the use of microbial antagonists to suppress diseases.

Trichoderma strains are the most known biocontrol agents, mostly against many soil pathogens. *Rhizoctonia solani* is a very destructive pathogenic fungus, the causing agent of a damping off in tobacco seedlings. Therefore, our aim was to examine the impact of several *Trichoderma* isolates obtained from rhizosphere of tobacco plants against this pathogen.

Investigations were carried out under in vitro conditions, using the method of dual cultures. Relative growth of the pathogen in the presence of biocontrol agent and the percentage of reduction of the radial growth of the pathogen were estimated. The relative growth was the weakest in PT1 and PT2 isolates (40.8 and 40.3%). These isolates showed the best results with the percentage reduction of pathogen 59.2 and 59.7%. PT3 and PT4 showed smaller effect (49.4 and 54.6% reduction).

These investigations confirmed the role of this biocontrol agent control of the pathogenic fungus *R. solani*. Further research should be lead to the true determination of the species, as well as intended biocontrol effect on this pathogen. We believe that this research open the way for the application of Trichoderma species, with mass multiplication or commercial products.

Key words: biocontrol, *Trichoderma* sp., *R. solani*, relative growth, inhibition of radial growth

ОЦЕНА НА БИОКОНТРОЛНИОТ ЕФЕКТ НА НЕКОИ *TRICHODERMA* ИЗОЛАТИ ВРЗ *RHIZOCTONIA SOLANI*

Производството на тутун поради строгите стандарди, во последниве години е засегнато од потребата за намалување на употребата на пестициди. Затоа, се вклучуваат сите методи и средства за контрола на штетните агенси со минимално влијание врз животната средина и економски последици.

Биолошката борба претставува современ, еколошки пристан во растителната заштита, која лесно се инкорпорира во системот на интегрална заштита. Во фитопатологијата, терминот биолошка борба најчесто се однесува на употреба на микробни анатагонисти за сузбивање на патогените.

Trichoderma видовите се најпознати биоконтролни агенси, најчесто против бројни почвени патогени. *Rhizoctonia solani* е мошне деструктивна патогена габа, предизвикувач на болеста сечење која тутунскиот расад. Затоа, нашата цел беше да се испита влијанието на неколку *Trichoderma* изолати добиени од

ризосферата на тутунски растенија врз овој патоген.
Испитувањата беа вршени во in vitro услови, по методот на двојни култури. Одредуван беше релативниот развој на патогенот во присуство на биоконтролниот агенс, како и процентот на редукција на радијалниот развој на патогенот. Релативниот развој беше најслаб кај двата изолати ПТ1 и ПТ2 (40,8 и 40,3%). Тие два изолати покажаа најдобри резултати, со процент на редукција 59,2, односно 59,7% во споредба со ПТ3 и ПТ4 (49,4 и 54,6%).
Со овие истражувања се потврди улогата на овој биоконтролен агенс во сузбувањето на патогената габа *R. solani*. Понатамошните истражувања треба да водат кон точната детерминација на видовите, како и одделниот биоконтролен ефект врз овој патоген. Сметаме дека со овие истражувања се отвора патот на примена на Trichoderma видовите, со масова продукција или како комерцијални препарати.

Клучни зборови: биоконтролен ефект, *Trichoderma* sp., *R. solani*, релативен развој, инхибиција на радијалниот развој

INTRODUCTION

Crop production bears the great losses because of a number of diseases caused by various pathogens. Tobacco production is also affected by several economically important diseases. Among them, the diseases caused by pathogenic fungi have a great part.
Concerning to damages, diseases of tobacco seedlings are of a greatest importance, especially the damping off caused by the pathogenic fungus *Rhizoctonia solani*. The importance of a healthy and quality tobacco seedlings for total production is known, and hence, the losses caused by this pathogen are huge. Also, *R. solani* is known for its great destructiveness specific to soil pathogens and a wide range of host plants (Nunez, 2005).
From the above, it is obvious the need for protection from the disease. In practice there are a limited number of fungicides, which are also used for a long time. The extended and excessive use of pesticides cause pathogen resistance and the control is not always efficient (Benitez et al, 2004; Hajieghrari et al., 2008). It also causes harmful effects to human health and environmental safety (Monte, 2001).
The total production of food, including agriculture and tobacco production in recent years is affected by the strict standards that require reducing the use of pesticides. Therefore, all methods and means of control of harmful agents with minimal impact on the environment are involved.
Biological control is a modern, environmentally friendly approach in crop protection, which can be easily incorporated into the Integrated Pest Management System. It stands out among the leading components in the development of many systems for sustainable agricultural production (Monte, 2001). According to Brimmer and Boland (2003), it is an alternative to synthetic pesticides because it provides higher level of security and minimal impact on the environment.
Biological control i.e. application of specific microorganisms that interfere with plant pathogens and pests is a natural, environmentally friendly approach to overcome the problems caused by the standard method of plant protection (Chet et al., 2006). In phytopathology, the term biological control often refers to the use of microbial anatagonists for control of pathogens.
Bacterial and fungal biocontrol agents with strong antagonistic abilities have the power to control many plant pathogens (Szekeres et al., 2006). Fungi of the genus *Trichoderma* are the most popular biocontrol agents. The success of *Trichoderma* species as biocontrol agents is due to their strong reproductive capacity, ability to survive in very unfavorable conditions, the efficiency of utilization of nutrients, the capacity to modify rhizosphere, stong aggressiveness

against phytopathogenic fungi and efficiency in stimulating the growth of the plant and its defense mechanisms. These properties make this genus an unique inhabitant with a high population densities in many life unions (Benitez et al., 2004).

As soil inhabitants, they live in the area of root system where they activate numerous biocontrol mechanisms that affect pathogen. Antibiosis, mycoparasitism and competition for food and space are the main in numerous mechanisms of biocontrol. These are complex, and what can be defined as a biocontrol, presents final result of various mechanisms that act synergistically to achieve protection from a disease (Howel, 2003).

But various biotic and abiotic environmental factors may influence the efficiency of *Trichoderma* spp. against phytopathogens (Handelsman and Stabb, 1996; Jaworska and Dlużniewska, 2007). Therefore, the various isolates show different biocontrol activity. Local isolates have the greatest antagonistic activity towards the pathogen in the many cases.

The first and quickest way for determining of mycoparasitism and producing of antibiotics is method of Petri boxes (Harman, 2006). Therefore, our aim was to investigate biocontrol activity of several local isolates of *Trichoderma* spp., to pathogen *R. solani* at *in vitro* conditions. It would allow selection of the best isolate for further application as biocontrol agent in tobacco protection from the damping off disease in tobacco seedling.

MATERIAL AND METHODS

Pathogenic fungus *Rhizoctonia solani* was isolated from infected plant material.

Trichoderma isolates were obtained from the root zone of the rhizosfere of healthy tobacco plants from region of Prilep, using the method of dilution. 1ml of dilution of 10^{-4} was was thrown into Chapeck agar as the most suitable medium for fungi. Reisolation and the maintainance of the pure cultures were on potatto medium.

In vitro investigations were conducted by the method of dual cultures. 5 mm fragments both from the 10-day culture of the pathogen and *Trichoderma* isolates were placed in the center of each half of the Petri dish on PDA (potato dextrose agar) as nutrient medium.

Pure cultures of *R. solani* and of each *Trichoderma* control agent were used as a check. Biocontrol effect of the four isolates (PT1-PT4) was researched.

The experiment was set up in three replications, with five Petri dishes for the check and dual cultures. Incubation was performed at 25º C and the diameter of the colony was measured each day during the 10-day incubation interval.

Relative growth of the pathogen was calculated by the method of Mello (2000), based on the values of pathogen's diameter in the presence of biocontrol agent.

$$RD = [(GP \text{ in the presence of BCA}) / (GP \text{ in the control})] \times 100$$

RD = relative development of a pathogen in a presence of biocontrol agent (%)

GP = growth of the pathogen

BCA = biocontrol agent

The percentage of reduction of pathogen's growth was determined according to the formula of Mishra (2010).

$$PIRG = [(C-T) / C] \times 100$$

PIRG = percentage inhibition of radial growth of the pathogen (%)

C = radial growth of pathogen in the absence of biocontrol agent (control)

T = radial growth of pathogen in the presence of biocontrol agent

Estimation was made by taking the values for diameter of pathogen's colony in the presence of biocontrol agent at the time of placing the pathogen in the control Petri dishes, i.e. on the sixth day. Evaluation was continuing to 10th day.

RESULTS AND DISCUSSION

Damping off disease in tobacco seedling causes significant economic losses. It is manifested by the appearance of infections in the small group of plants. Spreading of the disease, the percentage of infected area is increasing (Fig. 1). It is caused by the pathogenic fungus *R. solani* (Fig. 2).

Fig. 1 Symptoms of damping-off disease in tobacco seedlings

Fig. 2 Causing agent of damping off – R. solani (pure culture)

Biocontrol agent *Trichoderma* shows extremely fast radial growth (Table 1). It has be seen in all isolates. The poorest development is shown by the isolate PT3. Sporulation (beginning of forming the spores and intensity) is the lowest in the same isolate (PT3) (Fig. 3)

PT1 PT2 PT3 PT4

Fig 3. Pure cultures of the biocontrol agent Trichoderma – isolates PT1, PT2, PT3 and PT4

Table 1. Growth of colonies during incubation (mm)

Variant	Diameter (mm)									
	Days									
	1	2	3	4	5	6	7	8	9	10
R. solani in PT1	14,4	29,6	34,5	43,8	44,2	44,9	45,8	48,5	48,5	48,5
R. solani in PT2	13,8	30,0	41,9	43,7	44,0	44,3	45,0	45,1	45,1	45,1
R. solani in PT3	15,9	32,2	45,1	51,4	54,4	55,7	56,0	56,0	56,0	56,0
R. solani in PT4	14,9	28,7	39,5	43,6	45,6	49,9	52,4	52,5	52,5	52,5
Ø R. solani	12,9	45,8	59,8	83,7	106,6	110,0	110,0	110,0	110,0	110,0
Ø PT1	20,6	67,0	110,0	110,0	110,0	110,0	110,0	110,0	110,0	110,0
Ø PT2	13,0	54,1	109,1	110,0	110,0	110,0	110,0	110,0	110,0	110,0
Ø PT3	14,4	57,7	106,7	110,0	110,0	110,0	110,0	110,0	110,0	110,0
Ø PT4	19,4	62,1	109,1	110,0	110,0	110,0	110,0	110,0	110,0	110,0

In a pure culture, *R. solani* radially develops and fills Petri box on 6th day (Table 1). But in the dual cultures, in the presence of the biocontrol agent, its non inpediment development is seen only the first day. On the second day the contact of both cultures occurs (Fig. 4a-7a). From that moment the colony gets distorted form and development of the pathogen is difficult. The diameter of the colony is nearly 30% lower compared to that of control (Table 1). The pathogen continues to grow slightly, but measuring its diameter is nearly impossible, because of fulfilled Petri box by *Trichoderma. R. solani* colony seemed to "trapped by biocontrol agent. This situation is observed in the presence of tested four *Trichoderma* isolates.

Biocontrol agent continues to develope smoothly despite the presence of the pathogen. The first, its surrounds the pathogen and then „passes" through it, destroying and deforming his mycelia (Fig. 4b-7b). At the end of incubation, the Petri box is completely filled by the colony of Trichoderma (Fig. 4c-7c).

While the colony of pathogen in the check has got the maximum at 6th day, it is more than 50% lower in dual cultures, i.e. in the presence of the biocontrol agent. Thus, all the tested *Trichoderma* isolates showed the biocontrol activity against *R. solani*. The relative growth of the pathogenic fungus is the smallest in the presence of the isolate PT2, and the greates in the presence of isolate PT3 (Table 2).

Therefore, the percentage reduction of the of *R. solani* growth in the presence of *Trichoderma* ranges from 49.4% for the isolate PT3 to 59.7% for T2 isolate. Therefore, isolate PT3 showed the weakest, while isolate PT2 the strongest reducing effect on the development of *R. solani.*

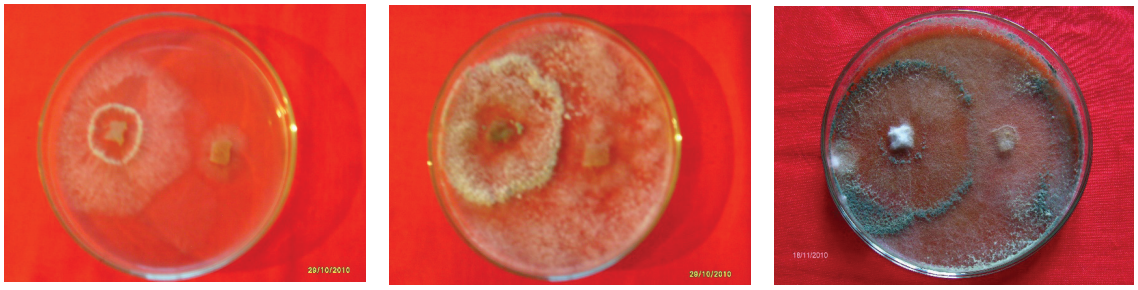
Table 2. Reduction of growth of *R. solani* with four *Trichoderma* isolates

Variant	Relative growth of the pathogen in the presence of *Trichoderma*	Percentage reduction of patho-gen's growth in the presence of *Trichoderma*
R. solani in PT1	40,8	59,2
R. solani in PT2	40,3	59,7
R. solani in PT3	50,6	49,4
R. solani in PT4	45,4	54,6

The results obtained in our investigations are in accordance with those of Rini and Sulochana (2007), in which there is a difference in the percentage inhibition of *R. solani*. Among examined 26 isolates of *Trichoderma*, 11 have efficacy in the control of the pathogen. In these studies, only *T. harzianum* TR 20 is characterized as a class 1 on the 6 th day of incubation. Despite these data, our tested isolates are included in class 1 of the mentioned scale in that paper, which is a good assessment of biocontrol activity of our local isolates.

Data for differences between isolates is presented by Foroutan (2013), in which is highlightened the different inhibition of mycelial development of *Fusarium graminearum* by different isolates as *T. harzianum*, as well as the *T. viride*. Also, the percentage inhibition of radial development of *Pythium aphanidermatum* is different by *Trichoderma* species, but different isolates of the same species, too. For eg. percentage of reduction among isolates of *T. harzianum* ranges from 52,2 to 72,0% (Mishra, 2010). According to Grondona et al. (1997), in a practical situation of biocontrol, differentiation is required to define the population in a range of species. Also, it is necessary to make a selection of the most effective isolate for each patosistem.

Mishra et al. (2011) pointed that *T. viride* isolate Tr8 due expressed antagonistic properties can be used for commercial purposes in local climatic conditions. According to the results of these investigations, the isolates PT2 and PT1 can be used for mass propagation and involvement in the system of integrated protection of tobacco from diseases.

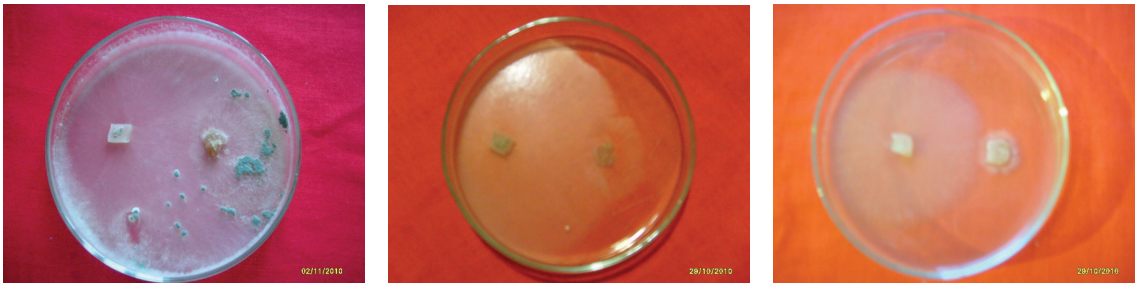


a **b** **c**

Fig. 4 Development of R. solani in a dual culture with Trichoderma - isolate PT1 (a= on the third, b=fourth day, c= the end of incubation)

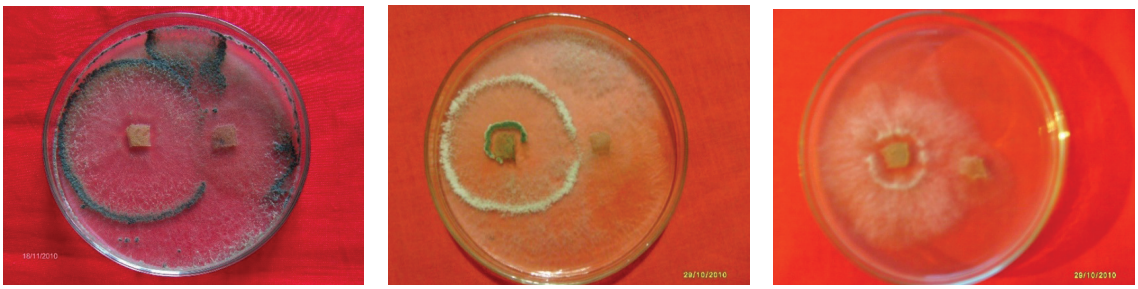
a b c

Fig. 5 Development of R. solani in a dual culture with Trichoderma - isolate PT2 (a= on the third, b=fourth day, c= the end of incubation)



a b c

Fig. 6 Development of R. solani in a dual culture with Trichoderma - isolate PT3 (a= on the third, b=fourth day, c= the end of incubation)



a b c

Fig. 7 Development of R. solani in a dual culture with Trichoderma - isolate PT4 (a= on the third, b=fourth day, c= the end of incubation)

CONCLUSIONS

- Four investigated *Trichoderma* isolates showed the biocontrol effect against the causing agent of damping off in tobacco seedlings- *R. solani*.
- Relative growth of *R. solani* at *in vitro* conditions ranged from 40,3% in the presence of isolate PT2 to 50,6% in the isolate PT3.
- Percentage of reduction of pathogen's growth ranged from 49,4% (PT3) to 59,7% (PT2).
- Isolate PT2 showed the highest inhibition of growth of *R. solani.*
- Isolate PT2 had the highest reducing effect on development of pathogenic fungus *R. solani*.
- Isolate PT1 had the good reducing effect, too.
- They can be used in the biological control against *R. solani* in tobacco seedling protection.

- Identification of Trichoderma species is needful for further development of methods of mass propagation.
- Preparations on the basis of the isolate with the best biocontrol effect have the biggest opportunities to use them in biological control of damping off in tobacco seedlings.

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Original Scientific paper

THE INFLUENCE OF SOME OF THE CHEMICALS TOWARDS TOBACCO SEEDS GERMINATION OF TOBACCO BURLEY VARIETY PELAGONEC

Milan Mitreski[1], Vladko Belovski[2] , Ana Korubin-Aleksoska[1]

[1]Scientific Tobacco Institute- Prilep, Kicevska bb, 7500 Prilep
University St. Kliment Ohridski "- Bitola, Republic of Macedonia
[2]Ministry of Agriculture, Forestry and Water Management of the Republic Macedonia
Aminta the Third No.2, 1000 Skopje, Republic of Macedonia
e-mail: vladko.belovski@gmail.com

ABSTRACT

All the research in 2012 were been performed on the seed material from the Burley tobacco variety Pelagonec, produced in year 2011. The seeds were treated with chemicals such as potassium nitrate (KNO_3) with concentration 0.2 and 0.4%, with gibberellic acid (GA_3) with concentration 0.05 and 0.08%, and they were also treated with an universal microbiological preparation Terra Biosa (ProBios) with two concentrations 100 ml and 150 ml diluted in 1l water (also there were two variants).
The research for the influence of the previously mentioned substances towards the germination of the seeds of tobacco variety Pelagonec, were being performed in the Laboratory for seed quality control of agricultural plants-L01. From the research we have established that the treatment with KNO_3 with concentration of 0.2% water solution gave the best results. The lowest seed germination was recorded in variant treated with Terra Biosa (150 ml in 1l water). The results presented here showed that KNO_3 with concentration of 0.2% water solution could be used to shorten the dormancy tobacco seed of the large leaf variety of Pelagonec which means increase germination energy and total germination.

Key words: tobacco seed, Burley-Pelagonec, potassium nitrate, gibberellic acid, Terra Biosa.

ВЛИЈАНИЕ НА НЕКОИ СРЕДСТВА ВРЗ 'РТЛИВОСТА НА ТУТУНСКОТО СЕМЕ ОД БЕРЛЕЈСКАТА СОРТА ПЕЛАГОНЕЦ

Истражувањата се вршеа во 2012 година на семенски материјал од берлејската сорта тутун-пелагонец, произведен во 2011 година. Семето се третираше со хемиските средства калиум нитрат (KNO_3) со концентрации од 0,2 и 0,4% (две варијанти), гиберелинска киселина (GA_3) со концентрации од 0,05 и 0,08% и со универзалното микробиолошко средство Terra Biosa (ProBios), во дози од 100 и 150 ml растворени во 1l вода (исто така две варијанти).
Испитувањата за влијанието на наведените средства врз 'ртливоста на семето од сортата пелагонец се изведоа во Лабораторијата за контрола на квалитетот на семето од земјоделски растенија - L01 при Научниот институт за тутун – Прилеп. Од истражувањата утврдивме дека најдобра варијанта е третманот со KNO_3 во концентрација од 0,2% воден раствор. Најслаба варијанта е третманот со Terra Biosa (150 ml во 1 l вода).
Со употребата на KNO_3 во концентрација од 0,2% р-р се скратува (прекинува) периодот на мирување (дормантноста) кај тутунското семе од крупнолисната сорта пелагонец односно се зголемуваат енергијата на 'ртење и вкупната 'ртливост.

Клучни зборови: тутунско семе, берлеј-пелагонец, калиум нитрат, гиберелинска киселина, Terra Biosa.

INTRODUCTION

The agriculture as one of the most important sources for diet of the population is based on the production of seed materials. The seed is a foundation of the agriculture and biodiversity.

In order to have stable and good quality tobacco production, there should be healthy seedling which is related with the usage of high quality tobacco seed.

The tobacco seed as well as all the other plants' seeds is the carrier and transfer of the inherited characteristics of the progeny. That's why the world gives such significance on the type of the seed used during sowing. One part of the seed production chain is the laboratory testing of seed quality. In our country there are used book of regulations, for all the methods used for seed material quality control, which are coordinated with the international standards for seed quality analysis, established by International Testing Association (ISTA).

After the harvest we cannot use the seeds from the large leaves variety types (Burley and Virginia) for sowing, because the germination is on low level, which means that it's on normal level or beyond normal level of usage. This means that the seeds are dormancy i.e. they acquire certain period of inactivity in order to perform the needed physiological processes, after which the seed can germinate on normal or higher level. In this period of "maturing" among the previously mentioned tobacco types' seeds, last for about a year. In order to have improvement in the germination of the seeds so that they can be used next year for sowing after the harvest is over; a seed treatment is being performed with different physical, chemical and microbiological substances.

Čirkovski (1954), has succeed with gibberellins treating to germinate for 10 days the seed of the wild type *Nicotiana miersii*, which hasn't shown any signs of germination when left in water for 258 days. Dima (2001), has pointed out that the seed germination can also be stimulated by performing ultrasound treatments, during which the vegetation period is being shorted, and the yield has been increased for 11-17%. She has also established that the treatment of the tobacco with indoleacetic acid with concentration of 0.01-0.05% which increases the germination energy up to 5-10% and the total germination for 8-13%. The thiourea of low concentrations has increased the total germination for 4-9%. Also the author emphasizes the fact that the seedling which is taken from the seed treated with these physiologically active substances is healthy and has given a significant increase of the yield.

These types of researches are very rare in our country because there is not enough information about the chemicals-stimulators which can be used for germination increase of the tobacco seeds. The purpose of our researches is to examine the influence of certain chemicals upon the period of dormancy of the seeds from the Burley tobacco variety-Pelagonec, there is improvement in the germination and great possibility of usage of the seed material in the following year after the harvest was performed.

MATERIAL AND METHODS

The research has used the tobacco seed type Burley more precisely the cytoplasmic male sterile variety Pelagonec, which was produced in year 2011, as a plant material. The both seeds from types Pelagonec as well as Virginia have hard covering and longer period of inactivity, so in the year of production they show lower results when it comes to the energy of germination and total germination.

According to Risteski (2011), the variety Pelagonec (Photo. 1), was patented in year 2010. The stable and good yield (3800 up to 4350 kg/ha), with the typical Burley quality makes this variety very attractive as well as for the producers and for the manufacturers. The seeds from this variety is very small, 0.085 g. per 1000 seed. (Photo. 2)

Photo 1. Burley variety Pelagonec

Photo 2. Tobacco seed from Pelagonec variety (adopted from Risteski, 2011)

During our researches we've used the following chemicals and microbiological preparation: potassium nitrate (KNO_3) gibberellic acid (GA_3) and Terra Biosa (ProBios).

- The potassium nitrate is colorless crystalline substance. It melts at temperature of 332 °C and at temperature of 400 °C it decomposes into potassium nitrate and oxygen. In certain concentrations can be used for breaking of the period of dormancy of the seed material.
- The gibberellic acid (GA_3) is used for regulation of the plants growth, and the low concentrations have high significance. Mainly it's used in the dosage between 0.01-10 mg/l. Higher concentrations have opposite effect. The gibberellins were established in 1898 by the Japanese scientists in a fungus, nowadays known as Gibberellafujikuroi. Up to now there are known 79 types of different gibberellins. GA_3 stimulates the growth of the stalk and root through fastening the mitotic cell partition in several plants, but also it influences with increase in the seed germination. Also, it can be used in the laboratories and greenhouse for germination stimulation in some of the plants which otherwise would stay in hibernation.
- Terra Biosa (Pro Bios), it's a soil conditioner and a microbiological preparation which production is based on live cultures from probiotic bacteria. USA, Germany and Denmark are the most famous producers of Terra Biosa. During our research we've used the one produced in Germany with a Polish license. Terra Biosa is made by lactic bacteria, yeast, photosynthetic bacteria, and molasses from sugar cane, extracts from two dozen plants and clean not chlorinated water. This substance is used for quality improvement and the health status of all biological systems. Also, it can be used to treating the seed and seedling material for quality properties improvement.

Before we've conducted the researches we've implement the following variations:

1. Untreated control (Ø), a standard procedure
2. Variation with KNO3 (0.2% solution)
3. Variation KNO3 (0.4% solution)
4. Variation GA_3 (0.05% solution)
5. Variation GA_3 (0.08% solution)
6. Variation Terra Biosa (100 ml/l water)
7. Variation Terra Biosa (150 ml/l water)

All variations were examined in 4 repetitions, the tobacco seed was placed in four Petri dishes containing 100 seeds. The dishes were left to germinate in thermostat-germination type "Sutjeska" with constant temperature of 25 ˚C. The samples were germinated and analyzed for 16 days since the day when they were left in the thermostat.

The potassium nitrate was moisturizing the filter paper in the Petri dishes where the seeds were put in and from time to time distillated water was being poured.

The gibberellic acid (GA_3), as a tobacco seed germination stimulator, which was used for soaking the seed (2g) in the solution placed in glasses according to the previously established variations (concentrations of GA_3 0.05-0.08%), with duration of 30 minutes. After which the seeds were placed on clean filter paper so that they could air dried and then we've placed them in Petri dishes for further testing of the germination. Also we've used the universal microbiological preparation Terra Biosa, we've used it for soaking for 30 minutes in a solution with already mentioned dosage. After the drying process the seeds were placed on filter paper in the Petri dishes, moisturized with distillated water. On Photo 3 we can see the Petri dish with tobacco seed used for germination test.

All the research conducted are according to the Rules on the modus of work, spatial and technical equipment of the authorized laboratories and methods for quality investigations of seed material in plants (Official Gazette of the Republic of Macedonia, No. 61/2007).

The researches were being conducted in 12th of May 2012, in the Laboratory for seed quality control of agricultural plants-L01 at the Scientific Tobacco Institute- Prilep, accredited according MKC EN ISO/IEC 17025:2006.

The given results from germination energy and total germination were being compared with the control and statistically were processed with analysis of variance and Least Significant Difference test (LSD).

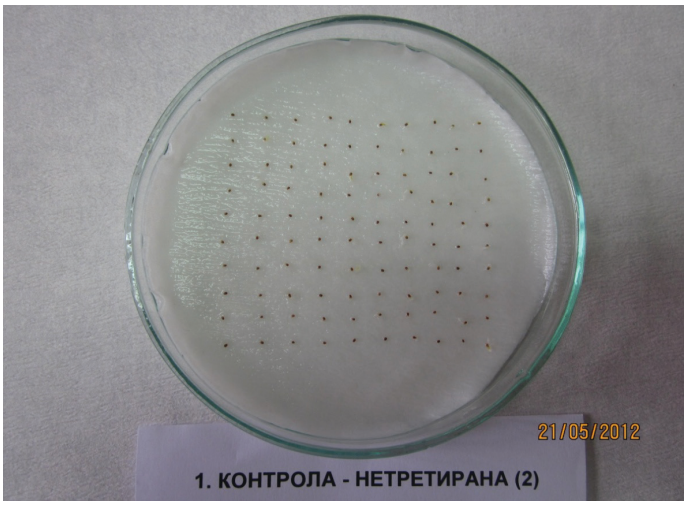

Photo 3. Petri dishes containing seeds for germination

RESULTS AND DISCUSSION

The results from the research are presented in their average values in the tables for easier comparison of all variants and having suitable and objective establishments.

Bogdančeski (1973), emphasizes that the treatment of the tobacco seeds from Prilep varieties Virginia and Burley have three different concentrations of the biological stimulators tryptophan, cortisone and gibberellins which can improve the germination energy and total tobacco seed germination of a certain concentration, while with the concentration increase the germination inhibition can be seen.

Jovičić et al., (2011), has cited Yamauchi, and he says that the previously activated phytochrome has influence on the synthesis of gibberellic acid which has positive influence on seed germination. Due to which the influence of the light towards seeds germination can be replace the treatment with phytochrome-gibberellins.

Dima (2001), has pointed out that the gibberellins pulling out the seeds from the inactive phase and it stimulates the germination process. The author researches the large leaf variety Baragan 132, and has established that the procaine-hydrochlorate with concentration of 0.05; 0.1 and 0.2%, increases the germination energy from 7 to 14% and the total germination from 9 to 11% when compared with the control. The nicotine acid with concentration of 0.05-0.1% increases the germination energy for 8-10% when compared with the control, when it comes to the concentration which is 0.3% has lower the germination energy for 4% and the total germination energy for 5%. Author also points out that the seedling given by the seeds treated with physiologically active substances is healthy, and has significantly increased the yield.

In Table 1 the average values of the tobacco seed variety Pelagonec germination energy are presented (the seventh day since setting of the germination samples)

Table 1. Average values of tobacco seeds germination energy (%)

Number	Variants	Germination energy	Variance		Rank
		%	Absolute	Relative	
1	Untreated Ø	78.25	/	100.00	5
2	KNO_3 (0.2%)	88.00^{+++}	+ 9.75	112.46	1
3	KNO_3 (0.4%)	76.25	- 2.00	97.44	6
4	GA_3 (0.05%)	87.00^{+++}	+ 8.75	111.18	2
5	GA_3 (0.08%)	78.75	+ 0.50	100.64	4
6	Terra Biosa (100 ml/1 вода)	80.25	+ 2.00	102.56	3
7	Terra Biosa (150 ml/1 вода)	67.25	- 11.00	85.94	7

LSD: 0.05 = 3.53% +
0.01 = 4.85% ++
0.001= 6.60% +++

We can see that the best option for treating the seeds is treatment with 0.2 % solution of KNO_3, where the average value of energy of germination is 88 %, when compared with the control (78.25 %), has a positive absolute difference of 9.75 %, while the relative difference showed a value of 12.46 %. in preference of this variant.

The variations of GA_3 (0.05%) are with germination energy of 87% and Terra Biosa (100 ml/1 water) with germination energy of 80.25%. The rest of the variant with the highly concentration from the used substances gave low results and two of them are much lower than the control.

The statistical processing of the results has shown the difference of the variations of KNO_3 (0.2%) and GA_3 (0.05%), when it comes to the control they are statistically signification, i.e. have higher significance because they are beyond the level of probability of 0.001%.

When it comes to the total tobacco seed germination (Table 2), the influence of the chemicals used is proportional with the germination energy, all variants have shown increase of the germinated seeds percentage in the period of total seed germination evaluation (at day 16, after the germination samples are set).

Table 2. Average values for the total tobacco seed germination (%)

Number	Variants	Total germination %	Variance		Rank
			Absolute	Relative	
1	Untreated Ø	82.75	/	100.00	5
2	KNO_3 (0.2%)	90.00^{+++}	+ 7.25	108.76	1
3	KNO_3 (0.4%)	81.25	- 1.50	98.19	6
4	GA_3 (0.05%)	88.75^{++}	+ 6.00	107.25	2
5	GA_3 (0.08%)	84.75	+ 2.00	102.42	4
6	Terra Biosa (100 ml/1 water)	88.25^{++}	+ 5.50	106.65	3
7	Terra Biosa (150 ml/1 water)	75.25	- 7.50	90.94	7

LSD: 0.05 = 3.69% $^{+}$
0.01 = 5.06% $^{++}$
0.001= 6.90% $^{+++}$

The treatment with KNO_3 (0.2%) can be seen as the most effective, because the seed has 90% germination, which is a positive absolute difference of 7.25% when compared with the control- untretated variant, which has total germination of 82.75%.

The results statistical processing has shown that: the treatment with KNO_3 (0.2%) is highly significant as well as the ones with GA_3 (0.05%) and Terra Biosa (100 ml/1 water) because the variance between the values on level of probability are 0.001 and 0.01%.

CONCLUSIONS

From the research we've made several conclusions:

- Out of all analyzed variants, the most intensive germination was seen during the fourth and seventh day since the seed samples for analyzes were set and the germination energy was determined.
- The lower concentration variants of chemicals have simulative effect on of the germination energy and total germination, which means that the period of dormancy, is lower.
- The variants with higher concentrations have shown negative influence, they were inhibitors of the energy and the total tobacco seed germination.
- During the research the best results of several treatments were established with the variations with KNO_3 with concentration of 0.2% (88% germination

energy and total germination of 90%), the ones with GA_3 with concentration of 0.05% (87% energy and total germination of 88.75%).

- The lowest seed germination was recorded in variant treated with Terra Biosa with dosage of 150 ml/1water (67.25% of which was germination energy and total germination of 72.25%).
- With usage of KNO_3 (0.2%) and GA_3 (0.05%), the period of dormancy of the Burley variety Pelagonec is shortening and there is a greater possibility to be used in the first year of production.
- According to us there should be more similar analyzes for the extended effect of the chemicals used during the process of seedling growth and tobacco transplantation in the fields.

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Original Scientific paper

RESULTS OF *IN VITRO* INVESTIGATIONS OF SOME NEW PESTICIDES UPON THE DEVELOPMENT OF SOIL BORNE PHYTOPATHOGENIC FUNGI

PetreTaškoski

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

ABSTRACT

Soil borne pathogenic fungi *Pythium debaryanum, Rhizoctonia solani* and *Phytophthora parasitica var. nicotianae* are important problem which causes serious damage in tobacco seedling production. The aim of the investigation was to evaluate the effectiveness of some new fungicides in the control of these pathogens. The paper presents the results obtained with the use of chemicals Orvego, Enervin and Signum, while Previcur and Top M. served as a standard. Investigations were performed during 2013 at *in vitro* conditions, in the phytopathological laboratory of Tobacco Institute – Prilep. Recommended rates of chemicals were added to the nutrition media infested with culture of the investigated pathogenic fungi and incubated for a period of ten days. The highest effectiveness of 100 % for all three pathogenic fungi was achieved by the chemical Enervin. Orvego showed 100 % effectiveness against the pathogen *P. parasitica var. nicotianae*, and the same effectiveness was achieved with Signumagainst *R. solani*. The new fungicides showed higher effectiveness than the standard products in control of the pathogenic fungi.

Key words: pathogens, *P. debaryanum, R. solani, P. parasitica var. nicotianae*, fungicides

РЕЗУЛТАТИ ОД ИСПИТУВАЊЕТО НА ПОНОВИ ФУНГИЦИДИ ВРЗ РАЗВОЈОТ НА НЕКОИ ПОЧВЕНИ ФИТОПАТОГЕНИ ГАБИ ВО IN VITRO УСЛОВИ

Во расадопроизводството кај тутунот посебен проблем претставуваат почвените фитопатогени габи *Pythium debaryanum*, *Rhizoctonia solani* и *Phytophthora parasitica var. nicotianae*, кои му нанесуваат огромни штети на тутунскиот расад. Заради тоа, целта на ова испитување беше да се провери ефикасноста на некои понови фунгициди за сузбивање на овие патогени. Во трудот се изнесени резултатите од испитувањето на препаратите Orvego, Enervinи Signum, а како стандардни се земенипрепаратите Previcuri Top M. Испитувањата се извршени во in vitro услови на хранлива подлога КДА во текот на 2013 година во фитопатолошката лабораторија на Научниот институт за тутун-Прилеп. Предвидената количина на препарат е додадена во хранливата подлога на која беше засеана култура од испитуваните патогени габи и е инкубирана за време од десет дена. Највисока ефикасност од 100% кај сите три патогени габи беше постигната со препаратот Enervin. Препаратот Orvego покажа 100% ефикасност спрема патогенот *P. parasitica var. nicotianae*, а со фунгицидот Signum исто таква ефикасност беше постигната спрема патогенот *R. solani*. Стандардните препарати покажаа добро фунгистатично дејство спрема патогените габи. Новите испитувани фунгициди покажаа повисока ефикасност во однос на стандардните препарати во сузбивањето на овие патогени габи.

Клучни зборови: патогени, *P. debaryanum*,*R.solani*, *P.parasitica var. nicotianae*, фунгициди

INTRODUCTION

Tobacco seedlings are frequently attacked by many pathogenic soil borne fungi that cause the damping-off disease. Due to favorable temperature and humidity

conditions in seedbeds which also favor the development of phytopathogenic fungi, the damages on tobacco can often reach over 50%. Symptoms that appear in seedbeds are similar and it is very difficult to visually determine the causing agent of the disease. The most common agents that attack tobacco seedlings are *Pythium debaryanum*, also known as *Pythium ultimum* - one of the main agents of damping-off disease in vegetable crops (Ivanović, 1992), *Rhizoctonia solani, Phytophthora parasitica var. nicotianae, Thielaviopsis basicola, Botrytis* sp., *Fusarium sp*. etc. Infestation is manifested through necrotization of seedlings root system and lower part of the stalk. Although the symptoms are similar, they are caused by different pathogens and therefore special attention should be paid to the choice of chemicals. Products that are used to control one causing agent will often not be effective against the other.Thus, before application of *fungicide* it is *essential*to *determine*the*cause*of the symptoms. Investigations were conducted with a number of standard chemicals offered by manufacturers. Taskoski (2001, 2005, 2009) obtained good results with propamocarb, metalaxyl and kaptan based chemicals in the control of *P. debaryanum*, with metalaxyl in the control of *P. parasitica var. nicotianae* and with thiophanate methyl in the control of *R. solani*. According to literature data (Ivanović, 1992), good protection in field conditions was achieved by application of fungicides based on chlorthalonil, thiram, kaptan, metalaxyl and promocarb. Some of the known fungicides, however, showed poor performance in practice. For that reason, our investigations include some newer products for seedlings protection from soil borne pathogens. The purpose of investigations was to estimate the effect of new fungicides on development of most frequently represented pathogens that cause serious damage to tobacco seedlings.

MATERIALS AND METHOD

In vitro investigations were made in phytopathological laboratory of the Scientific Tobacco Institute - Prilep. Tobacco seedlings were infested with pure culture of *P. debaryanum, R. solan i*and *P. parasitica var. nicotianae* – phytopathogenic fungi that cause damping off disease. The growth media used was potato dextrose agar (PDA). Culture of the pathogenic fungi was isolated from infested tobacco plants grown in Petri dishes using standard laboratory methods. The investigation included three new fungicides and two standard fungicides which have already been used in tobacco seedlings protection (Table 1).

Table 1.Investigated fungicides

Fungicide	Active ingredient	Concentration %
Orvego	Ametoctradin 300g/l + Dimethomorph 225g/l	0,1%
Enervin WG	Ametoctradin 120g/kg+ Metiram 440 g/kg	0,2%
Signum WG	Boscalid 267 g/kg + Pyraclostrobin 67 g/kg	0,1%
Previcur 607SL	Propamocarb 70%	0,25%
Top M 70WP	Thiophanate methyl 70%	0,1%

After autoclaving, different concentrations of fungicides were added to the media cooled at certain temperature. While the media was still warm it was placed in 90 mm Petri dishes sown with 3x3 mm fragment of the fungus culture and then incubated in a thermostat at 25°C for ten days.
Three tests were performed for each pathogenic fungus, with five rerplicates for each variant (chemical). Growth of the fungus colony in variants treated with fungicides was compared with the control, i.e. with the untreated fungus colony.
The readings were performed in a period of 10 days, with regular measuring of radial growth of the colonies. Average values from the five replicates were taken as end value for each variant. The percentage of the tested fungicides was calculated by the formula of Mudri (2000) and Siameto (2010):

Effectiveness % = (a - b / a) x 100,

where:
a = radial growth of the pathogen in the control
b = radial growth of the pathogen in the presence of fungicide

RESULTS AND DISCUSSION

Results of the experiments are presented in table, through the average values obtained from the five replicates.

Table 2 shows the results for daily growth of the pathogenic fungus *P. debaryanum*, obtained in the first experiment.

Table 2. Colony growth of the fungus *P. debaryanum*

Variant	Colony growth in mm by days									
	1	2	3	4	5	6	7	8	9	10
Control	12	40	45	45	45	45	45	45	45	45
Orvego0,1%	3	10	25	30	35	40	45	45	45	45
Enervin0,2%	-	-	-	-	-	-	-	-	-	-
Signum0,1%	3	12	22	40	42	45	45	45	45	45
Previcur0,25%	5	7	15	15	17	18	20	20	20	20
Top M 0,1%	5	28	45	45	45	45	45	45	45	45

24 hours after incubation, the radial growth of fungus colony in the control was 12 mm. Due to the fungus rapid development, the maximum increase of 45 mm was reached on the third day, which means that the Petri dish was full. Unlike control, somewhat slower growth was observed in variants treated with fungicides. Thus, in media treated with Orvego 0.1%, radial growth of the colony ranged from 3 mm after 24 hours to 45 mm on the seventh day of incubation. Similar results were obtained with Signum 0, 1 %. The highest fungal growth was recorded on the media treated with Top M 0,1% (5mm after 24 hours, and the maximum 45 mm on the third day). The lowest growth was registered with Previcur 0, 25 % (5 mm on the first day and 20 mm by the end of observation). Only in the media treated with Enervin 0, 2 % no colony growth of the pathogen P. debaryanum was observed.
Development of *R. solani* from the first experiment is shown in Table 3. This pathogenic fungus showed good growth, with the maximum of 45mm achieved on the fifth day of incubation. Somewhat lower growth was observed in the fungus grown on media treated with Orvego 0, 1 % and Previcur 0, 25 %. With both fungicides,

radial growth of 45 mm was measured on the seventh day of incubation. The lowest growth was measured on media treated with Top M 0,1% (3 mm on the second day, and only 15 mm by the end of observation). No mycelial growth of the fungus was observed in media treated with the fungicides Enervin 0, 2 % and Signum 0, 1%.

Table 3. Colony growth of the fungus *R. solani*

Variant	Colony growth in mm by days									
	1	2	3	4	5	6	7	8	9	10
Control	2	12	30	40	45	45	45	45	45	45
Orvego0,1%	-	5	15	20	30	40	45	45	45	45
Enervin0,2%	-	-	-	-	-	-	-	-	-	-
Signum0,1%	-	-	-	-	-	-	-	-	-	-
Previcur0,25%	-	5	20	30	35	40	45	45	45	45
Top M 0,1%	-	3	10	10	10	10	12	15	15	15

In the first experiment, pathogenic fungus *P. parasitica var. nicotianae* reached 30 mm by the end of observation in the control (Table 4). Somewhat poorer growth (20mm) was measured in the variant treated with Signum 0, 1%, and the lowest growth was observed with the fungicides Top M 0,1% (13 mm) and Previcur 0, 25% (15mm). No fungal growth was recorded in media treated with Orvego 0, 1% and Enervin 0, 2%.

Table 4. Colony growth of the fungus *P.parasitica var. nicotianae*

Variant	Colony growth in mm by days									
	1	2	3	4	5	6	7	8	9	10
Control	2	8	10	15	18	20	25	25	30	30
Orvego0,1%	-	-	-	-	-	-	-	-	-	-
Enervin0,2%	-	-	-	-	-	-	-	-	-	-
Signum0,1%	-	2	5	8	11	13	15	17	20	20
Previcur0,25%	-	4	6	8	10	12	12	15	15	15
Top M 0,1%	-	-	2	3	5	6	9	10	10	13

Results of investigations on the three pathogenic fungi obtained in the second experiment are presented in Tables 5, 6 and 7. In the second experiment, the fungus *P. debaryanum* showed rapid growth as in the first one. After 24 hours of incubation, the growth of the control was 10 mm, and the maximum radial growth of 45 mm was reached on the third day (Table 5). No major differences were observed in development of the colony grown in media treated with Orvego 0,1 %, Signum 0,1 % and Top M 0,1%. In all these treatments the fungus developed gradually and by the end of observation the colony growth reached 45 mm. Somewhat poorer growth was observed in the colony grown in media treated with Previcur 0,25 %, while absence of fungal growth was observed in media treated with Enervin 0,2 %.

Table 5.Colony growth of the fungus *P. debaryanum*

Variant	Colony growth in mm by days									
	1	2	3	4	5	6	7	8	9	10
Control	10	32	45	45	45	45	45	45	45	45
Orvego0,1%	5	15	30	45	45	45	45	45	45	45
Enervin0,2%	-	-	-	-	-	-	-	-	-	-
Signum0,1%	-	5	16	25	30	40	45	45	45	45
Previcur0,25%	5	10	12	15	16	18	20	25	30	36
Top M 0,1%	5	25	45	45	45	45	45	45	45	45

Pathogenic fungus *R. solani* which served as control had a successful growth in the second experiment (Table 6). The first day mycelial growth measured 2 mm and radial growth of 45 mm was measured on the sixth day. A similar growth was measured with variants treated with Previcur 0,25% and Orvego 0,1%, while the poorest growth (13 mm) was obtained with Top M 0,1%. No fungal growth was recorded in media treated with Enervin 0,2% and Signum 0,1.

Table 6.Colony growth of the fungus *R. solani*

Variant	Colony growth in mm by days									
	1	2	3	4	5	6	7	8	9	10
Control	2	8	14	22	35	45	45	45	45	45
Orvego0,1%	2	8	15	25	30	35	45	45	45	45
Enervin0,2%	-	-	-	-	-	-	-	-	-	-
Signum0,1%	-	-	-	-	-	-	-	-	-	-
Previcur0,25%	-	3	10	21	29	40	45	45	45	45
Top M 0,1%	-	2	5	7	8	10	10	10	12	13

Results on the development of pathogenic fungus *P. parasitica var. nicotianae* in the second experiment are presented in Table 7.

Table 7.Colony growth of the fungus *P. parasitica var. nicotianae*

Variant	Colony growth inmm bydays									
	1	2	3	4	5	6	7	8	9	10
Control	-	2	4	6	10	15	18	20	20	25
Orvego0,1%	-	-	-	-	-	-	-	-	-	-
Enervin0,2%	-	-	-	-	-	-	-	-	-	-
Signum0,1%	-	-	2	3	5	8	10	10	10	10
Previcur0,25%	-	1	3	4	5	8	10	10	10	11
Top M 0,1%	-	-	-	-	3	6	10	10	12	15

By the end of observation, radial growth of the fungus reached 25 mm in the control variant, which was the highest growth achieved. In treatments with Signum 0, 1

%, Previcur 0, 25 % and Top M 0,1%, the growth was 10 mm, 11 mm and 15mm, respectively. In this experiment too, no fungal growth was recorded in media treated with the fungicides Orvego 0, 1 % and Enervin 0,2%.

Results of investigations on pathogenic fungi *P. debaryanum, R. solani and P. parasitica var. nicotianae* obtained in the third experiment are presented in Tables 8, 9 and 10.

Data on growth of *P. debaryanum* are presented in Table 8. Due to the rapid growth of this fungus, 15 mm radial growth of the colony was measured after 24 hours, and the second day the Petri dish was full, i.e. radial growth was 45 mm. In media treated with Enervin 0, 2 % no mycelia growth was recorded until the last day of observation. Poor colony growth of 30 mm was observed in the variant treated with Previcur 0, 25 % on the tenth observation day. In variants treated with Orvego 0, 1 %, Signum 0,1% and Top M 0,1%, the growth of the colony started from the first day, to reach radial growth of 45 mm by the end of observation (Table 8).

Table 8. Colony growth of the fungus *P. debaryanum*

Variant	Colony growth in mm by days									
	1	2	3	4	5	6	7	8	9	10
Control	15	45	45	45	45	45	45	45	45	45
Orvego0,1%	-	8	20	25	30	40	45	45	45	45
Enervin0,2%	-	-	-	-	-	-	-	-	-	-
Signum0,1%	5	20	40	45	45	45	45	45	45	45
Previcur0,25%	5	8	11	16	18	20	22	25	27	30
Top M 0,1%	5	27	45	45	45	45	45	45	45	45

Mycelial growth of the phytopathogenic fungus *R. solani* was observed in the control from the first day, and the last day of observation it measured 45 mm (Table 9).

Media treated with Enervin 0, 2 % and Signum 0, 1 % showed no mycelial growth to the last day of observation. The poorest growth of 18 mm was measured in the variant treated with Top M 0,1%, while in the variants treated with Orvego 0,1% and Previcur 0, 25 % the mycelial growth started from the first or second day, and on the tenth day radial growth of 45mm was measured.

Table 9.Colony growth of the fungus *R. solani*

Variant	Colony growth in mm by days									
	1	2	3	4	5	6	7	8	9	10
Control	2	10	25	35	38	40	40	45	45	45
Orvego0,1%	2	8	15	25	30	37	40	43	45	45
Enervin0,2%	-	-	-	-	-	-	-	-	-	-
Signum0,1%	-	-	-	-	-	-	-	-	-	-
Previcur0,25%	-	4	12	22	30	38	45	45	45	45
Top M 0,1%	-	2	8	12	15	15	15	17	17	18

Colony growth of *P. parasitica var. nicotianae* is presented in Table 10. In the control variant, mycelial growth was observed on the second observation day,

but since this pathogenic fungus has poorer growth, only 20 mm were measured on the tenth day. No fungal growth was observed in media treated with Orvego 0,1 % and Enervin 0,2 % and the poorest growth of only 5 mm was recorded in fungicide treatments with Signum 0,1 %. In media treated with Previcur 0,25 % and Top M 0,1%, just like in the first and second experiment, there were no significant differences in colony growth of the fungus and it reached 12 mm and 13 mm, respectively.

Table 10.Colony growth of the fungus *P. parasitica var. nicotianae*

Variant	Colony growth in mm by days									
	1	2	3	4	5	6	7	8	9	10
Control	-	3	6	8	8	9	9	12	20	20
Orvego0,1%	-	-	-	-	-	-	-	-	-	-
Enervin0,2%	-	-	-	-	-	-	-	-	-	-
Signum0,1%	-	-	2	4	4	5	5	5	5	5
Previcur0,25%	-	3	5	7	10	11	12	12	12	12
Top M 0,1%	-	-	-	2	5	7	9	10	12	13

According to the results in the above tables, none of the three pathogenic fungi showed occurrence and growth of mycelia in media treated with Enervin 0,2 %. Also, there was no occurrence and growth of *R. solani* and P. *parasitica var. nicotianae*in media treated with Signum 0,1 %, and Orvego 0, 1 %, respectively.

The effectiveness of tested chemicals in the control of soil borne pathogenic fungi *P. debaryanum, R. solanii P. parasitica var. nicotianae*, i.e. their fungicidal and fungistatic activity in the I, II and III experiment is presented in Table 11.

Table 11.The effectiveness of tested fungicides, in %

Variant	*P. debaryanum*				*R.solani*				*P.parasitica var. nicotianae*			
	I	II	III	$\bar{x}$	I	II	III	$\bar{x}$	I	II	III	$\bar{x}$
Control	-	-	-	-	-	-	-	-	-	-	-	-
Orvego0,1%	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	100	100	100	100
Enervin0,2%	100	100	100	100	100	100	100	100	100	100	100	100
Signum0,1%	0,00	0,00	0,00	0,00	100	100	100	100	33,33	60,00	75,00	56,11
Previcur0,25%	55,55	20,00	33,33	36,29	0,00	0,00	0,00	0,00	50,00	56,00	40,00	48,66
Top M 0,1%	0,00	0,00	0,00	0,00	66,66	71,11	60,00	65,92	56,66	40,00	35,00	43,88

The highest effectiveness in the control of above soil borne phytopathogenic fungi during *in vitro* investigations was obtained with the fungicide Enervin applied in a concentration of 0.2 %. In all three experiments its effectiveness was 100 %, i.e. no occurrence and growth of these pathogenic fungi was recorded. High fungicidal effect (100 %) against pathogenic fungus *P. parasitica var. nicotianae* in all three experiments was obtained with the chemical Orvego in concentration of 0.1 %. The chemical Signum in concentration of 0.1 % showed 100% effectiveness in the control of pathogenic fungus *R. solani* and high fungistatic effect 75,00% in the control of *P . parasitica var. nicotianae* (in 3de replication, or 56,11% in average).

The standard chemicals confirmed their fungistatic effect against the investigated pathogens. By application of Previcur 0,25%, 55,55% effectiveness was obtained

in *P. debaryanum* in the Ist replication and 56,00% in *P. parasitica var. nicotianae* in the IInd replication.The obtained average values were 36,29% and 48,66% in *P. debaryanum* and *P. parasitica var. nicotianae* respectively. Similar results with 53.10 % to 69.46 % effectiveness were reported by Taskoski (2009), in his in vitro investigations of this fungicide in the control of *P. debaryanum*. The chemical Top M applied in concentration of 0.1 % showed high effectiveness (60,00 -71,11%) in the control of *R. solani* in the IIIrd and IIndreplication, with average value of 65,92%. This product also showed good fungistatic effect against *P. parasitica var. nicotianae* (35,00-56,66%, i.e.43,88% in average). Taskoski (2001) reported high effectiveness of chemicals with a.i. thiophanate methyl in the control of *R. solani* both at *in vitro* and in natural conditions of seedlings growing.

CONCLUSION

In our investigations, soil borne phytopathogenic fungi *P. debaryanum, R. solani and P. parasitica var. nicotianae* were successfully grown on potato dextrose agar (PDA) and in some of them maximum colony growth was obtained three days after incubation. The investigated chemicals showed big differences in colony growth, depending on the pathogenic fungus. Some fungicides showed high fungicidal effect against one pathogen and fungistatic effect against another.

Of the fungicides investigated, 100 % effectiveness was obtained with Enervin 0.2 % against all three pathogenic fungi and with Orvego 0.1 % against *P. parasitica var. nicotianae.*Signum 0.1% showed 100% effectiveness against *R. solani* and certain fungistatic effect against *P. parasitica var. nicotianae*.

Of the standard chemicals, fungistatic effect was confirmed with Previcur 0.25 % against pathogenic fungi *P. debaryanum*and *P. parasitica var. nicotianae*, and with Top M 0.1 % against *R. solani* and *P. parasitica var. nicotianae*.

Due to their high fungicidal effect on growth and development of *P. debaryanum, R. solani and P. parasitica var. nicotianae*, the chemicals Enervin, Orvego and Signum can find practical application in future, in protection of tobacco seedlings from these disease causing agents.

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Original Scientific paper

TOBACCO PRODUCTION IN THE REGION OF PELAGONIA -REPUBLIC OF MACEDONIA

Snežana Stojanoska**,**
University "St. KlimentOhridski -Bitola,
Scientific Tobacco Institute-Prilep,Republic of Macedonia
E-mail:snezana.stojanoska@yahoo.com

ABSTRACT

Analysis of tobacco production (yields and planted area) in the region of Pelagonia will be made in this paper. Pelagonia is traditional and largest producer of high-quality tobacco in the Republic of Macedonia. It is especially known for the production of oriental tobacco, which is the most interesting for the foreign market. In recent years,the production of oriental tobacco in R. Macedonia ranged from23.200 t in2009 to30.273t in2010, or in average27.040t.

In the period of investigation (2009-2013), the share of the Pelagonia region in the average tobacco production of R. Macedonia was 12.762tons.The highest production was recorded in the municipalities of Dolneni(4034,2tons) and Prilep(3124 ,0tons).

The average yield inPelagonia region ranged from1122,4kg/ha in 2009to 1297,4kg/ha in 2013. The highest yield was achieved in municipalities Krusevo-1346,6kg/ha, Dolneni-1341,6 and Mogila-1317,4kg/ha and in the other municipalities the yields were relatively lower.

The average area under tobacco in the same period amounted to 9946,8ha, the major part of which belonged to the municipalities of Dolneni (3006,2ha) and Prilep (2472,8 ha). In other municipalities the average area under tobacco was smaller and ranged about1000 hectares.

Keywords: tobacco, regions, production, area, yield

ПРОИЗВОДСТВО НА ТУТУН ВО РЕГИОНОТ НА ПЕЛАГОНИЈА - РЕПУБЛИКА МАКЕДОНИЈА

Во овој труд ќе извршиме анализа на производството- приносот и површините засадени со тутун во Пелагонискиот регион, кој претставува традиционален и најголем производител на ориенталски тутуни во Р. Македонија.

Пелагонија е најголемиот регион за производство на тутун со високи квалитативни вредности, особено типот прилеп кој е доста интересен за странскиот пазар.

Во последниве години просечното производство на ориенталски тутуни во Р. Македонија се движи од 23.200 тони во 2009 до 30.273 тони во 2010 година, или во просек27.040 тони.

Во просечното производство на тутун во Р. Македонија во периодот 2009-2013 година, Пелагонискиот регион учествува со 12.762 тони. Најголемо производство е остварено во општините Долнени (4.034,2 тони) и Прилеп (3.124 тони).

Просечниот принос на тутун во Пелагонискиот регион се движи од 1122,4 кг/ха во 2009 до 1297,4 kg/ha во 2013 година. Најголем просечен принос е постигнат во општините Крушево- 1346,6 kg/ha, Долнени- 1341,6 kg/ha и Могила-1317,4 kg/ha, а останатите општини имаат релативно понизок принос.

Просечната површина под тутун во овој регион во периодот 2009- 2013 година изнесува 9.946,8 хектари. Најголема површина под тутун има Општина Долнени- 3.006,2 хектари, понеа следи Општина Прилеп со

2.472,8 хектари а во останатите општини површините под тутун се помали и се движат околу 1000 хектари.

Клучни зборови: регион, површина, производство, принос, тутун

INTRODUCTION

Tobacco production has an important place in the agro-industrial complex of the Republic of Macedonia. According to our statistical nomenclature of territorial units, tobacco production in the country is present in 8 regions and 56 municipalities.

The largest region in the country is Pelagonia, in the southern part of the Republic Macedonia, covering the Pelagonia Valley and the basin of the Prespa Lake.

The region consists of the following municipalities: Dolneni, Prilep, Krusevo, Mogila, Krivogastani, Bitola, DemirHisar, Nobatsi and Resen. It is characterized by favorable soil and climate conditions for producing high quality oriental tobacco which is highly valued on the world market. We shall especially emphasize the production of the type Prilep, which averages about 11.681,4 tons and represents almost half of the total production in the country.

The aim of investigation was to make analysis on production, yield and planted areas in Pelagonia tobacco producing region. Results of the investigation will present the actual situation regarding the production, human and natural resources characteristic for this region.

MATERIALS AND METHOD

Data from the State Statistical Office of the Republic of Macedonia for the period 2009-2013 were used as material for this paper, along with the following publications Statistical Review: Field crops, orchards and vineyards in 2009-2013; Regional Yearbook: The regions in the Republic of Macedonia; scientific papers published in the journal Tutun/ Tobacco and other scientific and technical references. Secondary internal and external data sources were used in realization of this investigation.

The following methods were applied in the investigation: analytical, comparative, inductive, deductive, method of index and other mathematical-statistical methods common to agricultural research.

RESULTS AND DISCUSSION

The finest oriental, semi-oriental and tobaccos intended for export are traditionally produced in the area of the Republic of Macedonia.

In each region of the country, there are specific differences inthe relief, climate, altitude and soil conditions that favor the production of certain tobacco type.

The region of Pelagonia is the largest region in R. Macedonia and it is most suitable for production of oriental tobacco type Prilep.

The share of Pelagonia region in the total tobacco production of the country in the last five years ranged from 43.22% in 2011 to 50.79% in 2009, which makes an average of 47,29%. Approximate share was observed in 2012 and 2010 -46.96% and 46.17%, respectively (Table 1, Fig. 1).

Table 1. The share of Pelagonia region in the total tobacco production of R. Macedonia, in tons

Year	R. Macedonia	Pelagonia region	%
2009	23.200	11.783	50,79
2010	30.273	13.977	46,17
2011	26.537	11.469	43,22
2012	27.333	12.836	46,96
2013	27.859	13.746	49,34
Average	27.040	12.762	47,29

Source: State Statistical Office of the Republic of Macedonia. Regional Yearbook: the regions in R. Macedonia, 2014, Skopje

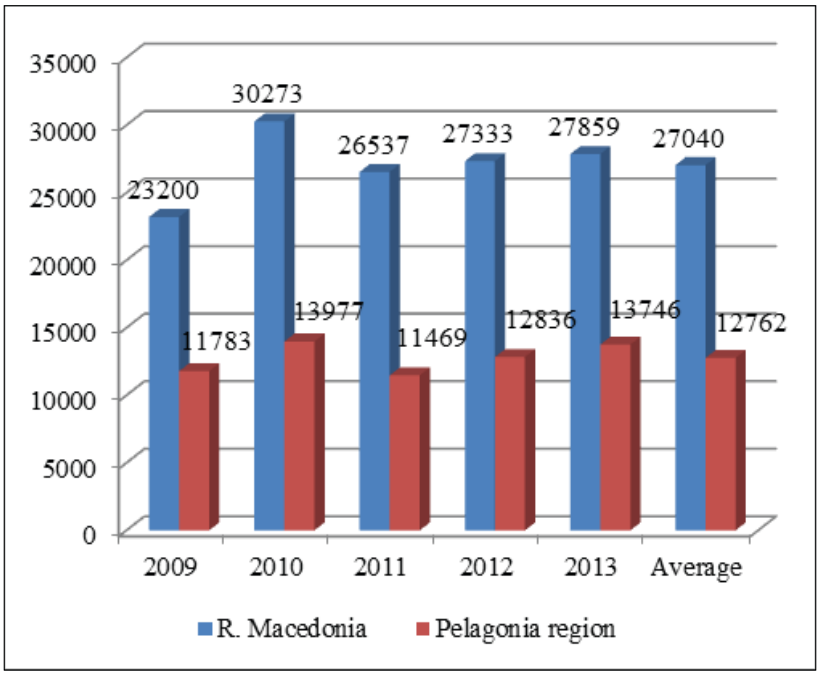

Figure1. The share of Pelagonia region in the total tobacco production of R. Macedonia, in tons

Table 2. Area sunder tobacco in the region of Pelagonia, by municipalities (ha)

Municipality	Areas under tobacco					Average	%
	2009	2010	2011	2012	2013		
Dolneni	2772	3121	3036	3040	3062	3006,2	30,3
Prilep	2156	2518	2440	2545	2705	2472,8	24,9
Krusevo	954	1024	968	989	1173	1021,6	10,3
Mogila	884	877	844	842	849	859,2	8,6
Krivogastani	747	796	808	824	844	803,8	8,0
Bitola	712	781	811	845	861	802,0	8,1
D. Hisar	740	767	733	736	751	745,4	7,5
Novaci	225	236	224	227	239	230,2	2,3
Resen	0	0	8	8	12	5,6	0,1
Total	9190	10120	9872	10056	10496	9946.8	100

Source: Statistical reviews -Field crops, orchards and vineyards

In the context of our research, will first present data on the areas under tobacco in Pelagonia region for the period 2009-2013by municipalities (Table 2, Fig. 2).

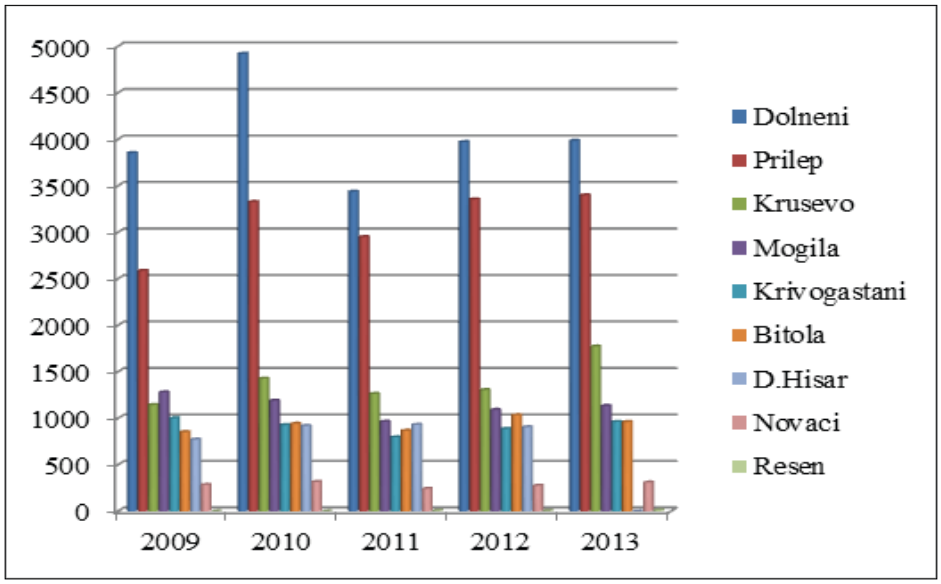

Figure 2. Area sunder tobacco in the region of Pelagonia(ha)

According to data presented in Table 2, the average area under tobacco in 2009-2013 was 9946.8 ha. The largest area was observed in municipalities Dolneni and Prilep (3006.2 and 2472.8 ha), which is around 55%, i.e. these two municipalities account for half of the area under tobacco.The area of all other municipalities is very small and ranges from only 0.1 to 10.3%.

Tobacco production of the Pelagonia region in 2009-2013 ranged from 11.469 tons in 2011 to 13.977 tons in 2010, i.e. an average of 12.521,4 tons. The largest production was observed in municipalities Dolneni-4034.2 tons, or 32.2% and Prilep- 3124.0 tons or 24.9%,which means that these two municipalities account for about 57%.In other municipalities, average production ranges from 6.0t in Resen to 1383.2 t in Krusevo. Higher production was also noted in the municipality Mogila(1133.2 tons), while the production in the other municipalities investigated was relatively low (Table 3, Fig. 3).

Table 3. Tobacco production in Pelagonia region, by municipalities (in tons)

Municipality	Tobacco production (t)					Average	%
	2009	2010	2011	2012	2013		
Dolneni	3854	4921	3438	3972	3986	4034,2	32,2
Prilep	2587	3328	2951	3355	3399	3124,0	24,9
Krusevo	1145	1428	1264	1305	1774	1383,2	11,0
Mogila	1282	1191	966	1092	1135	1133,2	9,0
Krivogastani	1003	928	797	887	963	915,6	7,3
Bitola	854	943	869	1035	961	932,4	7,4
D.Hisar	772	920	932	906	0	706,0	5,6
Novaci	286	318	243	276	311	286,8	2,3
Resen	0	0	9	7	14	6,0	0,3
Total	11.783	13.977	11.469	12.835	12.543	12.521,4	100

Source: Statistical reviews -Field crops, orchards and vineyards

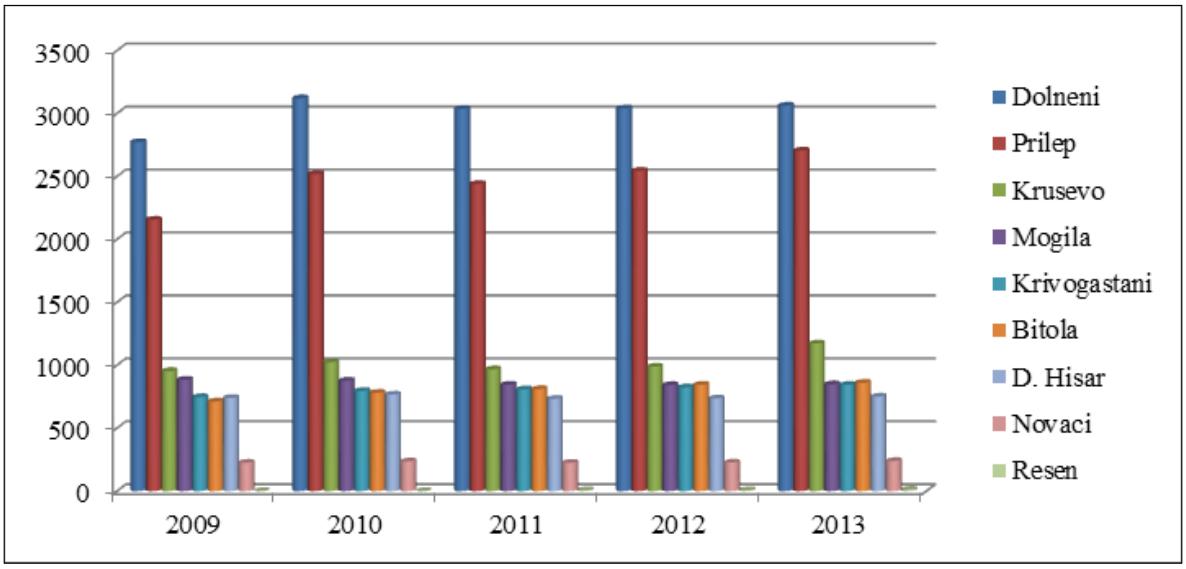

Figure 3. Tobacco production in Pelagonia region, by municipalities (in tons)

The average yield per unit area in the same period ranged from 1122.4kg/ha in 2009 to 1297.4 kg/ha in 2013, or 1189.9 kg/ha in average. The highest average yield was achieved in municipalities Krusevo(1346.6 kg/ha),Dolneni(1341.6 kg/ha) and Mogila(1317.4 kg/ha), while municipalities Prilep,Novaci and DemirHisarachieved almost equal average yield 1261.2, 1245.0 and 1227.8 kg/ha, respectively. Municipality of Resen has the lowest average yield of 632.6kg/ha (Tabela4, Fig. 4).

Table 4. Average tobacco yield in Pelagonia region, by municipalities (kg/ha)

Municipality	Yield (kg/ha)					Average	Rank
	2009	**2010**	**2011**	**2012**	**2013**		
Krusevo	1200	1394	1306	1320	1513	1346,6	1
Dolneni	1390	1577	1132	1307	1302	1341,6	2
Mogila	1450	1358	1145	1297	1337	1317,4	3
Prilep	1200	1322	1209	1318	1257	1261,2	4
Novaci	1272	1349	1084	1217	1303	1245,0	5
D. Hisar	1356	1209	1087	1205	1282	1227,8	6
Krivogastani	1034	1155	1154	1100	1425	1173,6	7
Bitola	1200	1208	1071	1225	1116	1164,0	8
Resen	0	0	1100	921	1142	632,6	9
Average	1122,4	1174,6	1143,1	1212,2	1297,4	1189,9	

Source: Statistical reviews -Field crops, orchards and vineyards

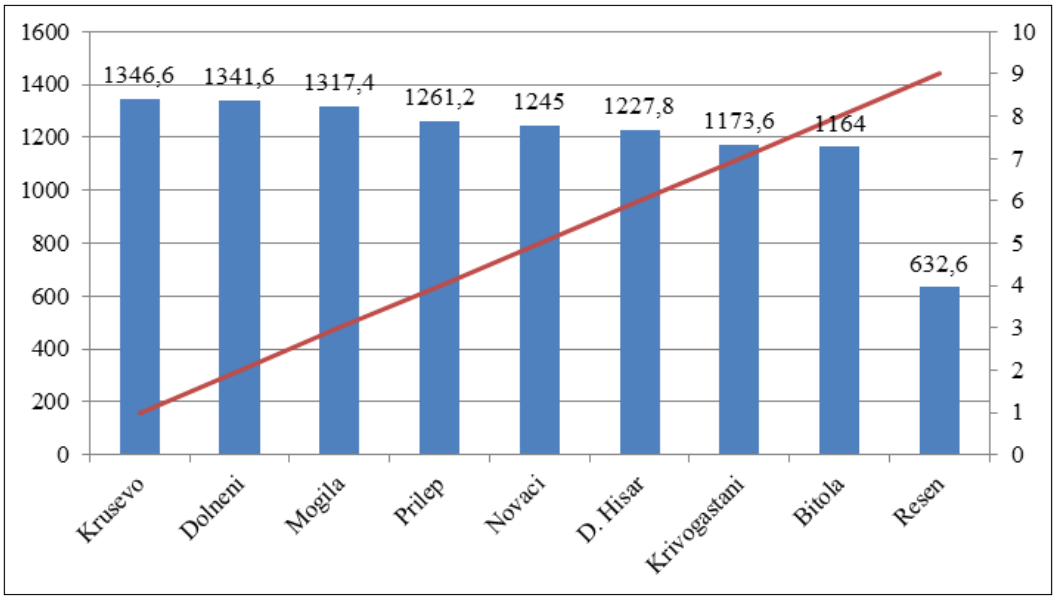

Figure 4. Average tobacco yield in Pelagonia region, by municipalities(kg/ha)

From the above data it can be seen that municipalities in the region of Pelagonia have favorable conditions for production of good quality tobacco.

CONCLUSIONS

Based onthe data presentedfortheproduct ionoftobaccoin the region ofPelagonia (R. Macedonia), the following conclusions can be drawn:

1. Asccording to the newstatisticalnomenclatureof the Territorial units, tobacco productionin the Republic of Macedoniaisrepresente din8regionsand 56municipalities.
2. The largestregionin the country isPelagoniaregion, with the follow ingmunicipalities:Dolneni, Prilep, Krusevo, Mogila,Krivogastani, Bitola, DemirHisar, NovatsiandResen.
3. The shareofPelagoniaregionin the total productionof the countryin the period2009-2013rangesfrom43.22% in 2011to 50,79% in 2009, which is an average of 47.29%. Similarpercentages were observedin 2012 and2010(46.96% and 46.17%).
4. The averageareas planted withtobaccoin the Pelagoniaregionin the same period amounted to9946.8hectares. The biggest planted areas were observed inmunicipalities Dolneni and Prilep(3006,2,2472,8 ha, i.e. 55%).
5. Tobacco production in Pelagoniaregionin the period2009-2013 rangesfrom11,469tons in2011 to13,977tonsin 2010, orin average 12,521.4tons. MunicipalitiesPrilepan dDolnenireached thehighesttobacco productionwith a shareof 57%.
6. The yield per unit area during the investigation period ranged from 1122.4kg/hain2009 to1297.4kg/ hain2013, averaging 1189.9 kg/ha. The highestaverage yieldwasachievedinmun icipalitiesKrusevoDolneni and Mogila. Somewhat lower yields were obtained in municipalities Prilep, Demir Hisar and Novaci.

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Original Scientific paper

DEVELOPMENT TRENDS OF TOBACCO PRODUCTION , COMPARED TO THE PRODUCTION OF WHEAT, CORN AND SUNFLOWER

Trajko Miceski , Silvana Pašovska

University,, Goce Delchev" Stip, Faculty Stip
e-mail:s_pasovska@yahoo.com

ABSTRACT

A retrospective look at the past period from 1973 to 2013, the tobacco production marked fluctuations in gradual decline.
Also there are perceive fluctuations in the production of wheat, corn and sunflower, in Macedonia, where the production from year to year oscillate with a tendency to a slight reduction in wheat production. The sunflower production is reduced as well there is a slight increase in the production of corn.
For better visibility, the labor movement of the crops is shown in absolute terms in the tons, and their annual average yields in kilograms per hectare (kg / ha) by separate cultures through spreadsheets and charts.

Key words: tobacco, sunflower, production, wheat

ТЕНДЕНЦИИ ВО РАЗВОЈОТ НА ПРОИЗВОДСТВОТО НА ТУТУН ВО СПОРЕДБА СО ПРОИЗВОДСТВОТО НА ПЧЕНИЦА, ПЧЕНКА И СОНЧОГЛЕД

Ретроспективен поглед на изминатиот период (1973-2013) открива забележителни флуктуации во производството на тутун, со тенденција за негово постепено опаѓање.
Исто така забележливи се и флуктуации во производството на пченица, пченка и сончоглед во Македонија, каде производството од година во година осцилира, со тенденција на благо намалување во производството на пченица. Производството на сончоглед исто така се намалува, а кај производството на пченка има мало зголемување.
За подобра видливост, даден е табеларен и графички приказ на движењето на овие култури во апсолутна вредност во тони, како и нивните годишни просечни приноси во килограми по хектар kg/ha

Клучни зборови: тутун, сончоглед, производство, пченица

INTRODUCTION

The traditional feature of Republic of Macedonia is the production of tobacco, wheat, sunflower and other agricultural and industrial crops.
heir production from year to year oscillates but recently, especially since 2000 there is a tendency of slight declension at almost all agricultural and industrial crops.

On this aspect the production of tobacco features a slight decrease, except for the last four years from 2010 to 2013 where the production is stable, from 27,000.00 to 30,000.00 little more than the average annual production within the last forty years, which is 26314.00 tonnes.
Following the world politics and intentions

of the EU, our country as a country applicant to EU and part of the world market must have clear image of the dynamic movement to the production of certain products.
In this paper, we provide tabular and graphical representations to the dynamic production of tobacco and to the appropriate agricultural and industrial crops, which will show the basis for the future production planning, according to the needs of national markets and bezels.

DEVELOPMENT TRENDS OD TOBACCO PRODUCTION IN R. MACEDONIA

In the last forty years, from 1973 to 2013, the production of tobacco oscillates with a tendency to gradually decline. In table 1 is shown the tobacco production in absolute values expressed in tonnes and registered (purified) forecasted data for the next four years with linear performance and oscillations that are certainly expected.

Table1. Dynamics in production of tobacco in R. Macedonia, (1973-2013)

Years	Tobacco production in absolute values	Tobacco production by purified and predicted values
1973	32.437	30.119
1974	27.978	29.929
1975	34.126	29.739
1976	33.721	29.548
1977	32.296	29.358
1978	31.154	29.168
1979	29.447	28.978
1980	23.587	28.787
1981	31.294	28.597
1982	34.000	28.407
1983	22.490	28.217
1984	30.719	28.026
1985	30.728	27.836
1986	35.020	27.646
1987	28.648	27.456
1988	22.259	27.266
1989	27.537	27.075
1990	16.452	26.885
1991	25.195	26.695
1992	26.502	26.505
1993	24.002	26.314
1994	18.862	26.124
1995	15.683	25.934
1996	15.412	25.744
1997	25.308	25.554
1998	32.746	25.363
1999	29.368	25.173
2000	22.175	24.983
2001	23.217	24.793
2002	22.911	24.602

2003	23.986	24.412
2004	21.630	24.222
2005	27.691	24.032
2006	25.036	23.841
2007	22.056	23.651
2008	17.087	23.461
2009	24.122	23.271
2010	30.280	23.081
2011	26.537	22.890
2012	27.333	22.700
2013	27.859	22.510
2014		22.320
2015		22.129
2016		21.939
2017		21.749

We can say that the linear trend shows the declining in the tobacco production in the last forty years which will continue in the future. But that does not mean that the progress will be according to the values of the regression line, there will be oscillations and would range around the current average annual production which is 26.314 tonnes for the last forty years. There have been few reasons such as the global market, various EU and other regulations, anti- tobacco propaganda and etc.

DEVELOPMENT TRENDS OF AGRICULTURALAND INDUSTRAL CROPS, COMPARED WITH THE PRODUCTION OF TOBACCO , IN R. MACEDONIA

Global world politics and policies of the EU constantly seek information for the production of agricultural and industrial corps in order to have a better perspective of their development dynamics. We as a country that prefers the global market also as an applicant for EU member must have information for the production dynamic of certain products. Therefore, in this paper are presented parallel data for the production of tobacco, wheat, corn and sunflower, in a period of forty years.

Table 2. Production of tobacco, wheat, corn and sunflower in R. Macedonia (1973-2013) in tonnes

Number	Years	Tobacco	Wheat	Maize	Sunflower
1	1973	32.437	275.936	107.755	23.569
2	1974	27.978	306.384	85.301	21.030
3	1975	34.126	286.696	96.857	27.162
4	1976	33.721	368.659	106.235	31.334
5	1977	32.296	226.293	83.065	28.527
6	1978	31.154	278.853	70.103	13.476
7	1979	29.447	280.056	90.999	25.195
8	1980	23.587	273.406	88.445	23.085
9	1981	31.294	235.730	91.520	25.052
10	1982	34.000	272.408	92.878	24.058
11	1983	22.490	245.566	98.992	24.807

12	1984	30.719	267.719	88.795	18.491
13	1985	30.728	288.455	79.194	19.515
14	1986	35.020	314.655	123.627	41.271
15	1987	28.648	292.226	95.419	32.951
16	1988	22.259	296.397	73.956	20.774
17	1989	27.537	313.752	136.700	46.345
18	1990	16.452	231.392	79.543	13.419
19	1991	25.195	340.747	134.958	38.685
20	1992	26.502	299.522	130.259	37.756
21	1993	24.002	249.789	101.063	18.841
22	1994	18.862	336.133	133.211	17.880
23	1995	15.683	381.226	165.652	22.290
24	1996	15.412	269.303	142.241	20.586
25	1997	25.308	293.762	157.234	14.902
26	1998	32.746	336.562	140.949	13.148
27	1999	29.368	319.419	160.550	13.937
28	2000	22.175	299.356	125.383	7.351
29	2001	23.217	246.208	117.070	5.475
30	2002	22.911	266.961	140.694	8.760
31	2003	23.986	225.300	136.492	6.794
32	2004	21.630	356.825	141.875	7.764
33	2005	27.691	333.850	148.234	6.711
34	2006	25.036	293.326	147.494	6.016
35	2007	22.056	218.076	118.378	3.579
36	2008	17.087	291.719	127.125	5.444
37	2009	24.122	271.117	154.237	7.774
38	2010	30.280	243.137	129.045	7.592
39	2011	26.537	256.103	126.096	8.497
40	2012	27.333	214.963	115.928	4.765
41	2013	27.859	258.960	131.043	3.832
Total		1.078.891	11.656.947	4.814.595	748.440
Annual average		26.314	284.316	117.429	18.255

Table 2 show that the production of tobacco and other industrial products can oscillate in the last forty years oscillate. In all examined cultures, except the corn, shows a gradual decline from year to year.

Thus, the tobacco production has reached the highest level of 35 020 tonnes in 1986 and the lowest level of 15 412 tons in 1996. But in the last four years (2010-2013) the production is above the annual average.

Without going into a separate analysis we will point out that the previous data for the production of tobacco, wheat, corn and sunflower could contribute and determinate the producers of the conceptualizing future entrepreneurial activities and development of their family businesses.

Considering the fact that the purpose of each manufacturer to grater quantity and quality in production by achieving high yields per unit area, and thus higher profits, then there must have be a recognition in the production, the impact of certain external factors, and the impact of the manufacturers of production.

CONCLUSION

Statistics for the development trends of tobacco production, wheat, corn and sunflower from 1971 to 2003 in Republic of Macedonia, shows cyclic movements that decrease with the exception of the production of maize which gradually increases.

Such development trends owe a number of factors, such as purchase prices, costs of production, weather, etc.

The presented data and analysis in the paper can be used for carrying out the findings and to guide the production of the industrial products in future, according to the strategic development of the agricultural policy of the EU and the world global agricultural policy.

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Original Scientific paper

CLUSTERING SMALL AND MEDIUM ENTERPRISES IN ORDER TO STRENGTHEN THEIR COMPETITIVENESS

Zoran Kalamanda

Tobacco Factory, Banja Luka
e-mail: obrenija2009@hotmail.com

ABSTRACT

Using clusters as a model for developing business has proved to be practical, especially in countries and regions that have a tradition of supporting the development of small and medium enterprises. The immediate precursors to the development of clusters are incubators. In general, where there is experience with incubators, clusters are developing rapidly.
The need for vertical and integrative connectivity allows the introduction of new firms and their adaptation to innovation –based networks. Localization allows the concentration of innovative activities, operational skills and entrepreneurial predispositions, which is of great importance for all interactive processes in the context of creating a new business.
The establishment and development of clusters result in significant effects that have a positive impact on the companies in the cluster and the whole region where the cluster is located. This implies the creation of more jobs, greater variety of manufacturing processes, lower procurement costs, expansion of the market and creation of opportunities for new business connections.

Keywords: clusters, small and medium enterprises, site.

КЛАСТЕРСКО ПОВРЗУВАЊЕ НА МАЛИ И СРЕДНИ ПРЕТПРИЈАТИЈА ЗА ЗГОЛЕМУВАЊЕ НА НИВНАТА КОНКУРЕНТНОСТ

Развојот на бизнисот по моделот на кластери се покажа практичен, особено во земјите и регионите кои имаат традиција во давањето поддршка за развој на малите и средните претпријатија. Непосреден претходник на развојот на кластери се инкубаторите и, во принцип, таму каде што постојат искуства со инкубаторите, кластерите брзо се развиваат.
Потребата за вертикално и интегративно поврзување овозможува воведување на нови компании и нивно прилагодување на мрежата врз база на иновации. Локализацијата овозможува концентрација на иновативните активности, оперативните способности и претприемачките предиспозиции, што е од големо значење за сите интерактивни процеси во контекст на создавање на нов бизнис.
Резултат на воспоставувањето и развојот на кластери се значајните ефекти кои позитивно влијаат на компаниите во кластерот и на целиот регион во кој се наоѓа кластерот. Тоа подразбира создавање на нови работни места, поголема разновидност на производните процеси, намалување на трошоците за набавка, проширување на пазарот и создавање на можности за нови деловни врски.

Клучни зборови: кластер, мали и средни претпријатија, локалитет.

INTRODUCTION

Clusters are geographic concentrations of interconnected companies and various related activities, specialized suppliers, service providers and related organizations for support (educational and research institutions, agencies, etc..) which compete or cooperate at a specific area of activity. The diversity of clusters forms and constant development of new types of associations make impossible to provide a common basis for all clusters, so they have no even a single definition.

The term cluster is derived from the English word cluster which means collection, group of the same or similar elements. In fact, clusters are networking model where firms are grouped in a flexible way, driving the development of small and middle enterprises and on this basis promote economic development at the local and regional level. However, it also indicates that the conceptual approach to the development of clusters is relatively complex and therefore can only be achieved by the simultaneous collaboration of representatives from governments, companies as candidates for the cluster, financial institutions, educational and other organizations.

THE OBJECTIVES OF THE ESTABLISHMENT OF CLUSTERS

The system clusters (economic clusters) is a new global model for small and medium-sized enterprises development. Clusters are developing where the small business development already reached a considerable level and where the system state measures are favorable. Clusters of companies classified in the same activity or activities create more comparative organization, development, market - marketing ventures at one region, or more closely related regions. In this way they contribute the company to be rapidly developed, to apply modern methods and, under modern management, derive the maximum out of the market environment, thereby creating opportunities for the economy of the region gain a competitive advantage over other regions.

The cluster as a specific network related profit and non-profit entities rapidly change market processes within the regional framework. This is because collective action participants in the cluster system clusters accelerate marketing effects in relation to suppliers, customers and the general distribution channels. At the same time, and because of the physiognomy of the cluster as upgrading of small and medium-sized enterprises, they accelerate the specialization of small firms and their classification into economic groups and the business sector.

The literature has still not sufficiently understood the phenomenon of clusters, but it recognized that they rapidly develop in specific locations, close to the most important resources for business, close to major industries and markets of goods for final consumption. One of the important features in the development of clusters is a high degree of novice companies (start - ups) and a high degree of innovation processes in them.

The most common joint objectives for which the clusters were established can be divided into six segments:

• Research and development of the network - creating a network within a cluster and between clusters (making a database of companies, regular visits, directory of suppliers and service providers, website, monthly information about updates in the industry and environment, periodicals, etc.);

• Training and education - analysis of the need for specific training, organization of training, regular meetings of companies in order to exchange experiences and contacts,

and so on.;
• Business cooperation - initiating and supporting projects of cooperation between companies, educational and research and development institutions, to connect with the funds to finance innovative projects;
• The impact on politics - lobbying and creating dialogue between industry, academic society and government;
• Innovation and technology – facilitating of innovation processes, trend monitoring, dissemination of new knowledge and the introduction of quality standards, improvement of technological processes;
• The growth of the clusters - the strengthening of regional identity, building national and international reputation, promote domestic and foreign investment.

The success of the cluster is based on mutual trust and respect of certain principles and rules. Clusters should be organized where the first results can be achieved. Certainly, it should be borne in mind that the cluster is a long - term project and, as such, it is very complex. The cluster is oriented strategy which involves the development of specific sectors, industries in a particular area for a particular company, members of the cluster. Cluster development is the most effective way to raise the economy of a region at a much higher level. Modern business is based on speed, quality, flexibility, innovation, connection and building a critical mass of capital and production (service) potential. This new style of business requires a team approach at the local level - the cluster approach. In many countries, especially in countries in transition, cluster development has been accepted as a fundamental strategy for economic development. Therefore, Michael Porter points out: " Clusters are the basis of new, productive economy."

Clusters are based on systemic connections between companies. Links can be built on common or similar products (eg, fruit producers, restaurants, tourist facilities, tourist agencies), production process (production of wheat, the production of agricultural machinery, reception and guest accommodation, organizing sightseeing tour), common technologies, the need for natural resources (agricultural land, waterways, forests and protected landscapes), requirements for certain professional qualifications (a common need for labor - translators, technicians, cooks).

There are two basic types of building clusters from the top down (top down) and from bottom to top (bottom up), and often the combination of the two. Model " from top to bottom" (top down) was developed in the countries in transition. As a rule, this model develops when the possibility exists for the formation of the cluster but is not recognized by the business sector and the need for the state to encourage the initiative of creating a cluster in the direction from top to bottom. The initiative of creating a cluster must include proper and related institutions as well as institutions for training, research, development and others. After the starting of the cluster state can remain as moderator of processes with certain incentives for cluster development.

Model "bottom-up" (bottom up) cluster development is related to the initiation of the development of the business sector. In this case, the government accepts the initiative and creates conditions for the development of clusters. This model is represented in Western Europe, where MSE are collaborating and competing each other and create a healthy economic structure whose final result is the creation of clusters. Benefits of association under the principle clusters showed the full value of the developed countries, but also in underdeveloped countries and countries in transition. Clustering means cooperation and innovation of active partner for companies of all sizes from a variety of areas, citing the education, development and introduction of new technologies into business processes and, above all, involvement in international development trends, creating opportunity not only to monitor the existing ones.

Cluster concept is not new, it was contemplated in the United States at the beginning of the twentieth century, by the constutuion of corporations and increase of productivity. Since then, the clustering approach has been considered in the context of the ability to take innovation not only at the corporate level, but also at the region and now the national and multinational level.

In short, within the cluster it is easier, faster and cheaper to achieve all these than in individually required companies:

- Obtaining of certificates of quality that ensures finding the right buyer for the right product;
- Product Branding or branches;
- Training of workers;
- Introduction of new technologies;
- Research;
- Improving of design .

According to research by the European Cluster Observatory from 2008, which comprised 31 European countries (of which 26 are the members of EU) it was found that all countries have adopted policies of clusters at the state or regional level. Half of them adopted the policy clusters in 1999. National clusters programs have over 80 % of the countries that are mainly related to industrial policy and company policy or the policy of science and technology .

Relevant institutions and funding sources, who are responsible for policy of clusters are mainly ministries of industry and trade or ministry of economy as well as the ministry of finance. Sources of funding are from the national budget 63 %, EU structural funds, 19 %, 7 % of the business sector, the regional budget of 3 % and 8 % other sources. Financing is done so that a group or cluster of potential clusters apply for grant support. Most applications (70 %) are made on the principle of bottom - up and the remaining 30 % by the principle of top - down.

The experience of the Republic of Serbia and Western Balkan countries in the field of the clusters are more than modest. Specific examples of clusters that exist or are under development are mainly experimental trials that were mostly developed without clearly defined rules, organization, mission and vision. One of the main problems is the lack of knowledge and lack of understanding of the term "cluster" and the importance of their institution for the development of the region. In the RS and the Western Balkans in general business conditions are extremely unstable. The system for facilitate and development of SME and their joining in clusters at the state level is not yet finished

In the Republic of Srpska and in Bosnia and Herzegovina the results are much more modest in this area, compared to the previously mentioned countries. The following table provides an overview to the now established clusters in the Republic of Serbia.

Table 1. Clusters in the Republic of Srpska

No	Name	City	Yar of estab.	No of members	Field of industry
1	Cluster „Drvo-PD"	Prijedor	2005	40	Vood industry
2	Cluster "Drvo-G"	Gradiška	2007	11	Vood industry
3	Cluser "Drina drvo"	Srebrenica	2007	n/a	Vood industry
4	Cluster "Drvo"	Banja Luka	2007	n/a	Vood industry
5	Cluster "Solargroup"	Banja Luka	2007	5	Solar systems
6	Cluster "Koža"	Banja Luka	2009	6	Leather proccesing industry
7	Cluster "Bilje-graf"	Trebinje	2007	n/a	Plant production
8	Association "Drvotehnika"	Doboj	2007	n/a	Vood industry

In accordance with the Strategy of SME development, Republic Agency for SME development in 2007 from its own funds supported the establishment of six clusters: Drvo PD-Prijedor, Drvo Banja Luka, Drvo G-Gradiška, Solar Klaster Banja Luka, Klaster Bilje graf Trebinje i Udruženje Drvotehnika Doboj. From all the above clusters, "Drvo -PD" Prijedor is the most successful cluster that has over 40 members. The cluster of wood and furniture industry "DRVO" was established in 2005 in Prijedor, on the initiative of local businesses and supported by the Agency for Economic Development of the Municipality of Prijedor - PREDA. Cluster "DRVO" Prijedor is an organization whose goals are business networking, education, information exchange and promotion of business enterprises and active entrepreneurs within the wood and furniture industry, and improving the conditions of the activity of wood processing, production of wood products and furniture, monitoring technical technological advances in this field and adjustment of interests.

The status of cluster organization was received in 2007, by signing the contract for the project clusters with the Republic Agency for SME development. If we take into account that members have a headquarters in five neighboring municipalities and cities (Banja Luka, Kostajnica, Banja luka, Novi Grad and Prijedor), it is reasonable to say that the cluster DRVO" has a regional character.

The aim of the cluster "DRVO"- Prijedor is to improve the competitiveness of its activities, wood and furniture industry, and offer support to companies in raising product finalization. In this sense, the organization of joint visits to trade fairs and equipment manufacturers, study visits, seminars and training to meet the needs of members, all significantly contribute to the achievement of the above objectives .

Association "DRVO - G" from Gradiška has 11 members from the Municipality of Gradiska. Association of wood Processing "WOOD - G" Gradiska gathers all the economic operators who want to preserve and improve the secret crafts of woodworking and preserve the forest as a source of much needed raw materials.

Association "Drvotehnika" - Doboj gathers enterprises and entrepreneurial activities in the field of wood processing, in order to improve operations, better promotion and joint market Cluster "Drina drvo"- Srebrenica brings together companies from the municipalities of Srebrenica, Bratunac and Milici. Cluster activities are to strengthen the representation of common interests of companies in the wood processing and forestry, easier access to government and other institutions and organs which largely depends on the performance of the company, reducing participation in joint participation in fairs and the effects of increased performance, cost reduction through joint marketing and research target markets, the organization of joint supply chain for certain raw materials, establishment of new business contacts through meetings organized by cluster and professional and legal advice that members can receive from the cluster.

Cluster "Solar Group" - Banja Luka is form from Mechanical Engineering Banja Luka and companies "Topling" Prnjavor, "Koming" from Gradiška and "Bemind" -Banja Luka. These entities have established cooperation in the design, manufacture and installation of solar systems and shared for the first time in Bosnia and Herzegovina manufactured solar heating system.

POTENTIAL CLUSTERS IN THE REPUBLIC OF SRPSKA

In addition to the established clusters in the Republic of Srpska, there are a number of associations that can quickly be developed into clusters of remarkable size and volume

of business. Table 2 provides an overview of the association and cooperative which in their scope and results can compete with the existing clusters in the Republic of Serbia and beyond.

Table 2. Potential clusters in the republic of Srpska

No	Name	City	Year of estab.	No of members	Field of industry
1	Association of wood processing	Mrkonjić Grad	2007	18	Wood industry
2	Association "Vinos"	Trebinje	2007	15	Wine industry
3	Cooperative "Žalfija"	Trebinje	2005	60	Beekiping
4	Association of poultry	Srbac	2007	24	Pultry and food processing

Association of Wood Processing Mrkonjic city was founded in 2007. There are 18 members whose primary activity is the final and wood processing. Company's own products are shipped mainly to foreign markets of Serbia, Croatia, Macedonia and Italy.

Association "Vinos" Trebinje aims to create a world - known brand, but also to control the origin of the grapes and on that basis to protect the wine made in this region. It is planned that this association grows into a cluster of growers and wine producers. The association has 15 members including the basement "Vukoje".

Beekeeping cooperative "Žalfija"-Trebinje aims to develop beekeeping sector by encouraging the production, marketing and sale of bee products originating and produced by its members, then supply cooperatives and other beekeepers raw materials necessary for modern beekeeping and beekeepers education on the principles of "good beekeeping practices". The cooperative has 60 cooperatives and 1,000 contractors.

Association of poultry of Republic of Srpska from Srbac is a business association for poultry and food production which brings together 24 members from 12 municipalities. Members of the association are mainly manufacturing companies in this field and Veterinary Station of Srbac and Veterinary Institute Vaso Butozan from Banja Luka. The Association offers its members training, literature, help with legal documents, interests advocating and promotion.

CONCLUSION

Clusters as the forms of association of companies have shown good results in many economies and those reasons are imposed as a solution to overcome the obstacles to doing business faced by SMEs. Clusters are geographically limited and based on a systematic links between companies. Links can be built on common or complementary products, production processes, core technologies, the need for natural resources, requirements for particular qualifications, distribution channels, etc.

In simple terms, a cluster is a group of related companies in one industry. This group includes producers of raw materials, governmental and non - governmental organizations and even the educational and scientific institutions that have joined together to solve common problems. Clusters are considered to increase the productivity and competitiveness of companies, which then become more powerful in the national and global terms.

Successful operation of the cluster leads to positive effects in companies that are members of clusters as well as in the region

where the clusters are placed: more jobs, greater diversity of processes, lower costs of acquisition, diffusion of technology, expanding markets, creating opportunities for new business relationships, ability performance of major investment projects in the region, etc.

Depending on the characteristics of the region and the company members, clusters set their priorities using resources of their region and establish formal links that enable to achieve common goals. In addition, the formation of clusters as a form of association is also significant because of the competitive advantage at the global marketplace.

As the networking of SMEs in clusters is becoming increasingly important to the economies of many countries and regions, and that is applicable in many areas of business, there is a need to further studies.

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Original Scientific paper

POSSIBILITIES OF VIRGINIA TOBACCO STALKS UTILIZATION

Vesna Radojičić[1], Olivera Ećim-Djurić[1], Marija Srbinoska[2], Nermina Djulančić[3], Gordana Kulić[1]

[1]University of Belgrade, Faculty of Agriculture, 11080 Belgrade, Republic of Serbia
e-mail: mntabacco@agrif.bg.ac.rs
[2]University St.Kliment Ohridski-Bitola, Scientific Tobacco Institute, 7500 Prilep, Republic of Macedonia
[3]University of Sarajevo, Faculty of Agriculture and Food Science, 71000 Sarajevo, Bosnia and Herzegovina

ABSTRACT

The aim of the study was to determine the possibility of using the stalks of Virginia tobacco. Special attention was payed to estimation of the energy potential, through prediction of higher heating value (HHV), based on the lignin and ash content.
The usage of tobacco stalks will have significant environmental impact: it can solve the problem with the waste, giving the possibility to include it in the total biomass of Serbia, together with other wastes of agricultural production.
The results of the research reveal that tobacco waste can be used as raw material for production of proteins and cellulose, and certain amount can be used in energetic purposes, for production of biogas, bio-ethanol, pellets and briquettes, affording environmentally acceptable and energy-valuable products. The fact that there are no written data about the chemical composition of tobacco stalks from the Republic of Serbia gives special significance to this study.

Key words: Virginia, tobacco stalks, biomass, higher heating value (HHV), lignin, cellulose

МОЖНОСТ ЗА ИСКОРИСТУВАЊЕ НА СТЕБЛА ОД ТУТУНОТ ТИП ВИРЏИНИЈА

Ова истражување има за цел да се утврди можноста за користење на стеблото од тутунот тип Вирџинија. Посебно внимание е посветено на испитувањето на енергетскиот потенцијал и утврдувањето на вредноста на горната топлотна моќ (HHV), врз основа на содржината на лигнин и пепел.
Користењето на тутунските стебла ќе има значително еколошко влијание, со што се решава проблемот со отпадот и се дава можност истите, заедно со други остатоци од земјоделското производсрво, да бидат вклучени во вкупната биомаса на Република Србија.
Од резултатите на ова истражување може да се заклучи дека остатоците од тутунот можат да се користат како суровини за производство на протеини и целулоза, а одредена количина од овој материјал може да се употреби за енергетски цели, во производството на биогас, биоетанол, пелети и брикети, при што крајниот производ ќе биде еколошки прифатлив и енергетски употреблив. Фактот дека не постојат податоци за хемискиот состав на стеблата од тутунот произведен во Република Србија му дава поголема важност на ова истражување.

Клучни зборови: вирџинија, тутунски стебла, биомаса, горна топлотна моќ (HHV), лигнин, целулоза

INTRODUCTION

According to the Europian Union Directive 2001/77/EC, the biomass is a biodegradable fraction of the product, it is the waste and remains of agriculture (including the plant and animal substances), in forestry and supporting industry, also it is part of the industrial waste.

Republic of Serbia has relatively great potential of biomass, mainly because of the waste from the primary agricultural production as the straw (wheat, barley, oats and soybeans) and corn waste (Jovanović and Parović, 2009). It is evaluated that there are 12.5 million tons of biomass produced in R. Serbia each year.

It should be emphasized that tobacco stalks have significant impact on the total biomass in agricultural production. Tobacco stalks, which according to the categorization (Radojičić et al., 2009) are classified in green tobacco waste, also have significant impact in the total biomass.

Each year, large amounts of tobacco stalks are left in the field after harvest. Smaller part of them (approx. one quarter) is usually ploughed and a large amount is used as a waste or is burned in the field, which results in loss of recourses and environmental pollution. According to the data of the Statistical Office (Statistical Yearbook, 2011), large-leaf tobacco is grown on 5407 hectares, of which 4410 hectares are planted with Virginia tobacco.

The average yield of Virginia tobacco is 20.000 – 25.000 stalks per ha and the average weight of dry stalks is 300 – 400 g. It means a yield of 6.000 – 10.000 kg dried stalks per hectare, which can be further used in production of a variety of products. In Serbia, about 76 000 tons of tobacco stalks remain in field each year and they don't have any economic value.

Tobacco is mainly produced because of its leaves, which can be used for production of a variety of products (cigarettes, cigars, pipe tobacco, etc.). Literature data show other ways of use of tobacco, for production of organic acids (citric, maleic, oxalic acid), nicotine acid, proteins, paper, bioethanol, biogas, as well as inorganic fertilizer (Sun and Cheng, 2002; Martín et al., 2002; Chaturvedi et al. 2008; Martin et al. 2008; Shakhes et al. 2011; Radojičić and Kulić, 2011; Kapadiya et al. 2010;).

It is important to mention that tobacco contains lignin and relatively high concentration of cellulose. The highest concentration of cellulose is in the tobacco stalks - 35-40% of dry substance (Pesevski et al. 2010). Such chemical composition is suitable for the production of biofuels.

In near future, the usage of lignocellulosic biomass in the process of biofuels production will be necessary, because it is expected that fossil fuels will be replaced by renewable sustainable alternatives (Semenčenko et al., 2011). In addition, the high cellulose concentration in the stalks is very important secondary raw material for production of paper, cardboard, textile, cotton, flax and other plant fibers (Radojičić et al. 2011; Gao et al. 2011).

Nowadays tobacco stalks are very interesting product for the textile industry. They can be used in the production of colors and textile fibers. Up to 30 shades of a color can be produced in low temperatures by using tobacco combined with relatively small amount of water. Fibers are antibacterial and can be combined with cotton, silk and kashmir (Besucher newsletter, 2012).

MATERIAL AND METHODS

The stalks of Virginia tobacco from the production area of Vojvodina (Srem – Golubnici), collected from the field after harvest are used as a material for this research. First, stalks were cut into smaller pieces to about 3-5 cm length and then ground by a Retsch ZM1 mill (Germany), than sieved through a series of vibrating sieves. Fractions of 0.5 – 1.0 mm particulate size were taken for analyses of chemical composition.

The methods developed by the National Renewable Energy laboratory (NREL) are used as the most commonly employed method for determination of the lignin and all extractive soluble substances in organic solvents, ashes and moisture in wood and non-wood samples (NREL/TP-510-42619, 42622, 2005 and NREL/TP-510-42618, 42621, 2008).

The moisture content was determined by drying at 105°C till constant mass. The ash content in stalk was determined by dry oxidation at 575 °C till constant mass.

The procedure of acid insoluble lignin consists of treating the stalk samples with 72% sulfuric acid, followed by 1h heating at 30°C, with stirring. After adjusting the acid concentration to 4 % w/w by adding deionized water, the mixture was autoclaved at temperature 125 °C and pressure 15 psi, for 1h.

Acid insoluble lignin was defined as the residue corrected for acid-insoluble ash, retained on a medium porosity filter crucible after the primary 72% and secondary 4% H_2SO_4 hydrolysis steps.

Reducing sugars were determined by the picric acid colorimetric method, while the nicotine level was determined using a UV spectrophotometry and procedure described by Wang et al. (1990).

The cellulose content was determined following the method of Kürschner-Hanack, by treating the sample with a mixture of nitric acid and acetic acid under reflux, during four cycles per 1h. Then the cellulose was filtered, washed, dried and weighed.

The nitrogen content (N) was determined by *Kjeldahl* method. The nitrogen was quantified by mineralization within a strong acid medium, containing 98% sulfuric acid, followed by steam distillation and titrimetric determination of NH^{+4}/NH_3.

The results of the chemical composition analyzes of the samples of tobacco stalks are estimated on dry weight basis. All analyses are performed in triplicate.

RESULTS AND DISCUSSION

Chemical analysis of the stalks of Virginia tobacco is presented in Table 1.

Table 1. Chemical analysis of the stalks of Virginia tobacco

Parameters	Moisture (%)	Protein (%)	Nicotine (%)	Sugars (%)	Cellulose (%)	Acid insoluble Lignin (%)	Ashes (%)
	5.21	13.87	0.343	7.54	35.30	15.99	6.19

According to the available literature data (Leffingwell, 1999), the average content of proteins in Virginia tobacco leaves is 8 - 10 %. The stalks have higher protein content. These high values can be a result of the use of nitrogen fertilizers, different climate conditions or other conditions during the process of tobacco cultivation. The fact that Virginia tobacco has such a high content of proteins gives a possibility of their

extraction from tobacco stalks, purification and use in therapeutic and other purposes.

The nicotine content in Virginia tobacco leaves is about 2 % (Leffingwell, 1999), while in the stalks it is significantly lower. The results of our research in Table 1 are in accordance with previous mentioned data. The nicotine can be extracted from the stalks and used in the production of pesticides in pharmaceutical industry. Therefore the toxicity of the biomass is reduced and allows further usage.

Sugar content in the leaves of Virginia tobacco is 13 – 22 % (Leffingwell, 1999). As expected, tobacco stalks have significantly lower content of sugar compared to the leaves (Table 1). However, this content (7.54 %) is much higher compared to other tobacco types (e.g. the leaves of Burley tobacco contain only 0.01 – 2 % sugar). Accordingly, the stalks of Virginia tobacco can be used in the production of alcohol and biogas.

According to literature data, tobacco stalks contain 35 – 36 % cellulose, the main rib 10 – 15 % and the lamina 10 – 12% (Leffingwell, 1999).

The results of our research are in accordance with literature data. The analysis of the obtained value for cellulose content and the reviewed data about the amount of stalks in Virginia tobacco reveal that up to 3.530 kg cellulose can be obtained from one hectare.

The content of lignin in tobacco is 4 – 5 % and the stalks may contain 20 – 30 % lignin (Leffingwell, 1999). The result shows that the stalk percentage of Virginia tobacco (Table 1) is lower than that found in literature.

According to Demirbas (2001) formula, the relation between the content of lignin and heating power is

$$HHV = 0,0889 \cdot (L) + 16,8218,$$

Where:

L is the lignin content.

We calculated high heating value in the stalks of Virginia tobacco (18.243 MJ/kg), which represents a significant energetic potential.

According to Brkić et al. (2007), the heating power of straw is about 15 MJ/kg, that of wood 18.6 MJ/kg, of fuel oil 42 MJ/kg and of diesel fuel 41 MJ/kg. Generally, the heating power of the biomass pellets, which are produced from the wastes of the agricultural products, is from 13 to 18 MJ/kg (Agroinfotel, 2014).

If we compare the data on the tobacco stalks heating power with the above values, it can be concluded that the usage of Virginia tobacco stalks for the production of briquettes and pellets is profitable because they have high calorific value.

Tobacco stalks contain lower ashes content compared to the leaves (the average value is 7.89 %). The tobacco stalks dried in controlled conditions have the lowest ashes content (Leffingwell, 1999). Most of the plants contain about 3 % mineral matter.

Tobacco contains higher content of ashes compared to the leaves of other plants. Based on the data of our experiment (Table 1), the ashes content is lower than expected (6.19 %), which can be considered as a good result.

In fact, the possibility of biomass combustion is very low and the ash quantity is insignificant, which is a positive feature, compared to the fossil fuels.

The formula developed by Sheng and Azevedo (2005) shows the correlation between the ashes content and heating power:

$$HHV = 19,914 – 0,2324 \cdot Ash$$

From this formula a high heating value of the Virginia tobacco stalks can be recorded, reaching up to 18.475 MJ/kg.

The heating power which is predictable on the bases of lignin content and the calculation of the ashes content indicate the possibility of using the stalks of tobacco type Virginia for energetic needs.

CONCLUSIONS

This work is related to determination of the possibility of using the stalks of Virginia tobacco, on the basis of chemical analyses. From the results obtained, the following conclusions can be drawn:

- The protein content (13.87%) is higher than expected; proteins can be isolated and part of them can be used for various purporses, the leftover can be used as a biomass.
- The nicotine content (0.343 %) is within expected range; it can be isolated from the stalks and used in pharmaceutical industry or in pesticide production, which lowers the toxicity of the biomass;
- The sugars content (7.54 %) is higher when compared to the other tobacco types; the stalks of Virginia tobacco are exceptionally useful for biogas and ethanol production;
- The cellulose content (35.30 %) is higher, which indicates that the stalks of Virginia tobacco have high potential for cellulose production;
- The heating power calculated on the basis of lignin (18.243 MJ/kg) and ashes content (18.475 MJ/kg) shows that the stalks of tobacco type Virginia are significant energetic potential.

The agricultural waste from tobacco is exploited in order to obtain a secondary product, which can be used in other industries, for various purposes. This fact is not well known in R. Serbia as in the other countries from the region. According to the results of the research, tobacco stalks can be used in production of some of the above mentioned products as well as for energetic needs. In this way, the wanted economic effect would be achieved, which is highly important for reduction of environmental pollution.

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“Tutun/Tobacco” is published biannually (double issues).

Since the publication is of an international character, all manuscripts should be submitted in English. Authorswhose native language is not English should have their paperschecked 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 andother 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.

Review articles -should contain critical surveys of the accomplishments in the fields encompassed in the Journal, papers by an individual researcher or a group of researchers with the purpose to undertake, analyze, evaluate or synthesize previously published information. They should present the latest ideas and theories or new scientific achievements.

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

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ABSTRACT-at the beginning of the paper, both in English and Macedonian, should not exceed150 words. It should mention the techniques used without going into methodological detailsand should summarize the most important results. Abstracts should not include citations toreferences.Font size 10, centered.

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

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RESULTS AND DISCUSSIO Nshould be presented in tables, figures, diagrams and photographs, whichmust accurately describe the findings of the study, orderedsequentially as they appear in the text;

Tables should be numbered with Arabic numeralsaccording to their sequence in the text. The table title should be always above the table,centered, in 10 pt font, with one empty row between the title and the table and another one between the table and the text.Tablesshould besimple and shouldnot duplicatethe informationgiveninfigures.

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.

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Units–measurements should be given in SI units.

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Mickoski J., 1988. Ispituvanje na infektivnata sposobnost na peronosporata i pepelnicata na tutunot. Tutun/Tobacco 1-2, 21-40, Institut za tutun-Prilep.

Weybrew J.A., Wan Ismail W. A., Long R. C., 1983. The cultural management of flue-cured tobaccoquality.Tob. Sci. 27, 56-61.

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Тутун/Tobacco Tobacco Institute 7500 Prilep

Kicevska bb

Republic of Macedonia

E-mail: tobaccotip@yahoo.com