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CONTENT OF TOTAL PHENOLIC COMPOUNDS AND ANTIOXIDANT POTENTIAL OF ORIENTAL TOBACCO VARIETIES (*NICOTIANA TABACUM* L.)

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ABSTRACT

During 2011 and 2012, experimental plots were set with oriental tobacco type Prilep (variety P-66), type Jaka (variety JK-125/3) and type Basma (variety Basma 82) on the experimental field at the Scientific Institute in Prilep. The research was conducted in order to analyze the selected oriental varieties of tobacco, by monitoring the parameters that determine their high antioxidant capacity. The antioxidant potential of the tobacco was measured by determining the content of total phenolic compounds, scavenging potential on DPPH (1,1-diphenyl-picrylhydrazyl), reducing ability on Cooper (II) neocuproine (CUPRAC method) and the activity of catalase. The results showed positive correlation between the content of phenolic compounds and the scavenging potential of the methanolic extract on both DPPH and Cu2+ radicals. The highest concentration of phenolic compounds was detected in Jaka variety harvested in 2011 (28,10± 0,48 mg/g), in which we also recorded the highest scavenging activity of the methanolic extract on the DPPH radical (78 ± 2,20%), the CUPRAC (183,99 ± 2,70 µM TE/g DW) and highest catalase activity (8,96 ± 0,11 mg decomposed H2O2). The results showed that the methanolic extracts from Jaka–125/3 have the highest antioxidant activity among the tested varieties.

KEYWORDS: *Nicotiana tabacum* L.; antioxidant activity; DPPH; CUPRAC; catalase activity.

INTRODUCTION

Reactive oxygen species (ROS), such as superoxid anion radical, hydroxyl radical and hydrogen peroxide represent natural molecules normally synthesized in various metabolic processes in the body and play an important role in signaling pathways in cells. Excessive production of ROS in the influence of various stress factors may cause oxidative stress by damaging the nucleic acids, protein carbonilation and lipid peroxidation of cell membranes resulting in disruption of the integrity of cellular structures. All these changes contribute to the emergence of a variety of chronic diseases in living organisms, such as cancer, diabetes, cardiovascular disease and other degenerative changes associated with aging.

Antioxidants are molecules that have a function in preventing cells from oxidative damage, preventing the process of lipid peroxidation and inhibit the process of oxidation of various food products.[1] Antioxidants act as a "scavenger" of ROS, the electron donor and hydrogen atoms enzyme inhibitors, synergists with other antioxidants and metal chelating compounds. Antioxidants are divided into two groups: enzymatic antioxidants (superoxide dismutase, peroxidase, catalase, ascorbate peroxidase and glutathione reductase) and non-enzymatic antioxidants (glutathione, ascorbic acid, carotenoids, tocopherols) which are included in the defense mechanism cells in detoxification of ROS. Medical plants are the source of many metabolites which are used for medicinal purposes and are widely used in the treatment of various chronic diseases. Plant metabolites, especially phenolic compounds constitute bioactive compounds with high antioxidant potential. Many clinical trials and epidemiological studies show a negative correlation between the use of natural antioxidants and the emergence of various chronic diseases caused by oxidative stress. *Nicotiana tabacum* L. belongs to the family Solanaceae. This kind of plant is considered as one of the most important commercial crop in the world due to its use in the tobacco industry. From phytochemical aspect, tobacco contains high concentrations of a number of bioactive compounds, such as alkaloids, terpenoids, essential oils and polyphenols.[2] Although many studies confirm the high antioxidant capacity of fruits, vegetables and other plant species, but the antioxidant compounds in the tobacco were not subject of extensive scientific research. According to current scientific knowledge, tobacco used for industrial purposes contains a high concentration of polyphenol compounds and carotenoids as important natural antioxidants. However, it is important to note that smoking by humans is associated with a reduction in antioxidant capacity of blood plasma[3] due to the

presence of other reactive molecules and carcinogenic compounds in cigarette smoke. Therefore, the main goal of this research is to determine the content of total phenolic compounds, antioxidant potential, and catalase activity in three varieties of oriental tobacco (Prilep 66, Basma 82 and Jaka 125/3) as potential sources of natural antioxidants. The results of this study will have a contribution to the further use of these varieties for obtaining various tobacco products that are less harmful to human health.

MATERIALS AND METHODS

Plant material

For this experiment were used three varieties of the most exploited types of tobacco in the Republic of Macedonia: type Prilep variety P-66, type Jaka variety JK-125/3 and type Basma variety Basma 82 (fig. 1). The fermented raw tobacco (leaf biomass) of the lower belt colected in two vintages (2011 and 2012) was used for analysis of phenolic compounds and antioxidant capacity.

Fig. 1. Oriental tobacco varieties: a) Prilep P-66, b) Basma 82, c) Jaka JK-125/3

Extraction of phenolic compounds: Extraction of phenolic compounds from lyophilized material was carried out in the presence of 80% CH3OH at a temperature of 4°C.[4] The obtained methanolic extracts were placed in an ultrasonic bath for 15 minutes. Then, the extracts were centrifuged at 12,000 rpm for 15 minutes and the resulting supernatant was used for further quantitative determination of the content of phenolic compounds and antioxidant capacity.

Total phenolic compounds: Quantitative determination of the content of total phenolic compounds in plant extracts were carried out by Folin-Ciocalteu method.[5] The principle of this method is based on electron transfer in an alkaline medium of phenolic compounds phosphomolybdenic / phosphotungstic acid complex, resulting in the formation of a blue colored complex. The reaction mixture was composed of: plant extract, diluted Folin-Ciocalteu reagent with distilled water (1: 9) and 0,7 M Na2CO3. Samples were incubated in a water bath at a temperature of 50ºC for 5 minutes. Then, the samples were cooled to room temperature and were measured spectrophotometrically at wavelength of 765 nm. For quantitative determination of total content of phenolic compounds was used a standard solution galic acid (0,4 mg• mL-1). The resulting values for the content of total phenolic compounds were expressed as mg galic acid equivalent per g dry weight (mg GAE • g-1 DW).

DPPH method: Antioxidant capacity of plant extracts was determined by using the DPPH method[6] with some modifications. The principle of the method is based on the reduction of DPPH • radical (2,2-diphenyl-1-pikrilhidrazil) by antioxidant compounds, resulting in discoloration of the radical of purple to yellow. The discoloration is followed by reduction of the absorbance at wavelength of 518 nm. Analysis were prepared by adding a volume of plant extract 0,25 mM methanolic solution of DPPH. The analyzes were incubated for 10 minutes at room temperature, then was measured their absorbance at wavelength of 518 nm. Parallel with the analyzes were prepared control analysis (80% CH3OH and 0,25 mM DPPH). The total antioxidant capacity using the DPPH method was calculated using a standard solution of trolox (0-1000 microns). The results were presented as trolox microns equivalent per unit dry weight of the plant material (microns TE • g-1 DW).

CUPRAC method: The total antioxidant capacity of plant extracts also was determined by using the CUPRAC method.[7] This method is based on measuring the absorbance of the chromophore Cu+ -neocuproine chelate formed as a result of the development of redox reaction between antioxidant compounds and Cu2+-neocuproine. The reaction mixture was composed of: plant extract, 1 M CH3COONH4 buffer (pH 7.0), 10 mM CuCl2 and 7.5 mM neocuproine. The analyzes were incubated for 30 minutes at room temperature and was then measured their absorbance at wavelength of 450 nm. Molar absorption coefficient of trolox (ε535=1.67x104 L·mol-1·cm-1) was used to determine the total antioxidant capacity of plant extracts using the CUPRAC method. The results were presented as trolox microns equivalent per unit dry weight of plant material (microns TE • g-1 DW).

Catalase activity: The activity of catalase enzyme was measured by the method of Bah and Oparin. The

principle of the method is based on extraction of the enzyme from the plant material and determination of catalase activity by titration with KMnO4. The reaction mixture consisted of plant extracts and 1% H2O2 (neutralized with 0.1 M NaOH). Parallel to the analysis was prepared control sample (dH2O and 1% H2O2). Assays were incubated for 30 min. at room temperature, the reaction was terminated by adding 10% H2SO4. After incubation the samples were titrated with 0.1 M KMnO4. The activity of catalase (Ak) expressed as mg H2O2 decomposed by the action of catalase (mg H2O2 • g-1 FW).

Statistical analysis: The results for total phenolic content and antioxidant capacity determined by different methods in different varieties of tobacco were presented graphically with a mean and standard deviation. Statistical data processing was carried out by applying Oneway ANOVA followed by Tukey-Kramer test for significance ($p < 0.05$).

RESULTS AND DISCUSSION

Total phenolic content: The content of total phenols in the tested varieties of tobacco from the harvest 2011 and 2012 is presented in Fig. 2 and 3. The concentration of phenolic compounds in all tested varieties colected in 2011 and 2012 is ranged from 19.03 to 28,10 mg GAE • g-1. The results of this study showed significant differences in the content of phenols among all three varieties of the crop in 2011, while the harvest in 2012 was insignificant difference observed between varieties Prilep 66 and Basma 82. According to the results, the highest concentration of phenols was determined in the variety Jaka 125/3 , harvest 2011 (28,10 mg GAE • g-1), while the lowest content of phenols was found in Prilep 66, harvest 2011 (19,03 mg GAE • g-1).

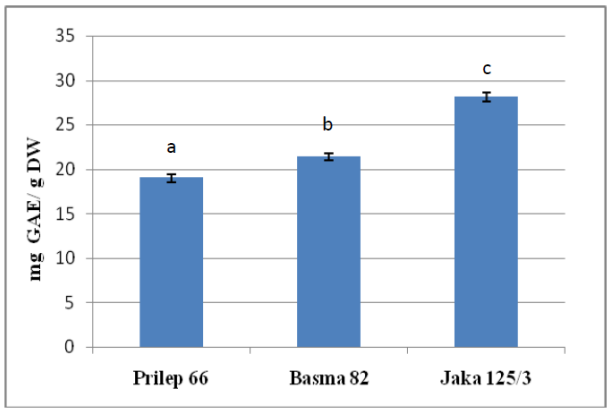

Fig. 2. Content of total phenols in varieties Prilep 66, Basma 82 and Jaka 125/3, harvest 2011 * Values marked with different letters indicate significant differences (p <0,05)

Comparing the content of phenols within each variety, statistically significant differences were found only in the variety Basma 82. These results are consistent with previous research showing that there are significant differences in the content of phenolic compounds between different varieties of tobacco. In fact, the process of growing and processing tobacco (fermentation, temperature, humidity) have great influence on the heterogeneity of the composition of foliage, resulting in changes in the content of phenolic compounds.

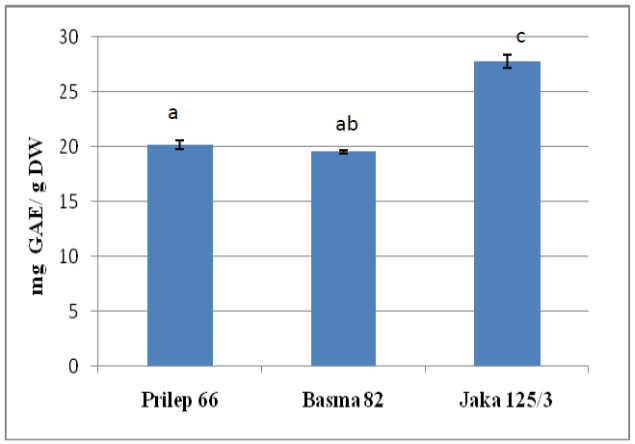

Fig. 3. Content of total phenols in varieties Prilep 66, Basma 82 and Jaka 125/3, harvest 2012 * Values marked with different letters indicate significant differences (p <0,05)

DPPH and CUPRAC methods

The application of rapid and accurate methods by using a small sample volume allows efficient screening of antioxidant potential of many plant species. A quick estimate of the capacity of the plant extracts to reduce synthetic nitrogen radical (DPPH) and Cu2 + can be used to determine the antioxidant capacity through phytochemical screening procedures.[8] DPPH and CUPRAC methods are one of the most effective methods by which can follow the neutralizing power of plant extracts on free radicals. DPPH stable radical receives an electron or hydrogen atom and becomes stable molecule. Neutralizing power of the extracts on the DPPH radical was tested by determining the degree of reduction of the absorbance at wavelength of 517 nm. The results showed that the extracts of the tested varieties of tobacco from the 2011 and 2012 harvests have different degrees of inhibition of DPPH (from 55.42 to 78.3%). The highest percentage of inhibition of the DPPH radical was established in Jaka 125/3 variety of vintage 2011 and 2012 (Fig. 4 and 5) compared to the other varieties tested. Also, the results of antioxidant activity expressed through CUPRAC value showed significant variation among the tested varieties coelcted of 2011 and 2012 (from 107.24 to 183,99 µM TE * g-1) (Fig. 6 and 7). The Jaka 125/3 variety of vintage 2011 and 2012 showed the highest antioxidant capacity compared with other tested varieties. Significantly lower antioxidant capacity determined by applying the DPPH and CUPRAC methods showed Prilep 66 and Basma 82 varieties.

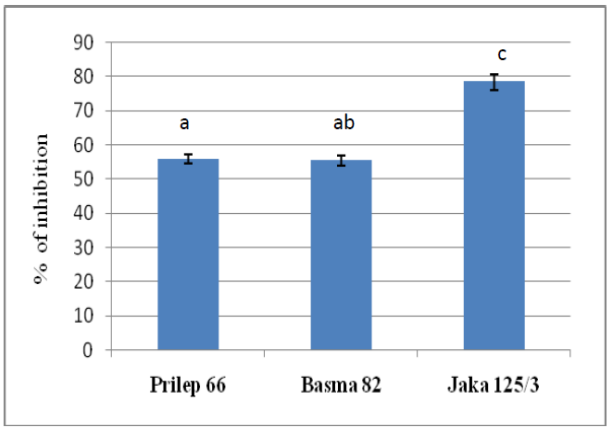

Fig. 4. Inhibition of DPPH radical for varieties Prilep 66, Basma 82 and Jaka 125/3, harvest 2011

* Values marked with different letters indicate significant differences (p <0,05)

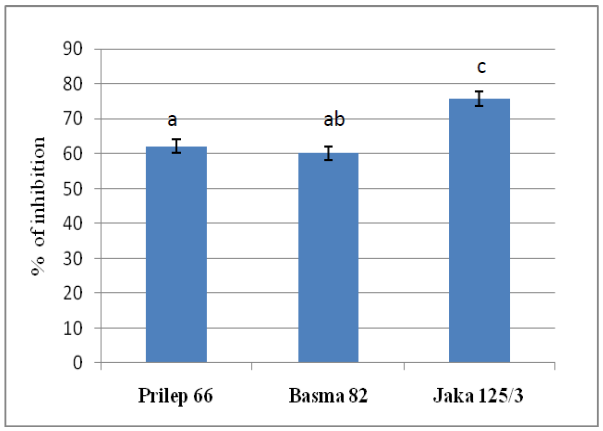

Fig. 5. Inhibition of DPPH radical for varieties Prilep 66, Basma 82 and Jaka 125/3, harvest 2012 * Values marked with different letters indicate significant differences (p <0,05)

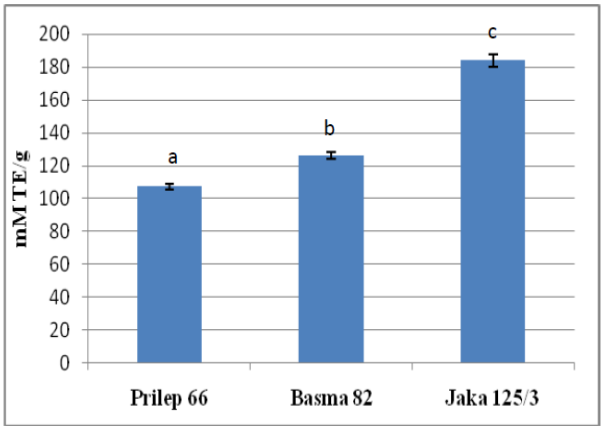

Fig. 6. CUPRAC values for antioxidant activity in varieties Prilep 66, Basma 82 and Jaka 125/3, harvest 2011 * Values marked with different letters indicate significant differences (p <0,05)

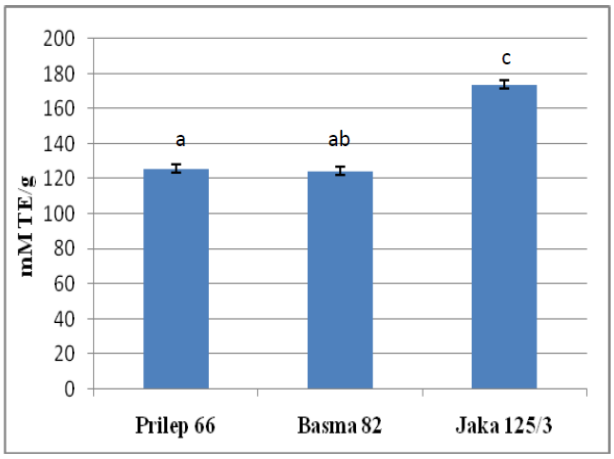

Fig. 7. CUPRAC values for antioxidant activity in varieties Prilep 66, Basma 82 and Jaka 125/3, harvest 2012 * Values marked with different letters indicate significant differences (p <0,05)

Catalase activity

During the survey was determined the activity of catalase, an enzyme that is particularly effective in the neutralization of the cytotoxic H2O2. Under normal conditions H2O2 accumulates in low concentrations, while the presence of free radicals determines its increased production. The activity of this enzyme is one of the defense mechanisms of the organism to stressful environmental conditions. Also, antioxidant capacity in tested varieties of tobacco was expressed by measuring the activity of the enzyme catalase. The results of the activity of catalase showed significantly higher activity of this enzyme in the variety Jaka 125/3 (7,9 mg decomposed H2O2) from harvest 2011 compared to varieties Prilep 66 and Basma 82 in which were found significantly lower values for the activity of the enzyme (0,94 and 0,77 mg decomposed H2O2). In 2012 harvest were not observed significant differences in the activity of catalase between tested varieties. The variety Basma 82 showed the highest activity of catalase compared to other tested varieties (Fig. 9). Previous scientific studies show that the activity of catalase in plants depends on the conditions of cultivation. In addition, some studies show that the activity of catalase increases in drought and increased salinity of soils. Also,[9] showed that low temperatures, wind and water erosion induce oxidative stress, which may result in increased activity of catalase. Accordingly, the obtained variations in the activity of catalase between different varieties of tobacco from different vintages probably due to different conditions of cultivation of plants.

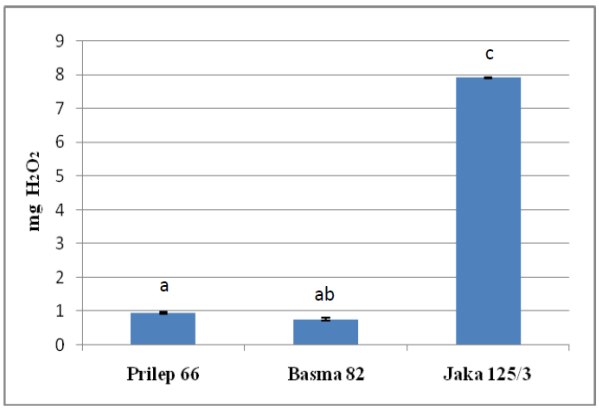

Fig. 8. Activity of catalase (mg neutralized H2O2) for varieties Prilep 66, Basma 82 and Jaka 125/3, harvest 2011 * Values marked with different letters indicate significant differences (p <0,05)

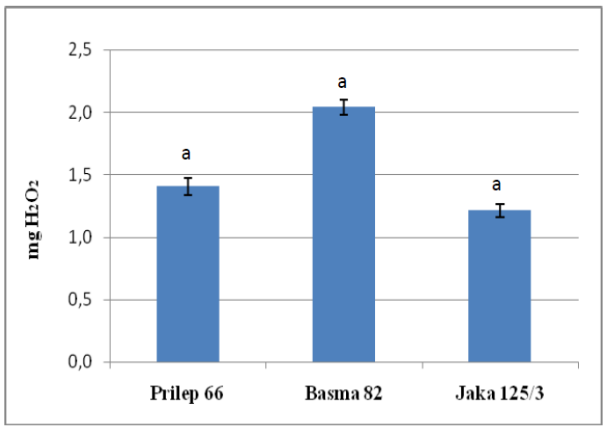

Fig. 9. Activity of catalase (mg neutralized H2O2) for varieties Prilep 66, Basma 82 and Jaka 125/3, harvest 2012 * Values marked with different letters indicate significant differences (p <0,05)

The participation of catalase in neutralizing H2O2 is another indication that the oriental tobacco varieties evaluated in this study may represent a good source of natural antioxidants and used for the production of tobacco products in the food and pharmaceutical industries.

Correlation between the content of phenolic compounds and antioxidant capacity

The results of Pearson's corelation coefficient between the content of phenols and antioxidant capacity determined by various methods in tested varieties of tobacco are shown in Table 1. According to the results of statistical data processing, the content of phenolic compounds was significantly positively correlated with DPPH (0,938; p <0,001) and CUPRAC (0,982; p <0,001) methods indicating that the antioxidant capacity of the tested varieties of tobacco in a significant rate due to the presence of phenolic compounds. Also significant positive correlation was found between DPPH and CUPRAC method (0,970; p <0,001), indicating that these methods can be used to determine the antioxidant capacity of different varieties of tobacco.

Table 1 Pearson's correlation coefficient (r) between the content of phenols, DPPH and CUPRAC method, and catalase activity among varieties Prilep 66, Basma 82 and Jaka 125/3 of Nicotiana tabacum, harvest 2011 and 2012 (n = 6). *

Pearson-s coefficient (r)	Total phenols	DPPH method	CUPRAC method
DPPH method	0,938***		
CUPRAC method	0,982***	0,970***	
Catalase activity	-0,238	-0,052	-0,162

Values marked with *** p <0.001 show a significant correlation between the examined parameters The results of this study showed that extracts from tobacco can be used to produce a variety of commercial products that would participate in the protection of biomolecules (lipoproteins, nucleic acids, fatty acids, proteins, amino acids, polysaccharides) by the action of ROS in biological systems. The results indicate the possibility of utilization of the tested varieties of tobacco as a potential source of natural antioxidants.

CONCLUSION

This study was first presented research on the content of total phenols, reducing power against free radicals and the activity of the enzyme catalase in three varieties of oriental tobacco (Prilep 66, Basma 82 and Jaka 125/3). Significant differences in the content of phenolic compounds and antioxidant potential were found between different varieties of tobacco. Also, the results showed that the tested varieties of tobacco with the capacity to neutralize free oxygen radicals. Of the tested varieties of tobacco, Jaka 125/3 was selected as an elite breed due to the high content of phenolic compounds and antioxidant capacity. Additional research on the interaction of phenolic compounds and other antioxidant compounds could be an effective alternative in the use of the tested varieties of tobacco as a source of bioactive components in the food and pharmaceutical industries.

REFERENCES

1. Nićiforović N, Mihailović V, Masković P, Solujić S, Stojković A, Pavlović Muratspahić D. Antioxidant activity of selected plant species; potential new sources of natural antioxidants, Food Chem. Toxicol., 2010; 48: 3125-3130.
2. Yong-Kuan Chena, Xue-Sen Lia, Guang-Yu Yanga, Zhang-Yu Chena, Qiu-Fen Hub, Ming-Ming Miaoa. Phenolic compounds from Nicotiana tabacum and their biological activities, Journal of Asian Natural Products Research May 2012; 14(5): 450–456.
3. Traber MG, Vliet A, Reznick AZ, Cross CE. Tobacco-related diseases. Is there a role for antioxidant micronutrient supplementation? Clin. Chest Med. 2000; 21: 173-187.

4. Gadzovska S, Maury S, Ounnar S, Righezza M, Kascakova S, Refregiers M, Spasenoski M, Joseph C, Hagège D. Identification and quantification of hypericin and pseudohypericin in different *Hypericum perforatum* L. *in vitro* cultures. Plant Physiol. Biochem., 2005; 43: 591–601.
5. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 1965; 16: 144-158.
6. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity, LWT-Food Sci. Technol., 1995; 28: 25-30.
7. Apak R, Güçlü K, Özyürek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols, vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method, J. Agric. Food Chem., 2004; 52: 7970-7981.
8. Tusevski O, Kostovska A, Iloska A, Trajkovska Lj, Gadzovska S. (2013). Phenolic production and antioxidant properties of some Macedonian medicinal plants. European Journal of Biology.
9. Clarke HJ, Siddique KHM. Response of chickpea genotypes to low temperature stress during reproductive development. Field crop research, 2004; 90: 232-334.