Journal of Experimental Biology and Agricultural Sciences, December - 2020; Volume – 8(Spl-2- AABAS) page S292 – S297

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

EFFECT OF SOIL AND GROWTH CLIMATIC CONDITIONS ON VITAMIN CONTENT OF RED CLOVER (*Trifolium pratense* L.)

Alexander Leonidovich Mikhailov[1], Olga Arnoldovna Timofeeva[1], Uliana Aleksandrovna Ogorodnova[1], Nikita Sergeevich Stepanov[1]

Department of Biological Sciences, Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia

Received – September 25, 2020; Revision – November 09, 2020; Accepted – December 16, 2020
Available Online December 15, 2020

DOI: http://dx.doi.org/ 10.18006/2020.8(Spl-2-AABAS).S292.S297

KEYWORDS

Red Clover

Biologically Active Substances

Vitamin C

Provitamin A

Carotene

Soil

Climate

ABSTRACT

The current study was conducted to estimate the effect of soil and growth climatic conditions on the vitamin content of red clover (*Trifolium pratense* L.). Further, in vitamins, the content of ascorbic acid and vitamin A (provitamin – carotene) was estimated from the aerial parts of the clover. Ascorbic acid is a powerful antioxidant, antiviral, and antitumor vitamin while vitamin A is a useful vitamin for eyesight. Ascorbic acid content in the red clover tissues was determining potassium hexacyanoferrate method while Provitamin A and total carotenoid was estimated by spectrophotometric method. Results of the study revealed that the highest content of vitamins C was found in the plant grown under the climatic conditions of the southern taiga subzone of the Atninsky and in the zone of deciduous forests (Apastovsky and Kamsko-Ustinsky districts) while the plant is grown under the coniferous-deciduous forests (Zelenodolsky district) climatic conditions are a rich source of vitamin A. From the results of the study, it can conclude the soil and growing conditions especially temperature regime and soil moisture affected the level of vitamins in red clover.

* Corresponding author

E-mail: almihailov@bk.ru (Alexander Leonidovich Mikhailov)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI]
(http://www.horizonpublisherindia.in/).

1 Introduction

Plants are a good source of various therapeutical importance biologically active compounds, these biochemical substances are not only have medical value but also widely used for industrial purposes. Vitamins are one of these compounds. Vitamins are a large group of organic compounds with a relatively low molecular weight, different chemical nature, and simple structure. Ascorbic acid, also known as vitamin C, is a unique polyfunctional compound. Vitamin C is a recognized antioxidant due to its ability to reversibly oxidize (Andersen 2003; Ageevaet al., 2016), it also participates in most important energy processes of the plant cell, namely respiration and photosynthesis (Booth et al., 2006). The great interest in vitamin C as a drug is due to its antiviral and antitumor effects (Valieva &Abdrakhimova, 2010). Like vitamin c, vitamin A provitamin – carotene, which belongs to the carotenoids group, also play important role in various biochemical cycles. Retinol, or vitamin A, involved in redox reactions, protein synthesis regulation, and metabolism and also play an important function in the cell and subcellular membranes system. Like ascorbic acid, it also exhibits strong antioxidant properties (Chen & Chen, 2014; Kawaguchi et al., 2015).

Red clover (*Trifolium pretense* L family Fabaceae .) is a green, perennial forage crop which widely grown for animal feed and green manure. Biochemically it is a good source of isoflavonoids, flavonoids, cyanogenic glycosides, volatile oil, saponins, and trace vitamins and minerals. This plant has anticarcinogenic, hepatoprotective, and antiviral activities, antioxidant, anti-inflammatory, antispasmodic, antineoplastic, and antifungal properties. Traditionally it is used for the treatment of various remedies such as ease menopausal symptoms, eye problem, psoriasis, eczema, gout, and chronic fever (Çölgeçen et al., 2011; Oza & Kulkarni, 2018).

It has been well reported that plant growth, its physiological conditions, and biochemical profile are affected to the greatest extent by environmental and soil factors (Andersen, 2003; GoriunovaIu, 2009). Further, the synthesis of biologically active substances in plants is a dynamic process, and it noticeably changes with the course of plant ontogenesis and growing conditions. Further, Ageeva et al. (2016) suggested that the same plant species grown in different environmental conditions differ greatly in the intensity of biochemical synthesis and accumulation of various compounds. Considering the above facts, the study of the variability in biologically active substances is highly important to characterize the same plant species which are collected from different growing areas. So, the aim of this was to access the effect of soil and climatic conditions on the accumulation of vitamins in red clover plants growing in the Republic of Tatarstan.

2 Materials and Methods

Leaves and stem of red clover (*Trifoium pretense* L.) was collected from the various geographical location of the Republic of Tatarstan, climatic conditions of the sample collection are Deciduous forests (Kamsko-Ustinsky district, Apastovsky district, Tetyushsky district, Laishevsky district, Verkhne-Uslonsky district); Coniferous-deciduous forests (Zelenodolsky district); Southern taiga (Atninsky district); and Forest-steppe zone (Spassky district).

Quantification of ascorbic acid content was carried out by using spectrophotometric potassium hexacyanoferrate (Fe^{2+}) method given by Burger & Karas-Gašparec (1973). In this, in an acidic medium, ascorbate reduces potassium hexacyanoferrite (Fe^{3+}) to potassium hexacyanoferrate (Fe^{2+}), which occurred in the presence of ferric ions, and forms iron hexacyanoferrate (Berlin blue); a blue colour solution is formed and the intensity of this blue colour was measured with the help of spectrophotometer at 680 nm. For this, 50 mg raw material was ground in 1.5 ml of citrate buffer (0.1 M, pH 3.69), followed by transfer to Eppendorf tube, vigorously shaken and heated at 40°C for 20 minutes. Then it was centrifuged for 5 min at 12500g and the supernatant was used for subsequent measurements.

From this, 500 µl of the supernatant/extract was added to 25 µl of 1% $K_3[Fe(CN)_6]$ solution, then 25 µl of 2% NaF solution, and this mixture was stirred for 5 minutes. After this, distilled water and ferric chloride solution were added. The resulting reaction mixture was kept for 7 minutes, periodically shaken, and measurements were taken with the help of a spectrophotometer at 680 nm and results were compared with the control solution (buffer instead of extract).

The concentration of Provitamin A was measured from the content of carotenoids isolated from the aerial part of red clover. For determination of carotenoids, 100 mg red clover leaves sample was ground in a porcelain mortar with 2 ml of 85% acetone (Rebbelen). This extract was filtered through a paper filter, and the absorbance of the extracts was measured on a UV / VIS spectrophotometer at wavelengths of 662, 644, 452.5 nm. The concentration of pigment was calculated using the following formula (Yakushenkova & AL-Hussein, 2019).

$$x = 100 \times B/A$$

where:

A - is the mass of raw leaves taken for analysis (mg)

B - is the amount of chlorophyll in the extract (mg);

100 - coefficient for expression in percent.

All the collected data were analyzed with the help of the Microsoft Excel program.

3 Results and Discussion

The level of ascorbic acid varies with plant species and climatic conditions. Furthermore, factors that determine the accumulation of ascorbic acid in plants are dependent on the various biochemical pathways and the enzymes responsible for activation of these biochemical pathways are dependent on the soil and geographic conditions (Valieva & Abdrakhimova, 2010; Catarino et al., 2017). Within a species, the accumulation of ascorbate depends largely on the growing conditions. If the plant develops normally in the absence of nutritional deficiencies and all other conditions favorable, the content of vitamin C insignificantly changes (Kolodziejczyk-Czepas, 2016). There is evidence that the synthesis and content of ascorbate are associated with climate and weather conditions. In the conditions of high temperatures and lack of moisture, the ascorbic acid content in fruits is less than the lower temperatures and at optimal humidity (Korzhet al., 2011; Rehman et al., 2018). Further, high precipitation and dry hot weather affect or decreased the vitamin C content in many plant species. Together with precipitation during the growing season, the distribution pattern during the season is of great importance (Atkinson & Urwin, 2012). Results of the current study revealed that the vitamin C content of red clover depending on soil and climatic conditions (Figure 1 & 2).

The content of vitamin C was higher in red clover plants grown in the Atninsky district (Isleitar forestry) located in the southern taiga subzone (180 µg/g dry weight) with a moderately warm climate and moist soils. Also, red clover grew here on a forest edge where the lighting conditions were limited (about 50% of full illumination), did not allow the plants to “overheat” have a higher content of ascorbic acid. The least content of ascorbic acid was observed in the Laishevsky district of broad-leaved forests (Figure 1).

The diagrams (Figure 3-4) show the results of vitamin A assays in the samples of red clover plants collected in ecologically different regions of the Republic of Tatarstan. The study found that plants collected in different environmental conditions differ in vitamin A content. Based on the data obtained in the course of the study, we concluded that the highest content of provitamin A (0.36 mg/g dry weight) is observed in the location of the village of Kamskoye Ustye of the Kamsko-Ustinsky district of the Republic of Tatarstan in the zone of broad-leaved forests. The least content of all provitamin A was observed in red clover in the Laishevsky district of deciduous forests (Figure 3).

Provitamin A accumulates significantly in the plant body, and it is usually high at high temperatures (Karomatov & Abdulkhakov, 2016). Soil moisture is also an important factor. With water deficiency, the content of provitamin A decreases. Thus, the result of the study revealed that red clover plants are quite different in chemical composition, and it depends on the place of growth and climatic conditions. Moreover, we were unable to identify an area in which the content of all studied compounds was maximum. Some areas are characterized by the highest content of ascorbic acid, and provitamin A. As the value of medicinal plants consists, first of all, in a complex of biologically active substances when choosing an area most suitable for harvesting clover, one should consider the balance of their composition.

Current studies agree with the literature data that the content of biologically active compounds for the same species differs within geographic zones this might be due to variation in the climate conditions (Carvalho et al., 2010). Within natural zones, the composition of metabolites of plant raw materials depends on the type of soil, its physical and chemical properties, landscape, ecological-cenotic conditions, and fluctuations in temperature and precipitation during the season (Goriunova, 2009). Important factors influencing the synthesis of biologically active metabolites are the optimal, balanced level of illumination, moisture, heat, and soil composition for each plant species.

Conclusion

The highest content of vitamins C is observed in southern taiga of the Atninsky district, in deciduous forests (Apastovsky and Kamsko-Ustinsky districts,) and in coniferous-deciduous forests (Zelenodolsky district) while vitamin A content was observed in plant grown in Zelenodolsky district, this depends on temperature conditions and soil moisture. Based on the total content of the studied compounds, plants *T. pretense* from Apastovsky and Kamsko-Ustinsky districts of deciduous forests and Zelenodolsky district of coniferous-deciduous forests can be recommended for collection.

Analyzing the results of the current study suggested that red clover is a source of ascorbic acid and vitamin A, which can serve as an active interesting compound in pharmacology. This provides for prerequisites for its more in-depth and detailed study as a valuable object of phytotherapeutic methods and treatment methods since this species belongs to plant sources of natural medicines.

Acknowledgments

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University. The paper was published with the financial support of the RFBR and the Government of the Republic of Tatarstan within the framework of the scientific project No.18-44-160015.

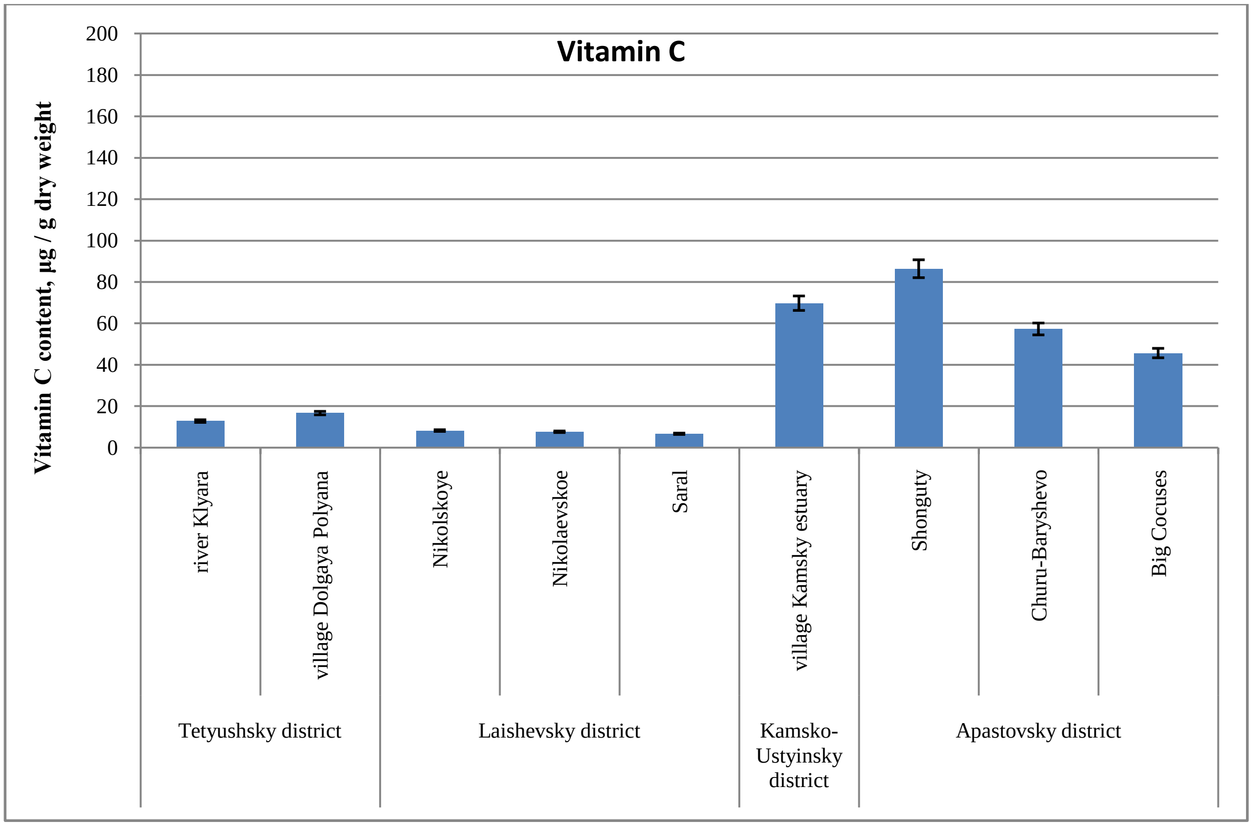

Figure 1 The content of vitamin C in red clover plants of the meadow zone of broad-leaved forests of the Republic of Tatarstan ($p<0.05$).

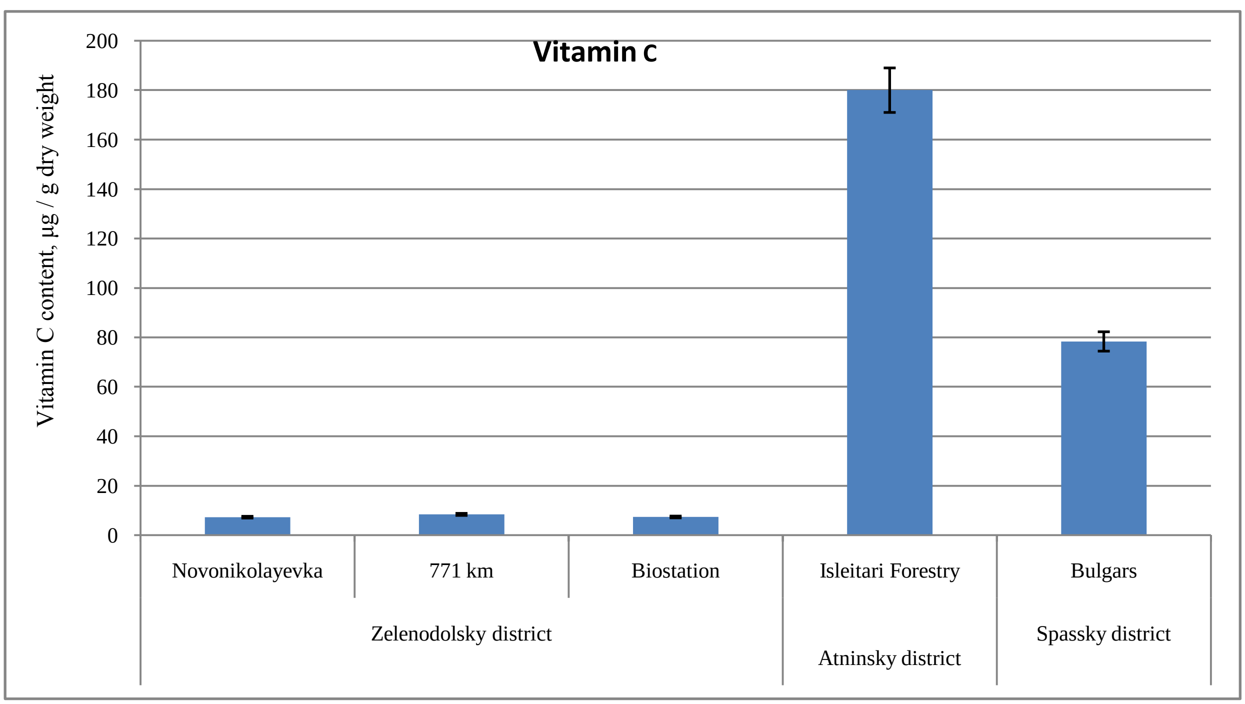

Figure 2 The content of vitamin C in red clover plants from different growth zones of the Republic of Tatarstan ($p<0.05$).

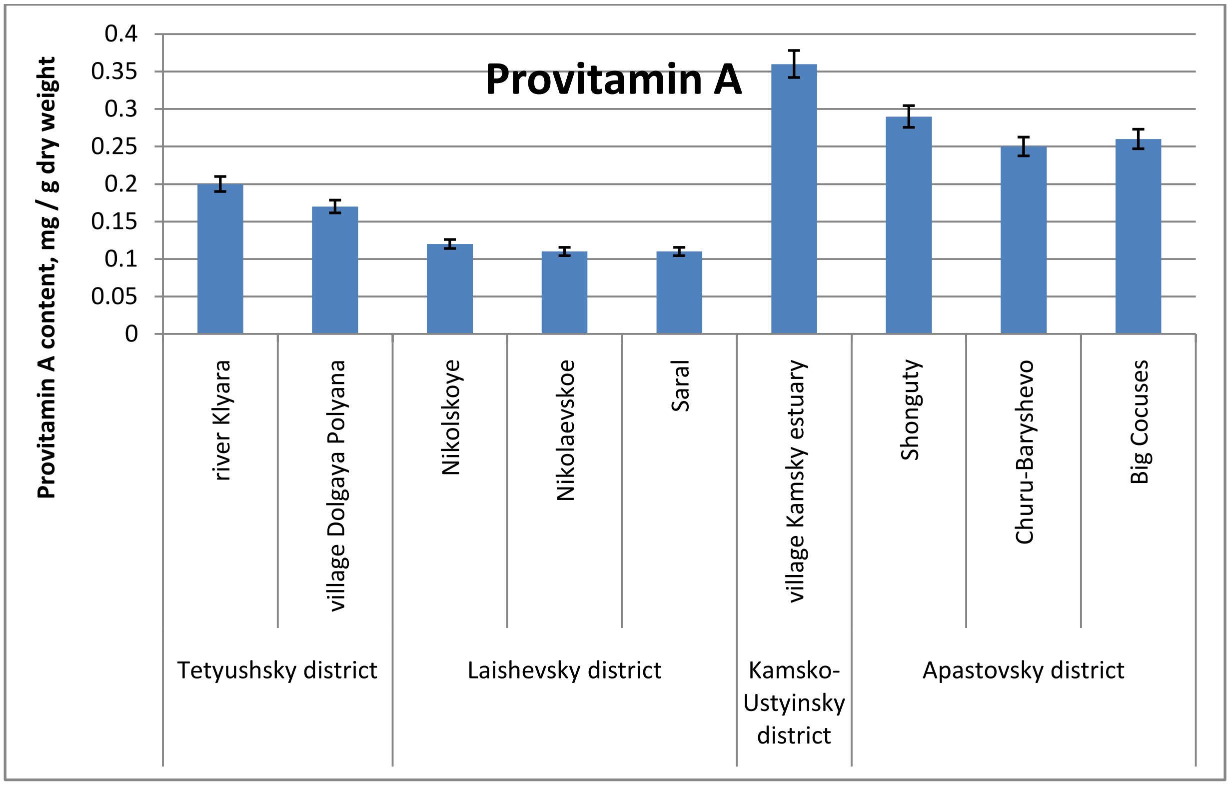

Figure 3 The content of provitamin A in red clover plants of the meadow zone of broad-leaved forests of the Republic of Tatarstan ($p<0.05$).

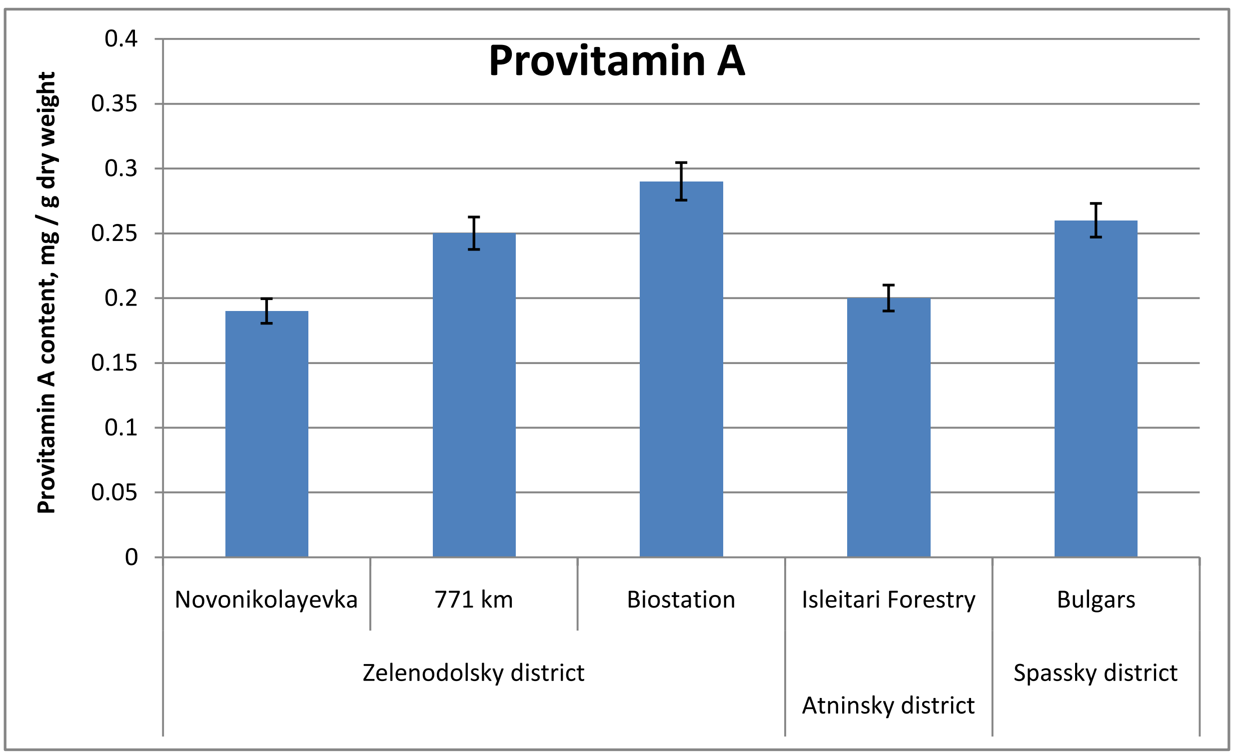

Figure 4 The content of provitamin A in meadow red clover plants from different growth zones of the Republic of Tatarstan ($p<0.05$)

Conflict Of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

References

Ageeva EE, Iamashev TA, Reshetnik OA (2016)Investigation of antioxidant and antiradical properties of clover herb extracts (*Trifolium pretense* L.) [Text]. Bulletin of the Technological University 19(16): 86-88.

Andersen CP (2003) Source-sink balance and carbon allocation below ground in plants exposed to ozone. New Phytologist 157(2): 213-228.

Atkinson NJ, Urwin PE (2012) The interaction of plant biotic and abiotic stresses: from genes to the field. Journal of Experimental Botany 63(10):3523-43.

Booth NL, Overk CR, Yao P, Burdette JE, Nikolic D, Chen SN, Bolton JL, Breemen RB, Pauli GF, Farnsworth NR (2006) The chemical and biologic profile of a red clover (Trifolium pratense L.) phase II clinical extract. Journal of Alternative & Complementary Medicine 12(2):133-9.

Burger N, Karas-Gašparec V (1973) Spectrophotometric determination of ascorbic acid with potassium hexacyanoferrate(III). Talanta 20 (8): 782-785.

Carvalho IS, Cavaco T, Carvalho LM, Duque P (2010) Effect of photoperiod on flavonoid pathway activity in sweet potato (*Ipomoea batatas* (L.) Lam.) leaves. Food chemistry118(2):384-90.

Catarino MD, Silva AM, Cruz MT, Cardoso SM (2017) Antioxidant and anti-inflammatory activities of Geranium robertianum L. decoctions. Food & Function 8(9):3355-65.

Chen W, Chen G (2014) The Roles of Vitamin A in the Regulation of Carbohydrate, Lipid, and Protein Metabolism. Journal of Clinical Medicine 3(2): 453–479. doi: 10.3390/jcm3020453.

Çölgeçen H, Koca U, Büyükkartal HN (2011) Use of Red Clover (*Trifolium pratense* L.) seeds in human therapeutics. In: Preedy VR, Watson RR, Patel VB (Eds.), Nuts & Seeds in Health and Disease Prevention (1st ed.) London, Burlington, San Diego: Academic Press is an imprint of Elsevier, Pp 975-980.

GoriunovaIu D (2009) The influence of environmental factors on the content of some antioxidants in plants. Author's abstract, Cand. Biology.Kaliningrad, Pp. 24 .

Karomatov ID, AbdulkhakovIU (2016) Red clover - application in medicine. Biology and Integrative Medicine 5:95–109.

Kawaguchi R, Zhong M, Kassai M, Ter-Stepanian M, Sun H (2015) Vitamin A Transport Mechanism of the Multitransmembrane Cell-Surface Receptor STRA6. Membranes (Basel) 5(3): 425–453. doi: 10.3390/membranes5030425.

Kolodziejczyk-Czepas J (2016) Trifolium species–the latest findings on chemical profile, ethnomedicinal use and pharmacological properties. Journal of Pharmacy and Pharmacology 68(7):845-61.

Korzh AP, Guriev AM, Belousov MV, Iusubov MS (2011)The chemical composition of water-soluble polysaccharides from red clover (*Trifolium pretense* L.). Chemistry of Vegetable Raw Materials 2: 47-50.

Oza MJ, Kulkarni YA (2018) A novel contribution to quality standards of Trifolium pratense L., a dietary supplement. Indian Journal of Natural Products and Resources 9(4): 311-330.

Rehman A, Farooq M, Naveed M, Nawaz A, Shahzad B (2018) Seed priming of Zn with endophytic bacteria improves the productivity and grain biofortification of bread wheat. European Journal of Agronomy 94:98-107.

Valieva AI, Abdrakhimova JR(2010) Secondary metabolites of plants: physiological and biochemical aspects (Part 3. Phenolic compounds). Study guide. - Kazan: Kazan Federal University, - 40 p.

Yakushenkova TP, AL-Hussein D (2019) Biochemical Status of Plants Brassica oleracea var. sabellica, Ocimum basilicum and Petroselinum crispum Induced by a Different Spectrum of Light. Plant Archives 19 (2)2792-2796.