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THE COMPARATIVE MORPHO-ANATOMICAL STUDY OF NEW CULTIVARS AND SOME SPECIES OF BLACKBERRY

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Abstract. The morphological and anatomical characteristics of sp. *Rubus fruticosus* L., *R. laciniatus* and new cultivars of blackberry (Thornless Evergreen, Thornfree, Arapaho, Chester), multiplied by *in vitro* micro-technologies in the Laboratory of Embryology and Biotechnology of the Botanical Garden (I) of Academy of Sciences of Moldova, planted in the greenhouse and in the field, were studied. The morpho-technological analysis was performed based on characteristics like: stem position and viability; period of maturation and fruit firmness; plant productivity and adaptability. The study was carried out on a complex of anatomical indices: cuticle type, size, degree of packing of cells, types of trichomes and their distribution, the presence and the way of distribution of calcium oxalate druses, the correlation index of thickness of the upper/lower epidermis, epidermis/mesophyll, leaf/mesophyll. The screening of results has highlighted the morphological and anatomical characteristics of the studied taxa and of those with adaptive characteristics that are suitable to the soil and the climate of the Republic of Moldova, which may provide support for the development of strategies of management of blackberry plantations in our country on more extensive areas.

Keywords: blackberry, species, varieties, morphology, anatomy.

INTRODUCTION

The fruit trees are a valuable fruit plant category, which provides fruits, rich in various natural chemical compounds, for balanced nutrition. Due to the presence of biologically active substances (vitamins, anthocyanins, flavonoids, tannins, organic acids, and alimentary fibers), fruits are required more frequently as a source of raw material for the production of pharmaceuticals, cosmetics and for zoo veterinary [6, 15, 16]. To satisfy the increasing demand, in recent years, breeders have created new cultivars of blackberry with new biological peculiarities (without thorns, erect stem), resistant to drought and frost and with commercially important qualities (transport and storage firmness, large sized fruits, high and special taste qualities, flavors etc.) [2, 6].

For this purpose, in the Laboratory of Embryology and Biotechnology of the Botanical Garden (Institute), biotechnological works were undertaken for determining the optimal conditions for *in vitro* propagation of planting material without viruses, of some new cultivars of blackberry, produced in the USA and in Europe [8], and a field collection was created from 11 new cultivars of blackberry [9].

The purpose of this paper is the morphological and anatomical comparative study of blackberry species and new cultivars derived from them, for elucidating the indices with diagnostic character for identification and those with adaptive role to the climatic conditions in the R. Moldova.

MATERIALS AND METHODS

Blackberry leaves, collected during flowering from the collection of the Botanical Garden (I) of ASM of, have served as botanical material (fresh and dried) for study: spontaneous species *Rubus fruticosus* var. *ulmifolius* Schott (s. Susleni, r. Orhei, R. Moldova) [12], sp. *R. laciniatus*, *Thornless Evergreen* cultivar (cultivated in Romania, Transylvania) and 3 cultivars of blackberry with American origins, *Thornfree* (patented in Moldova) and cultivars *Arapaho*, *Chester*, obtained from sp. *R. fruticosus* [15]. The studied leaves dried naturally, arranged in thin layers in well-ventilated rooms. The anatomical study was conducted according to the classical methods [8, 10, 13]. The microscopic examination was performed at the binocular optical microscope *Micros* (Austria)

with digital camera, coupled to the computer, on the cross sections obtained from fresh/dried leaves and superficially preparations, cleared with chlorhydrate or 3% NaOH [10] at the Department of pharmacognosy and pharmaceutical botany of the State University of Medicine and Pharmacy "Nicolae Testemitanu". The measurements of anatomical structures were performed on cross sections, at 4×, 10× and 40 × magnification. The results were statistically processed by the Statistical Programme 7.

RESULTS AND DISCUSSIONS

The Morpho-Technological Characteristics of Species and Cultivars of Blackberry

The morpho-technological analysis of sp. *R. fruticosus* and *R. laciniatus*, and blackberry cultivars was carried out based on the following indicators: biological features of the stem, technological indices of fruits, the period of maturation of fruits, productivity and adaptability (tab. 1).

The morphology of the studied taxa is similar, only with some differences: all of them are characterized by vigorous aspect of shrubs, exception – *Arapaho* cultivar. The stem position in space is different: upright for *Arapaho* cultivar, repent – specific for sp. *R.lacinatus*, *Thornless Evergreen* and *Thornfree* cultivars; semi-erect – *Chester* cultivar. This biological feature of the plant is an important indicator for the management, location and organization of blackberry plantations. A common feature of all the studied taxa is the presence of leaves in winter too, but *Thornless Evergreen* cultivar differs by even more obvious leaf persistence.

Table 1. The morpho-technological characteristics of species and varieties of blackberry

VARIETY	Biological characters	Morpho-technological characteristics of fruits	Period of maturation (month)	The level of firmness of fruit	Productivity kg/plant	Adaptability in the Republic of Moldova's conditions
Thornless Evergreen	vigorous; repent stem.	weight – 4-5g; shape – spherical; taste – sweetish flavor.	VII-IX	++	4-5	good growth and development; resistant to drought and frost.
Chester	vigorous; semi-erect stem.	weight – 5 g; shape – oval-spherical; taste – sweet-sour.	VIII-IX	+++	5-6	moderate growth and development; resistant to frost.
Arapaho	semi-vigorous; erect stem.	weight - 6-7g; shape - cylindrical; taste - sweet-sour.	VI-VII	+++	5-6	good growth and development; resistant to frost.
ThornFree	vigorous; semi-erect stem.	weight – 5g; shape – oval; taste – sweet-sour.	VI-VIII	++	4-5	good growth and development; resistant to drought and frost.
Rubus fruticosus **(spontaneous)**	vigorous; repent stem.	weight – 4g; shape – oval; taste – sweetish slightly astringent.	VII-VIII	+	0,5-1	good growth and development; resistant to drought and frost.

Note: the level of firmness of fruit: + - reduced; ++ - moderate; +++ - increased.

The biological and technological characteristics of the fruits are of the greatest interest to growers and consumers. The mass of one fruit ranges from 4 g to 7 g; 4 g – *Thornless Evergreen* cultivar sp. *R. fruticosus* (spontaneous); 5 g – *Chester* cultivar; 6-7 g – *Arapaho* cultivar. The studied taxa also differ in fruit shape: cylindrical –Arapaho, spherical – *Thornless Evergreen*, and *R. fruticosus*, oval – *Thornfree* and intermediate, oval-spherical – *Chester.* The fruits possess a broad range of tastes: from sweet (*Thornless Evergreen*) to sweet sour (*Chester* and *Arapaho*) and sweet sour, astringent taste (*Thornfree* cultivar and sp. *R. fruticosus*), which allow the consumer the opportunity to choose blackberry fruits according to preferences and needs.

The blackberry cultivars differ in the fruit maturation period: early – *Arapaho*, followed by *Thornless Evergreen* and *Thornfree* and sp. *R. fruticosus* (spontaneous), the most belated maturation period is characteristic of *Chester* cultivar. The duration of ripening is different, the longest being characteristic of *Thornless Evergreen* and *Chester* cultivars. This index, which characterized these new cultivars, could make fresh blackberry fruits available over a long period, starting from June and ending in September, but the maximum – still remains for August.

The studied taxa develop polydrupe fruits (an aggregate of multiple drupelets), characterized by juiciness, determined by the high degree of vacuolation of mesocarp cells [14]. Thus, drupelets can easily lose their integrity at mechanical manipulations performed during harvesting, sorting, packing, transporting [16]. The presence of sclereids and the endocarp thickness, the degree of compaction of the parenchyma cells and vacuolation ensure fruit quality. The firmness of fruits is determined by their integrity, which is ensured by the development of superficial structures such as the presence of a waxy epicuticular layer, the cuticle thickness and its depth among epidermal cells [1]. The analysis of these anatomical indicators shows that the fruits of *Chester* and *Arapaho* cultivars have the highest firmness (+++), followed by *Thornless Evergreen* and *Thornfree* (++) and those with reduced firmness from the spontaneous sp. *R. fruticosus*.

According to phenological observations made on blackberry plants from the collection created in the field, all taxa show good growth and development in the climatic conditions of our country, only on plants of the *Chester* cultivar, we noted yellowing of leaves during drought. To ensure plant vigor and high productivity, all cultivars require irrigation. Resistance to both drought and frost characterizes the studied taxa, but *Arapaho* and *Chester* cultivars are only frost resistant. According to morphological and technological characteristics, all analyzed cultivars can be recommended for cultivation in large areas of the Republic of Moldova, but need to be considered: the stem position in space, the degree of firmness of fruits, plant productivity and adaptability. Given the results of the comparative morpho-technological study, the most recommended blackberry cultivars would be *Arapaho* and *Chester.*

Anatomical Study

Sp. *R. fruticosus*. The analysis of superficial preparations and cross sections (fig. 1) of the leaf shows that the upper and lower epidermis are single-layered, composed of cells that are well wrapped, polygonal, with external walls slightly thickened. The epidermis is covered by a layer of cuticle, which easily penetrates between the epidermal cells. On the superficial preparation of the upper epidermis, cells with wavy walls were observed. Both epidermises develop unicellular trichomes in cluster and covered with thick membrane. Pubescence is more pronounced on the lower epidermis, trichomes being distributed both along the veins and leaf surface. We note the presence of anomocytic stomata on both epidermises, but, numerically, more on the lower one. Leaf mesophyll is differentiated, dorsal-ventral, the palisade tissue consists of two rows of cells, slightly elongated, well arranged under the upper epidermis. The spongy tissue consists of parenchymal, and lobed cells.

Collateral closed bundles, accompanied by sclerenchymatic sheath, cross the mesophyll. The presence of cells with druses of calcium oxalate, both along the veins and dispersed in the mesophyll, is obvious. In addition, mechanical, collenchyma tissue was observed under both epidermises, on cross sections, in the region of the ribs.

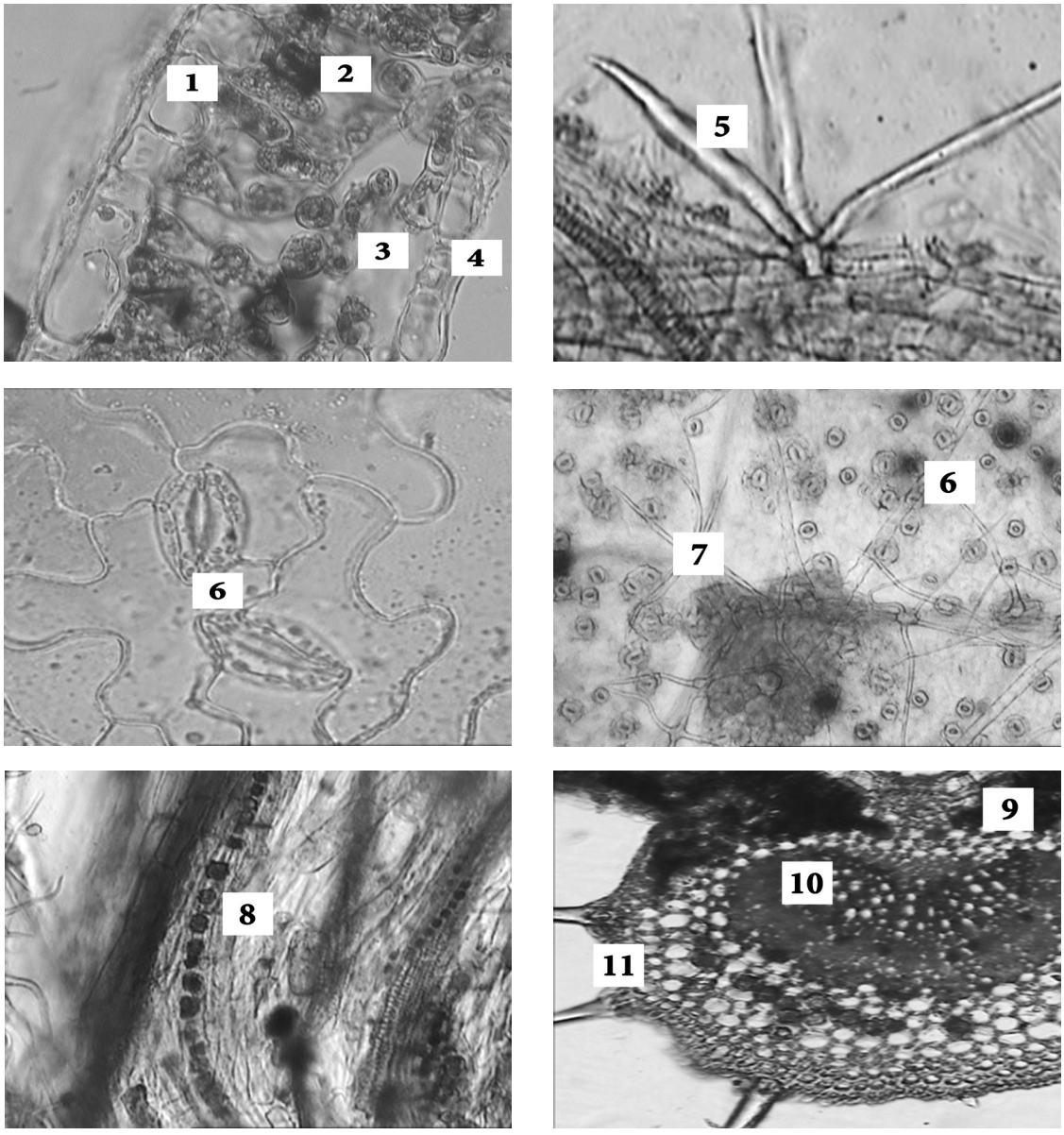

Fig.1. Leaf anatomy of sp. *R. fruticosus:* A (40×) – transversal section of leaf lamina; **B** (40×), **D, E** (10×) – superficial preparation of lower epidermis; **C** (40×) – superficial preparation of upper epidermis; **G** (40×) – transversal section trough main rib: 1 – upper epidermis, 2 – lower epidermis, 3 – palisade tissue, 4 – spongy tissue, 5 – unicellular trichomes in cluster, 6 – stomates, 7 – unicellular trichomes in cluster (view from above), 8 – druse of calcium oxalate along the vein, 9 – mechanical tissue, 10 – vascular bundle, 11– unicellular trichome.

***Arapaho* cultivar.** The leaves of this cultivar are characterized by the same anatomical structure as the sp. *R. fruticosus*, with only minor differences (fig. 2). Alike, the epidermis consists of compactly arranged cells, but its shape is slightly flattened. We mention the presence of trichomes, unicellular, but solitary. We

distinguish trichomes that are short, straight and thickened at the base and trichomes that are long, slightly curved, included into the basal socket. Cells with an abundance of big druses of calcium oxalate are dispersed in the leaf blade. Spongy tissue is more developed.

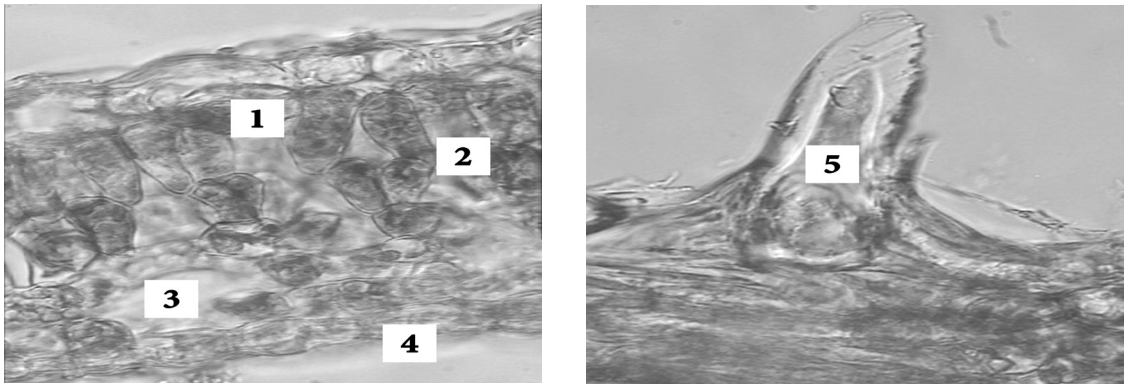

Fig.1. Leaf anatomy of *Arapaho* cultivar: A (40×) – transversal section of leaf lamina; **B** (40×) – unicellular trichome in longitudinal section: 1 – upper epidermis, 2 – palisade tissue, 3 – spongy tissue, 4 – lower epidermis, 5 – cellular wall.

***Chester* cultivar.** The microscopic analysis of multiple preparations (fig. 3) shows that the leaves of this cultivar develop the same anatomical type as sp. *R. fruticosus* with some specifications: cuticle does not penetrate between epidermal cells and epidermal cells have oval shape. The pubescence is not so evident, with unicellular, solitary trichomes, but long and rare, entwined and fixed in the basal socket and the calcium oxalate druses are arranged in the sheath of the vascular bundle. Palisade tissue cells are elongated, arranged in two rows with small intercellular spaces, spongy parenchyma cells are lobed with large intercellular spaces.

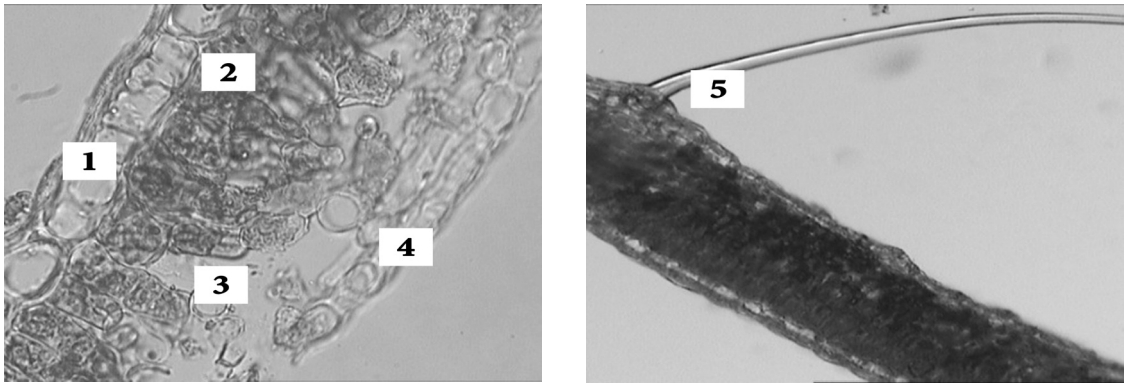

Fig. 3. Leaf anatomy of *Chester* cultivar: A (40×), **B** (10×) – transversal section of leaf lamina: 1 – upper epidermis, 2 – palisade tissue, 3 – spongy tissue, 4 – lower epidermis, 5 – unicellular solitary trichome.

***Thornfree* cultivar.** The leaf anatomy of this cultivar (fig. 4) is characterized by the presence of trichomes: in clusters or solitary, long and unicellular, sharp at the peak, relatively large, with a dilated and rounded base, which is included in a multicellular socket with browning content, with more pronounced pubescence. Epidermal cells are isodiametric. There are also druses of calcium oxalate.

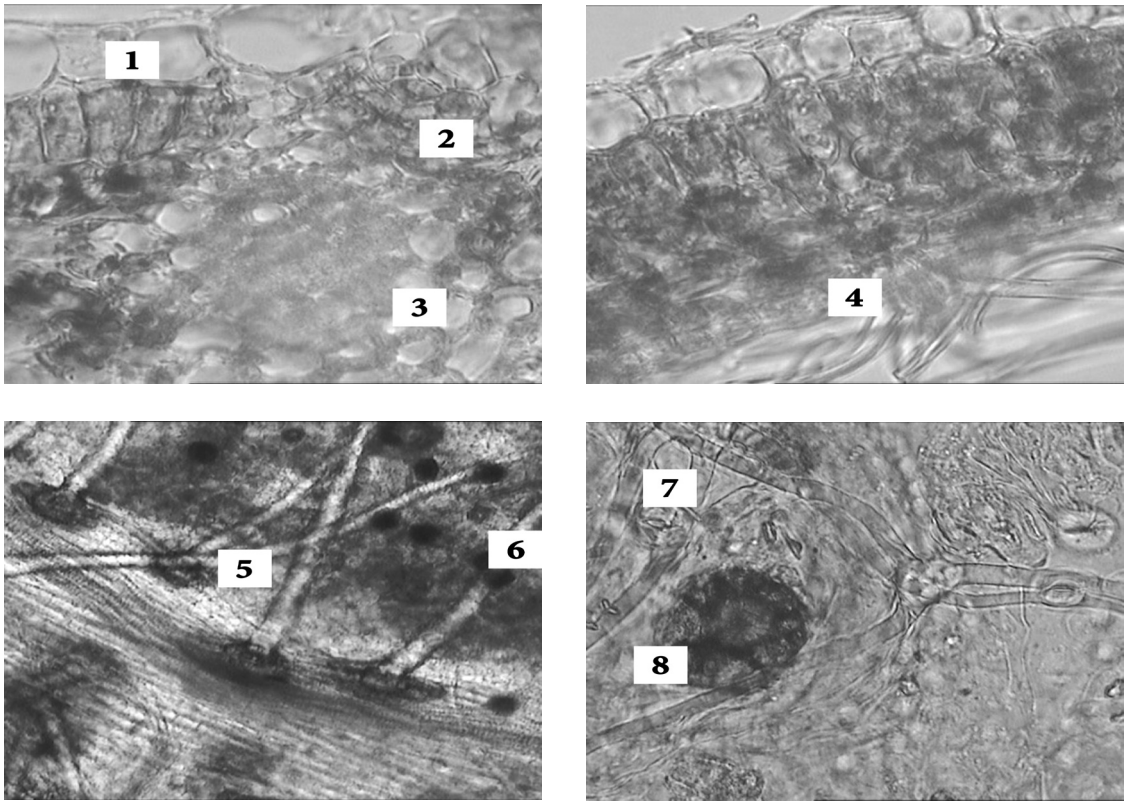

Fig. 4. Leaf anatomy of *Thornfree* cultivar: A, B (40×) – transversal section of leaf lamina; **C, D** (40×) – superficial preparation of lower epidermis: 1 – upper epidermis, 2 – palisade tissue, 3 – vascular bundle; 4 – trichomes arranged in cluster; 5 – unicellular, solitary trichomes; 6 – calcium oxalate druses, 7 – stomata; 8 – the base of one trichome with brownish content.

***Thornless Evergreen* cultivar (obtained from sp. *R. laciniatus*).** The anatomical structure of the leaf is of dorsal-ventral type, which is characterized by spongy tissue with large intercellular spaces. The cells of both upper and lower epidermises are slightly flattened and well packed. The stomata are present only on the lower epidermis. The upper epidermis consists of cells with regular contour with almost straight sidewalls on the upper epidermis of leaf and tortuous visible walls on the lower epidermis. Tector trichomes are distinguished; they are unicellular, solitary or anastomosed by 2 or 5, often located on veins. The venation is well pronounced on the lower epidermis of the leaf.

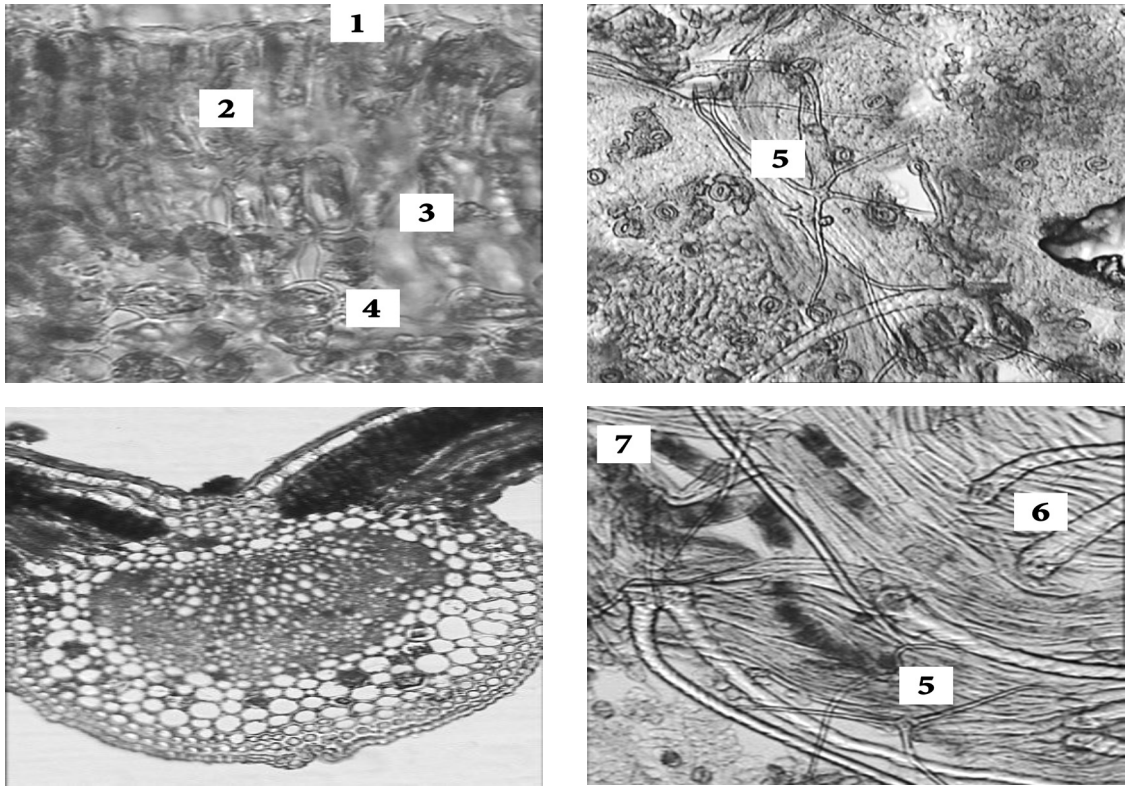

Fig. 5. Leaf anatomy of *Thornless Evergreen* cultivar: A(40×) – transversal section of leaf lamina; **B, D** (40 ×) – superficial preparation of the lower epidermis; **C** (40×) – section trough central vein: 1 – upper epidermis, 2 – palisade tissue, 3 – lacunar tissue; 4 – lower epidermis, 5 – anastomosed trichomes, 6 – unicellular and solitary trichomes; 7 – calcium oxalate druses.

Comparative Anatomical Study of the Leaves of Studied Taxa

The comparative anatomical study was conducted based on the following indicators: the thickness of the leaf, the epidermis (upper and lower), the mesophyll and their correlation; the cuticle type (cuticle penetration between the epidermal cells); the presence, type and distribution of trichomes; the presence and the distribution of stomata; the mesophyll type and anatomical characteristics, the type of vascular bundle, the presence and location of calcium oxalate druses.

According to the results of the analysis of different structural indicators (tab. 2), the lowest values of leaf thickness (114 μm) are in the sp. *R. fruticosus* (spontaneous). The comparative analysis of anatomical parameters of the cultivars of the sp. *R. fruticosus* and *R. laciniatus* shows that, for all cultivars, leaf thickness is greater in plants grown in open field than in the greenhouse. Comparatively, the leaves of *Arapaho* cultivar develop the greatest thickness (240.0 μm), followed by *Thornfree* (191.5 μm), then *Thornfree Evergreen* (170.0 μm) and the smallest – *Chester* cultivar (148.0 μm). A common characteristic of the leaves of the studied taxa is that the epidermis develops cuticle of external-internal type, except the leaves of *Chester* cultivar – with external cuticle type. The extent of the cuticle is an informative index with key role in plant adaptation to environmental conditions and in their resistance against excessive insolation, radiation, increased temperature, and lack of humidity [1, 3, 5], which suggests that the new studied dewberry cultivars have a well-developed protective potential, which provides resistance to unfavorable climatic conditions of the R. Moldova.

Table 2. Anatomical characteristics of the leaves of spontaneous species and new cultivars of blackberry

Species, cultivars	Thikness of leaf (µm±n)	Type of cuticle	Thikness of epiderma (µm±n)		Thikness of mesophyll (µm±n)	The correlation of thickness index		
			Upper	Lower		Upper / lower epidermis	Epidermis /leaf	Mezophyll /leaf
R.fruticosus	114.0±4.1	External-internal	20.95±1.51	8.53±1.19	83.74±4.23	2.45	3.87	0.73
Arapaho (open ground)	240.0±14.01	External-internal	44.3±2.73	35.4±2.03	168.2±11.0	1.2	3.0	0.67
Arapaho (greenhouse)	121.0±7.72	External-internal	29.7±4.39	21.14±2.62	71.4±3.0	1.33	2.32	0.58
Chester (open ground)	148.0±3.84	External	27.4±0.87	22.3±1.13	98.9±3.50	1.22	2.97	0.66
Chester (greenhouse)	130.0±6.23	External	26.6±1.73	22.63±1.34	77.5±3.80	1.17	2.64	0.59
Thornfree (open ground)	191.5±2.62	External-internal	34.3±1.35	29.1±0.87	125.0±2.41	1.17	3.01	0.65
Thornfree (greenhouse)	117.0± 2.03	External-internal	21.4±1.16	11.88±0.41	80.0±5.20	1.8	3.51	0.68
Thornless Evergreen (open ground)	170.0±7.71	External-internal	35.0±1.6	27.7±0.7	107.0±7.43	1.26	2.7	0.62
Thornless Evergreen (greenhouse)	142.0±5.4	External-internal	25±1.5	18.95±1.5	96.0±4.54	1.31	3.23	0.67

Note: n – deviation

For all blackberry taxa studied, including the spontaneous sp. *R. fruticosus*, it is specific that the upper epidermis is thicker than the lower. The epidermis with the highest values (i. e., upper and lower epidermis – 44.3 and 35.4 µm) characterizes the leaves of *Arapaho* cultivar. In the lineage, we mention the values of the leaf epidermises of the cultivars *Thornless Evergreen* (35.0 and 27.7 µm), *Thornfree* (34.3 and 29.1 µm). The leaves of the spontaneous sp. *R. fruticosus* have the thinnest epidermises (20.9 and 8.5 µm). However, we must highlight the value of the correlation between the thickness of the epidermises, which is the highest (2.4) in the spontaneous species and twice as high as in the other cultivars *Arapaho, Chester, Thornfree*, and *Thornless Evergreen* (tab. 2). This is a criterion indicating the anatomical plasticity and flexibility of the epidermis as a first barrier to external factors. It is known that [3, 5, 11, 14] the thickness of the epidermis determines the protection of the leaf mesophyll, and the increased value of the correlation between the thickness of the upper/ lower epidermises for sp. *R. fruticosus* is a measure of adaptability to adverse conditions, especially resistance to drought, cold and radiation, which have been very pronounced during the last two decades [1, 3]. For all analyzed cultivars, the upper epidermis is thicker in the plants grown in open ground than in the plants grown

in the greenhouse, which is an indicator of the adaptability of plants to environmental conditions and ensure a more effective protection for the leaf mesophyll from the action of unfavorable conditions in summer and winter.

The leaves of the cultivars of blackberry, cultivated in field, differ from the spontaneous sp. *R. fruticosus* in the thickness of leaf mesophyll, which is better developed than in the latter. Comparatively, the leaves of the field cultivars *Arapaho* (168.2 μm) *Thornfree* (125.0 μm) and *Thornless Evergreen* (107.0 μm) form a thicker mesophyll than *Chester* cultivar (98.9 μm). The values of epidermis and mesophyll thickness correlate with the values of the ratio of mesophyll thickness and leaf thickness. Thus, the highest value is characteristic of the leaves of sp. *R. fructiosus* (0.73), followed by the varieties (decreasingly): *Arapaho* (0.67), *Chester* (0.66) and *Thornfree* (0.65), which shows high photosynthetic assimilation efficiency. According to experimental data, obtained by a group of authors [7, 14], the better the mesophyll is developed, the greater the photosynthetic activity of the plant is. Thus, the varieties of blackberry have a more efficient capacity of assimilation, which is an important source for the development of reproductive organs, which, in turn, ensure the higher productivity of plants, expressed in larger fruits and higher yields (tab. 1).

The leaves of all the analyzed taxa are pubescent, especially on the lower epidermis and along the veins, constituting a protective cover of leaves against insolation and dehydration. A common anatomical feature of taxa is the presence of tector unicellular trichomes. The sp. *R. fruticosus* has trichomes that frequently form clusters, *Arapaho* cultivar – solitary trichomes, short with thick membrane, *Chester* cultivar – long, solitary and rare trichomes, *Thornfree* cultivar – trichomes that are solitary or in clusters, by 2 or 3, with dilated base in the socket of cells with brownish content, *Thornless Evergreen* cultivar – solitary trichomes anastomosed by 2 or 5, often on the veins. These morphological differences of trichomes represent a distinctive anatomical feature that helps to indentify the taxa.

Another common anatomical criterion of the studied taxa is the development of anomocytic stomata on both epidermises, mainly on the lower epidermis. The only exception is *Thornless Evergreen* cultivar, with stomata only on the lower epidermis, which represent a distinctive anatomical feature.

The blackberry species and the cultivars derived from them are characterized by the development of salts and the formation of calcium oxalate druses, and their differences relate to the manner of distribution: in the sheath of vascular bundles and in mesophyll (sp. *R. fruticosus, Thornfree* cultivar), only in mesophyll (*Arapaho* cultivar) and only in vascular bundle (*Chester* cultivar). In addition to the diagnostic role of the calcium oxalate druses, they have also a physiological role, demonstrated in the last few decades [1, 4], giving them a role in increasing the protective potential of leaves under unfavorable conditions and, especially, responding to the action of stress conditions. Thus, we highlight sp. *R. fruticosus* and *Thornfree* cultivar with a more pronounced capacity of adaptation to the action of unfavorable growth conditions, as compared with other studied taxa of blackberry.

Proceeding from the qualitative and quantitative anatomical data, reported above, the comparative anatomical study of the leaves of taxa, carried out according to a complex of anatomical indicators, shows that all taxa develop a structural adaptive potential to the action of environmental conditions, supplanted by external structures: thick, external-internal cuticle, tector trichomes, the size and the degree of packing of the epidermal cells, and internal ones: the degree of mesophyll development, the presence and the distribution of calcium oxalate druses, the degree of development of mechanical tissue. These structures work synergistically and make up a protector compensator hysto-anatomic complex of leaf to external factors (lack of moisture, high temperatures, of soil and air, during summer and low – in winter). The development of pubescence (the number and the distribution of trichomes on the epidermis) and the calcium oxalate druses (number and location), depending on taxa and growing conditions (field or greenhouse), and reveal structural-adaptive plasticity of the leaf apparatus to the environmental conditions.

CONCLUSIONS

1. The morphological and technological analysis of the studied taxa shows that they differ in stem position in space, vitality, technological characteristics of the fruits (period of maturity, firmness, productivity and the adaptability to the climatic conditions of the R. Moldova).
2. The anatomical comparative study of taxa resulted in the anatomical characteristics of leaves of each taxon and revealed structures with diagnostic character to identify species and new blackberry cultivars (cuticle type, trichome type – simple, short or long, with or without socket and their distribution – solitary, anastomised by 2-3 or in clusters and the location on the epidermis, the distribution of calcium oxalate druses).
3. The anatomical comparative study of the new cultivars and some species of dewberry, grown in open ground and in greenhouse, revealed anatomical indicators, with adaptive character to unfavorable conditions, such as: the degree of pubescence, the degree of development of the epidermis and its derivatives, the correlation index of epidermal/leaf thickness, upper/lower epidermis, mesophyll/leaf, level of development and distribution of the calcium oxalate druses.
4. The morphological, technological and anatomical characteristics, of the new blackberry cultivars and 2 species of g. *Rubus*, may provide complex and conclusive support for the elaboration of strategies for the management of dewberry plantations on large areas in the R. Moldova.

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INFLUENCE OF GROWTH SUBSTRATUM ON BIOMETRIC AND PHYSIOLOGICAL PARAMETERS IN *COTONEASTER DAMMERI* "SKOGHOLM" SAPLINGS

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Abstract. The goal of this study was to assess the influence of the growth substratum structure on growth and development in *Cotoneaster dammeri* "Skogholm" saplings during the first vegetation stages. The large share of garden soil (50-60% of the volume) and of peat (30%) had a negative influence on some biometric and physiological parameters of the saplings through the correlation coefficient (leaf area – LA, r = -0.940; sapling height – H, r = -0.922; sapling diameter – D, r = -0.976). Increasing the share of sand in the mixture (40-50%) had a positive influence on the same biometric and physiological parameters (LA, r = 0.927; H, r = 0.899; D, r = 0.971). Between physiological parameters at leaf level that evolved in direct relationship with the growth substratum, were identified statistically ensured interdependence relations (leaf length – leaf area, $R^2 = 0.993$; $p << 0.001$; leaf width – leaf area, $R^2 = 0.984$; $p << 0.001$; leaf number – leaf area, $R^2 = 0.991$; $p << 0.001$). Based on the photosynthetic area of each variant, the saplings accumulated differently the biomass at stem level, in correlation with the leaf area resulted from the number of leaves and their size (LA – H, $R^2 = 0.932$; $p << 0.001$; LA – D, $R^2 = 0.978$; $p << 0.001$).

Keywords: Biometric parameters, Cotoneaster, growth substratum, physiological parameters, saplings

INTRODUCTION

The Genus *Cotoneaster* has about 90 species, different taxonomic studies being made on *Cotoneaster dammeri* (Zhou et al., 2000; Lu et al., 2005). *Cotoneaster dammeri* C. K. Schneid. is a creeping shrub native from China and it decorates due to both its branches laying on the ground and its dark-red, globe-like fruit that resists for a long time on the plant (Iliescu, 1998). It is also an important ornamental species due to its anatomy, high ecological plasticity, and ability of fitting niches in landscaping.

The plant behaviour has been studied under different soil and climate conditions due to the interest in cultivating it within landscape arrangements. Though it adapts to various soil conditions, its requirements from the nutrition medium during its first vegetation stages are high. Numerous studies have assessed the species relationship with different growth environments, nutrient mixtures, water regime, and nutrients aiming at optimising these growth factors (Gilliam et al., 1980; Kraus and Waren, 2002; Owen Jr. et al., 2008; Owen et al., 2009). Barker and Pilbeam (2007) have seen that the best growth of the roots and sprouts of Cotoneaster (*Cotoneaster dammeri* C. K. Schneid.) occurred when adding dolomite to the nutrient medium compared to other sources of calcium and magnesium.

Some studies have assessed the influence of the form, design, and quality of the container material on plant growth and of root deformation in ornamental species among which *Cotoneaster* species (Privett and Hummel, 1992; Amoroso et al., 2010).

The rooting and growth substratum is important for the multiplication of these shrubs (*Cotoneaster* plants) during the first multiplication stages because it strongly influences the formation of the root system and, hence, of vigorous plants.

The goal of this study was to assess the influence of the share of some components of the substratum for the optimisation of the nutritive component mixture aiming at increasing sapling growth in *Cotoneaster*

dammeri "Skogholm".

MATERIAL AND METHOD

The goal of the study was to assess the influence of the growth substratum structure on growth and development in the saplings of Cotoneaster during the first stages of vegetation.

The biological material was represented by the species *Cotoneaster dammeri* "Skogholm", a Swedish selection with a creeping habitus and leaves persisting over the winter, with a higher height than that of the basic species (0.8-1 m); the stalks are curved and reach the soil, with elongated elliptic, dark-green, glossy leaves (Iliescu, 1998). Was used cuttings 10±0.5 cm long with a diameter of 0.35 cm, aged 100 days, rooted in river sand and treated with the rooting biostimulator Radistim 2 (Poşta, 2012a).

The growth substratum mixture was prepared in four variants (Poşta, 2012b) from garden soil, peat and sand in variable proportions (volumes) as follows: V_1 – 60% garden soil; 30% peat; 10% sand; V_2 – 50% garden soil; 30% peat; 20% sand; V_3 – 40% garden soil; 20% peat; 40% sand; V_4 – 30% garden soil; 20% peat; 50% sand. The volume of growth jars was 1000 cm^3.

Biometric and physiological parameters assessed to describe the evolution of the cuttings on the growth substratum prepared focused on the sapling collar diameter (D), sapling height (H), leaf size (length – L; width – W), leaf number (Lnr) and leaf area (LA). Sapling height and leaf size were determined by measuring with a ruler with a precision of ±0.5 mm. Diameter was determined by measuring with electronic callipers with a precision of ±0.001 mm. The number of leaves was obtained by numbering and the leaf area was determined after the model proposed by Sala et al. (2015) based on leaf size and leaf area constant.

Statistical analysis of experimental data. Assessing results variance was done with the ANOVA single factor test and the differences significance limits were calculated through variance analysis. Statistic analysis of correlation was used to assess the degree of interdependence between the components of the growth substratum and the physiological parameters and their interrelations. The statistic safety parameters taken into account were the correlation coefficients r and R^2 and the statistic safety parameter p. For the statistic analysis, were used the EXCEL application of the Office 2007 suite and the PAST Programme (Hammer et al., 2001).

RESULTS AND DISCUSSION

Growth substratum mixtures with different component shares have influenced in a different way the rooting, growth and development of the Cotoneaster cuttings, which has been pointed out through the prism of the biometric and physiological parameters that were determined.

Sapling height had values ranging between 11.58 cm in the variant V_1 (control variant) and 25.56 cm in the variant V_4, with statistically ensured differences. Sapling diameter varied between 0.800 cm in the control variant (V_1) and 1.388 cm in the variant V_4, with statistically ensured differences, as shown in Table 1.

Table 1. Variations of Cotoneaster sapling stem parameters under the influence of the growth substratum

Growth substratum	Trial variant	Sapling height		Sapling diameter	
		H (cm)	Differences (cm)	D (cm)	Differences (cm)
Garden soil 60% + peat 30% + sand 10%	V_1	11.58	-	0.800	-
Garden soil 50% + peat 30% + sand 20%	V_2	14.40	2.81^{*}	0.946	0.146^{***}
Garden soil 40% + peat 20% + sand 40%	V_3	17.48	5.89^{***}	1.165	0.365^{***}
Garden soil 30% + peat 20% + sand 50%	V_4	25.56	13.96^{***}	1.388	0.588^{***}

Limits the significance of differences		$LSD_{5\%}$ = 1.98 cm; $LSD_{1\%}$ = 2.85; $LSD_{0.1\%}$ = 4.19	$LSD_{5\%}$ = 0.059; $LSD_{1\%}$ = 0.084; $LSD_{0.1\%}$ = 0.124

Leaf size oscillated between 2.60 and 5.93 cm (in length) and between 1.33 and 2.99 cm (in width), increasing with the increase of the share of sand in the growth substratum, all of it statistically ensured (Table 2).

Leaf number and leaf area in saplings also varied depending on the nutrient mixture. Values ranged between 6.15 and 19.15 (leaf number) and between 14.01 and 222.81 cm^2 (leaf area), all statistically ensured, as shown in Table 3.

Table 2. Size of Cotoneaster sapling leaf under the influence of the growth substratum

Growth substratum	Trial variant	Leaf length		Leaf width	
		L (cm)	Differences (cm)	W (mm)	Differences (cm)
Garden soil 60% + peat 30% + sand 10%	V_1	2.60	-	1.33	-
Garden soil 50% + peat 30% + sand 20%	V_2	3.80	1.20***	1.81	0.48***
Garden soil 40% + peat 20% + sand 40%	V_3	4.77	2.17***	2.37	1.04***
Garden soil 30% + peat 20% + sand 50%	V_4	5.93	3.33***	2.99	1.66***
Limits the significance of differences		$LSD_{5\%}$ = 0.09; $LSD_{1\%}$ = 0.14; $LSD_{0.1\%}$ = 0.20		$LSD_{5\%}$ = 0.14; $LSD_{1\%}$ = 0.20; $LSD_{0.1\%}$ = 0.30	

Table 3. Number of Cotoneaster sapling leaves and leaf area under the influence of the growth substratum

Growth substratum	Trial variant	Leaf number		Leaf area	
		Lnr	Differences	LA (cm^2)	Differences
Garden soil 60% + peat 30% + sand 10%	V_1	6.15	-	14.01	-
Garden soil 50% + peat 30% + sand 20%	V_2	7.75	1.60*	35.24	21.23*
Garden soil 40% + peat 20% + sand 40%	V_3	13.22	7.07***	98.84	84.83***
Garden soil 30% + peat 20% + sand 50%	V_4	19.15	13.00***	222.81	208.80***
Limits the significance of differences		$LSD_{5\%}$ = 1.51; $LSD_{1\%}$ = 2.18; $LSD_{0.1\%}$ = 3.20		$LSD_{5\%}$ = 18.22; $LSD_{1\%}$ = 26.20; $LSD_{0.1\%}$ = 38.54	

The ANOVA single factor test pointed out the existence of variation of experimental data under the influence of the mean nutrition factor, statistically ensured – $p << 0.001$, $F > Fcrit$, for Alpha = 0.001 (Table 4).

Table 4. ANOVA test, single factor

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	100468	5	20093.61	16.62088	1.34E-11	4.526032
Within Groups	108804.4	90	1208.938			
Total	209272.4	95				

Alpha = 0.001

Growth substratum, through the share of the components sued (garden soil, peat and sand) influenced considerably the growth rate of the saplings, which is notable in the biometric and physiological parameters

were determined. Garden soil, due to its large share in the nutrient mixture (V_1 – 60%; V_2 – 50%, in volumes) influenced negatively the growth and development of the saplings, with negative and highly intensive correlations between biometric parameters and garden soil (R^2 = -0.922, -0.996). Large shares of peat in the mixture (V_1 and V_2 – 30%, in volumes) also had a negative influence on sapling growth, with negative correlations with the sapling physiological parameters, but of lower intensity compared to garden soil (R^2 = -0.781, -0.888). Sand, in exchange, due to its large share in the mixtures, had positive correlations with the physiological parameters of the saplings (R^2 = 0.899, 0.981). These interdependence relations between biometric and physiological parameters of the saplings and the components of the nutrient mixture show that *Cotonaester* saplings need, in the process of multiplication, a growth substratum with a light, sandy texture, with lower supply of organic matter and nutrients, because of the low consumption of nutrients during vegetation stages (Sala, 2011), which facilitates the development of both the root system and of the other parameters.

Under the experimental growth substratum conditions, Cotoneaster saplings developed with variable intensity as shown by the physiological parameters among which were identified a set of interdependence relations.

The interdependence relation between leaf area and leaf size was assessed based on 2nd grade polynomial functions and it had a high degree of correlation depending on both leaf length, equation (1) (R^2 = 0.993; $p << 0.001$) and on leaf width, equation (2) (R^2 = 0.985; $p << 0.001$). The graphic distribution of the particular values of leaf area depending on L and W is shown in Figures 1 and 2, respectively. The variation of some leaf parameters depending on plant nutrition status was also recorded during the study of other horticultural species (Blidariu and Sala, 2013; Jivan and Sala, 2013, 2014; Camen et al., 2016).

$$LA = 20.51\ L^2 - 112.1\ L + 165.8 \quad (1)$$

$$LA = 63.41\ W^2 - 150.1\ W + 100 \quad (2)$$

where: LA – leaf area; L – leaf length; W – leaf width.

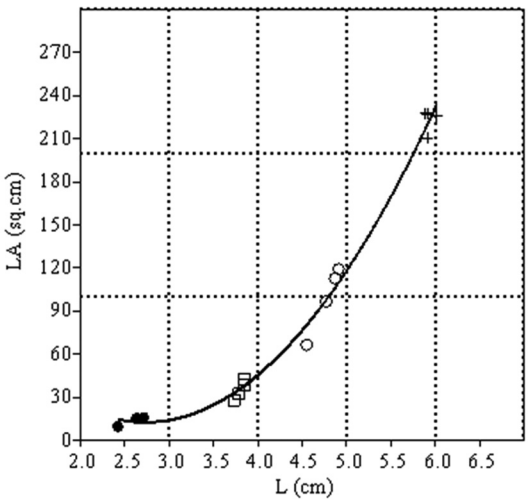

Figure 1. Graphic distribution between leaf area (LA) and leaf length (L); · – V_1; □ – V_2; ○ – V_3; + - V_4

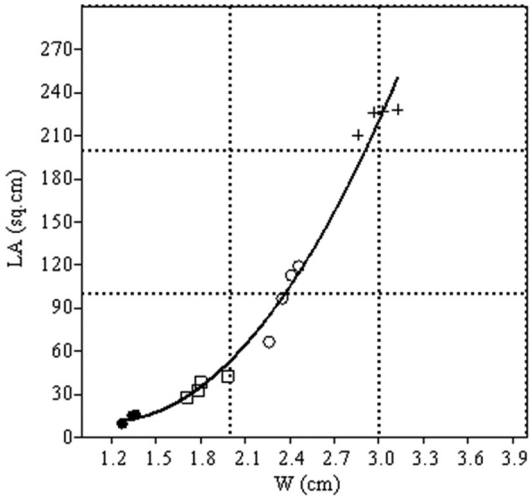

Fig. 2. Interdependence relation between leaf area (LA) and leaf width (W); · – V_1; □ – V_2; ○ – V_3; + - V_4

Based on the photosynthesis area of each variant, saplings accumulated, in a different way, biomass at stem level, in correlation with leaf area determined through leaf number and leaf size. The relation between

leaf area and the diameter of the sapling stem collar, and sapling height was described by 2nd grade polynomial, equations (3) and (4), all statistically ensured (LA – D, $R^2 = 0.978$; $p << 0.001$; LA – H, $R^2 = 0.932$; $p << 0.001$).

$$D = - 1.207\ E - 05\ LA^2 + 0.005593\ LA + 0.7419 \quad (3)$$

$$H = - 3.516\ E - 05\ LA^2 + 0.07271\ LA + 11.05 \quad (4)$$

where: D – sapling diameter; H – sapling height; LA – leaf area.

Between sapling diameter and sapling height, there was a relation of interdependence described by a 2nd grade polynomial equation (5), statistically ensured ($R^2 = 0.978$; $p << 0.001$), as shown in Figure 3.

$$H = 226.98\ D^2 - 36.36\ D + 23.76 \quad (5)$$

where: H – sapling height; D – sapling diameter.

Between leaf number and leaf area, the relation of interdependence was described by a 2nd grade polynomial equation (6) ensured statistically ($R^2 = 0.991$; $p << 0.001$), as shown in Figure 4.

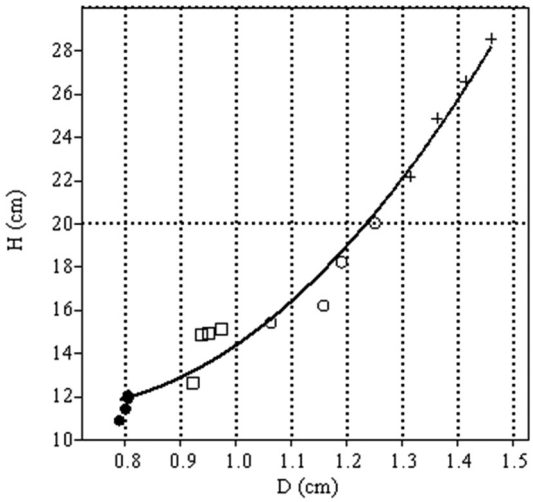

Figure 3. Graphic distribution of the relation between sapling diameter and sapling height; · – V_1; □ – V_2; ○ – V_3; + - V_4

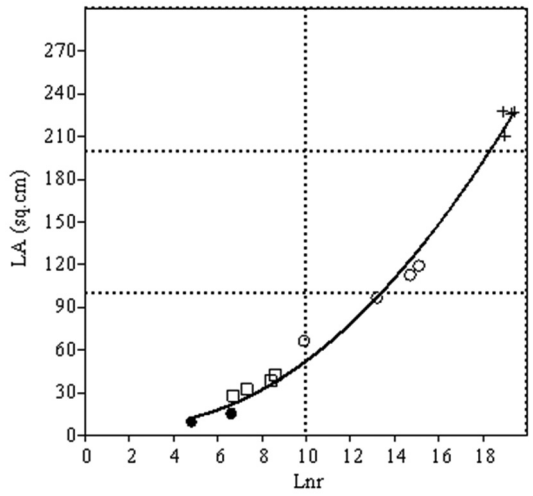

Figure 4. Graphic distribution of the relation between leaf area and leaf number; · – V_1; □ – V_2; ○ – V_3; + - V_4

$$LA = 0.7458\ Lnr^2 - 3.349\ Lnr + 11.21 \quad (6)$$

where: LA – leaf area; Lnr – leaf number.

The nutritive component mixture used to grow the seedlings of woody ornamental species needs to ensure a high degree of fertility and optimum moisture and aeration regime to allow the development of the root system.

The influence of the growth substratum on some physiological parameters and on ornamental plant growth and development has been noted in numerous studies.

Tyler et al. (1993) have noted a decrease of the root dry matter volume in two ornamental species (*Cotoneaster dammeri* C. K. Schneid. "Skogholm" and *Hemerocallis* sp. "Red Magic") cultivated on a substratum of pine bark with increased compost rate in the growth substratum, while leaf dry matter volume was not affected. In the same studies, they recorded the variation of some physiological parameters depending on watering rate and foliar nutrient supplementing.

The influence of the volumetric share of the components in the growth substratum on some growth parameters in Cotoneaster was also studied by Chong and Cline (1994).

The increasing variation of the root dry matter in Cotoneaster and of the sprouts in *Rudbeckia* was recorded depending on the growth rate between 0 and 4 g N when growing plants on a substratum of pine bark amended with 8% (by volume) composted turkey litter. The watering rate did not affect the growth rate of the fresh substance in *Cotoneaster*, but it generated changes in *Rudbeckia* (Tyler and Warren, 1996).

The different shares of the basic components of nutrient mixtures were studied depending on volume share and species requirements in *Weigelia rosea* Lindl (Poșta et al., 2014).

CONCLUSIONS

Growth substratum influenced in a different way the formation of the root system, the growth and development of Cotoneaster saplings depending on peat, garden soil and sand share in mixtures within the tested limits. The large share of peat (30%) and garden soil (60%) had a negative influence on sapling biometric and physiological parameters, while an increase of the share of sand to 40-50% (in volumes) had a positive influence. For Cotoneaster saplings, growth substratum, with large shares of components ensuring light textures (sand or other neutral mineral components) are recommended to obtain highly vigorous saplings.

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INFLUENCE OF SEED TREATMENT ON GERMINATION IN *LABURNUM ANAGYROIDES* MED.

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Abstract. This study focuses on the evaluation of the effect of pre-germination seed treatment in *Laburnum anagyroides* Med. on sapling germination and status during the first growth stages. Seeds were moistened for 24 h with cold water (V_1), hot water 90°C (V_2), stratification (V_3) and scarification (V_4). The best effects on medium germination was with scarification (98% germinated seeds), followed by stratification (87% germination), hot water (76%) and cold water (70%). The effect of the treatments was also in sapling status 37 days after sprouting, the proper age for transplanting. From the perspective of the biometric and physiological parameters, were studied collar diameter (D), sapling height (H), root number (Rn), root length (Rl), leaf number (Ln) and leaf area (LA), the best effects were generated by scarification, followed by stratification, with statistically ensured differences. Cluster analysis of results facilitated the grouping of experimental variants based on Euclidian distances into two clusters with statistic safety (Cophenetic coefficient = 0.887).

Keywords: common laburnum, propagation, seed treatment, sapling

INTRODUCTION

Laburnum anagyroides Medic. is an indigenous species widespread in southern Europe; in Romania, it is rarely found in spontaneous flora, in Oltenia. The species has high ecological plasticity: it is resistant to frost, drought, salinity, and air pollution (Percival, 2005; Roloff et al., 2009). It can grow on poor, dry, limey soils with proper aeration. Because of the alkaloid cystine present in its bark, leaves and seed, it has certain toxicity (Gray et al., 1981). Leaf consumption by certain animal species makes the milk toxic (Poșta, 2009a).

The species' ornamental features are represented by the erect port, flowers, and leafage shape and aspect. The flowers are yellow, 2 cm long, grouped into pendent racemes up to 30 cm long; its blooming period is May-June (between 2.5 and 4 weeks); it is also an important honey species (Stawiarz and Wróblewska, 2013). Depending on the type of landscape architecture, it can be planted alone on lawns, in groups or as pergola tunnels; it is very appreciated for its golden flowers (Poșta, 2009b).

Some studies have assessed the place, role and importance of the species *Laburnum* within natural ecosystems in relation with pollinator species (Suso et al., 2016), while other studies have assessed the invasive feature of the species within areas occupied by tree species (Křivánek and Pyšek, 2008).

The propagation of the species *Laburnum anagyroides* has been made most commonly through seeding or grafting; treating with electromagnetic microwave is beneficial for the increase of the germination percentage (Aladjadjiyan, 2002), which is also common in other species (Talei et al., 2013). Recent studies have reported the first *in vitro* micropropagation of *Laburnum anagyroides* starting from buds explanted from mature trees (Timofeeva et al., 2014, 2016).

This study aimed at assessing seed treatment by moistening, stratification and scarification on germination and on some sapling biometric and physiological parameters during the first vegetation stages.

MATERIAL AND METHOD

The aim of this study was assessing the influence of seed treatment in the propagation of *Laburnum anagyroides* and the effect on saplings during the first vegetation stages.

The biological material was the species *Laburnum anagyroides* Medic. Fruits of common laburnum were harvested in September 2014 from the same biological source within the Didactic Horticultural Research Station of the Banat's University of Agricultural Science and Veterinary Medicine "King Michael I of Romania" from Timisoara, Romania. The fruits were dried under aeration and shadow conditions. Seed extraction was done in the laboratory of the Department of Arboriculture and Landscaping; after harvesting, the seeds were kept in tight close vases at 4°C. Prior to the treatments, the seeds were immersed to remove empty seeds. After seed selection through immersion, were obtained 200 even seeds for each variant (50 seeds/replication) according to the experimental protocol.

Experimental variants. Because of the difficult germination under natural conditions (tegument features), the seeds were treated with cold water for 24 h (V_1), with hot water 90°C for 24 h (V_2), and they were stratified (V_3) and scarified (V_4). The experiments were randomised with four replications. Seed stratification was made in sand in November 2014, keeping the sand moist at 3-4°C. Seeding was done on February 2015 for all experimental variants, in an even substratum made up of 50% garden soil and 50% sand.

Biometric and physiological parameters. To assess the influence of the seed treatment on germination and on saplings, were determined, in each variant, the percentage of germinated seeds, the collar diameter in the saplings (D), sapling height (H), the number of roots (Rn), the length of the roots (RL), the number of leaves (Lnr) and the leaf area (LA). Sapling collar diameter was measured with electronic callipers (precision: ±0.001 mm). Sapling height was measured with a ruler (precision: ±0.5 mm). The number of leaves was determined by numbering per sapling and variant. Leaf area was measured based on leaf size and area constant, according to the model proposed by Sala et al. (2015).

Statistic analysis of experimental result. Experimental data were analysed through the ANOVA test to assess variance and through variance analysis to assess the significance limits of the differences (LSD 5%, 1% and 0.1%). Multivariate statistic analysis of the Cluster-type analysis was used to classify the variants based on Euclidian distances, and the safety parameter used was the Cophenetic coefficient. Statistic analysis of the results was done based on the EXCEL application, suite Office 2007, and on the PAST Programme (Hammer et al., 2001).

RESULTS AND DISCUSSION

Seed treatment with cold and hot water (90°C) for 24 h and through stratification and scarification had different, specific effects on seed germination, even in first germination stage, saplings from the perspective of biometric and physiological parameters measured 37 days after sprouting, the proper age for transplanting.

As for germination, preparatory treatments of the seeds influenced germination differently. The percentage of germinated seeds, 10 days after germination started, was 70% in the variant V_1 (moistening with cold water), 76% in the variant V_2 (moistening with hot water 90°C), 87% in the variant V_3 (stratification) and 98% in the variant V_4 (scarification) (Figure 1).

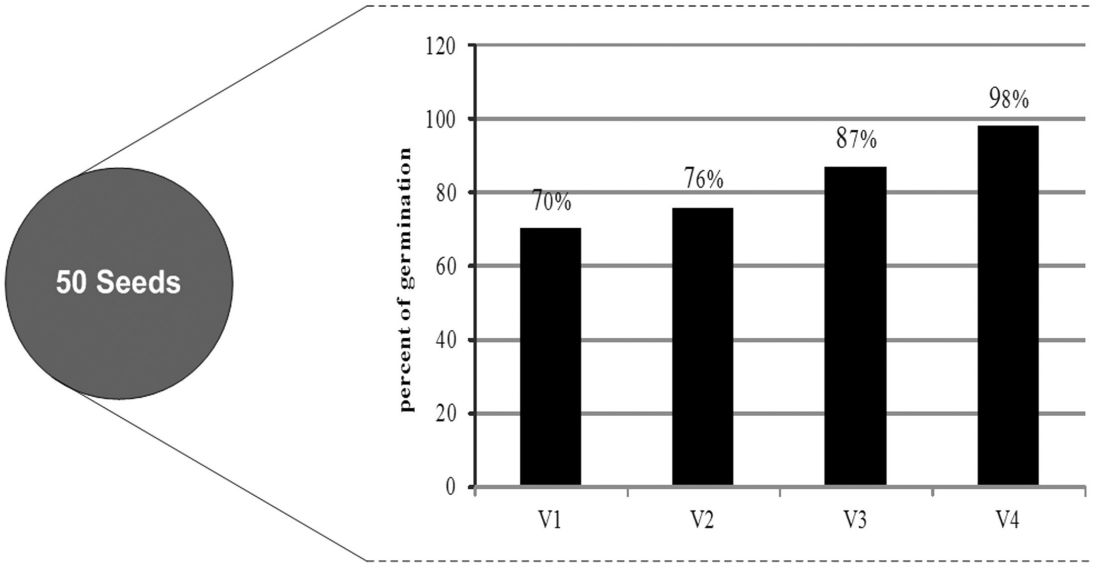

Figure 1. Percentage of germination depending on seed treatment

Taking into account the specificity of common laburnum seed tegument, scarification produced the best results in seed germination, followed by stratification, which reflected in the growth of the saplings during the first vegetation stages.

Sapling collar diameter ranged between 0.191 cm in the control variant (V_1) and 0.378 cm in the variant V_4 (scarification). Differences between the control variant and the other variants were significant only in the variant V_4 (Table 1). Sapling height ranged between 3.76 cm in the variant V_1 and 6.31 cm in the variant V_4, with statistically ensured differences in the variants V_3 and V_4 (stratification and scarification, respectively) (Table 1).

Table 1. Variations of common laburnum sapling stem parameters depending on seed treatment

Seed treatment	Trial variant	Sapling collar diameter		Sapling height	
		Mean diameter (cm)	Differences (cm)	Mean height (cm)	Differences (cm)
Moistening with cold water	V1	0.191	-	3.76	-
Moistening with hot water	V2	0.201	0.010	4.11	0.34
Stratification	V3	0.288	0.097	5.63	1.87^{***}
Scarification	V4	0.378	0.186^{**}	6.31	2.55^{***}
Limits of the significance of differences		$LSD_{5\%} = 0.116$; $LSD_{1\%} = 0.167$; $LSD_{0.1\%} = 0.245$		$LSD_{5\%} = 0.53$; $LSD_{1\%} = 0.76$; $LSD_{0.1\%} = 1.12$	

The root system also had differences depending on seed treatment. The saplings from seeds treated through scarification (V_4) and stratification (V_3) had a more developed root system (in root number and root length). The mean number of roots ranged between 1.92 in the variant V_1 and 2.80 in the variant V_4, with significant differences in the variants V_3 and V_4. Root length ranged between 0.53 cm in the variant V_1 and 1.73 cm in the variant V_4, with significant differences in the variants V_2, V_3 and V_4 (Table 2).

Table 2. Common laburnum sapling root size depending on seed treatment

Seed treatment	Trial variant	Mean root number		Mean root length	
		Root number	Differences (N)	Root length (cm)	Differences (cm)
Moistening with cold water	V1	1.92	-	0.53	-
Moistening with hot water	V2	2.10	0.17	0.75	0.22*
Stratification	V3	2.55	0.62**	1.29	0.76**
Scarification	V4	2.80	0.87***	1.73	1.20***
Limits of the significance of differences		$LSD_{5\%} = 0.39$; $LSD_{1\%} = 0.57$; $LSD_{0.1\%} = 0.84$		$LSD_{5\%} = 0.16$; $LSD_{1\%} = 0.23$; $LSD_{0.1\%} = 0.34$	

With a more developed root system and early growth, the saplings of the variants V_4 and V_3 developed a different leaf system with a larger number of leaves and larger leaf area than in the variants V_2 and V_1. The mean number of leaves ranged between 2.15 in the variant V_1 and 2.70 in the variant V_2 with significant differences in the variant V_4 (Table 3). Leaf area, in exchange, varied wider between 0.576 cm^2 in the variant V_1 and 1.148 cm^2 in the variant V_4, with significant differences in the variants V_3 and V_4 (Table 3).

Table 3. Leaf number and leaf area in common laburnum saplings depending on seed treatment

Nutrient mixture	Trial variant	Leaf number		Leaf area	
		Leaf number	Differences (N)	Leaf area (cm^2)	Differences(cm^2)
Moistening with cold water	V1	2.15	-	0.576	-
Moistening with hot water	V2	2.00	-0.15	0.681	0.10
Stratification	V3	2.32	0.17	0.873	0.29*
Scarification	V4	2.70	0.55**	1.148	0.57**
Limits of the significance of differences		$LSD_{5\%} = 0.32$; $LSD_{1\%} = 0.46$; $LSD_{0.1\%} = 0.69$		$LSD_{5\%} = 0.27$; $LSD_{1\%} = 0.40$; $LSD_{0.1\%} = 0.59$	

Graphic distribution and variation of biometric and physiological parameters depending on seed treatment are shown in Figure 2 below.

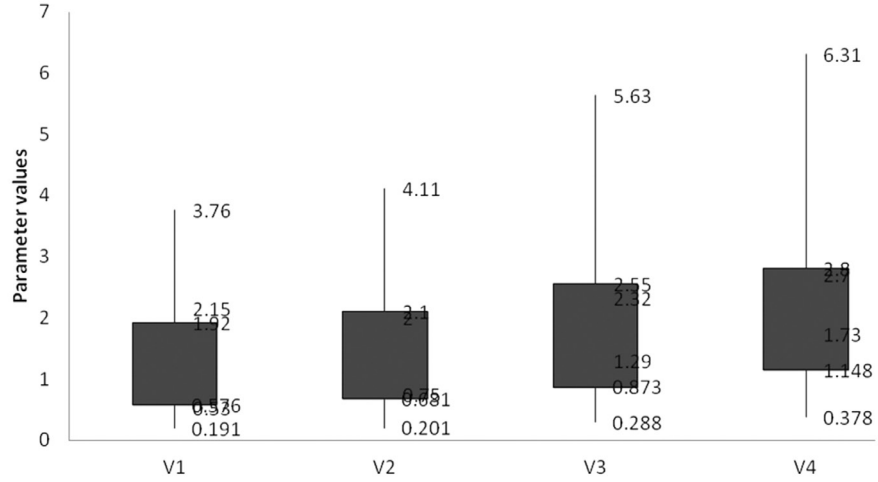

Figure 2. Graphic distribution and variation of biometric and physiological parameters depending on seed treatment

Multivariate analysis of the Cluster-analysis type facilitated the grouping of the variants based on Euclidian distances depending on biometric and physiological parameters with a high degree of statistic safety (Cophenetic coefficient: 0.887). There were two distinct clusters, one in which were grouped the variants V_1 and V_2 and one in which were grouped the variants V_3 and V_4, depending on the values of the analysed parameters (Figure 3).

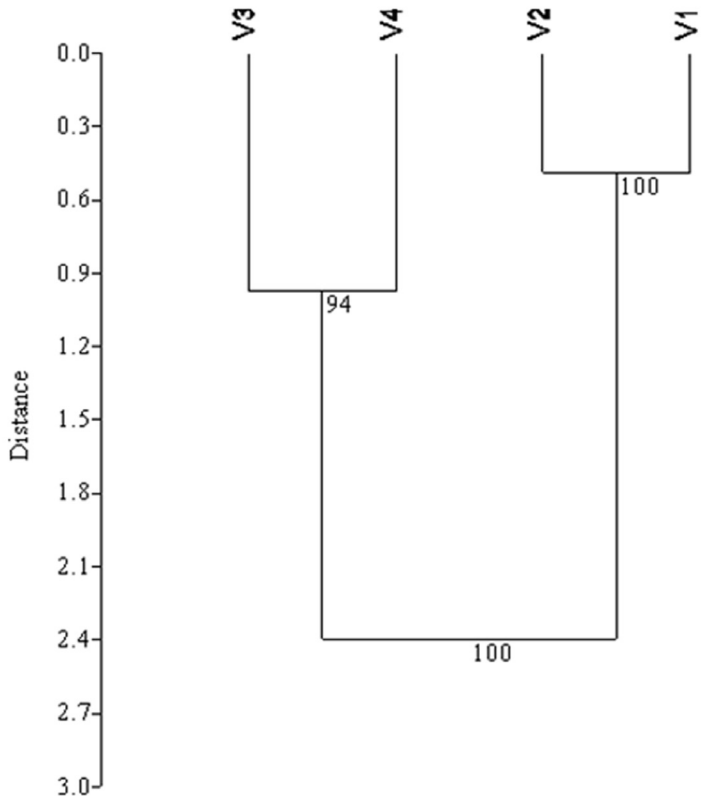

Figure 3. Cluster grouping of experimental variants based on Euclidian distances

Physiologically mature seeds have a balanced content of reserve substances that make possible germination and plant growth during the first stages of vegetation. An important role is that of plant vegetation and nutrition conditions (Sala, 2011), crop health state, stressors, etc. However, certain seed features make germination difficult, which asks seed pre-termination techniques, methods and treatments to get proper germination in certain species (Bewley, 1997).

They have carried out studies on physiological dormancy seed preparation and on the influence of nutrient mixtures on sapling growth in other ornamental woody species (Poşta, 2013; Poşta, 2015; Poşta and Camen, 2015). Aladjadjiyan (2002) reported results regarding the positive influence of electromagnetic wave treatments on seed germination. Another study assessed the effect of gibberellic acid on germination and sprouting ability in sycamore maple (*Acer pseudoplatanus* L.) treated seeds (Stejskalová et al., 2015). In an ample study, Fredrick et al. (2016) monitored the influence of seed pre-treatment on germination in six provenances of *Faidherbia albida* (Delile) A. Chev. They use five seed treatment methods – cutting, acid dipping, cold water dipping, hot water dipping, and a control variant – and saw that the highest percentage of germinated

seeds was in the cut seed variants. Acid dipping produced a high germination percentage and the best mean germination time. Overall, comparative analysis pointed out seed cutting and cold water dipping for 24 h as accessible, cheap, and less dangerous methods. Together with the classical treatments already presented, they also studied other species seed treatments with magnetic nanofluids that enhanced germination (Sala, 1999) changed plant growth (favourable sugar content and plant water regime) (Sala et al., 2017).

In this study, the scarification variant (V_4) generated the best results in germinated seeds and in sapling development from the perspective of biometric and physiological parameters. Hot water seed treatment (90°C) for 24 h (V_3) also generated good results, with statistically ensured differences.

CONCLUSIONS

Pre-germination treatments of *Laburnum anagyroides* seeds with cold water and with hot water (90°C) for 24 h, stratification and scarification had a different influence on the percentage of germinated seeds and on the values of biometric and physiological parameters in the saplings. Scarification produced the best results on both germination and sapling status, followed by stratification. In both methods, the differences between the biometric and physiological parameters were statistically ensured. With the other two methods, the treatment with hot water produced better results than the treatment with cold water.

ACKNOWLEDGEMENT

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Physocaulis nodosus
The Red Book of the Republic of Moldova, Third edition. 2015

ASPECTS OF THE *IN VITRO* ORGANOGENESIS OF THE SPECIES *LYCIUM BARBARUM* L. (GOJI)

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***Abstract**:* For the initiation of *in vitro* cultures of goji, shoot apex pieces, inoculated in culture medium Murashige-Skoog (MS), supplemented with hormones of different concentrations (α-NAA – alpha-naphthalene acetic acid and BAP – benzylaminopurine) were used (tab. 1). When higher doses of NAA and BAP were used, there was a proliferation of shoots and an increase in their length in the Ningxia N1 variety. The disinfection of the plant material was performed according to the method optimized in the laboratory. The obtained results denote that the growth medium used for the propagation of shoots derived from meristems can significantly influence the process of in vitro rhizogenesis. The experiments aimed at determining the rooting capacity of plantlets in vitro were organized depending on the culture medium. Thus, irrespective of the combination and concentration of growth hormones, it had a positive influence on the rooting capacity of the shoots, the number and length of the roots formed. In the case of the plantlets that regenerated in V4 and V6 medium, for which the concentration of IAA was increased to 2.0 mg/l and IBA – to 1.0 mg/l, the rooting rate was 73% and 93%, respectively.

INTRODUCTION

Clonal micropropagation by *in vitro* culture is a popular and appreciated method, both theoretically and practically, used for the vegetative propagation of some recalcitrant species and varieties. Its purpose is the rapid propagation of plants, the production of healthy and virus-free planting material, the creation of new varieties with desired characters, resistant to various physical and chemical stress factors, when conventional propagation by seeds, cutting and grafting does not yield satisfactory results [2].

Due to its numerous valuable properties, *Lycium barbarum* L. (boxthorn, goji berry, wolfberry) is of practical interest at national and international level. This fact outlines the need to develop and optimize the technology of rapid production of propagation material by *in vitro* culture, highlighting the morphogenetic potential of various types of explants by testing the phases of initiation, reproduction and rooting *in vitro* and *ex vitro*, in various culture media, associated with the creation of optimal conditions for cultivation [4].

Lycium barbarum L., a species in the family Solanaceae, has been known for about 2000 years in Tibet. The cultivation of boxthorn is advantageous from alimentary, economical and medicinal point of view. Yearly, it produces high amounts of fruits and thus, under optimal weather conditions, the average yield is over 6 tons per hectare. Goji berries have many health benefits due to their rich content of minerals such as magnesium, iron, calcium, potassium, copper or selenium, 18 amino acids, vitamins A, C, B (B2, B6) and E, polysaccharides, unsaturated fatty acids, carotenoids, phytosterols and phenols [3]. Goji is the most powerful antioxidant of all the existing foods in the world and contains more carotene than any other food known so far [6, 9]. In order to obtain planting material, used in the agri-food sector, it is necessary to enrich and diversify the sources that are widely used in the country's economy. One of the ways to achieve this goal is the acclimatization and cultivation of a new variety with special and valuable characters.

MATERIALS AND METHODS

The experimental work was carried out in the Embryology and Biotechnology Laboratory of the Botanical Garden (Institute) of ASM (in 2015-2016).

The cultivar *Ningxia N1* [2, 4], which was offered by the Fruit Research Station Cluj, Romania, was used as biological material.

Shoot apex pieces of about 2 cm were taken in May, from plants cultivated in the greenhouse. The asepsis of the biological material was achieved in two steps, the first step consisting in pre-disinfecting for 15 minutes in a weak solution of $KMnO_4$ (0.05%) and 2-3 drops of Tween-20, to reduce superficial tension at the surface of

the explant tissue, thus favouring the correct asepsis of the inocula, followed by 3 rinses with distilled water. In the second step, the explants were immersed in sterilizing solution. For this purpose, 0.1% diacid was used for 7 minutes, continuously stirring. To remove the sterilizing agent, three washings with sterile distilled water were performed for 10 minutes each, for the complete elimination of the diacid at tissue level; otherwise, the diacid would affect the apical meristem, causing its necrosis. The sterilization of the culture media and of the water used to wash the biological material was carried out at 120°C (at the pressure of 1 atmosphere), by autoclaving, for 45 minutes. After the culture media cooled, the inoculation of the plant material was performed. All explant sterilization operations, as well as inoculation, were carried out under aseptic conditions inside the hood with sterile laminar air flow.

Experimental variants were organized according to the combination and concentration of growth regulators introduced in culture media, as well as according to the objective, as follows:

1. For the initiation of the *in vitro* culture, we used shoot apexes, inoculated in MS 100% agar medium, supplemented with 0.5 mg/l BAP, in glass test-tubes with a diameter of 2 cm and length of 20 cm, covered with gauze plugs, in each test-tube inoculating a micro-shoot (Fig. 1, A). The amount of growth medium in each test-tube constituted 14 ml. The plantlets grew up to 5-8 cm during 30 days.
2. The micro-shoots obtained after the phase of initiation of the *in vitro* culture were divided and transferred in the MS 100% agar medium and were supplemented with different concentrations of BAP and NAA, in six experimental variants determined by the different combinations and concentrations of growth hormones (Tab. 1). The growing tip of the stem, together with 1-2 developing leaves, was detached and inoculated in the culture medium. The best time for inoculation is the period of intense growth of shoots. The amount of growth medium in each test-tube constituted 14 ml.
3. The *in vitro* rooting of the obtained shoots was stimulated by the addition of MS 100% liquid nutrient media, provided with filter paper bridges, hormone free (control variant) and with different concentrations of IBA and IAA auxins (Tab. 2).
4. The statistical interpretation of the results was done in Microsoft Excel program.

The containers with inocula were placed in the growth chamber on illuminated shelves (with white light) having an illuminance of 1700 lux and a 16/24 hour photoperiod, temperature – 24-26°C. Observations were made periodically, 3-7 days after inoculation.

RESULTS AND DISCUSSIONS

The analysis of the results of applying *in vitro* tissue culture methods to obtain high quality planting material has shown that *Lycium barbarum* is easy to initiate *in vitro*, has positive reactivity, especially starting from the apical meristem with primary foliar primordia.

Table 1 The influence of various concentrations of BAP and NAA on the proliferation and propagation in vitro of Lycium barbarum L., Murashige & Skoog 1962 (MS)

Experimental variants	Growth regulators (mg/L)	Percentage of viable inocula	Average length of shoots (cm) SE*	Number of shoots SE *
V1	***0.2mg/l BAP***	80	3.0 ± 0.4	2.4 ± 0.2
V2	***0.4mg/l BAP***	85	4.0 ± 0.3	5.4 ± 0.3
V3	***0.6mg/l BAP***	90	5.0 ± 0.6	5.2 ± 0.4
V4	***0.6mg/lBAP+0.6mg/l NAA***	95	6.0 ± 0.3	8.7 ± 0.5
V5	***0.4mg/lBAP+0.4mg/l NAA***	75	3.5 ± 0.3	6.9 ± 0.4
V6	***0.2mg/lBAP+0.2mg/l NAA***	65	2.0 ± 0.3	5.3 ± 0.4

* Average ± Standard Error

For the mass growth and propagation of plants, the media V.4, V.3 and V.2 (Tab.1) were highlighted.

***In vitro* propagation**

The influence of BAP and NAA growth regulators on the propagation process. Analyzing the obtained data, we have found that with the increase of the BAP concentration the average length of the shoots increases. At the BAP concentration of 0.6 mg/l, the shoot length reached 5 cm, and at the combination of hormones with the concentration of 0.6 mg/l BAP and 0.6 mg/l NAA, the maximum length reached 6 cm. This fact demonstrates the possibility to stimulate the development of adventitious buds; the increase of the concentration of benzylaminopurine (BAP) greatly increases the development of multiple shoots, and the increase of the concentration of α-naphthaleneacetic acid (NAA) in MS culture media favours the faster growth in length of the micro-shoots [8].

NAA has very complex effects on plant development. It influences the cell growth in length, facilitates the permeability of membranes to water and various ions and has rhizogenic action, that is why it is often used to stimulate rooting [5, 10].

The best result was achieved in the medium supplemented with 0.6 mg/l BAP, where about 20 adventitious shoots grew. Experimentally, the optimal variant was detected – with 0.4 mg/l BAP, obtaining 15 adventitious shoots. In the medium with a lower concentration of cytokinins, 0.2 mg/l BAP, the number of shoots was lower, namely, six shoots per inoculum (Fig. 1, B).

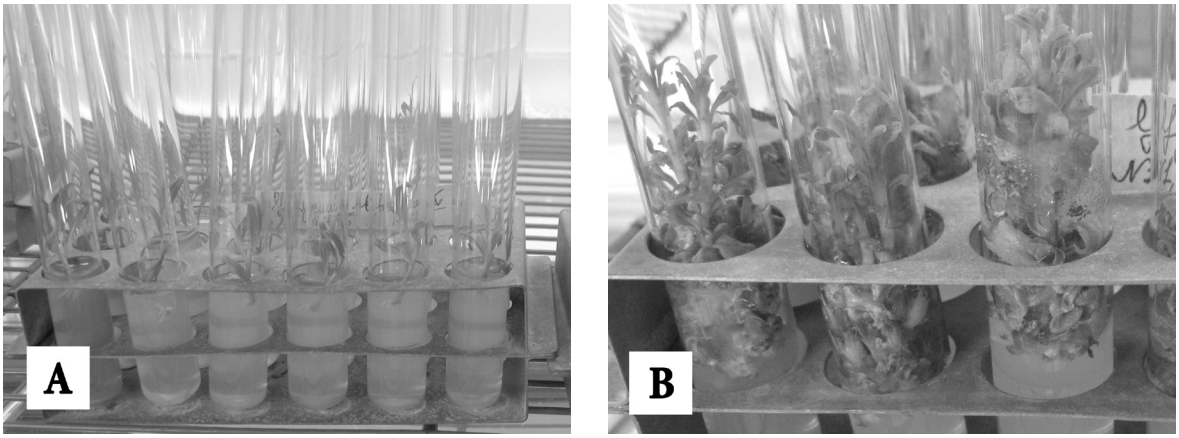

Figure 1. Plantlets regenerated from shoot apexes: A – *initiation of boxthorn culture in vitro*, B – *plantlets cultivated in vitro, in MS 100% medium supplemented with 0.6 mg/l BAP+0.6 mg/l NAA*.

The influence of the amount of sugar on the growth of the shoots. In order to optimize the process of shoot development *in vitro*, it was determined the possibility to replace sucrose with commercial sugar, which proved to be more cost effective and efficient. Sucrose was used only in the inoculation phase. In the variant when the concentration of 30 g/l of sugar was used, the shoots were more vigorous; lustre and more vivid colour of the leaves were observed. In 30 days, the shoots, in the variant with a higher amount of sugar, reached a length of 6 cm, 2 cm higher than in the control variant and 3 cm lower than in the variant with 15 g/l of sugar.

In vitro rhizogenesis. For the rooting of plantlets obtained *in vitro*, the liquid medium Murashige-Skoog 1962, 100%, was tested in seven experimental variants: supplemented with indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA) growth regulators, comparatively, at different concentrations (Tab. 2). In the sixth variant, a difference between the growths of the plantlets was observed in comparison with the control variant, the concentration of the medium was directly correlated with the growth of the plantlets. It was determined that the Murashige-Skoog medium 100%, 1 mg/l IBA, was optimal for the initiation of the rhizogenesis of

plantlets, with a success percentage of 93%. In this medium, the appearance of rootlets was observed within 14 days after the *in vitro* transfer to the nutrient medium, and over 20-30 days, it was observed a considerable growth of the plantlet, which could be used as material for cutting, for further propagation, and the basal part was transferred to *ex vitro*. The complete growth of rootlets was achieved over a period of 30 to 35 days, in the same medium (Fig. 2).

For micropropagation, as cutting, we used a segment consisting of two or three internodes. It is important that the explant segment is as vigorous and healthy as possible, so we get high quality planting material. The length of the cutting for micropropagation was 1.5-2.2 cm. Once the shoots reached the length of 10-12 cm, they were subjected to cutting and transferred to another nutrient medium for subsequent micropropagation.

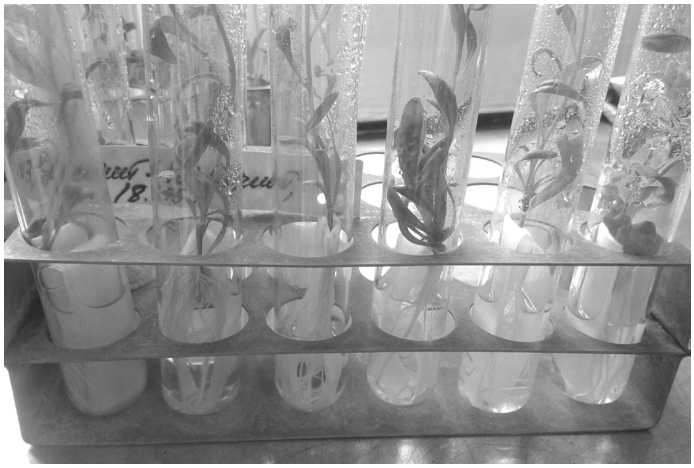

Figure 2. Root development of the regenerated micro-shoots of *Lycium barbarum* L.

The plantlets were grown in rooms with photoperiod of 16 hour light and 8 hours dark, with an average temperature of 23±20°C. In 30-35 days, the length of the explant reached 10-12 cm.

Table 2. In vitro rooting capacity of shoots regenerated from the culture of explants cultivated in MS medium supplemented with IAA and IBA

Experimental variants	Growth hormones (mg/l)		Response ercentage	Number of roots (S.E.) **
	IAA	**IBA**		
V1	00	00	23	1.0 ± 0.12
V2	0.5	-	60	2.3 ± 0.37
V3	1.0	-	70	3.2 ± 0.38
V4	2.0	-	73	5.6 ± 0.38
V5	-	0.5	54	4.3 ± 0.36
V6	-	1.0	93	8.3 ± 0.87
V7	-	2.0	70	6.3 ± 0.36

** Average ± standard error

The transfer of micro-plantlets from in vitro to ex vitro conditions and their acclimatization. The process of root development *in vitro* ran without major deviations. Similarly, the process of adapting to *ex vitro* conditions largely went normally for the cultivar under study. After the initiation, stabilization, multiplication and rooting of the explants, the acclimatization process followed. The process of acclimatization of the shrub *Lycium barbarum* (boxthorn), transferred in *ex vitro* from *in vitro* conditions, resulted in a viability of 90-95%,

the plants survived successfully depending on the season when they underwent adaptation. In this process, only the basal part of the shoot, which had roots, was involved, the medial and apical parts were further micropropagated in nutritive media (Fig. 3, B).

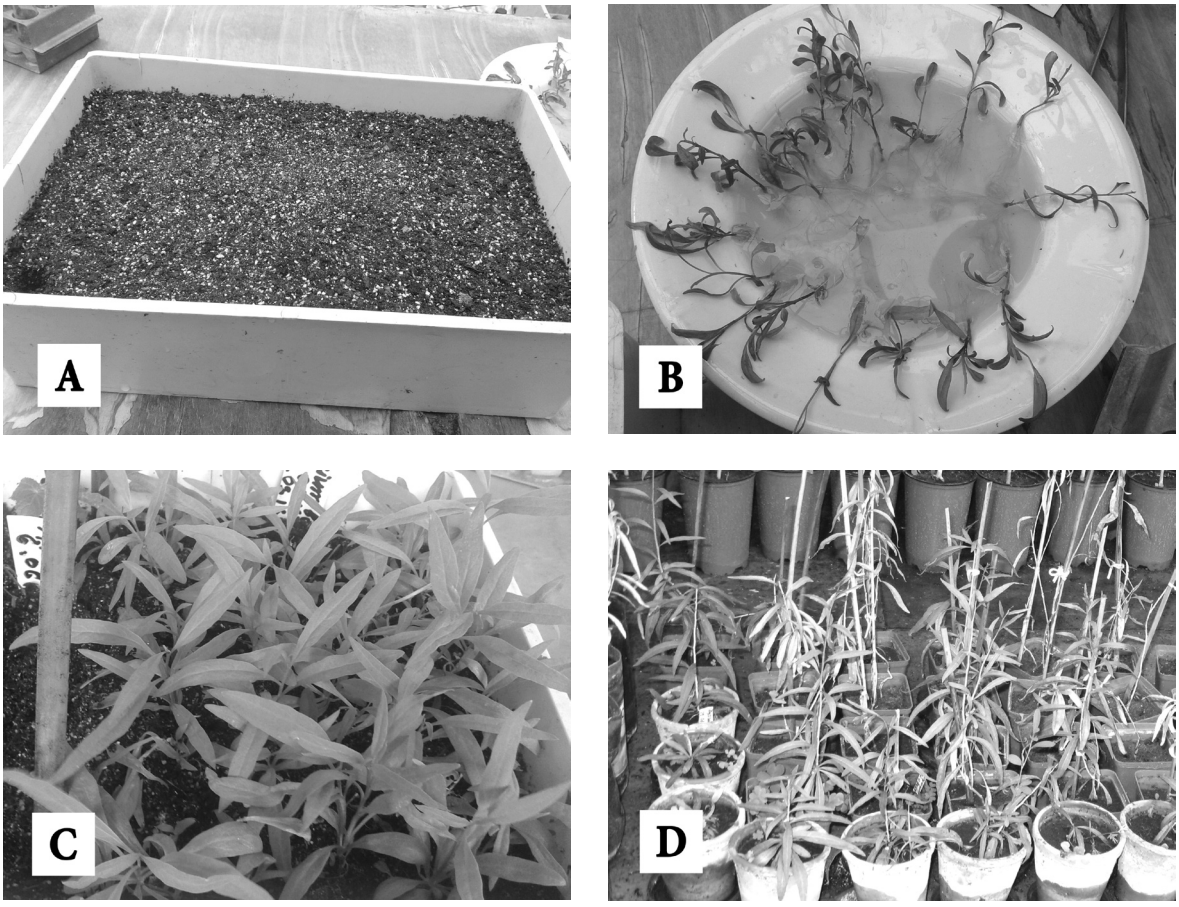

Figure 3. Aspects of the transfer of micro-plantlets of *Lycium barbarum* L. from *in vitro* to *ex vitro* conditions and their acclimatization: A – *substrate*; B – *KMnO4 solution;* C – *well-developed and rooted plantlets;* D - *plants transferred to vegetative pots with nutrient mixture.*

The plants, previously soaked in pale pink solution of $KMnO_4$ (Fig. 3, B), were transferred to a solid substrate comprised of: peat and sand in a ratio of 1:1 (Fig. 3, A), where the sand was sterile, autoclaved at 2 atm for 30 min. The plantlets cultivated *in vitro* were planted in trays and were covered with transparent sheets in order to maintain humidity. After 10-14 days, the sheet was removed and, after 15 days, the plants were transferred into vegetative pots with a nutrient mixture represented by: manure, leaf soil, sand and perlite, in proportion of 1:1:1:1:0.5 (Fig. 3, C, B). Acclimatization was carried out in the greenhouse.

The results of the obtained investigations, evaluated and analyzed in the light of the data from the literature, suggest that the production of planting material from meristematic apexes, established and optimized as a result of our research, constitutes a profitable, substantial and reproducible propagation method, which can be efficiently applied in practice, as confirmed by other researchers [1, 9].

CONCLUSIONS

1. The research on the initiation of *in vitro* culture of *Lycium barbarum* L. revealed defining aspects of culture conditions in terms of the development of an effective and rapid propagation protocol.
2. The supplementation of the inoculation medium with phytohormones such as cytokinins (BAP) leads to good results in the rapid growth of the shoots, the development of buds and of multiple adventitious shoots.
3. Besides, the high concentration of BAP cytokinin – 0.6 mg/l and NAA auxin – 0.6 mg/l, respectively, used in the culture medium, resulted in a high percentage of 95% viable explants of *Ningxia N1* variety.
4. The analysis of the data presented in Table 2 reveals that the rooting process takes 21-25 weeks, the rooting percentage is over 90% and results in well-developed, 5-cm-long plantlets with well-developed root system.

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Pimpinella titanophila
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II. CONSERVATION OF BIOLOGICAL DIVERSITY

THE RARE PLANT SPECIES OF DOWNY OAK FORESTS (*Quercus pubescens* Willd.) FROM REPUBLIC OF MOLDOVA

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Abstract: In downy oak forests, it was studied over 90 rare plant species with a conservation value. We have inventoried their habitats and distribution, they grow in specific habitats in steppe clearing that is characteristic for this kind of ecosystem. The rare plant species from downy oak forests make three main groups. The most of them are located at the North-Eastern limit of its spreading area, for their conservation, it is necessary to take serious scientific measures for maintaining the most propitious conditions of habitats.

Keywords: downy oak, conservation, rare, species.

INTRODUCTION

The influence of human activity over the environment that has lead to the detriment of population and diversity of plant species from downy oak forests (*Quercus pubescens* Willd.), by over exploitation of natural resources or a general shift away from the land, two processes that have had different but equally harmful consequences for the conservation of species and habitats. If some time ago the rare (endangered) plant species from Flora of Republic of Moldova represented 14 percent of the total number of species, today, the percentage increased to 25 percent. The *Stipa* genus and the relict vegetation communities of *Chrysopogon gryllus* (L.) Trip are threatened, that is caused by extreme condition at the limit of the spreading area, result of deforestation, human activity, overgrazing. The aim of this study is to **make evident the problem of rare plant species with different status like:** endangered plant species, critically endangered and vulnerable plant species and to establish ecological and chorological peculiarities of it, this is an actual issue about conservation of species and they habitats, and rational exploitation of natural resources.

The first layer of stands consists, mainly, of downy oaks. The distribution of woody species is uneven; trees are arranged in groups, forming groves alternating with glades. Rarely, in the first layer, pedunculate oak, sessile oak, Tatarian maple, common pear and sweet cherry can be found [6].

MATERIALS AND METHODS

To achieve the objectives set, the research has resorted to office and field methods the evaluation of the plant species after the method of Braun-Blanquet (1964) has been used, bibliographic documentation and the floristic investigation were performed in 2015. The floristic inventories and plant collection in diverse vegetation periods were made. The rare plant species of downy oak forests was identified, employing specialty scientific works [1, 3, 5], ecological indices according to the [7] source were taken.

RESULTS AND DISCUSSIONS

Taxonomical analysis. The investigation attested 97 rare plant species in downy oak forests, were emphasized: **Alliaceae:** *Allium guttatum* L., *Nectaroscordum bulgaricum* Janka.; **Amaryllidaceae:** *Galantus plicatus* Bieb., *Sternbergia colchiciflora* Waldst. et Kit.; **Apiaceae:** *Seseli tortuosum* L., *Silaum silaus* (L.) Schiny et Thell., *Torilis multicaulis* (Poir) Schischk.; **Asparagaceae:** *Asparagus officinalis* L., *Asparagus tenuifolius* Lam., *Asparagus verticillatus* L., **Asteraceae:** *Centaurea angelescui* Grinț., *Centaurea marshalliana* Spreng., *Centaurea thirkei* Sch.Bip., *Centaurea trinervia* Steph., *Doronicum hungaricum* Reichenb. fil., *Helichrysum arenarium* (L.) Moench, *Hieracium robustum* Fries., *Scorzonera purpurea* L., *Scorzonera austriaca* Willd., *Serratula bulgarica* Acht. et Stojan., *Serratula lycopifolia* (Vill.) A. Kernes; **Berberidacea:** *Berberis vulgaris* L.; **Brassicaceae:** *Crambe tataria* Sebeok, *Hesperis tristis* L.; **Boraginaceae:** *Pulmonaria officinalis* L.; **Caryophillaceae:** *Dianthus capitatus* Bolb. ex DC., *Dianthus deltoides* L., *Gypsophila collina* Stev. ex Ser., *Pleconax conica* (L.) Sourcova, *Silene dichotoma* Ehrh., *Silene exaltata* Friv., *Silene nemoralis* Waldst. et Kit., **Corylaceae:** *Carpinus orientalis* Mill.; **Cyperaceae:** *Carex caryophyllea* Latourr., *Carex praecox* Schreb., *Carex supina* Wahlenb.; **Euphorbiaceae**: *Euphorbia villosa* Waldst. et Kit. *Mercurialis ovata* Sternb et Hoppe; **Fabaceae:** *Astragalus dasyanthus* Pallas, *Astragalus ponticus* Pallas, *Astragalus pubiflorus* DC., *Astragalus subuliformis* DC., *Chamaecytisus ruthenicus* (Fisch. Ex Woloszcz.) Klaskova, *Lathyrus venetus* (Mill.) Wohlf., *Medicago rigidula* (L.) All., *Trifolium vesiculosum* Savi; **Fumariaceae:** *Corydalis cava* (L.) Schweigg. Korte, **Geraniaceae:** *Erodium ciconium* (L.) L'Her; **Hyacinthaceae:** *Hyacinthella leucophaea* (C. Koh) Shrur; **Iridaceae:** *Crocus reticulatus* Stev. ex Adam, *Iris aphylla* L., *Iris halophila* Pallas., *Iris hungarica* Waldst. et Kit, *Iris variegata* L.; **Lamiaceae:** *Nepeta parviflora* Bieb.; **Liliaceae:** *Bulbocodium versicolor* (Ker-Gawl.) Spreng., *Bellevalia sarmatica* (Georgi) Woronow, *Colchicum triphyllum* G. Kunze, *Convallaria majalis* L., *Fritillaria meleagroides* Patrin ex Schult. et Shult. fil, *Lilium martagon* L., *Muscari neglectum* Guss. ex Ten., *Ornithogalum refractum* Schlecht., *Ornithogalum fimbriantum* Willd. *Ornithogalum oreoides* Zahar., *Ornithogalum kochii* Parl., *Tulipa biebersteiniana* Schult. Et Shult. fil.; **Paeoniaceae:** *Paeonia peregrine* Mill.; **Primulaceae:** *Primula veris* L.; **Poaceae:** *Briza media* L., *Chrysopogon gryllus* (L.) Trin., *Stipa dasyphylla* (Lindem.) Tratv., *Stipa pennata* L., *Stipa pulcherrima* C. Koch., *Stipa ucrainica* P. Smirn, *Stipa tirsa* Stev.; **Ranunculaceae:** *Adonis vernalis* L., *Adonis wolgensis* Stev., *Clematis vitalba* L., *Delphinium fissum* Waldst. et Kit., *Pulsatila grandis* Wend., *Pulsatila montana* (Hoppe) Reichb.; **Rosaceae:** *Agrimonia pilosa* Ledeb., *Amygdalus nana* L., *Crataegus microphylla* C. Koch, *Crataegus pentagyna* Waldst. et Kit., *Potentilla canescens* Bess., *Potentilla micrantha* Ramond ex DC., *Potentilla thyrsiflora* Huels. Ex Zimmeter, *Pyrus elaeagnifolia* Pall., *Sorbus domestica* L., *Spiraea crenata* L.; **Rutaceae:** *Dictamnus gymnostylis* Stev, *Haplophyllum suaveolens* (DC.) G. Don fil.; **Rubiaceae:** *Cruciata pedemontana* (Bell.) Ehrend., *Galium volhynicum* Pobed.; **Scrophulariaceae:** *Euphrasia pectinata* L.

In downy oak forests it was studied over 90 rare plant species with a conservation value. We have inventoried their habitats and distributed they grow in specifically habitats in steppe clearing that is characteristic for this kind of ecosystem. We delineated 27 families and 56 genera, some of them including from 6 to 10 plant species (*Fabaceae* family 10 species, *Rosaceae* family 9 species, *Poaceae* family 7 species), but the most of them included just 1 to 2 species. A lot of rare plant species are multiannual and are part of hemicryptophites (54%) and geophytes (30%). The number of phanerophytes is minor (7 species or 8%). Mezophytes plant species included 47 percent and xerophytes included 37 percent; the major percent of geographical elements is from Pontian taxa. From total number of rare

plant species, 22 are included in the Moldavian Red Book and other national and international lists of protected species and are protected by law. The rare plant species of downy oak forests have a lot of limitation factors. Some of them located at the limit of the spreading area, isolated population, reduced specifically habitats, plantation of forest crops in clearings, overgrazing, collecting of blooming plants, bulb extraction. The geographical elements are varied, this is caused by the geographical position of Republic of Moldova that is situated in contact of three botanical and geographical districts. The rare species from downy oak forests make three main groups.

- Species that are located at the North-Eastern limit of its spreading area. This group included 18 plant species or 21 percent from total number of endangered species from downy oak woods. A dominant number of endangered plant species from southern area of spreading are Mediterranean species as *Chrysopogon gryllus* (L.) Trin., *Crataegus pentagina* Walds. et Kit., *Carpinus orientalis* Mill., *Ornitogalum fibriantum* Willd., *Ventenata dubia* (Leers.) Cross; Balkan species as *Centaurea thirkei* Sch. Bip., *Paeonia peregrina* Mill., *Serratula bulgarica* Acht. et Stojan., *Digitalis lanata* Ehrh.; Pontic species with specific feature of Mediterranean species are *Nectaroscordum bulgaricum Janca*., *Delphinium fissum* Waldst. Kit., *Trifolium vesiculosum* Savi., Pontic elements are represented by *Colchicum triphyllum* G. Kunze., *Ornitogalum oreoides* Zahar; European species are represented by *Pulsatila grandis* Wend., *Centaurea angelescui* Grint., *Sorbus domestica* L. [5]. The most species from this group are included in the Moldavian Red Book (the third edition) [2].
- The rare plant species with large area of spreading not just from the territory of the Republic of Moldova. This group includes Pontic, Mediterranean and Panonian plant species as *Astragalus ponticus* Pall., *Bellevalia sarmatica* (Georgi) Woronow, *Crupina vulgaris* Cass., *Pleconax conica* (L.) Sourcova, European plant species with a large areal as *Pulsatilla grandis* Wend., Euroasiatic plant species as *Agrimonia pilosa* Ledeb., *Astragalus varius* S. G. Gmel., *Serratula coronata* L. p. p., *Spirea crenata* L. One of the most rare plant species from this group is *Bulbocodium versicolor* (Ker Gawl) Spreng., its range included Hungary, Romania, the steppe region of Ucraine; and can be found it just in four places of habitats in our Republic, the stability of the population is in danger for this species. Distinct protection is required for *Pulsatilla grandis* Wend. and *Fritillaria meleagroides* Patrin ex Schult. fil., these plant species are included in European Red Lists of Vascular Plants [9] and List from Bern Convention. This species needs to be protected in all countries where have had sign the international convention of the biodiversity. It is also necessary to protect the population of *Crupina vulgaris* Cass., *Pleconax conica* (L.) *Sourkova,* which are growing near Rascaeti village. This species is met not so frequently in South Bugeac steppe.
- The species with a large spreading area from all the territory of the Republic of Moldova. These species are protected by law (*Adonis vulgaris* L. *A. wolgensis* Stev.), the population of *Amigdalus nana* (L.) that is not so numerous.

CONCLUSIONS

We can mention that the investigation attested 97 rare plant species in downy oak forests, the taxa of the rare plant species of the downy oak forests of the Republic of Moldova are reported to the Mediterranean and Balkan area. The most of them are located at the North-Eastern limit of their spreading area, and for their conservation it is necessary to take serious scientific measures for maintaining the most propitious conditions of habitats. It is necessary to take protection measures: monitoring the status of the existing population and identification of new places of growth; conservation

of the plant species' *ex-situ* condition. Only extensive reserves can guarantee the development of normal distribution of plant species. Therefore, it seems best to set up systems incorporating large unbroken reserves (of at least several hundred hectares) surrounded by buffer zones, making up a mosaic with differently managed areas [4].

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Seseli peucedanifolium
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RICHNESS OF RARE PLANT SPECIES IN DIFFERENT HABITAT TYPES IN THE SOUTHERN OPILLYA, WESTERN UKRAINE

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Abstract. Rare plant species richness in different habitat types of the Southern Opillya are studied. Meadow steppes appeared to have the highest species richness. Occurrence and population size of rare species in habitats are analyzed. Species of rare occurrence and small populations predominate in almost all habitat types. However, wetland species are most threatened due to melioration.

Keywords: habitat type, rare plant species, species richness, frequency of occurrence, population size, the Southern Opillya, Western Ukraine

INTRODUCTION

Anthropogenic transformation of natural landscapes causes a decrease in population size and even extinction of populations of many plant species. This process of habitat degradation starts of rare species extinction. The rare species compose 20-25% or more of local species diversity in many regions. There are several important steps to be done for successful conservation of biological diversity: to find rare species in flora of a region that need conservation; to determine number and state of populations of rare species; to create management-plans for their conservation and restoration.

Populations of rare species that grow in one habitat all face the same future in the case of its degradation. Thus, creation of management-plans of their conservation needs integral assessment of populations of rare species in each habitat type.

Natural and semi-natural landscapes of the Southern Opillya are highly fragmented and isolated due to transformation of 60-70% of the territory into agricultural lands. Forests (mainly oak and beech ones) cover from 16% to 30% of the territory, whereas other habitat types have very small areas (meadows – 4.5%; wetlands – 0.4%; ponds – 0.4%; meadow steppes – 0.2%) [35, 36]. Additionally many habitats have experienced transformation due to continuous anthropogenic pressure.

Conservation strategy of populations of rare plants varies depending on habitat type. Thus, the present study aimed to investigate rare plant species richness in different habitat types of the Southern Opillya, trends in the species occurrences and patterns in population size in each habitat type.

MATERIAL AND METHODS

According to Meusel et al. [20], the Southern Opillya is situated within Central European Province of Deciduous Forest region. According to the Geomorphological zoning of Western Ukraine [43], the territory belongs to the Southern Opillya Undulating Eminence of Wolyn-Podillya region. The Southern Opillya occupies the area of 3500 km^2 on North of Ivano-Frankivsk Region, Southwest of L'viv Region and West of Ternopil Region (Western Ukraine). Relief of the region is undulating accept for valleys of Dniester, Bystrytsya, Gnyla Lypa etc. rivers.

Main criteria of the rare species determination were as follows: 1) conservation status of species at international, national or regional scales; 2) limited distribution of species within the Southern Opillya; 3) the small size of most populations.

We studied distribution of rare plant species in the years 2009-2016 [7-9, 11, 13, 28, 31-34, 38] using route methods. We also examined herbarium materials of M. G. Kholodny Institute of Botany, NAS of Ukraine (KW), Taras Shevchenko National University of Kyiv (KWU), Institute of Ecology of the Carpathians NAS of Ukraine (LWKS), Ivan Franko National University of L'viv (LW) and Vasyl Stefanyk Precarpathian National University and considered literature data [1, 3-4, 5-6, 15-19, 22-24, 26-27, 30, 37, 39-45].

Rare species were classified by their habitat types on the following groups: forest, forest margin, wet meadow, dry meadow, meadow steppe, gypsum outcrops, wetland and aquatic species [16]. We studied rare species number, also number and size of populations in each habitat type.

Small populations were counted entirely. Large populations were estimated using sampling counts. Populations of rare species were divided into ten classes depending on their size: A – from one to ten individuals; B – from eleven to fifty; C – from 51 to a hundred; D – from 101 to 500; E – from 501 to a thousand; F – from 1001 to 5000; G – from 5001 to ten thousand; H – to 100000; I – to a million; J – more than a million.

The nomenclature follows Red Data Book of Ukraine [29] and the Nomenclatural Checklist of S. L. Mosyakin and M. M. Fedoronchuk [21].

RESULTS AND DISCUSSIONS

Flora of the Southern Opillya comprises 259 rare species. Out of them 91 species are listed in the Red Data Book of Ukraine [29], thirteen in the Annex I of Bern Convention and Revised Annex I of Resolution 6, twelve in EU Habitats Directive (Annex II; Annex IV), 94 in Red Lists of Ivano-Frankivsk, L'viv and Ternopil Regions [25].

Forests are habitats for 50 rare plant species, 23 of which are listed in the Red Data Book of Ukraine [29]. High occurrence and large populations are typical only for three forest species (*Allium ursinum* L., *Galanthus nivalis* L. and *Leucojum vernum* L.). *Lilium martagon* L., *Neottia nidus-avis* (L.) Rich., *Epipactis purpurata* Smith, *Listera ovata* (L.) R. Br., *Phyllitis scolopendrium* (L.) Newman etc. also have high occurrences but their populations include from few to several hundred individuals. Other forest species are scarce and have small populations (e. g. *Atropa belladonna* L., *Lycopodium annotinum* L., *Cephalanthera rubra* (L.) Rich., *Staphylaea pinnata* L., *Orthylia secunda* (L.) House). Several species were found in one locality (*Campanula latifolia* L., *Phyteuma spicatum* L., *Orchis purpurea* Huds.). *Aconitum besserianum* Andrz. ex Trautv. is likely to become extinct in the Southern Opillya [6].

There are 24 species of forest margins, five of which are listed in the Red Data Book of Ukraine [29] and one (*Cypripedium calceolus* L.) in Annexes of Bern Convention and EU Habitats Directive. Several species have scattered distribution within the region (*Festuca heterophylla* Lam., *Dactylorhiza maculata* (L.) Soó s. l., *Astrantia major* L., *Melittis sarmatica* Klokov) but their populations do not comprise more than several hundreds of individuals. There are many species (*Cypripedium calceolus, Aconitum moldavicum* Hacq. ex Rchb., *A. hosteanum* Schur, *Crepis sibirica* L., *Avenella flexuosa* (L.) Drejer, *Cimicifuga europaea* Schipcz., *Lathyrus latifolius* L.) with limited distribution and population size. Several margin species are threatened due to their rare occurrences and extremely small populations (e. g. *Rhamnus tinctoria* Waldst. et Kit., *Laserpitium prutenicum* L., *Festuca tenuifolia* Sibth.). A single known population of Tephroseris papposa (Rchb.) Schur counts only eight individuals.

Meadows are habitats for 38 rare plant species. Seventeen species are listed in the Red Data Book of Ukraine [29], three in Annex I and Resolution 6 of Bern Convention and two in Annexes of EU Habitat Directive. Species *Dactylorhiza majalis* (Rchb.) P. F. Hunt & Summerhayes and *Trollius europaeus* L. occur relatively often. Instead, other species are scarce due to degradation of suitable habitats (melioration, overgrazing). Wet meadows of the river valleys are habitats for *Colchicum autumnale* L., *Fritillaria meleagris* L., *Carex hartmanii* Cajand, that occur in several localities but have large populations. Other species are also very rare and have small populations (*Allium angulosum* L., *Dianthus stenocalyx* Juz., *Gentiana pneumonanthe* L., *Iris sibirica* L.). *Crepis mollis* (Jacq.) Asch. is known from a single locality.

Population size of species growing in moderately humid and dry meadows usually do not exceed 500 individuals. Very few species are widely distributed among meadow habitats (*Platanthera bifolia* (L.) Rich., *Potentilla alba* L., *Gladiolus imbricatus* L.). The majority of meadow species have limited distribution (*Traunsteinera globosa* (L.) Rchb., *Aconitum lasiocarpum* (Rchb.) Gáyer, *Adenophora lilifolia* (L.) Ledeb.

ex A. DC *Senecio schvetzovii* Korsh., *Scorzonera humilis* L.). Several species were found in a single locality (*Coeloglossum viride* (L.) C. Hartm., *Thesium ebracteatum* Hayne, *Potentilla thyrsiflora* Huels. ex Zimmeter).

Meadow steppes have the highest plant species richness among different habitat types. There are 83 rare species, 29 of which are red-listed [29]. Seven else are conserved at international scale (all seven species are listed in Annexes II and IV of EU Habitat Directive; five species in Annex I and Resolution 6 of Bern Convention; *Carlina onopordifolia* Besser ex Szafer, Kucz. & Pawł. in European Red List [2] and the IUCN Red List as Vulnerable species). Populations of *Stipa* species (*S. capillata* L., *S. pennata* L., *S. pulcherrima* K. Koch and *S. tirsa* Steven), *Sesleria heufleriana* Schur and *Poa versicolor* Besser are large in number, hence these species often dominate in vegetation. Several species are present in almost all localities studied (*Adonis vernalis* L., *Chamaecytisus blockianus* (Pawł.) Klásk., *Echium russicum* J. F. Gmel., *Iris aphylla* L. subsp. *hungarica* (Waldst. & Kit.) Hegi, *Pulsatilla grandis* Wender., *P. patens* (L.) Mill. s. l., *Trifolium rubens* L., *Cirsium pannonicum* (L. f.) Link), however their populations are not big. Most species have rare occurrence and small populations. Many plants are known from a few locations (*Euphorbia volhynica* Besser ex Racib., *Daphne cneorum* L., *Aconitum pseudanthora* Błocki ex Pacz., *Dictamnus albus* L., *Carlina onopordifolia, Verbascum phoeniceum* L., *Salvia nutans* L., *S. dumetorum Andrz., Trifolium lupinaster* L., *Scorzonera hispanica* L., *Muscari neglectum* Guss. ex Ten) or even from a single one (*Ophrys apifera* Huds., *Trifolium lupinaster* var *albiflorum* Ser., *Trifolium ochroleucon* Huds., *Galium tinctorium* (L.) Scop.). Populations of some species are extremely small comprising several individuals (*Neotinea ustulata* (L.) R. M. Bateman, Pridgeon et M. W. Chase, *Hypericum elegans* Stephan ex Willd., *Allium rotundum* L.).

Populations of eleven rare species were found on gypsum outcrops. Two of them (*Sedum antiquum* Omelcz. et Zaverucha and *Festuca pallens* Host.) are listed in the Red Data Book of Ukraine [29]. Rare specialist species of this group often have limited distribution and small populations (*Jurinea calcarea* Klokov, *Gypsophila fastigiata* L., *Campanula rotundifolia* L. etc.). A single known population of *Minuartia thyraica* Klokov comprises over a million individuals.

The majority of wetlands have been meliorated. The remaining patches often have small areas with changed hydrologic regime. Despite their transformation, wetlands support populations of 37 rare plant species, thirteen of which are listed in the Red Data Book of Ukraine [29]. Also *Eleocharis carniolica* W. D. J. Koch and *Lindernia procumbens* (Krock.) Borbás are conserved at international level (Annexes of EU Habitat Directive and Bern Convention).

Species inhabiting floodplain wetlands occur in 1-4 localities. The majority of populations are small with several tens of individuals. Most threatened species are *Eleocharis carniolica, Pedicularis sylvatica* L., *Thelypteris palustris* Schott, *Dryopteris cristata* (L.) A. Gray, *Triglochin palustre* L., *Limosella aqutica* L. etc.

There are four calcareous fens known in the Southern Opillya. Populations of *Carex davalliana* Smith, *C. hostiana* DC., *Cladium mariscus* (L.) Polh s. l., *Schoenus ferrugineus* L. differ in size depending on area and state of habitats. *Pinguicula bicolor* Woł. was found in two of them, whereas *Sesleria caerulea* (L.) Ard. – in one. Four species (*Salix starkeana* Willd., *Tofieldia calyculata* (L.) Wahlenb., *Viola palustris* L., *V. uliginosa* Besser) are considered extinct.

We have found eleven aquatic rare species in the region. Three of them are listed in the Red Data Book of Ukraine [29], two in Annex I of Bern Convention. Oxbow lakes are habitats for *Nuphar lutea* (L.) Smith, *Nymphaea alba* L., *N. candida* C. Presl. whereas *Nymphoides peltata* (S. G. Gmel.) Kuntze and *Potamogeton gramineus* L. grow in ponds. *Salvinia natans* (L.) All. and *Trapa natans* L. s. l. inhabit both oxbow lakes and ponds. *Najas marina* L. was found in Burshtyn Water Reservoir, other three species (*Naumburgia thyrsiflora* (L.) Rchb., *Hottonia palustris* L., *Cicuta virosa* L.) in shallow water. Populations vary in size from extremely small (species of shallow water) to relatively big (*Najas marina, Nymphoides peltata, Salvinia natans*).

Therefore, the species of rare occurrence predominate in each habitat type (fig. 1 A). Aquatic and wetland species showed extremely limited occurrence. Eutrophication of lakes and melioration of wetlands caused loss of most suitable habitats. As a result, 97.4% of wetland species are known from 1-5 localities.

Very few species occur in 30-70 localities. Most of them are species of forests, meadow steppes and dry meadows. Significant area of forests and number of meadow steppe patches explain relatively high occurrence of these species. However, degradation of most habitats caused extinction of populations of other species [10, 12]. Thus, despite big number of patches and high richness of these habitat types, most species occur in 1-10 localities.

Population size of rare species generally is small (fig. 1 B). The share of populations of A-D classes vary from 72.4% to 92% among different habitat types. Only aquatic species have larger populations; 60.9% of them are of E-G classes.

Most numerous populations (H-J classes) comprise 0-12% of all populations in studied habitat types. More often they occur in forests and wet meadows, whereas only one large population is in gypsum outcrops and no one in forest margins and dry meadows.

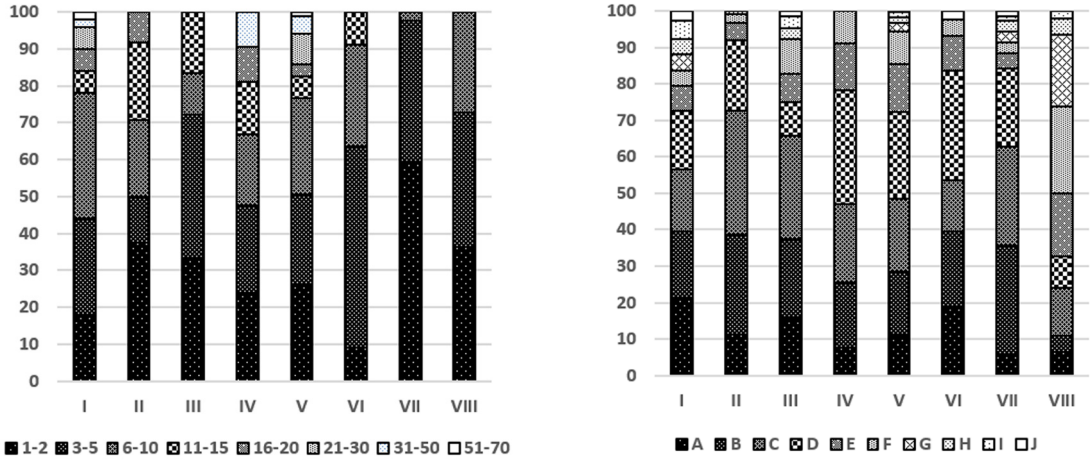

Fig. 1. Number (A – left diagram) and size (B – right diagram) of populations of rare species (percentage)

Comparing species richness and general population number among habitats, meadow steppes appeared to be the richest in rare species and their populations among habitats of the Southern Opillya (fig. 2 A, B). Forests cover area 80-150 times bigger, however comprise 1.7 times lower rare species richness and 1.8 times less populations than meadow steppes. Wetlands being on the third place on species richness comprise low number of populations. On the contrary, dry meadows are relatively poor in rare species but the habitats comprise two times more populations than wetlands. Number of populations of other habitat types is symmetrical to their species richness.

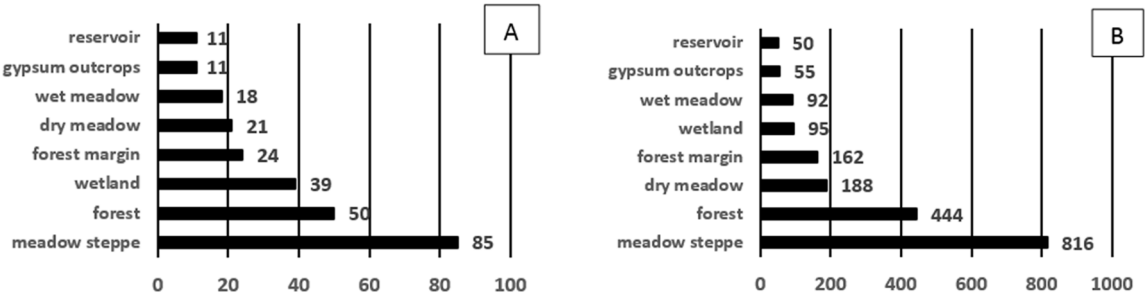

Fig. 2. Rare species number (A) and general number of the populations (B) in different habitat types

CONCLUSIONS

Each type of natural and semi-natural habitats in the Southern Opillya comprise species conserved at international, national and regional scales. Due to transformation, fragmentation and isolation of natural landscapes, species of rare occurrence predominate in all habitat types. Most wetlands were meliorated resulting in total decrease in the rare species occurrence. Most populations of rare species are small. Only populations of aquatic species often are of medium size. Rare species richness vary from 11 to 85 species in different habitat types and reaches its maximum in meadow steppes. For successful conservation of rare species, it is necessary to work out management-plans for each habitat type considering patterns of species occurrence and population size.

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FLORA OF "CÎZLAR" STEPPE MEADOW: RARE AND THREATENED VASCULAR PLANTS

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Abstract: This article presents data on floristic composition and value of the steppe meadow "Cîzlar", positioned near village Cîzlar, Leova district, as well as the results on investigations on high vascular flora, which comprises 353 wild spontaneous growing species, belonging to 131 genera and 44 families. The lists of rarest and the most threatened species are given. The unique growing site of critically endangered species – *Rosa inodora* Fries is found on the territory which is proposed to be included in the next edition of the Red Book of the Republic of Moldova and in the List of Vascular Plants protected by national law, as well as the management issues on habitat are given.
Keywords: flora, steppe, biodiversity, rare species, *Rosa inodora*, Republic of Moldova

INTRODUCTION

The vegetation of Republic of Moldova is under permanent transformations by human impact. In the last three decades plant diversity in the Republic of Moldova has been facing increasing anthropogenic pressure, which causes deterioration and loss of habitats and direct destruction of species populations by trampling, grazing, infrastructural development, stone-pitting, etc.

The dry grassland habitats in Republic of Moldova are located within the boundaries of the Ponto-Sarmatic steppes – *62C0 [7], belonging to the Steppic Biogeographical Region of the European continent, which has only a small foothold in the European Union [6], but it develops into a vast band of vegetation that stretches out from the eastern parts of Romania and incorporates the entire region known as Dobrogea over southern parts of Republic of Moldova, Ukraine, Russia and western Kazakhstan.

The Ponto-Sarmatic steppes display special habitat conditions, due to the various climatic effects and the unique biogeographical position. They offer many highly-specialized animal and plant species a valuable habitat and are characterized in general by a high biodiversity. Along with Romania and Ukraine, Republic of Moldova bears responsibility for the preservation of the numerous species of animals and plants of the Ponto-Sarmatic area, because many of these species have their most important range of distribution here or because the Ponto-Sarmatic steppes is their only home [10, 15].

For these reasons the European Union placed the Ponto-Sarmatic steppes on the list of "priority habitats". The member states of the EU have thus committed themselves to preserve such habitats and to provide support by specific programs [7].

The objective of the study was to investigate a valuable sector of a steppic vegetation in the vicinity of Cîzlar village, Leova district, in ordet to evaluate the endangerment of the rare and threatened floristic element and to bring awareness and scientific arguments in order to confer the status of State natural protected area.

MATERIALS AND METHODS

The study was conducted during 2015-2017. The designation of Habitat type was made according to the Interpretation Manual of EU Habitats, Directive 92/43/EEC on the basis of scientific criteria defined in Annex III of the Directive [7]. Description of the associations was made based on characteristic, self-evident, dominant and differential species, according to the phytosociological research method of the central European school, based on the traditional ecological-floristic systems developed by Tüxen, 1955 [17] and J. Braun-Blanquet, 1964 [1].

The species identified on site were collected, dried, conditioned and inserted in the Herbarium of the Botanical Garden (Institute) of ASM. In parallel with the collection of the material for herbarium, the specialty

literature was studied. All detected plant species are native to local flora and the taxonomy followed by the recent taxonomical literature [2, 3, 13]. The estimation of the threat status of the species is made according to the IUCN Red List Categories and Criteria (2001, 2003) [8, 9].

RESULTS AND DISCUSSIONS

The sector of steppe vegetation is situated in the southern part of village Cîzlar, Leova district (figure 1), mean geographic coordinates: N 46°39'7.62"; E 28°32'28.91". The estimated surface of the site is 198.3 hectares.

The field investigations of the steppe vegetation site and comparative studies demonstrated the presence of xerothermic species group in the following plant communities: *Stipetum lessingianae, Potentillo arenariae Agropyretum pectiniformae, Bothriochloetum ischaemi.* More specifically, the alliances of *Stipion lessingianae* Soo 1947 (with ass. *Stipetum lessingianae* Soo 1947) and *Festucion rupicolae* Soo 1940 corr. 1964 (with ass. *Potentillo arenariae-Stipetum capillatae* (Hueck 1931) Libbert 1933; *Agropyretum pectiniformae* (Prodan 1939) Dihoru 1970 and *Bothriochloetum ischaemi* (Kristiansen 1937) I. Pop 1977), conserve relic vegetation enclaves, characterizing valuable European priority habitats of the steppic grassland group, the „Ponto-Sarmatic steppes" [7], which many European botanists indicate that they probably persist continuously from the Holocene [5, 11].

Figure 1. The sector of steppe vegetation (vill. Cîzlar, Leova district)

As a result of field investigations, the high vascular flora comprises 353 wild spontaneous growing species, belonging to 131 genera and 44 families. The rare floristic component of the vegetal communities of the site is represented by a list of rare and threatened with extinction species, included in the several red lists (including the lists of adjacent territories):

Figure 2. *Achillea ochroleuca* Ehrh.– Critically Endangered species

21 rare taxa (*Stipa dasyphylla* (Lindem.) Trautv., *Stipa lessingiana* Trin. et Rupr., *Stipa pennata* L., *Stipa ucrainica* P. Smirn., *Hyacinthella leucophaea* (C. Koch) Schur, *Iris variegata* L., *Ornithogalum refractum* Schlecht., *Adonis wolgensis* Stev., *Astragalus dasyanthus* Pall., *Polygala sibirica* L., *Goniolimon besserianum* (Schult.) Kusn., *Allium inaequale* Janka, *Carex liparocarpos* Gaudin, *Astragalus albidus* Waldst. et Kit., *Adonis vernalis* L., *Crambe tataria* Sebeok, *Helichrysum arenarium* (L.) Moench, *Asparagus officinalis* L., *Colchicum arenarium* Waldst. et Kit., *Bellevalia sarmatica* (Georgi) Woronow, *Crocus reticulatus* Stev. ex Adams.) are protected by the state in the Republic of Moldova [12].

Eight rarest and the most threatened species are included in the Red Book of the Republic of Moldova [16]: 1 Critically Endangered (CR) species – *Achillea ochroleuca* Ehrh. (figure 2); 4 species of the category

Endangered (EN) – *Stipa dasyphylla* (Lindem.) Trautv. (figure 3), *Crambe tataria* Sebeok (figure 4), *Colchicum arenarium* Waldst. et Kit. (figure 5), *Stipa tirsa* Stev. (figure 6) and 3 species of category Vulnerable (VU) – *Bellevalia sarmatica* (Georgi) Woronow (figure 7), *Nepeta parviflora* Bieb. (figure 8) and *Adonis wolgensis* Stev. (figure 9).

Figure 3. *Stipa dasyphylla* (Lindem.) Trautv. – Endangered species

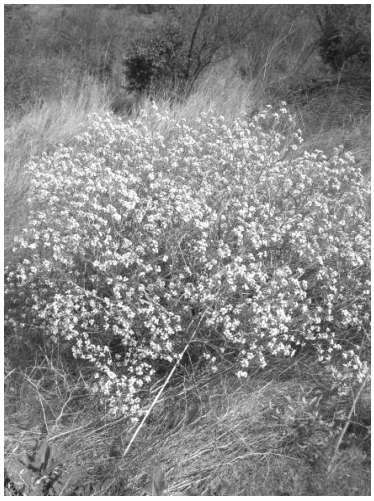

Figure 4. *Crambe tataria* Sebeok..– Endangered species

Figure 5. *Colchicum arenarium* Waldst. et Kit.– Endangered species

Figure 6. *Stipa tirsa* Stev. – Endangered species

Figure 7. *Bellevalia sarmatica* (Georgi) Woronow – Vulnerable species

Figure 8. *Nepeta parviflora* Bieb. – Vulnerable species

14 species (*Adonis wolgensis, Astragalus corniculatus, Colchicum arenarium, Stipa dasyphylla, Stipa ucrainica, Bellevalia sarmatica, Nepeta parviflora, Centaurea besseriana, Myosotis discolor, Otites exaltata, Centaurea trinervia, Polygala sibirica, Dianthus campestris* and *Rumex thyrsiflorus*) included in the Red Book of Romania [4].

16 species (*Adonis wolgensis, Stipa lessingiana, Stipa ucrainica, Adonis vernalis, Crocus reticulatus, Stipa*

capillata, Carex liparocarpos, Stipa dasyphylla, Crambe tataria, Colchicum arenarium, Stipa tirsa, Stipa pennata, Ornithogalum refractum, Astragalus dasyanthus, Bupleurum tenuissimum and *Astragalus ponticus*) are included in the Red Book of Ukraine [14].

Figure 9. *Adonis wolgensis* Stev. – Vulnerable species

In addition, there were registered three species of Community interest – *Echium russicum* J. F. Gmel., *Crambe tataria* Sebeok and *Colchicum arenarium* Waldst. et Kit. These species are listed in the Annex II of the Council Directive 92/43/CEE on the conservation of natural habitats and of wild fauna and flora whose conservation requires the designation of Special Areas of Conservation [7].

An analysis of the geographical distribution of species found on the site showed that 21 species are growing in the region at the limits of their natural distribution areas.

On the basis of estimated conservation status, according to IUCN Red List Categories and Criteria [8, 9], we propose *Rosa inodora* Fries [CR B2ab(ii, ii); D] (figure 10) to be included in the next edition of the Red Book of the Republic of Moldova and in the List of Vascular Plants protected by national law.

Management issues on Cîzlar steppe grassland

Comprehensive management plans need to be prepared for the sustainable development of the region. These should include care and development plans, as well as grazing concepts, because the preservation of the habitats is closely coupled to the development of an agricultural use compatible with the environment. The following suggestions for monitoring and research on the site are proposed:

Figure 10. *Rosa inodora* Fries – Critically Endangered species

- development of the site's management plan based on the current situation;
- promoting the conservation and increase of the number of characteristic species in primary steppes – *Stipa ucrainica* and *S. lessingiana*;
- the regulations development of economic use of the site to preserve the steppic vegetal communities, taking into account the optimal timing of haying and grazing;
- securing the sides of ravines by planting shrubs of native flora (species of *Caragana*, *Amygdalus* and *Chamaecytisus* genera);
- establishing of a long-term monitoring of flora and vegetation of steppe communities;
- the allocation of special areas for organized recreation;
- laying of trails for eco-tourism routes;
- removal of the dried trees and shrubs to prevent the occurrence of fire hazards;
- reconstruction of the steep walls of the quarry for sand and clay mining, consolidation of wall sides by plantations of native trees and shrubs.

CONCLUSION

Steppe and steppic species of animals and plants are an important component of the landscape and biological biodiversity, natural heritage of our country. The danger is that from the former extended steppe carpet of republic only shreds and fragments have still survived. And until recent times, the efforts to conserve natural steppic grasslands are not adequate enough and do not contest to the threats which they are facing.

Indeed, it is difficult to ensure proper protection to the steppe ecosystems, because of their natural specificity, the way of their usage and the absence of consensus in the society about the value of the steppes. It is well understood perception that at the Pan-Eurasian level the speppic bioms are the most vulnerable and they become most close to the dangerous limits, from which the reverse could be impossible. (Kovacz 2003, 2008-2009, Izverscaia & Ghendov 2009, Izverscaia *et al.* 2009, Shabanova 2012, Shabanova *et al.* 2013c).

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ANALYSIS OF WILLOW (*SALIX* L.) FLORA IN UKRAINIAN CARPATHIANS

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Abstract. On the basis of the of the published data analysis, herbarium specimens and our route studies, we have found out that the natural flora of the genus Salix in the Ukrainian Carpathians has 22 species. The alpine and subalpine florocentic complexes comprise six species of *S. alpina Scop., S. herbacea* L., *S. retusa* L., *S. Kitaibeliana* Willd., *S. rhaetica Anders., S. silesiaca* Willd. It is doubtful that two species of *S. hastata* L. and *S. reticulate* L. are present on the territory of Ukrainian Carpathians and need the chorology clarification. The most numerous is boreal flora Salix element in the Carpathians, covering 16 species.

Keywords*: Salix,* willow, flora, Ukrainian Carpathians, alpine, subalpine and boreal species

INTRODUCTION

Genus willow (*Salix* L.) is one of the largest in the boreal flora of Ukraine and according to various sources, it comprises 24-25 species (Nazarov et al., 1952; Fuchylo, Sbytna, 2009, Ishchuk, 2015b). Most willows are phanerophytes, but there are also micro-, meso- and nanophanerofytes and hamefytes among them. Genus *Salix* species, due to its plasticity, can change a tree form into bush one and vice versa depending on soil and climatic conditions. Willows are widely used in the national economy for the extraction of tannins, salicine, as well as energy crops. They also perform polliniferous, milliferous, decorative, hydroregulating and phytomeliorative functions, and they have been widely used in folk crafts recently for basket weaving and flower arrangement (Ishchuk, 2015a). However, the number of willows is steadily declining due to both high demand for raw materials and due to excessive anthropogenic impact on the riverine and highland ecosystems. The exact number of the introduced species and cultivars of genus *Salix* in Ukraine is not known since more and more cultivars are imported from Europe, and this process is almost uncontrollable currently (Ishchuk, 2015a, 2015b). There are also some disputable issues concerning willows' horology in the natural flora.

The aim of our research was to analyze the taxonomic composition of species of the genus Salix in the Ukrainian Carpathians, which represents the largest variety of willows, including a number of relict species that need protection.

MATERIALS AND METHODS

We conducted the research on the genus Salix species chorology by routing methods in the Ukrainian Carpathians and by analyzing the literature and herbarium samples of the genus Salix species in the herbarium of the Institute of Botany of NAS of Ukraine (KW), Uzhgorod National University (UU), I. Franko L'viv National University (LW) and the State Natural History Museum of Ukraine (L'viv) (LWS). The nomenclature of the plant species is given in accordance with the checklist by S. M. Mosyakin and M. M. Fedoronchuk (Mosyakin, Fedoronchuk, 1999) and the international nomenclature Index (IPNI, 2005). When analyzing the genus Salix species chorology, we used floristic zoning by B. Chopyk and S. Mosyakin (2015), under which the following areas are isolated on the territory of the Ukrainian Carpathians: The Carpathians, the Eastern Beskids and low meadows, Gorgans, Svydovets, Chornohora, Chyvchyno-Hrynyavski mountains Maramorosh Alps, Volcanic Carpathians, Transcarpathian foothills and Transcarpathian plains.

RESULTS AND DISCUSSION

M. I. Nazarov, M. I. Kotov, P. I. Herzhedovych (1952) analyzed most fully and summarized flora of the genus Salix species in the Ukrainian Carpathians in the paper "Flora of the USSR", in which they cite 29 species of willows for Ukraine in general and 25 species for the Carpathians. Of these, three species are common only in the *S. babylonica* L. culture in the south, *S. elegantissima* Koch. – in the western regions of Ukraine and Poland, *S. dasyclados* Wimm. – in the western regions of Ukraine. I. Ivanitskii processed herbarium specimens of willows in scientific herbariums of L'viv during the 1963-1964, but no records or papers on the review were left.

Following him, A. K. Skvortsov did the fundamental systematic review of *Salix* genus flora in his monograph (1968). The scientist analyzed critically the flora of Salix genus species in Ukraine once more (1987), describing this genus for plant determinant, Ukraine, in 1987. The last critical analysis of willow flora in Ukraine A. K. Skvortsov (Skvortsov, 1999) made in 1999 in the book «Willows of Russia and Adjacent Countries. Taxonomical and Geographical Revision». M. I. Nazarov et al. (1952), Ye. M. Bradis (1965) and V. Krichfalushiy (1982) referred willow Kitaibeleve (*S. Kitaibeliana* Willd.) to a separate genus while A. K. Skvortsov (1968, 1987, 1999) considers it a variety of blunt-leaf willow (*S. retusa* L.) and he did not even refer it as a subspecies. Willow Xerophilous (*S. xerophila* Floder.) was not confirmed to belong to the flora of Ukraine by more recent studies. Fuchylo Ya. D. and Sbytna M. V. (2009) believe that it is replaced by Stark willow or bluish willow (*S. Starkeana* Willd. = *S. livida* Wahl.) on the territory of Ukraine. *S. Starkeana* samples, examined in the herbarium of the Institute of Botany of NAS of Ukraine (KW), had prolapses in the ribs on the underside of the leaf which made them looked like a willow Xerophilous.

Bradis Ye. M. (1965) in the "Determinant of the flora of Ukraine" describes 26 kinds of willows in the natural flora of Ukraine. Of these, A. K. Skvortsov (1968, 1987, 1999) does not consider *S. Kitaibeliana* to be a separate species. Herbarium specimens of *S. repens* L. he refers to *S. rosmarinifolia* L. The "Flora of the USSR" (Nazarov et al., 1952) and "Determinant of the flora of Ukraine" (Skvortsov 1987) also described *S. Jacquinii* Host. and *S. nigricans* Sm., which A. K. Skvortsov, Ya. D. Fuchylo, N. V. Sbytna (2009) considered synonyms for *S. alpina Scop*. and *S. myrsinifolia* Salisb. respectively. A. K. Skvortsov (1968, 1987, 1999) considers gray willow (*S. incana* Schrank) synonymous for *S. eleagnos* Scop.

The Carpathian region willow flora was also studied by K. A. Malynowskiy (1980), V. Krichfalushiy (1982) during the last century. In particular, V. V. Krichfalushiy (1982) singles out four florocenotic complexes in the flora of the Carpathians willows: alpine, sub-alpine, mountain-subalpine and supplementary species. Also, V. V. Krichfalushiy (1982) considers *S. Kitaibeliana* and *S. retusa* to be separate species, as *S. Kitaibeliana* differs from *S. retusa* with a straighter trunk, large leaves, thicker branches and more flowers on the catkins. In addition, *S. Kitaibeliana* is wide spread in acidic flysch rocks, while *S. retusa* – on typical calcicole.

We also share the opinion of K. A. Malinowskiy (1980), V. V. Krichfalushiya (1982) on the probability of *S. reticulata* L. absence in the Ukrainian Carpathians, as we did not discover this species in our route studies and in the analysis of herbarium specimens of the Ukrainian Carpathians herbarium in Kyiv, L'viv and Uzhgorod.

Chopyk V. I., Fedoronchuk M. M. (2015) in the "Flora of the Ukrainian Carpathians" cited 25 species for the Carpathian willow flora, including *S. babylonica* L. and *S. blanda* Anders., which can be found only in the culture. The authors also express doubts about the availability of *S. hastata* L. and *S. reticulata* in the Carpathians flora, since the latter one is not confirmed with herbarium specimens. The same paper also reveals Rhaetian willow (*S. rhaetica* Anders.) = *S. phylicifolia auct. non* L. According to the latest data, V. I. Chopyk, M. M. Fedoronchuk (2015) share the opinion of A. K. Skvortsov (1968, 1987, 1999), and consider *S. retusa* to be a synonym of *S. Kitaibeliana*, *S. elegans* Bess. – synonymous with *S. myrtilloides* L., *S. nigricans* as a synonym of *S. myrsinifolia* Salisb., S. *phylicifolia auct. non* L. – synonymous with *S. rhaetica,* and *S. incana* – synonymous with *S. eleagnos.*

However, special attention should be paid to rare and endangered species of willows, whose populations

are steadily declining and need additional detailed studies (Kobiv, 2010; Ishchuk, 2014a, 2014b). M. I. Nazarov et al., (1952), Ye. M. Bradis (1965), V. V. Krichfalushiy (1982) indicate in their description of *S. retusa, S. jacquinii*, *S. reticulata* and *S. lapponum* L. the spread, that these species can be found in the alpine zone of the Carpathians extremely rarely. However, no species of the genus Salix are available in the first edition of the Red Book of the USSR (1980). Yet, Grass willow (*Saliceta herbaceae*) and Kytaybeleve willow (*Saliceta kitaibelianae*) are listed in the first category of the Green Book of the USSR (1987) as a rare surviving group that survived in the upper reaches of the Carpathians, especially in the subalpine zone of Svydovets Chornogory, since the Ice Age.

Five species of willows are listed in the second edition of the Red Book of Ukraine (1996). The category of endangered species comprises Reticulate willow (*S. reticulata*). The category of vulnerable ones comprises Grassy willow (*S. herbacea* L.), the category of rare species list Bilberry willow (*S. myrtilloides* L.), Starke willow (*S. starkeana*) and Retuse willow (*S. retusa*).

The last edition of the Red Book of Ukraine (2009) lists six species of the genus Salix, of which *S. alpina* Scop. is classified as endangered, *S. lapponum, S. myrtilloides* and *S. starkeana* – vulnerable, and *S. herbacea, S. retusa* are categorized as rare.

The analysis reveals that the natural flora of the Ukrainian Carpathians is represented by 22 species of the genus *Salix*.

1. *S. alpina Scop*. (*syn. S. jacquiniana* Willd., *S. jacquinii* Host.) – Relict species with disjunctive habitat in the Central Europe. In Ukraine, the species is spread in the Carpathians alpine zone of Svydovets mountain area at a height of 1880 m above sea level. Alpine willow is a dioecious gametophyte, mezohygrophyte and microtermal species growing in damp limestone rocks in the structure of *Salicion retuse* and *Androsation alpine* union groups. The species is represented by only a small isolated population with small area and the small number of individuals. It has a narrow ecological and coenotic amplitude limited by specific ecological conditions and the impact of the recreation and it is protected in the Carpathian biosphere reserve.
2. *S. alba* L. – indifferent boreal Eurasian species, widespread in the Carpathian up to 1000 m above sea level. It grows in all areas of the Carpathians along rivers, forms moist forests as well as dense woods along with *S. fragilis*, *Ulmus scabra* Mill., *Populus nigra* L. and *P. alba* L.
3. *S. daphnoides Vill*. – indifferent Atlantic and Central European species. A. K. Skvortsov (1968) distinguishes it only for area of Kolomyia. *S. daphnoides* can be rarely found on the banks of mountain streams in Chornohora, Chyvchyny-Hrynyavsk mountains and Marmarosh Alps.
4. *S. aurita* L. – indifferent boreal Eurasian species whose southern boundary in Ukraine is Sumy-Kyiv line and beyond, capturing forest-steppe, wedged in the Carpathians, where the tree occasionally grows in the Carpathian Mountains, Eastern Beskid, Gorgany and Svydovets and rises to 1400 m above sea level. The species refers to calceophytes.
5. *S. Kitaibeliana* (*syn. S. retusa*) – Central European species with disjunctive habitat on the eastern boundary of distribution in Chernohora. Krichfalushiy V. V. (1982) considers *S. Kitaibeliana* and *S. retusa* to be different species. In Ukraine *S. Kitaibeliana* grows in the alpine and subalpine zones of the Carpathians ranges of Svydovets, Chernohora. *S. Kitaibeliana* is protected in the Carpathian Biosphere Reserve and the Carpathian National Park.
6. *S. caprea* L. – indifferent boreal Eurasian species, widespread throughout the Carpathian Mountains up to 1400 m above sea level with the exception of the Transcarpathian plain. It grows in moist enough, but non-bogged areas in clearings, forest edges, and as undergrowth or second tier in deciduous forests.
7. *S. x fragilis* L. – indifferent Caucasian-Asia Minor type, spread in Ukrainian Carpathians up to the height of 900-1100 m above sea level. It grows in damp non-bogged areas, in floodplain forests, often in settlements.
8. *S. lapponum* – relict boreal eurosiberian species, which is on the verge of its range in Ukraine, its southern boarder goes through Kovel - Lutsk - Rivne – Novograd-Volynskiy – Ovruch line. Further mentioned are isolated island habitats in the Ukrainian Carpathians in the range of Chornohora and in the left-bank

Polissya. The species is protected in Poland and the Czech Republic. Normally, *S. lapponum* grows in individual samples or in small groups in open or sparse growth of trees, in sedge-sphagnum areas. The species is protected in the Carpathian national park.

9. *S. eleagnos* – South and Central European mountainous species that rises up to 800 m above sea level in the Carpathian Mountains. Occasionally found over mountain streams in the Carpathians, the Eastern Beskid, Gorgan and Svydovets.
10. *S. myrsinifolia* – indifferent Eurasian boreal species, the southern boundary of which in Ukraine is in the Left-bank on the line Sumy-Chernihiv. Later this species was confirmed by A. K. Skvortsov (1987) for the Right-bank of Polissya. In the Carpathians, its habitat was found here and there in L'viv region (Zhydachiv district, Novi Strilyshcha). It grows in wetland areas, forest edges, along roads and ditches.
11. *S. cinerea* L. – indifferent boreal Eurasian species, common in all parts of the Carpathians floral areas up to 1,000 meters above sea level. It grows on the edges of lowland and transitional bogs, wet meadows and other moist low flow areas. It forms dense shrub with *S. triandra* in floodplains.
12. *S. viminalis* L. – indifferent boreal Eurasian species, which rises in the Carpathians up to 900-1000 m above sea level. Distributed in occasional parterres along rivers and lakes in the pre-Carpathian Mountains, Eastern Beskydy, Gorgany, Svydovets, Chyvyno -Hrynyavski mountains, Volcanic Carpathians and Transcarpathian foothills. Recently, a decline in the number of species is observed due to excessive use of this type of willow in basket weaving by the local population.
13. *S. purpurea* L. – Atlantic and Central European boreal species, distributed in all areas of the Carpathians flora, to a height of 1200 m above the sea level. It grows along rivers and streams in wetland meadows.
14. *S. pentandra* L. – indifferent Eurasian boreal species common in the Carpathian Mountains, Eastern Beskid, Gorgan, Svydovets, Chyvchyny-Hrynyavskyh mountains Volcanic Carpathians and Transcarpathian foothills to a height of 800 meters above the sea level. It grows in bogs, wet meadows and along mountain rivers. Prefers acidic soils ph = 4,0-4,5.
15. *S. rhaetica* (syn. *S. phylicifolia*) – indifferent Eurasian boreal species. It occurs to an altitude of 1700-1850 meters above the sea level. In arrays Svydovets Chornohra and that is confirmed by herbarium specimens Uzhgorod National University (UU) and the Natural History Museum in L'viv (LWS). Distributed sporadically along the banks of streams, in marshy areas, sometimes penetrates the forest zone.
16. *S. rosmarinifolia* L. – indifferent boreal Eurasian species. Rarely grows in marshy meadows and sand in the forest zone, particularly in the herbarium of L'viv Natural History Museum (LWS) we found a herbarium specimen of *S. rosmarinifolia* collected in 2010 by L. Borsukevych around with. Black Oslavy, Nadvirna district of Ivano-Frankivsk.
17. *S. silesiaca* Willd. – Central European indifferent boreal species common in the Eastern Beskid, Gorgan, Svydovets, Montenegro, Chyvchyny-Hrynyavskyh mountains, the Alps and Maramorosh Volcanic Carpathians in the upper mountain and subalpine zones. The natural habitat of *S. silesica* is disjunctive in Europe and covers the Sudetenland, the Carpathians, the mountains of Bosnia and Herzegovina, Montenegro, Albania and Western Bulgaria (Skvortsov, 1999). In the Ukrainian Carpathians is the eastern boundary of this species. Typically, *S. silesica* creates habitats covered in forests, forest edges, clearings, bushes, on both sides of the mountain streams, ditches, Roads in the middle and upper forest belt to a height of 1700-1800 meters above the sea level.
18. *S. starkeana* – indifferent Eurasian boreal relict species, which is on the southern edge of the range, which runs through the Kharkiv - Poltava - Lubny - Kano - Vinnytsia - Rohatyn - L'viv - Mostysk. In Ukraine, it occurs sporadically. Occasionally, in the Eastern Beskid, Gorgan, Volcanic Carpathians and Transcarpathian foothills. It grows in eutrophic and mesotrophic marshes, peatlands, wet floodplain and lowland meadows, meadow steppes, forest edges, in bushes, woodlands, light woods, on railway embankments. In the Carpathians, it needs protection. It is also protected in Germany, Romania, Slovakia and the Czech Republic.

19. *S. herbacea* – relict species with disjunctive arctic-alpine habitat. In Ukraine, the area covers a population isolated in mountain ranges Maramorosh Alps and Montenegro. It grows on rocky hillsides and the upper alpine and subalpine zone at an altitude of 1750-2000 meters above the sea level, mainly in areas with long kept snow. *S. herbacea* is protected on the territory of the CBR and the Carpathian National Nature Park.
20. *S. triandra* L. – indifferent boreal Eurasian species, distributed in the Carpathian Mountains, Eastern Beskid, Gorgan, Svydovets, Montenegro, Chyvchyny-Hrynyavskyh Transcarpathian mountains and foothills to a height of 1200 m above the sea level. It grows in wet meadows, swamps and along rivers. At an altitude of 1000 m, it is rare.
21. *S. retusa* – Central European species with disjunctive habitat on the eastern limits of distribution in the array of Montenegro. It grows in alpine and subalpine zones, mountain rocky slopes and rocky tops, at an altitude of 1750-2000 meters above the sea level. *S. retusa* is protected in the Carpathian Biosphere Reserve and Carpathian National Park.
22. *S. myrtilloides* L. – relict boreal-subarctic species common on the southern edge of its range. In Ukraine, it occupies Carpathian Woodlands, wastes, occasionally grows in Carpathian steppe extended to 1000 m above the sea level. Sometimes - small clumps, there is a sharp decrease in population. It grows in bogs, wet meadows, spruce forests under a tent. It occurs very rarely and is in need of protection in the Ukrainian Carpathians. *S. myrtilloides* is protected in Polesie, as well as Belarus, Latvia, Lithuania, Germany, Poland, Romania, Slovakia and the Czech Republic.

According to the literature, in the Alps Marmarosh, there is very rare mesh willow (*S. reticulata*), and in the array of Montenegro – *S. hastata* L. (Krichfalushiy, 1982, Chopyk, Fedoronchuk, 2015). However, the evidence in the Herbarium of the Institute of Botany of NAS of Ukraine (KW), Uzhgorod National University (UU), L'viv National University. AND I. Frank (LW) and the State Natural History Museum of Ukraine (m. L'viv) (LWS) is not revealed, so we believe that these species are absent in the Ukrainian Carpathians.

CONCLUSIONS

Thus, based on the analysis of publications, herbarium specimens and their study route, it can be argued that the natural flora of the genus Salix in the Ukrainian Carpathians has 22 species. By alpine and subalpine floro-coenatic complexes were found six species *S. alpina, S. herbacea, S. retusa, S. Kitaibeliana, S. rhaetica, S. silesiaca.* Two types of *S. hastata* and *S. reticulata* raise doubts about their presence on the territory of Ukrainian Carpathians and their chorology need clarification. The most numerous boreal flora Salix element in the Carpathians, covering 16 species: *S. alba, S. x fragilis, S. pentandra, S. triandra, S. myrtilloides, S. myrsinifolia, S. caprea, S. cinerea, S. aurita, S. starkeana, S. viminalis, S. lapponum, S. daphnoides, S. rosmarinifolia, S. eleagnos, S. purpurea.* All boreal species belong to mesohygrophytes or hygrophytes. It was also found that all types of subalpine and alpine zone and boreal *S. starkeana, S. lapponum* need protection.

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Achillea ochroleuca
The Red Book of the Republic of Moldova, Third edition. 2015

MULLEIN SPECIES *(VERBASCUM* L., *SCROPHULARIACEAE* JUSS.*)* IN DNIESTER-PRUT RIVER REGION

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Abstract: The article brings the list of one of the biggest genera in the *Scrophulariaceae* family – *Verbascum* L., which embodies 11 species in the Dniester-Prut region. The dichotomic key for genus *Verbascum*, as well as brief ecological and habitat characters for each species are given.

Keywords: flora, *Scrophulariaceae*, *Verbascum*, biology, ecology

INTRODUCTION

The maintenance of floristical diversity is based on fundamental monographic studies on separate taxonomic groups. Genus *Verbascum* L. – variable in morphology and ecology, takes tone of the central position in system of *Scrophulariaceae* Juss. family. The common name of *Verbascum* L. is "mullein" (also known as "velvet plant") [4], is a genus of about 400 species of flowering plants in the figwort family *Scrophulariaceae*. [8] The name "mullein" probably comes from the Latin word *mollis*, meaning soft, referring to the plant's woolly stem and leaves. The name also might relate to the Latin *malandrium*, meaning malanders, a cattle disease for which mullein was used as a remedy. They are native to Europe and Asia, with the highest species diversity in the Mediterranean region. [4] It plays a significant role in vegetation cover and is an important component in structure of the open grassland habitats of the Dniester-Prut river region. There are many melliferous, decorative, fodder and medicinal taxa in the given genera. The mullein plants have a long history of use as herbal remedies. *Verbascum* flowers have been used in traditional medicine internally (as tea) or externally (as ointment, tea, baths or compresses) for treatment of disorders of the respiratory tract, veins, gastrointestinal tract, and the locomotor system. Mullein leaves have been used in cosmetic preparations to soften skin.

MATERIAL AND METHODS

During our investigation concerning genus *Verbascum* for the flora of Dniester-Prut region we performed all necessary research on field and laboratory examination. Firstly, we reviewed all published information on the presence of species in the territory, and consulted specimen materials in different scientific herbaria (Herbarium of the Botanical Garden (Institute) of ASM, Herbarium of the State University of Moldova, Herbarium of the Botanical Institute Komarov (Sankt-Petersburg, Russian Federation). The geographical elements and chromosome numbers are given according to literature data. [3, 8, 11] The illustrations were performed by Teleuta S.

RESULTS AND DISCUSSIONS

In the flora of Dniester-Prut river region, the genus *Verbascum* embodies 11 species.

Genus ***VERBASCUM*** L. **– MULLEIN – LUMÂNĂRICĂ – КОРОВЯК**

Linnaeus, 1753, Sp. Pl.: 177; id. Gen. Pl., ed. 5: 83. – *Celsia* L. 1753, Sp. Pl.: 621; id. Gen. Pl., ed. 5: 272

L e c t o t y p u s: ***V. thapsus*** L.

The high morphological diversity among species of the genus *Verbascum* causes problems in the delimitation of the species. Therefore, an identification key for these species is prepared.

Key to species

1a. Corolla violet, rarely yellow. Stem almost without leaves ..... **11. *V. phoeniceum*.**
1b. Corolla yellow. Stem leafy ..... **2.**
2a. Flowers solitary. Inflorescence simple ..... **3.**
2b. Flowers are grouped by 2-7 dichasia. Inflorescence branched ..... **4.**
3a. Flowers sessile, with suborbicular bracts and 2 bracteoles at the calyx base. Corolla with pellucid glands. Filament-hairs yellow or orange. Plant indumentum yellowish-tomentose. Bazal leaves long-petiolate ..... **4. *V. ovalifolium*.**
3b. Flowers with pedicels of 5-20 mm and linear bracts; bracteoles absent. Corolla without pellucid glands, but with purple papills inside. Filament-hairs purple. Plants glabrous below and with stalked or sessile glands above. Bazal leaves shortly-petiolate or sessile ..... **5. *V. blattaria*.**
4a. Anthers of 2 lower stamens decurrent and of 3 upper reniform ..... **5.**
4b. Anthers all reniform ..... **7.**
5a. Upper cauline leaves ±shortly-decurrent, with cordate base ..... **1. *V. phlomoides*.**
5b. Uper cauline leaves decurent along internodes, with narrow base ..... **6.**
6a. Pedicel connate with inflorescence axis. Corolla 12-25 mm in diameter, with pellucid glands, tomentose outside. Upper 3 filaments with white hairs, the lower 2 glabrous. Stigma capitate ..... **3. *V. thapsus*.**
6b. Pedicel not connate with inflorescence axis. Corolla 25-55 mm in diameter, almost without pellucid glands, with setellate hairs outside. Upper 3 filaments with yellow hairs, the lower 2 glabrous. Stigma spathulate, decurrent ..... **2. *V. densiflorum*.**
7a. Filaments with white or yellow hairs ..... **8.**
7b. Filaments with purple hairs ..... **10.**
8a. Bazal leaves lanceolate, cauline – ondulate, with cordate-amplexicaul base. Corolla 18-30 mm in diameter ..... **7. *V. speciosum*.**
8b. Bazal leaves obovate, obong-eliptic or oblong-ovate, cauline – plane, sessile or with short petiole, not amplexicaul. Corolla (10)12-22 mm in diameter ..... **9.**
9a. Bazal leaves with petiole 0,5-5 cm long. Corolla (10) 12-15 mm in diameter. Lower 2 filaments glabrous above, upper 3 filaments with white hairs ..... **6. *V. lychnitis*.**
9b. Bazal leaves with petiole 5-10 cm long. Corolla 15-22 mm in diameter. All filaments with white hairs ..... **9. *V. banaticum*.**
10a. Bazal leaves ovate-oblong, with petiole 2-10 cm long. Calyx gray-tomentous ..... **8. *V. marschallianum*.**
10b. Bazal leaves cordat-ovate or cordat-oblonge, with petiole 12-20 cm long. Calyx pubescent ..... **10. *V. nigrum*.**

Section 1. *Verbascum*. – Sect. *Thapsus* Griseb. 1844, Spic. Fl. Rumel. 2: 48. – Sect. *Blattaria* Griseb. 1844, Spic. Fl. Rumel. 2: 41. – Sect. *Fasciculata* (Murb.) B. Fedtsch. subsect. *Heterandra* (Franch.) B. Fedtsch. 1955, Фл. СССР, 22: 123. – Sect. *Singuliflora* (Murb.) B. Fedtsch. 1955, Фл. СССР, 22: 155, p. p. – The section includes species with the following characteristics: stamens 5; anthers different: those of the 2 anterior (lower) stamens decurrent on the filament, those of the 3 posterior (upper) stamens are always reniform and transversely medifixed; stigma spatulate, rarely capitate.

1. *V. phlomoides* L. 1753, Sp. Pl.: 1194; Б. Федченко, 1955, Фл. СССР, 22: 123; Котов, 1960, Фл. УРСР, 9: 409; Ghişa, 1960, Fl. R. P. Române, 7: 407; Ferguson, 1972, Fl. Europ. 3: 210; Иванина, 1981, Фл. евр. части СССР, 5: 213; Гейдеман, 1986, Опред. высш. раст. Молд. ССР, изд. 3: 474; Котов, 1999, Опред. высш. раст. Укр., изд. 2: 283; Васильева и Коваленко, 2003, Консп. флори Півден. Бессарабії: 172; Negru, 2007, Determ.

pl. fl. R. Moldova: 204; Ciocârlan, 2009, Fl. ilustr. a României: 679. **лекарственный.** 2n = 32, 34.

Is a biennial plant (figure 1), distributed in steppe zone of Eurasia and Mediterranean region. [3, 8] Mediterranean-Pannonian-Sarmatian geographical element. The species is widely distributed in the Dniester-Prut river region in margins and clearings of forests, on steppe and calcareous slopes, dry meadows, pastures, as a weed along roadsides and in field margins. The plants bloom in June-August, propagate by seeds. Plants are used as medicinal, ornamental, vitamin-bearing, technical etc.

Figure 1. *Verbascum phlomoides* L.

2. *V. densiflorum* Bertol. 1810, Rar. Lig. Pl. 3: 52; Ferguson, 1972, Fl. Europ. 3: 210; Иванина, 1981, Фл. евр. части СССР, 5: 213; Гейдеман, 1986, Опред. высш. раст. Молд. ССР, изд. 3: 474; Котов, 1999, Опред. высш. раст. Укр., изд. 2: 283; Васильева и Коваленко, 2003, Консп. флори Півден. Бессарабії: 172; Negru, 2007, Determ. pl. fl. R. Moldova: 204; Ciocârlan, 2009, Fl. ilustr. a României: 680. – *V. thapsiforme* Schrad. 1813, Monogr. Verbasci, 1: 21; Б. Федченко, 1955, Фл. СССР, 22: 126; Котов, 1960, Фл. УРСР, 9: 410; Ghișa, 1960, Fl. R. P. Române, 7: 411. **– Dense-flowered mullein. – L. densifloră. – К. густоцветковый.** 2n = 32, 36.

Figure 2. *Verbascum densiflorum* Bertol.

Is a biennial plant (figure 2), distributed in the Mediterranean region and Europe. [3, 8] European geographical element. The species is widely distributed in the region. Grows in margins and clearings of forests, on steppe and calcareous slopes, dry meadows, pasture, as a weed along roadsides and in field margins. The plants bloom in July-August, propagate by seeds. Dense-flowered mullein used in traditional medicinal practice.

3. *V. thapsus* L. 1753, Sp. Pl.: 177; Б. Федченко, 1955, Фл. СССР, 22: 128; Котов, 1960, Фл. УРСР, 9: 411; Ghișa, 1960, Fl. R. P. Române, 7: 412; Ferguson, 1972, Fl. Europ. 3: 211, quoad subsp. *thapsus*; Иванина, 1981, Фл. евр. части СССР, 5: 213; Котов, 1999, Опред. высш. раст. Укр., изд. 2: 283; Васильева и Коваленко, 2003, Консп. флори Півден. Бессарабії: 172; Negru, 2007, Determ. pl. fl. R. Moldova: 204; Ciocârlan, 2009, Fl. ilustr. a României: 680. **– Great mullein, common mullein. – Ciucurică. – К. обыкновенный, Медвежье ухо.** 2n = 32, 36.

Is a biennial plant (figure 3). The area of distribution includes Mediterranean region, Europe (except the extreme north), Asia Minor, the Caucasus, Western and Eastern Siberia (west), Mongolia; introduced in China, Japan, New Zealand and Americas. [3, 8] Eurasian geographical element. In the region it is met only in the southern zone. Grows in margins and clearings of forests and on calcareous slopes. The plants bloom in June-July, propagate by seeds. Great mullein is used as a medicinal plant.

4. *V. ovalifolium* Donn ex Sims, 1807, Curtis's Bot. Mag. 26: tab. 1037; Б. Федченко, 1955, Фл. СССР, 22: 156; Котов, 1960, Фл. УРСР, 9: 421; Ghișa, 1960, Fl. R. P. Române, 7: 438; Ferguson, 1972, Fl. Europ. 3: 208, quoad subsp. *ovalifolium*; Иванина, 1981, Фл. евр. части СССР, 5: 213; Гейдеман, 1986, Опред. высш. раст.

Figure 3. *Verbascum thapsus* L.

Молд. ССР, изд. 3: 475; Котов, 1999, Опред. высш. раст. Укр., изд. 2: 284; Васильева и Коваленко, 2003, Консп. флори Півден. Бессарабії: 172; Negru, 2007, Determ. pl. fl. R. Moldova: 204; Ciocârlan, 2009, Fl. ilustr. a României: 679. – *V. compactum* Bieb. 1808, Fl. Taur.-Cauc. 1: 159. **– Oval-leaved mullein. – L. ovalifolie. – К. овальнолистный.**

Is a biennial plant (figure 4). The area of common distribution covers the territory of Central (Romania) and East (south) Europe, Balkan Peninsula, the Caucasus. [3, 8] Pontic-Balkan geographical element. In the region occurs in the Dniester river basin, central and southern zone as a weed along roadsides, in the ruderal habitats in the edges of arid forests, on steppe and calcareous slopes, dry meadows. The plants bloom in June-July, propagate by seeds.

It is rare on the territory of neighboring Romania and has been included in the Red Book of vascular plants in Romania [1] as a vulnerable taxon (**VU**).

5. *V. blattaria* L. 1753, Sp. Pl.: 178; Б. Федченко, 1955, Фл. СССР, 22: 167; Котов, 1960, Фл. УРСР, 9: 427; Ghişa, 1960, Fl. R. P. Române, 7: 441; Ferguson, 1972, Fl. Europ. 3: 208; Иванина, 1981, Фл. евр. части СССР, 5: 215; Гейдеман, 1986, Опред. высш. раст. Молд. ССР, изд. 3: 475; Котов, 1999, Опред. высш. раст. Укр., изд. 2: 284; Васильева и Коваленко, 2003, Консп. флори Півден. Бессарабії: 171; Negru, 2007, Determ. pl. fl. R. Moldova: 204; Ciocârlan, 2009, Fl. ilustr. a României: 679. – *V. repandum* Willd. 1809, Enum. Pl. Horti Berol.: 226. **– Moth mullein. – L. obscură. – К. тараканий.** 2n = 18, 30.

Figure 4. *Verbascum ovalifolium* Donn ex Sims

Is a biennial, or sometimes annual, plant (figure 5). distributed in the Western Eurasia; introduced in North America. [3, 8] West-Eurasian geographical element. The species is widely distributed in the region. Grows in meadows, margins and clearings of flooded forests, damp and wet pastures, as a weed along roadsides in wet habitats, sometimes on saline soil. The plants bloom in (May) June-July, propagate by seeds. Moth mullein is used as a medicinal and honey-bearing plant.

Figure 5. *Verbascum blattaria* L.

Sectio 2. *Lychnitis* Griseb. 1844, Spic. Fl. Rumel. 2: 45. – Sect. *Fasciculata* (Murb.) B. Fedtsch. subsect. *Isandra* (Franch.) B. Fedtsch. 1955, Фл. СССР, 22: 122. – Sect. *Singuliflora* (Murb.) B. Fedtsch. 1955, Фл. СССР, 22: 155, p. p. – The section includes species with the following characteristics: stamens 5 or 4; all anthers reniform and transversely medifixed; stigma capitate, rarely spathulate.

6. *V. lychnitis* L. 1753, Sp. Pl.: 177; Б. Федченко, 1955, Фл. СССР, 22: 142; Котов, 1960, Фл. УРСР, 9: 416; Ghişa, 1960, Fl. R. P. Române, 7: 422;

Ferguson, 1972, Fl. Europ. 3: 215; Иванина, 1981, Фл. евр. части СССР, 5: 215; Гейдеман, 1986, Опред. высш. раст. Молд. ССР, изд. 3: 474; Котов, 1999, Опред. высш. раст. Укр., изд. 2: 283; Васильева и Коваленко, 2003, Консп. флори Півден. Бессарабії: 172; Negru, 2007, Determ. pl. fl. R. Moldova: 206; Ciocârlan, 2009, Fl. ilustr. a României: 682. – *V. pulverulentum* Bieb. 1808, Fl. Taur.-Cauc. 1: 160, non Vill. 1779. **– White mullein. – L. farinacee. – К. мучнистый.** 2n = 26, 32, 34.

Is a biennial plant (figure 6), distributed in the Mediterranean region, Europe (except the extreme north), Asia Minor, the Caucasus, Western Siberia (west); introduced in Scandinavia, The Baltic States, North America. [3, 8] West-Eurasian geographical element. The species is widely distributed in the region. Grows on steppe and calcareous slopes, in margins and clearings of forests, dry meadows, pastures, as a weed along roadsides and in field margins. The plants bloom in July-August, propagate by seeds. White mullein is used as a tinctorial and honey-bearing plant.

7. *V. speciosum* Schrad. 1809, Hort. Gotting.: 22; Б. Федченко, 1955, Фл. СССР, 22: 135; Котов, 1960, Фл. УРСР, 9: 413; Ghişa, 1960, Fl. R. P. Române, 7: 418; Ferguson, 1972, Fl. Europ. 3: 213, quoad subsp. *speciosum*; Иванина, 1981, Фл. евр. части СССР, 5: 215; Гейдеман, 1986, Опред. высш. раст. Молд. ССР, изд. 3: 474; Котов, 1999, Опред. высш. раст. Укр., изд. 2: 283; Negru, 2007, Determ. pl. fl. R. Moldova: 206; Ciocârlan, 2009, Fl. ilustr. a României: 683. – **Hungarian mullein, showy mullein. – L. uimitoare. – К. великолепный.**

Figure 6. *Verbascum lychnitis* L.

Is a biennial plant (figure 7). The area of common distribution covers the territory of Mediterranean region, Central and Eastern Europe (south-west), Asia Minor, Iran, the Caucasus; introduced in North America. [3, 8] Mediterranean-Pontic-Caucasian geographical element. In the region occurs sporadically in the northern, central and southern zone in the ruderal habitats in the edges of forests, in steppes and calcareous slopes, dry meadows, as a weed along roadsides, in pasturelands, at the margins of fields and vineyards. The plants bloom in July-August, propagate by seeds.

8. *V. marschallianum* Ivanina et Tzvel. 1985, Новости сист. высш. раст. (Ленинград), 22: 272. – *V. orientale* Bieb. 1808, Fl. Taur.-Cauc. 1: 160; Б. Федченко, 1955, Фл. СССР, 22: 147; Котов, 1960, Фл. УРСР, 9: 417. – *V. austriacum* auct. non Roem. et Schult. 1819: Гейдеман, 1986, Опред. высш. раст. Молд. ССР, изд. 3: 474; Котов, 1999, Опред. высш. раст. Укр., изд. 2: 283; Васильева и Коваленко, 2003, Консп. флори Півден. Бессарабії: 171; Negru, 2007, Determ. pl. fl. R. Moldova: 204. – *V. chaixii* Vill. subsp. *orientale* (Bieb.) Hayek, 1929, Prodr. Fl. Penins. Balcan. 2: 127; Ferguson, 1972, Fl. Europ. 3: 216; Иванина, 1981, Фл. евр. части СССР, 5: 217; Ciocârlan, 2009, Fl. ilustr. a României: 682. – *V. chaixii* Vill. var. *orientale* (Bieb.) Murb. 1933, Monogr. Gatt. *Verbascum*: 418; Ghişa, 1960, Fl. R. P. Române, 7: 426. – **Marshall's mullein. – L. Marşal. – К. Маршалла.**

Is a biennial or perennial plant (figure 8). Area of distribution covers the Central (southern part of Romania) and Eastern Europe (center and south), the Caucasus, Middle Asia, Western Siberia. [3, 8]

Figure 7. *Verbascum speciosum* Schrad.

Pontic-Sarmatian geographical element. The species is widely distributed in the region. Grows in margins and clearings of forests, on steppe and calcareous slopes, pastures, as a weed in ruderal habitats along roadsides and in field margins, occasionally in dry meadows. The plants bloom in June-September, propagate by seeds.

Figure 8. *Verbascum marschallianum* Ivanina et Tzvel.

Figure 9. *Verbascum banaticum* Schrad.

9. *V. banaticum* Schrad. 1823, Monogr. Verbasci, 2: 28; Б. Федченко, 1955, Фл. СССР, 22: 134; Котов, 1960, Фл. УРСР, 9: 412; Ghişa, 1960, Fl. R. P. Române, 7: 417; Ferguson, 1972, Fl. Europ. 3: 215; Иванина, 1981, Фл. евр. части СССР, 5: 217; Котов, 1999, Опред. высш. раст. Укр., изд. 2: 283; Васильева и Коваленко, 2003, Консп. флори Півден. Бессарабії: 171; Ciocârlan, 2009, Fl. ilustr. a României: 680. **– Mulleinof Banat. – L. bănăţeană. – К. банатский.**

Figure 10. *Verbascum nigrum* L.

Is a biennial plant (figure 9). The area of distribution includes Mediterranean region, Central and Eastern (south-west) Europe, Minor Asia. [3, 8] Pannonian-Pontic-Mediterranean geographical element. In the region it is met only in the littoral zone of the Black Sea on the sands along river banks. The plants blooms in June-July, propagate by seeds.

10. *V. nigrum* L. 1753, Sp. Pl.: 178; Б. Федченко, 1955, Фл. СССР, 22: 149; Котов, 1960, Фл. УРСР, 9: 418; Ghişa, 1960, Fl. R. P. Române, 7: 426; Ferguson, 1972, Fl. Europ. 3: 216, quoad subsp. *nigrum*; Иванина, 1981, Фл. евр. части СССР, 5: 217; Гейдеман, 1986, Опред. высш. раст. Молд. ССР, изд. 3: 474; Котов, 1999, Опред. высш. раст. Укр., изд. 2: 283; Васильева и Коваленко, 2003, Консп. флори Півден. Бессарабії: 172; Negru, 2007, Determ. pl. fl. R. Moldova: 206; Ciocârlan, 2009, Fl. ilustr. a României: 682, quoad subsp. *nigrum*. – **Dark mullein, black mullein. – L. neagră. – К. черный.** 2n = 30, 34.

Is a biennial or perennial plant (figure 10), distributed in the Mediterranean region, Europe, the Caucasus, Siberia. [3, 8] European-Siberian geographical element. The species is widely distributed in the region. Grows in margins and clearings of forests, on steppe and

calcareous slopes, dry meadows, pastures, as a weed along roadsides and in field margins. The plants bloom in June-July, propagate by seeds. Dark mullein is used as a medicinal, tinctorial, insecticidal and honey-bearing plant. Plants are toxic.

11. *V. phoeniceum* L. 1753, Sp. Pl.: 178; Б. Федченко, 1955, Фл. СССР, 22: 168; Котов, 1960, Фл. УРСР, 9: 429; Ghişa, 1960, Fl. R. P. Române, 7: 445; Ferguson, 1972, Fl. Europ. 3: 209; Иванина, 1981, Фл. евр. части СССР, 5: 218; Гейдеман, 1986, Опред. высш. раст. Молд. ССР, изд. 3: 475; Котов, 1999, Опред. высш. раст. Укр., изд. 2: 284; Васильева и Коваленко, 2003, Консп. флори Півден. Бессарабії: 172; Negru, 2007, Determ. pl. fl. R. Moldova: 204; Ciocârlan, 2009, Fl. ilustr. a României: 679. – **Purple mullein, temptress purple**. – **L. violacee. – К. фиолетовый.** 2n = 32, 36.

Is a perennial plant (figure 11). Common area covers the Mediterranean region, Central and Eastern Europe, the Caucasus, Minor and Middle Asia, Iran (north), Western Siberia. [3, 8] Mediterranean-Pontic-Sarmatian geographical element. The species is widely distributed in the region. Is a typically steppe plant. Grows on steppe and calcareous slopes, in margins and clearings of dry forests, occasionally in dry pastures. The plants bloom in May-July, propagate by seeds. Plants are decorative and ornamental.

Figure 11. *Verbascum phoeniceum* L.

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QUALITATIVE AND QUANTITATIVE STRUCTURE OF THE ALGAL COMMUNITIES OF THE RIVER BIC

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Abstract: As a result of investigations on algal communities of the river Bic (the sector crossing Chisinau) there were found 253 species and varieties of algae, producing a biomass of 50 g/m^2 up to 4-3 kg/m^2. Diatoms are developing intensively in the autumn-winter-spring period, *Chlorophyta* – duing spring-summer-autumn and *Cyanophyta* and *Euglenophyta* – during summer and autumn. The species belonging to the *Dinophyta* and *Xanthophyta* phyla are rarely encountered in the periphyton of the river.

Keywords: periphyton, phytoplankton, algal communities, biodiversity, eutrophication.

INTRODUCTION

The river Bic is one of the main tributaries of the right side of the river Dniester and has a length of 155 km. The river's spring is on the slope of the beech forest near the village Temeleuti (Calarasi district) and flows into the river Dniester near the village Gura-Bicului. It is qualified as the most degraded river in the Republic of Moldova, which starting from Chisinau, it was transformed into a wastewater channel. In the river basin, there were built 284 waterbeds (ponds), which are mud silted and partially covered with hydrophilic vegetation. On the river course there is the Ghidighici reservoir, on which depends the river flow in the place where it enters the municipality of Chisinau [1; 4].

The accumulation of biogenic elements in large quantities can cause an intense development of aquatic vegetation, as well as some species of algae indicating the high level of eutrophication, manifested by activating the phenomenon of "water blooming" [5; 6].

MATERIALS AND METHODS

During the years 2015-2016 samples of periphytonic algae from the river Bic were collected and studied. The collection and processing of algae samples were performed according to the unified methods of collecting and processing of the hydrobiological samples from the land and experimental [3; 9; 10]. A part of the collected material was brought in the laboratory and analysed fresh with the microscope MBL 2100, while another part was fixed in solution of formalin or ethanol. A total of more than 30 qualitative and quantitative algal samples were studied. For the identification of the species, there were used well recognized identification keys.

RESULTS AND DISCUSSIONS

The study of the qualitative-quantitative dynamics of algal communities has a particular significance in terms of continuous eutrophication and pollution of the water reservoirs. The wastewater discharged in the river Bic contributes to the degradation of the specific algoflora composition. As a result, there develop intensely cyanophyta and euglenophyta, belonging to genera *Anabaena, Aphanizomenon, Microcystis, Oscillatoria, Phormidium* and *Euglena*, which may cause the phenomenon of "water blooming". In such conditions there also begin to develop intensely species of diatom and chlorophyta resistant to increased concentrations of organic substances dissolved in water. In the coastal zone of the river and on various substrates, filamentous algae grow abundantly (species of the genera *Cladophora, Rhizoclonium, Oedogonium, Enteromorpha, Stigeoclonium, Ulothrix, Zygnema, Mougeotia*), forming a biomass of up to 10-5 kg/m^2 [7].

As a result of investigations on algal communities of the river Bic, there have been highlighted representatives belonging to the following phyla: *Cyanophyta* – 51 taxa (20,2%), *Bacillariophyta* – 104 (41,3%), *Xanthophyta* – 3 (1,2%), *Dinophyta* – 3 (1,2%), *Chlorophyta* – 72 (28,6%) and *Euglenophyta* – 19 (7,5%) (tab. 1).

Table 1. Taxonomic structure of river Bic algoflora

Phylum	Number					
	Classes	Order	Family	Genera	Species and varieties	%
Cyanophyta	2	3	14	17	51	20,2
Bacillariophyta	2	4	8	30	104	41,1
Xanthophyta	2	2	2	2	3	1,2
Dinophyta	1	1	1	2	3	1,2
Chlorophyta	4	6	19	37	73	28,8
Euglenophyta	1	1	1	5	19	7,5
Total	12	17	45	93	253	100

***Cyanophyta* Phylum:** *Anabaena affinis* Lemm., *A. catenula* Kutz. Born. et Flah., *A. spiroides* Kleb., *A. variabilis* Kutz., *Aphanizomenon elenkinii* Kissel., *A. flos-aquae* (L.) Ralfs, *Calothrix brevissima* G. S. West., *Coelosphaerium dubium* Grun., *Dactylococcopsis elenkinii* Roll., *D. irregularis* G. M. Sm., *Gloeocapsa magma* (Breb.) Kutz., *G. minor* (Kütz.) Hollerb., *G. turgida* (Kutz.) Holerb., *Gomphosphaeria lacustris* Chod., *Homoeothrix varians* Geitl., *Lyngbya cryptovaginata* Schkorb., *L. kuetzingii* (Kutz.) Schmidle, *L. lagerheimii* (Mob.) Gom., *L. limnetica* Lemm., *Merismopedia elegans* A. Br., *M. glauca* (Ehr.) Kütz., *M. punctata* Meyen, *M. tenuissima* Lemm. *Microchaete tenera* Thur., *Microcystis aeruginosa* Kutz., *M. pulverea* (Wood) Forti, *Oscillatoria agardhii* Gom., *O. amphibia* Ag. ex Gom., *O. amoena* Kutz., *O. brevis* (Kutz.) Gom., *O. chalybea* (Mert.) Gom., *O. geminata* (Menegh.) Gom., *O. irrigua* Kutz., *O. limnetica* Lemm., *O. limosa* Ag., *O. ornata f. planctonica* Elenk., *O. planctonica* Wolosz., *O. simplicissima* Gom., *O. tenuis* Ag., *O. terebriformis* (Ag.) Elenk., *Phormidium ambiguum* Gom., *Ph. bohneri* Schmidle., *Ph. foveolarum* (Mont.) Gom., *Ph. fragile* Menegh.ex Gom., *Ph. frigidum* Fritsch., *Ph. molle* (Kutz.) Gom., *Ph. valderiae* (Delp.) Geitl., *Pseudanabaena catenata* Lauterb., *Schizothrix lacustris* A. Br., *Spirulina laxa* Smith, *S. major* Kutz.

***Bacillariophyta* Phylum:** *Achnanthes affinis* Grun., *A. hungarica* Grun., *Amphipleura pellucida* Kutz., *Amphora ovalis* Kutz. *var. ovalis, A. ovalis var. constricta* Skv., *A. ovalis var. pediculus* Kutz., *A. perpusilla* Grun., *Anomoeoneis sphaerophora* (Kutz.) Pfitz., *A. sphaerophora var. sculpta* (Ehr.) O. Mull., *Asterionella formosa* Hass., *Bacillaria paradoxa* Gmelin., *Biddulphia laevis* Ehr., *Caloneis amphisbaena* (Bory) Cl., *C. bacillum* (Grun.) Mer., *C. silicula* (Ehr.) Cl. *var. silicula*, *C. silicula var. peisonis* Hust., *Cocconeis pediculus* Ehr., *C. placentula var. euglipta* (Ehr.) Cl., *C. placentula* Ehr. *var. placentula, Coscinodiscus lacustris* Grun., *Cyclotella kuetzingiana* Thw., *C. meneghiniana* Kutz., *Cymatopleura eliptica* (Breb.) W. Sm., *C. solea* (Breb.) W. Sm., *C. solea var gracilis* Grun., *Cymbella cistula* (Hemp.) Grun., *C. lanceolata* (Ehr.) V. H., *C. prostrata* (Berkeley) Cl., *C. tumida* (Breb.) V. H., *C. turgida* (Greg.) Cl., *C. ventricosa* Kütz., *Diatoma elongatum var. tenue* (Ag.) V. H., *D. vulgare* Bory., *D. vulgare var. lineare* Grun., *D. vulgare var. productum* Grun., *Diploneis ovalis* (Hilse) Cl., *Epithemia sorex* Kutz., *E. zebra* (Ehr.) Kutz., *Fragilaria brevistriata* Grun., *F. capucina var. mesolepta* Rabenh., *F. intermedia* Grun., *Gomphonema acuminatum* Ehr. *var. acuminatum, G. acuminatum var. trigonocephalum* (Ehr.) Grun., *G. angustatum var. productum* Grun., *G. augur* Ehr., *G. constrictum* Ehr. *var. constrictum, G. constrictum var. capitatum* Cl., *G. olivaceum* (Lyngb.) Kutz., *G. olivaceum var. calcareum* Cl., *G. parvulum* Kutz., *Gyrosigma acuminatum* (Kutz.) Rabenh., *G. acuminatum var. curtum* Grun., *Hantzschia amphioxys* (Ehr.) Grun., *H. amphioxys var. vivax* Grun., *Melosira varians* Ag., *M. granulata* (Ehr.) Ralfs., *Navicula bacillum* Ehr., *N. confervacea* Kutz., *N. cryptocephala* Kutz. *var. cryptocephala, N. cryptocephala var. venete* Grun., *N. cuspidata* Kutz., *N. gracilis* Ehr., *N. hungarica*

var. capitata Cl., *N. gregaria* Donk., *N. menisculus* Schum., *N. pupula* Kütz., *N. pygmaea* Kutz., *N. radiosa* Kutz., *N. rhynchocephala* Kutz., *N. viridula* Kutz., *N. vulpina* Kutz., *Nitzschia acicularis* (Kutz.) W. Sm., *N. amphibia* Grun., *N. apiculata* (Greg.) Grun., *N. dissipata* (Kutz.) Grun., *N. dubia* W. Sm., *N. fonticola* Grun., *N. frustulum* (Kutz.) Grun., *N. gracilis* Hantzsch., *N. hungarica* Grun., *N. linearis* W. Sm., *N. obtusa* W. Sm., *N. palea* (Kutz.) W. Sm., *N. recta* Hantzsch., *N. sigma* (Kutz.) W. Sm., *N. sigmoidea* (Ehr.) W. Sm., *N. vermicularis* (Kutz.) Hantzsch in Rabenh., *N. tryblionella* Hantzsch., *Pinnularia viridis* (Nitzsch.) Ehr., *Pleurosigma elongatum* W. Sm. *Rhoicosphenia curvata* (Kutz.) Grun., *Stenopterobia intermedia* Lewis, *Stephanodiscus dubius* (Fricke) Hust., *Surirella biseriata* Breb., *S. linearis* W.Sm., *S. ovalis* Breb., *S. ovata* Kutz. var ovata, *S. ovata var. pinnata* (W. Sm.) Hust., *S. ovata var. salina* (W. Sm.) Hust., *S. peisonis* Pant., *Synedra acus* Kutz., *S. tabulata* (Ag.) Kutz., *S. ulna* (Nitzsch) Ehr. *var. ulna, S. ulna var. amphirhynchus* (Ehr.) Grun.

***Xanthophyta* Phylum:** *Chloridella neglecta* (Pasch. et Geitler) Pasch., *Tribonema affine* (Kutz.) G. S. West, *T. viride* Pasch.

***Dinophyta* Phylum:** *Glenodinium pulvisculus* Stein., *Peridinium bipes* Stein., *P. cinctum* (O. F. M.) Ehr.

***Chlorophyta* Phylum:** *Ankistrodesmus falcatus* (Corda.) Ralfs., *Ankyra ancora* (G. M. Smith) Fott., *A. ocellata* (Korsch.) Fott., *Carteria multifilis* (Fres.) Dill., *C. pallida* Korsch., *Characium acuminatum* A. Br., *C. sieboldii* A. Br., *C. strictum* A. Br., *Chlamydomonas ehrenbergii* Gorosch., *C. globosa* Snow., *C. monadina* Stein., *C. reinhardtii* Dang., *Chlorella vulgaris* Beier., *Chlorhormidium subtile* (Kutz.) Starmach., *Cladophora glomerata* (L.) Kutz., *Closterium acerosum var. elongatum* Breb., *C. tumidulum* Gay., *C. venus* Kutz., *Coelastrum microporum* Nag., *Coenochloris fottii* (Hind.) Tzar., *Coleochaete scutata* Breb., *C. soluta* Pringsh., *Cosmarium formosulum* Hoff., *C. impressulum* Elfs., *C. laeve* Rabenh., *C. meneghinii* Breb., *C. undulatum* Corda., *C. venustum* (Breb) Arch., *Crucigenia rectangularis* (A. Br.) Gay., *C. tetrapeda* W. et G. S. West., *Dictyosphaerium granulatum* Hind., *D. pulchellum* Wood., *Didymocystis planctonica* Korch., *Enteromorpha intestinalis* (L.) Link., *E. pilifera* Kütz., *Eudorina elegans* Ehr., *Gonium pectorale* Mull., *Micractinium pusillum* Fres., *Monoraphidium arcuatum* (Kors.) Hind., *M. contortum* (Thur.) Kom.-Legn., *M. irregulare* (G. M. Smith) Kom.-Legn., *Mougeotia sp., Oedogonium sp., Oocystis borgei* Snow., *O. solitaria* Wittr., *Pandorina morum* (Mull.) Bory, *Pediastrum boryanum* (Turp.) Menegh., *P. boryanum var. longicorne* Reinsch., *P. duplex* Meyen, *P. simplex* Meyen, *P. tetras* (Ehr.) Ralfs., *Rhizoclonium hieroglyphicum* (Ag.) Kutz., *Scenedesmus acuminatus* (Lagerh.) Chod., *S. acutiformis* Schröd., *S. acutus* Meyen, *S. arcuatus* Lemm., *S. falcatus* Chod., *S. obliquus* (Turp.) Kütz., *S. quadricauda* (Turp.) Breb., *S. spinosus* Chod., *Schroederia robusta* Korsch., *Spirogyra rivularis* (Hass.) Rabenh., *Staurastrum gracile* Ralfs, *Stigeoclonium prostratum* Fritsch., *S. tenue* (Ag.) Kutz., *Tetraedron minimum* (A. Br.) Hansg., *T. caudatum* (Corda) Hansg., *T. triangulare* Korsch., *Treubaria crassispina* G. M. Sm., *Ulothrix subtilissima* Rab., *U. variabilis* Kutz., *Uronema confervicolum* Lagerh., *Zygnema sp.*

***Euglenophyta* Phylum:** *Euglena acus* Ehr., *E. limnophila* Lemm., *E. oblonga* Schmitz., *E. oxyuris* Schmardo., *E. polymorpha* Dang., *E. texta* (Duj.) Hubner, *E. viridis* Ehr., *Lepocinclis globosa* France, *L. playfairiana* Defl., *Phacus acuminatus* Stokes, *P. caudatus* Hübner, *P. curvicauda* Swir., *P. ovalis* Woronich., *Strombomonas acuminata* (Schmarda) Defl., *S. fluviatilis* (Lemm.) Defl., *Trachelomonas hispida* (Perty) Stein, *T. planctonica* Swir., *T. rotunda* Swir., *T. verrucosa* Stokes.

Thus, according to the number of species and varieties of algae observed in the river periphyton, dominate the diatoms, which list 104 species from 30 genera, belonging to 8 families, 4 orders and 2 classes. The representatives of the class *Pennatophyceae* with 97 taxa (93,3% of the total number of highlighted diatoms) have an important role in shaping the algocenosis. From this class the most numerous in species are the: *Naviculaceae* family with 45 species and varieties of algae; *Nitzschiaceae* – with 20 species; *Fragilariaceae* with 12 species and *Surirellaceae* – with 11 species. Frequently found in the periphyton have been the species of the genera *Cymbella, Gomphonema, Navicula, Pleurosigma, Pinnularia, Gyrosigma, Amphora, Bacillaria, Hantzschia, Nitzschia, Diatoma, Fragilaria, Synedra, Cymatopleura* and *Surirella*, which develop intensely, especially in the cold season.

Centrophyceae class is represented by 7 species (6,7%), belonging to 2 families - *Coscinodiscaceae* with 6

species and *Biddulphiaceae* with one species *Biddulphia laevis*.

The bacillariophyta *Biddulphia laevis,* usually lives in the sublittoral areas near the sea and in the river delta, where the river flows into the sea, where the mineralization level is increased. This species has previously been detected in the accumulation basin Cuciurgan. In the river Bic it was detected in the periphyton samples collected throughout the whole study area, especially from the station situated upstream from the city of Chisinau. It develops more intense during the warm season, in the benthos (on the surface of the mud), as well as on the surface of the submerged plants, submerged rocks, etc. It represents colonies of massive cells, with cylindric form, united in „zigzag" chains, having the walls strongly silicified. The cells have dimensions of 150-200 mm length (fig. 1).

On the second place according to the number of species are green algae with 73 taxa, belonging to 37 genera, 19 families, 6 order and 4 classes. Taxonomically reacher is *Chlorococcophyceae* class, with 38 species, or 52,1% of all species of this phylum. More numerous in species proved to be the families *Scenedesmaceae* (11 species) and *Chlorococcaceae* (6 species). The families *Coelastraceae, Micractiniaceae, Radiococcaceae* and *Treubariaceae* include only one species. The most representatives from *Chlorococcophyceae* class are met frequently in the river plankton [2; 8].

There were found 13 species of algae from the class *Ulothrichophyceae,* the majority belonging to *Chaetophoraceae* (5 species) and *Ulothrichaceae* (4 species) families. An important role in forming the algal biomass have the representatives of the family *Cladophoraceae* with the genera *Cladophora* and *Rhizoclonium,* which during the warm season form clusters of filaments attached to the substrate or swimming freely. Among their filaments, at the station located upstream of the city Chisinau, are frequently encountered species of the genera *Oedogonium, Stigeoclonium, Coleochaete, Chlorhormidium, Ulothrix, Uronema,* as well as *Enteromorpha,* producing a biomass of up to 3-4 kg/m^2 (fig. 2). Downstream from Chisinau, where the water is polluted, the periphyton total number in the warm seasons is 2-3 times lower than in the upstream from the city and constitutes 87500-109640 mln. cells/m^2 with a biomass up to 500-700 g/m^2.

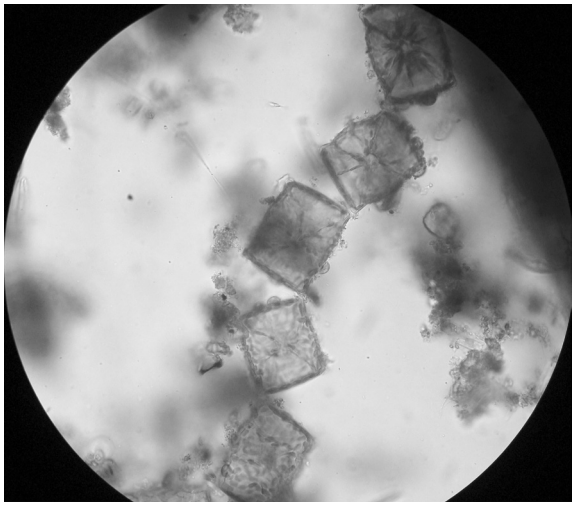

Fig. 1. Diatoms *Biddulphia laevis* 400 x

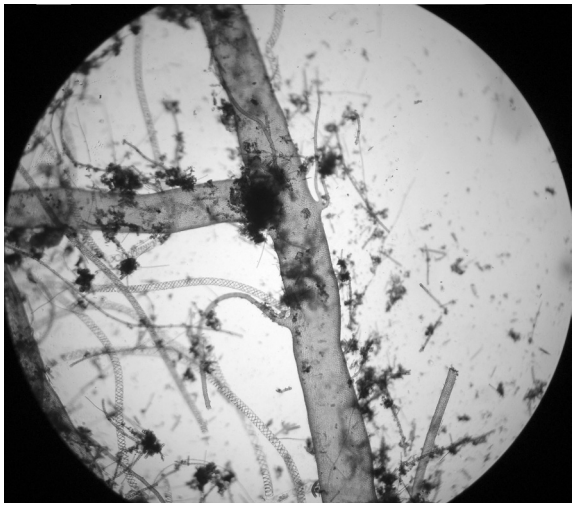

Fig. 2. Chlorophyta *Enteromorpha pilifera* 100 x

The *Zygnematophyceae* class includes 13 species of algae. The most numerous in taxa is the *Desmidiaceae* family (10 species), with the *Closterium, Cosmarium* and *Staurastrum* genera, whose species are frequently found in river plankton and in benthos. From the *Zygnemataceae* family there were highlighted species belonging to the genera *Mougeotia, Spirogyra* and *Zygnema,* which grow abundantly in the quiet flow places and among

thickets of aquatic higher plants.

The *Volvocophyceae* class includes 9 taxa of five genera, belonging to 2 families: *Volvocaceae* and *Chlamydomonadaceae* and to 2 order (*Volvocales* and *Chlamydomonadales*). During summer, the species of the genera *Chlamydomonas* and *Carteria* develop more intensely, which sometimes causes the phenomenon of "water blooming".

There were found 51 species and varieties of algae of the *Cyanophyta* phylum, which belong to 2 classes, 3 order and 14 families. Them most numerous in species is *Hormogoniophyceae* class, which includes 38 taxa or 74,5% of total number of cyanophyta highlighted in the river Bic periphyton. From this class, the most numerous in taxa is the family *Oscillatoriaceae* with 27 species, quantitatively predominating those of *Oscillatoria* and *Phormidium* genera, producing a biomass of about 40-70 g/ m^2. The species of the family *Anabaenaceae* were also developing abundantly, causing the phenomenon of "water blooming" (fig. 3). The *Homoeothrichaceae, Nodulareaceae, Rivulariaceae, Pseudonostocaceae* and *Schizothrichaceae* families, each include a single species and develop with abundance indexes of "rare" and "very rare". The Chroococcophyceae class is represented by 13 species, which are frequently found in the river plankton. More numerous is the *Merismopediaceae* family (4 taxa), while *Coelosphaeriaceae* and *Gomphosphaeriaceae* each include a single species. The *Cyanophyta* appear in periphyton in spring and develop intensely during the warm season.

The *Euglenophyta*, from the waters of the river Bic, including 19 species, belong to the *Euglenophyceae* class, *Euglenales* order and *Euglenaceae* family. Most of the species belong to the genera *Euglena* (7 taxa), *Phacus* (4) and *Trachelomonas* (4).

Euglenophyta together with *Cyanophyta* were forming on the surface of the water mud large colonies (120-200 cm^2, and in some places with the smooth water flow – 1,3 cm^2), mucilaginous, of 0,2-0,8 cm thick, having blue-green color. Fragments of these colonies, removed from water currents from the surface of the mud, were also met among thickets of aquatic higher plants.

Xantophyta and *Dinophyta* are present in the river Bic periphyton with three species each. These were highlighted in periphyton during summer, with abundance indexes of "rare" and "very rare", therefore have a minor role in determining the quantitative parameters of the river algoflora. Frequently are met only the species belonging to the *Tribonema* genera (fig. 4).

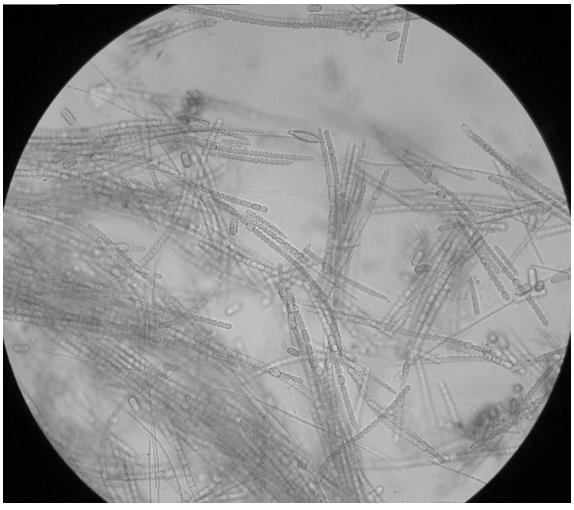

Fig. 3. Cyanophyta *Anabaena catenula* 400 x

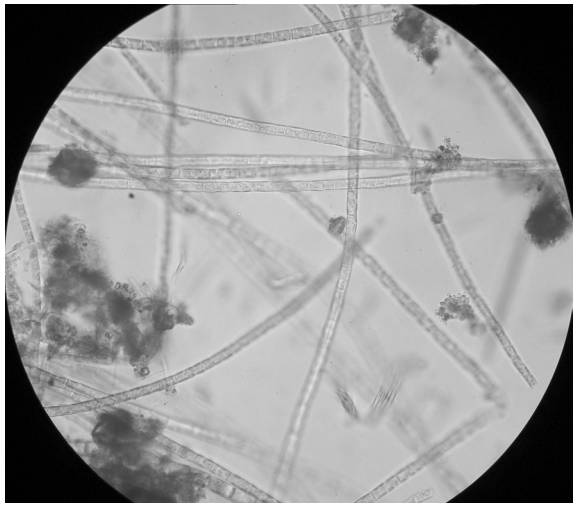

Fig. 4. Xanthophyta *Tribonema viride* 400 x

CONCLUSIONS

In the periphyton algoflora of the river Bic 253 species and varieties of algae were identified: *Cyanophyta* – 51, *Bacillariophyta* – 104, *Xanthophyta* – 3, *Dinophyta* – 3, *Chlorophyta* – 73 and *Euglenophyta* – 19. The species belong to 93 genera, 45 de families, 17 order and 12 classes.

Communities of algae develop abundantly on various types of substrates, forming a biomass from 40 g/m^2 up to 3-4 kg/m^2, with the prevalence of diatoms, clorophyta, cianophyta and euglenophyta. In the river develop intensely algal species resistant to increased concentrations of organic substances, such as the *Oscillatoria, Phormidium, Melosira, Cyclotella, Navicula, Nitzschia, Synedra, Carteria, Chlamydomonas, Chlorella, Gonium, Enteromorpha, Stigeoclonium, Euglena* genera etc., which sometimes cause the phenomenon of "water blooming". Downstream from the city of Chisinau, where the water is polluted, the quantity of periphyton is 2-3 times lower than upstream.

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GYMNOSPERM PLANTS OF THREATENED CATEGORIES' SPECIES IN THE CONIFERETUM OF GRISHKO NATIONAL BOTANICAL GARDEN

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Abstract: The Coniferetum of National botanical garden has been founded in 1945. As of 2017, there are plants of 66 species. These plants belong to five plant families (*Cupressaceae, Ephedraceae, Ginkgoaceae, Pinaceae, Taxaceae*) and 22 plant genera. In accordance with The IUCN Red List of Threatened Species, these species are facing a higher risk of global extinction Critically Endangered (CR), Endangered (EN) and Vulnerable (VU), and would be threatened without an ongoing taxon-specific conservation programme (Near Threatened (NT)). The threats are a combination of the extensive exploitation, the spread of the introduced pathogen and a result of climate change. In order to indicate the plants' growth characteristics, we use the diameter of trunks parameter (DBH - Diameter at breast height). There are eight trees from CR category, *Abies numidica* De Lannoy ex Carrière, which were planted in 1953 from seeds obtained from Adler (Russian Federation). Now these plants' DBH are 44 - 71 cm. The grafts of *Picea koyamae* Shiras. from the same category were selected in the arboretum "Goverla" (Ivano Frankivsk region) in 2006; now we have four under stocks. The EN category is represented by the following plants. Thirteen *Ginkgo biloba* L. trees from Bucharest, planted in 1949 with DBH 30-50 cm; ten trees from Odessa planted in 1956 with DBH 25-50 cm. One *Abies koreana* E.H.Wilson young tree purchased in 2009 from Belgium. 21 trees *Metasequoia glyptostroboides* Hu & W.C.Cheng from Lviv botanical garden were planted in 1964 and one tree from Leningrad (Saint-Petersburg) was planted in 1955. These plants' DBH are 25-50 cm. *Picea omorika* (Pančić) Purk., obtained from Germany in 1947, have DBH 28 cm. From the VU category there are three plants *Picea asperata* Mast. taken from Fomin Botanical garden (Kiyv) and planted in 1970; their DBH are 15-23 cm. Two trees *Picea likiangensis* (Franch.) E. Pritz. taken from Yalta in 1971, their DBH are 22,5 cm. *Picea breweriana* S.Watson young tree bought in 2009 from Belgium. Tree *Pseudolarix amabilis* (J. Nelson) Rehder from Beregomet dendropark (Chernivci region) planted from seeds in 2010. From the NT category there are seventeen plants *Chamaecyparis lawsoniana* (A.Murray bis) Parl. obtained from Uzhgorod in 1962; DBH 11-28 cm. There are six trees *Platycladus orientalis* (L.) Franco grown from China seeds since 1964 year with DBH 23-41 cm. Two plants *Chamaecyparis obtusa* (Siebold & Zucc.) Endl. grown from Hiroshima seeds since 2010. *Cryptomeria japonica* (Thunb. ex L. f.) D. Don taken in 1971 from Uzhgorod. Plants taken from Trostyanets dendropark comprise four trees *Pinus peuce* Griseb. obtained in 1966; DBH 22-46 cm, five trees *Tsuga canadensis* (L.) Carrière obtained in 1954; DBH 15-21 cm and *Thuja standishii* (Gordon) Carrière taken in 1952; DBH 35 cm.

Keywords: Gymnosperms, threatened plant species, Kyiv.

INTRODUCTION

In accordance to Christenhusz et al. [3], we consider each of the currently existing four gymnosperm groups (Cycadidae, Ginkgoidae, Gnetidae, Pinidae) as subclasses of class Equisetopsida. Overall there are about 1026 gymnosperms species: the three 'non-conifer' groups comprise ca 310 species of cycads in 10 genera, one extant ginkgphyte and 80–100 gnetophytes in three genera; there are a total of 615 species of conifers in 70 accepted genera [3]. About half of them are threatened species: Cycadidae - 255 species, Ginkgoidae - 1, Gnetidae - 11, Pinidae – 301 [17]. The threats are a combination of the extensive exploitation, the spread of the introduced pathogen and a result of climate change. The main reasons for that have nothing to do with the air pollution. These are issues accompanying the planet overpopulation and its impact on the natural environment: exploitation, conversion of woods to farmland and urbanization, degradation of woodland vegetation by excessive gathering of firewood, grazing of livestock, burning etc. What little is left after large-scale exploitation by the affluent portion of humankind is further destroyed by the larger and less well-off resident populations [4].

Botanic garden collections are also being proposed as refuge for threatened species to investigate and combat the effects of climate change. The subject of 'assisted migration' (intentionally relocating plants to new habitats) is a controversial one, but botanic gardens can assist by providing test sites for assisted migrations [16].

The Coniferetum of M. M. Grishko National botanical garden National Academy of science of Ukraine (NBG) has been founded in 1945. As of 2017, there are plants of 66 species. These plants belong to 22 plant genera from five plant families (*Cupressaceae*, *Ephedraceae*, *Ginkgoaceae*, *Pinaceae*, *Taxaceae*). Geographically,

the National botanical garden is located in Kyiv, a city with a continental climate. The coldest temperature ever recorded in the city was −32,9°C, the highest - +39,4°C. The average precipitation is 621 mm, the average annual temperature is +8,4°C [25].

MATERIAL AND METHOD

In accordance with The IUCN Red List of Threatened Species, these species are facing a higher risk of global extinction Critically Endangered (CR), Endangered (EN) and Vulnerable (VU), and would be threatened without an ongoing taxon-specific conservation programme (Near Threatened (NT)) [10]. Resistance to the lower temperature limits is showed according to The Gymnosperm Database (2015) [19]. In order to indicate the plants' growth characteristics, we use the diameter of trunks parameter (DBH -Diameter at breast height).

RESULTS AND DISCUSSIONS

The Coniferetum's seventeen species are threatened in the natural habitat. The basic information about this issue is presented in the Table 1.

Table 1. Main information about threatened categories species plants in the Coniferetum

Species	In the natural sites		In the garden		
	Threat-ened categories	Resistance to the lower temperature limits	Year of planting	DBH	Number of plants
Abies numidica de Lannoy ex Carrière Algerian Fir	CR	-28.8°C	1953	44-71	8
Picea koyamae Shiras. Koyama's Spruce	CR	-28.8°C	2006	-	4
Ginkgo biloba L. Maidenhair Tree	EN	-34.3°C	1949 1956	30-50	23
Abies koreana E. H. Wilson Korean fir	EN	-28.8°C	2009	-	1
Metasequoia glyptostroboides Hu &W. C. Cheng Dawn Redwood	EN	-28.8°C	1955; 1964	25-50	21
Picea omorika (Pancic) Purk. Serbian spruce	EN	-28.8°C	1947	28	1
Picea asperata Mast. Dragon Spruce.	VU	-23,2°C	1970	15-23	3
Picea likiangensis (Franch.) E. Pritz. Likiang Spruce	VU	-12,1°C	1971	22,5	2
Picea breweriana S.Watson Brewer's Spruce	VU	-23.2°C	2009	-	1
Pseudolarix amabilis (J. Nelson) Rehder Chinese Golden Larch	VU	-23.2°C	2010	-	1
Chamaecyparis lawsoniana (A. Murray bis) Parl. Port-Orford-cedar	NT	-23.2°C	1962	11-28	17
Platycladus orientalis (L.) Franco Oriental Arbor-vitae	NT	-23.2°C	1964	23-41	6
Chamaecyparis obtusa (Siebold & Zucc.) Endl. Hinoki Cypress	NT	-23.2°C	2010	-	2
Cryptomeria japonica (Thunb. ex L. f.) D. Don Japanese Cedar	NT	-23.2°C	1971	-	1
Pinus peuce Griseb. Balkan Pine	NT	-28.8°C	1966	22-46	4
Tsuga canadensis (L.) Carrière Eastern Hemlock	NT	-34.3°C	1954	15-21	5
Thuja standishii (Gordon) Carrière Japanese Arbor-vitae	NT	-23.2°C	1952	35	1

Algerian fire's nature side is located on the Petite Kabylie Mountain range which runs parallel to the Mediterranean coast of Nord Africa. The total extent of occurrence of forests containing *Abies numidica* is estimated to be less than 30 km^2. The actual area of occupancy is estimated to be less than 1 km^2. Even though the area is protected by a Djebel Babor Nature Reserve, there has been a continual loss in nature side and a decline in the quality of habitat as a result of forest fires, collection of firewood and grazing. These species are therefore listed as Critically Endangered [22]. These species' plants are found in the botanical collections only in Ukraine. Trees *Abies numidica* were planted from seeds obtained from Adler (Russian Federation) in the NBG. Periodically these plants produce cones with empty seeds.

Koyama's Spruce is Endemic to Central Honshu, Japan; Prefectures: Nagano (Akaishi Mountains) and Yamanashi (Yatsugatake Mountains). *Picea koyamae* is a relic species that is at the southern extremity of the range for the genus. It has an extent of occurrence of a less than 100 km^2. Historical events such as logging, fire, typhoons and landslides have resulted in the relatively small, fragmented stands. Recent genetic studies indicate that there is little genetic exchange between these populations. A change in precipitation, snowfall, temperature, and the frequency of severe weather events associated with climate change is also having an effect on the quality of habitat and regeneration. There has been a reduction of the area of occupancy and there continues to be a loss of mature individuals. This species is therefore listed as Critically Endangered [12]. Plants of this species are very rare in the Ukrainian dendrological collections. The grafts of *P. koyamae* were selected in the arboretum "Goverla" (Ivano Frankivsk region) in 2006; now we have four under stocks.

Maidenhair Tree is the only species in the genus. It has an ancient geological record, appearing in the Jurassic [18]. Extant *Ginkgo* populations in the Dalou Mountains (SW China) represent fragments of the original natural *Ginkgo* range. Among 572 individual trees of *G. biloba*, 33 had ages of 366–878 years. The ratio of female to male adult trees of *G. biloba* is 3 to 2. A key factor in the survival of the ancient *Ginkgo* forests must have been the belief in feng shui [19].The species has been widespread in cultivation for several centuries. NBG contains thirteen *G. biloba* trees from Bucharest planted in 1949, ten trees from Odessa planted in 1956 with DBH 30-50 cm. Only four trees are female; each year they produce germinal seeds.

The Korean Fir, endemic to South Korea, has an estimated area of occupancy of about 12 km^2. It occurs in four fragmented locations on the mainland and on the remote Jeju Island. The distances between each location range from 40–250 km and are likely to be too great to allow for effective gene flow. There is clear and documented evidence of a continuing decline in the area of occupancy and quality of habitat due to a number of factors which include the effects of climate change, pathogen attack and, on Mountain Halla, the invasion of pines and bamboo (Sasa). For these reasons *A. koreana* has been assessed as Endangered [13].This fir is often used in ornamental horticulture. In the Coniferetum, there is one *A. koreana* young tree purchased in 2009 from Belgium.

Dawn redwood is endemic to central China. Metasequoia's extent of occurrence is well within the threshold for Endangered. Although there are 18 locations, the population is severely fragmented. There has been a recent decline in the quality of habitat and a number of mature individuals [7]. 21 trees *M. glyptostroboides* from Lviv botanical garden were planted in 1964, and one tree from Leningrad (Saint-Petersburg) was planted in 1955. The cons have formed annually on these trees, but the seeds never germinated.

Serbian spruce is restricted to four locations in the Tara National Park in Serbia and the almost adjacent areas in Bosnia and Herzegovina. There is a continuing decline in the extent of occurrence, area of occupancy, quality of habitat, and the number of mature individuals in some locations. This is primarily due to the poor regeneration and inability to compete with associated tree species. *Picea omorika* is widely grown in gardens in northern Europe. A well coordinated *ex-situ* conservation programme could play a significant role in conserving its genetic diversity. An extensive programme of seed-banking would also be advantageous [14].There is *P. omorika*, obtained from Germania in 1947 in the Coniferetum. Serbian spruce seed orchard has created in Kyiv region [26].

Dragon Spruce occurs in the high mountains of West central China (Sichuan) and is an important timber

tree in China.The logging ban should at least have slowed the reduction substantially, but the suspicion is that it has not stopped it entirely. The species as a whole is therefore considered to be Vulnerable [1].*Picea asperata* was taken from Fomin Botanical garden (Kiyv) and planted in 1970. These trees bear cons with periodically germinating seeds. It is damaged by a spider mite.

Likiang Spruce is from Bhutan and China. There has been more than a 30% population reduction in the past 75 years due to the impacts of logging. The Government of China has recently imposed a logging ban in western China [24]. Plants of this species are in some botanical gardens only. Two trees *Picea likiangensis* in the Coniferetum taken from Yalta in 1971.

Brewer spruce, a relict of the widespread Arcto-Tertiary forests, is now restricted to a highly fragmented range in the northwestern California and southwestern Oregon near the Pacific coast. Forest fires are the ongoing threat; these could increase if climate change causes longer spells of drought so fires would intensify. It is therefore projected that further decline will ensue, and this species therefore meets the criterion for Vulnerable [15].This species' plants don't grow in other Ukrainian botanical gardens. We have *Picea breweriana* young tree bought in 2009 from Belgium.

Chinese Golden Larches endemic to China: recorded from the lower Chang Jiang. It has a fairly restricted range and it is estimated that the area of occupancy may be less than 500 km², in which case it would qualify for Endangered. The population is severely fragmented and there is continuing decline in the quality of the habitat and number of mature individuals [23]. Plants of this species are rare in Ukrainian botanical collections. In the Coniferetum, there is a tree *Pseudolarix amabilis* from Beregomet dendropark (Chernivci region) planted from seeds in 2010.

Port-Orford-cedar is native to a limited area along the California Pacific Coast. Its range extends from the coast to about 50 miles inland. There is also a small disjoint population in the Scott Mountains of California. International trade of timber has previously put enormous pressure on the remaining old growth stands. The spread of the introduced pathogen *Phytophthora lateralis* continues and limits the successful regeneration in many areas. It is assessed as Near Threatened on the basis that its recent decline almost meets the criterion for listing as threatened [5]. Plants of this species are in all Botanical gardens and used in landscaping. Coniferetum contains the *Chamaecyparis lawsoniana* obtained from Uzhgorod in 1962. There are full seeds on the Chamaecyparis trees annually.

The original extent of occurrence of Oriental Arbor-vitae, before people planted them everywhere, is small and difficult to establish. From what is known, it is likely that mature trees in natural forests are quite rare. It is therefore most likely to be Near Threatened. This species native from China, Korea and Russian Far East. It is apparently quite widespread but the geographic and altitudinal range is confused by populations which may have been planted or have become naturalized [9]. *Platycladus orientalis* is used in landscaping in Central Ukraine but it can be damaged by low temperature. In the Coniferetum, it was grown from China seeds since 1964.

Hinoki Cypress is native from Japan. It has been assessed as Near Threatened in Japan (*var. obtusa*) and Vulnerable in Taiwan (*var. formosana*). The Japanese variety is the more widespread and numerous so the species as a whole is also assessed as Near Threatened, on the basis of a population decline which may approach 30% [6]. There are two trees *Chamaecyparis obtusa* grown from Hiroshima seeds since 2010.

Within Japan, the Japanese cedar natural distribution is discontinuous and scattered in limited areas as a result of the extensive exploitation of this species during the past 1,000 years. Yakushima is one of the few areas where natural forest still occurs and here there are many trees that are over 1,000 years old. Cryptomeria has also been identified as susceptible to changes in precipitation. Identifying and distinguishing between natural forests and plantations makes the calculation of an extent of occurrence and area of occupancy problematic. However, the extent of occurrence of the natural populations is likely to be within or close to the threshold for Vulnerable [21]. In Kyiv region, *Cryptomeria japonica* was damaged by low temperature. The plant taken in 1971 from Uzhgorod died from frost – 35°C in the winter of 2005-2006. Now, we have a young plant bread by

cuttings from the deceased.

Balkan Pine is probably a Tertiary relict that has survived severe contractions of its range due to Alpine glaciations during the Pleistocene. Its current range consists of two disjoint populations, one in the west centered in Albania and one in the east in West Bulgaria. Its extent of occurrence is large, but its area of occupancy is below the threshold for Vulnerable. Balkan Pine, in contrast with several other species, is resistant to the pathogenic fungus *Cronartium ribicola* (Basidiomycota). In horticulture, it has long been used [8]. In the Coniferetum, there are four trees *Pinus peuce* obtained in 1966, from Trostyanets dendropark (Chernigiv region). These trees annually produce quality seeds.

Eastern Hemlockis recorded from eastern North America. It is very widespread and abundant; occurs in many forests. An introduced insect pest, the Hemlock Woolly Adelgid (*Adelges tsugae*), is causing substantial dieback in many areas. Moderating temperatures associated with climate change could allow the spread of this pest into areas where it has so far been prevented from infesting due to low winter temperatures. This causes the flagging of this species as Near Threatened [10]. Five trees *Tsuga canadensis* obtained in 1954 from Trostyanets dendropark (Chernigiv region) are in the Coniferetum. They have produced empty seeds every year. We have not observed any insects on these plants.

The protected status in the historical past of Japanese Arbor-vitae in Japan is assumed to have had a positive effect preventing over-exploitation. However, its valuable timber must have led to some reduction in abundance of mature trees [12]. Coniferetum possesses the *Thuja standishii* taken in 1952 from Trostyanets dendropark. It breads cons annually.

CONCLUSIONS

Seventeen from sixty six species of Coniferetum (25%) have the threatened status in the natural habitat.

Plants of eighth species (*Abies numidica, Ginkgo biloba, Metasequoia glyptostroboides, Picea asperata, Chamaecyparis lawsoniana, Platycladus orientalis, Pinus peuce, Tsuga canadensis*) are in group (3 and more trees) and they could produce full seeds but *Tsuga canadensis* and *Abies numidica* bear cons with empty seeds.

Trees of five species (*Ginkgo biloba, Abies koreana, Picea omorika, Chamaecyparis lawsoniana, Platycladus orientalis*) are used in different type of landscaping. The plants of these species are more common in the culture than in the natural habitats

A spider mite inhabits *Picea asperata* and *Tsuga canadensis* hence weakens them. These plants need special care.

Plants of five species (*Picea asperata, P. likiangensis, Chamaecyparis lawsoniana, Platicladus orientalis, Thuja standishii*) demonstrated the resistance to the lower temperature than -23°C listed in [20]. The only exception is *Cryptomeria japonica*, which did not survive -35°C in the winter of 2005 - 2006.

Pinus peuce is not damaged by *Cronartium ribicola* and it is best for landscaping compared to other five-needled pines.

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III. INTRODUCTION OF PLANTS AND SUSTAINABLE USE OF PLANT RESOURCES

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PHENOLOGICAL STUDIES OF MEDICINAL *TEUCRIUM* L. (*LAMIACEAE*) SPECIES IN THE BOTANICAL GARDEN (INSTITUTE) OF ASM

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Abstract: This paper refers to four medicinal *Teucrium* L. (*Teucrium flavum*, *T. hircanicum, T. polium* and *T. chamaedrys*) species cultivated at experimental fields in the Botanical Garden (Institute) of ASM. Phenological studies of investigated species under *ex situ* conditions have been undertaken. Under the conditions of cultivation, the rhythm of seasonal development of spontaneous *T. polium* and *T. chamaedrys* is generally the same as in natural habitat. During three vegetation seasons, allochthonous *T. flavum* and *T. hircanicum* species proved an excellent growth with high vegetative expansion and abundant flowering.

Keywords: medicinal plants, *Lamiaceae*, *Teucrium*, *ex situ* experiment, phenological phases.

INTRODUCTION

Teucrium L. (*Lamiaceae* family) is a large genus including more than 300 species widespread on all continents of the world, from which almost 50 species are distributed in Mediterranean region [6, 31]. In the flora of the Republic of Moldova *Teucrium* L. genus is represented by 6 species: *Teucrium chamaedrys* L., *T. montanum* L., *T. pannonicum* A. Kerner, *T. polium* L., *T. scordium* L. and *T. scordioides* Schreb. [20]. Previous field investigations, survey of scientific references and the ethnobotanical studies allowed identifying the main important and usable species of this genus, naturally growing in the republic [9, 10].

The members of this genus represent mostly perennial herbs, shrubs or subshrubs. They have erect or ascending, leafy stems. Leaves petiolate or sessile, not divided, with subentire to crenate-dentate margins. Inflorescence – pedunculate cymes or verticillasters, distant or condensed into spikes or heads. Nutlets ovoid or obovoid rounded.

A large number of species belonging to the genus *Teucrium* L. are used in folk medicine. They are mainly used in treatment of digestive disorders and respiratory problems. Externally some *Teucrium* species are very useful in the treatment of diverse skin diseases, such as purulent skin eruptions, furuncles, wounds, mycosis, and skin abscesses. A large number of compounds such as iridoids, sescvi-, di- and triterpenoids, flavonoids, steroids, carbohydrates, phenols, phenolic acids, amino acids, tannins, vitamin C, coumarins with a wide spectrum of biological properties have been isolated from the *Teucrium* L. plants [12, 25, 26, 33, 35, 36]. These constituents have been found to have hypoglycaemic, cicatrizing, hypolipidemic, hemostatic, diuretic, antispasmodic, anti-inflammatory, detoxifying, antiseptic, purgative, antifungic, antibacterial, antioxidant effects [1, 8, 24, 30]. Modern pharmacological studies suggest that some *Teucrium* L. species are found to be rich natural sources of new anticancer compounds. Their plant extracts possess strong anticancer activity [28].

The essential oil studies of several *Teucrium* L. species (*T. polium, T. chamaedrys, T. arduini, T. flavum, T. hircanicum*) have been performed [14, 16, 19]. These oils are characterized by the presence of caryophyllene, caryophyllene oxide, α-humulene, germacrene D, α-murolene, (E)-β-farnesene, carvacrol [4]. The essential oil of *Teucrium* L. species possesses antimicrobial [15], antioxidant [11, 18], antiphytoviral [4] and antibacterial properties [23].

Along with medicinal value, many *Teucrium* L. due to antimicrobial, antioxidant and antifungal activities,

are useful as natural preservative ingredients very demandable today in food industry. *T. polium* has also an important insect antifeedant activity against different species of insects [5]. In addition, some *Teucrium* L. species are valued as ornamental plants and an important pollen source. All these data reveal a significant medicinal and economic importance of *Teucrium* L. species such that in recent decades they are the subject of different studies.

MATERIALS AND METHODS

Phenological studies of four medicinal *Teucrium* L. (*Teucrium flavum*, *T. hircanicum, T. polium* and *T. chamaedrys*) species have been undertaken. These new (for our collection) species have been obtained from seeds received by international seed exchange (*Teucrium flavum* and *T. hircanicum*) and from spontaneous flora (*T. polium* and *T. chamaedrys*). Voucher specimens are lodged in the Herbarium of Botanical Garden (Institute) of ASM. An extensive literature survey of studied species was made according to their therapeutic importance and utilization in popular and modern medicine. The experiments aimed at seed germination were performed in February – March. The seeds received by *Delectus Seminum* (*Teucrium flavum* and *T. hircanicum*) were sown in special substrate under greenhouse conditions and in Petri dishes in laboratory conditions.

The *ex situ* experiment was conducted during three vegetation periods (years 2014-2016) at Experimental Field of the Botanical Garden (Institute) of ASM, geographically located at N 46° 58' 25.43", E 28° 52' 47.16". Climate data regarding the years during which the experiment was conducted are provided in Table 1 (State Hydrometeorology Service). The soil textual class of the experimental area is chernozem.

Table 1. Temperature and rainfall data for 2014, 2015, and 2016 years

Year and period		Mean Temp. (°C)	Max. Temp. (°C)	Min. Temp. (°C)	Total rainfall (mm)
2014	spring	+10.9..+12.4	+32	-7	160-205
	summer	+19.3..+22.4	+39	+6	140-260
	autumn	+9.0..+11.5	+33	-13	100-190
2015	spring	+9.9..+11.4	+31.4	-6.8	100-140
	summer	+21.6..+23.8	+38.3	+7	80-160
	autumn	+10.4..+12.7	+38.6	-8	100-200
2016	spring	+10.9..+12.2	+30.6	-7.2	108-138
	summer	+20.8..+23.0	+37.0	+3.5	160-260
	autumn	+8.5..+11.1	+34.0	-9	115-220

The experimental design used was randomized complete blocks, where seedlings (*T. flavum* and *T. hircanicum*) at the 2-6 true leaf stage were transplanted into the beds at experimental field. For *T. polium* and *T. chamaedrys* were chosen locations with similar conditions as in natural habitats.

The phenological observations were registered using standard methods [27, 32, 34]. The following phenological stages (initial growth, budding, start of flowering, full flowering, and seed maturity) were recorded on a weekly basis.

RESULTS AND DISCUSSIONS

***Teucrium flavum* L**. is an evergreen, dense sub-shrub widespread in the Mediterranean region growing on rocky sites, but also on roadsides and wasteland.

Figure 1. *Teucrium flavum*

Commonly named Yellow Germander, *T. flavum* (figure 1) is a perennial, densely branched shrub with flower stems up to 50 cm high, woody at the base. The leaves opposite, ovate, with serrate margins, dark green, glossy above. In inflorescences, the hermaphrodite flowers are arranged in whorls. The calyx is 8-9 (circa 6) mm long, hispid to villous hairy, covered with glands. The individual calyx lobes are about two-thirds as long as the tube, triangular and provided with a distinct midrib. The corolla is cream to lemon-yellow, sparsely hairy. Fruit – schizocarp consisting of four ovoid nutlets.

The essential oil studies of *T. flavum* are reflected in literature data [11, 16]. Its constituents have been found to have antioxidant [11], antidiabetic [12], cardiovascular [7], analgesic and CNS actions [3].

***Teucrium polium* L.**, known popularly as Golden Germander (figure 2), is a sub-shrub native to the Mediterranean region and southwestern Asia. In the spontaneous flora of the Republic of Moldova is distributed throughout the country occurring in steppe habitats, on stony-calcareous, dry slopes [17].

Teucrium polium is a perennial plant with tomentose, lanate or rarely pilose-hispid stems reaching about 30-50 cm in height. The opposite, simple, sessile, cuneate-oblong or linear leaves are about 0.7-2.5 cm in length. White to pale cream-colored flowers form simple paniculate or corymbose inflorescences. The seeds are brown, reticulate nutlets.

The traditional uses of the *T. polium* include the treatment of fever and stomach pain in children, hypertension, bacterial infections, inflammation, anorexia and diarrhea [29]. Crushed leaves are applied as compresses for wounds [26]. The plant is also used to flavor alcoholic drinks with a bitter base which have digestive qualities.

Scientific reports have indicated that *T. polium* extracts possess antioxidant [12, 18], hypoglycaemic [8], antimicrobial [15], antibacterial [2, 23] hypolipidemic [24], anti-inflammatory [30], antispasmodic and antinociceptive [21] and analgesic [13] effects.

***Teucrium chamaedrys* L.** (Wild Germander) is an aromatic and medicinal plant widespread from Central Europe to Asia and North Africa. It is naturalized in the United States.

In the flora of Republic of Moldova *T. chamaedrys* (figure 3) is widely distributed throughout the country occurring in habitats with forest-steppe vegetation, forming small groups in grassy openings of arid types of forests [17, 20].

Figure 2. *Teucrium polium*

Figure 3. *Teucrium chamaedrys*

Figure 4. *Teucrium hircanicum*

Teucrium chamaedrys L. is a perennial, pubescent, evergreen plant with a creeping rootstock and upright to spreading stems that reach about 10-30 cm in height. The opposite oblong-ovate, deeply veined, pubescent, dark green leaves, approximately 1.3-3.8 cm long, resembling small oak tree. The tubular, labiate, rose-colored flowers are arranged in axillary whorls on leafy, terminal spikes. The seeds are ellipsoidal nutlets, 1.0-1.8 mm long with dark reddish-brown and reticulate surface.

T. chamaedrys has been used medicinally since ancient Greek time. It is a bitter, astringent plant that reduces inflammation; stimulates the digestion and lowers fever. It has antiseptic, diuretic and decongestant effects. Internally it is used for loss of appetite, gall bladder, digestive disorders, diarrhea, rheumatoid arthritis and bronchitis. Externally is used for skin eruptions and injures. Leaves are used for flavor liqueurs, vermouths and tonic wines.

Modern scientific reports have indicated that *T. chamaedrys* essential oil possess antimicrobial, analgesic and anti-inflammatory effects [14, 22].

***Teucrium hircanicum* L.** (Caucasian Germander) is native to western Asia and Caucasus region. It is a perennial species (figure 4) with hairy stems, woody at base. Leaves are ovate or oblong-ovate, crenate; base truncate to cordate; apex obtuse. Inflorescence is a dense terminal spike-like raceme circa 15 cm long; bracts subulate, pedicels 1-2 mm long. Calyx 3-4 mm long, campanulate, densely hairy, glandular-scaly, prominently veined at fruiting. Corolla tube 3-5 mm long, hairy; limb deflexed and concave, hairy outside, reddish purple; terminal lobe approximately 3-4 mm in diameter, rounded; lateral lobes 1-1.5 mm long, ovate, rounded at apex. Nutlets circa 1 mm long, wider than long, brownish, reticulate.

Unlike *T. flavum, T. chamaedrys* and *T. polium*, *T. hircanicum* was less studied for its applicability in phytotherapy but it deserves attention in sense of more phytochemical and pharmacological researches, because of neo-clerodane diterpenoids with antifeedant activity [5, 14].

The phenological studies on all four species of *Teucrium* showed, that the resting period under *ex situ* conditions is observed during winter and spring (from October to March). During the all three vegetation periods there was not noted significant difference in the starting and duration of phenological phases of *Teucrium* L. species. For the 2015 vegetal period plants were less vigorous and with lower values of morphometric parameters due to the weather conditions (lesser precipitation level and higher temperature during June and July) (table 1).

The shoot regrowth is observed in the last decade of March (*T. polium, T. hircanicum* and *T. chamaedrys*) and in the first decade of April for *T. flavum*. The period from regrowth to the start of generative period of investigated species varies within 57 to 76 days.

For *T. chamaedrys* and *T. polium* the budding phase occurs during May and June (table 2) and lasts for about 20 days. In the second decade of June *T. chamaedrys* starts flowering. *T. polium* starts flowering a decade later. Mass flowering of both *T. chamaedrys* and *T. polium* species under *ex situ* conditions is observed in June – July and duration of flowering stage lasts for 36 to 40 days, respectively.

Table 2. Phenological spectrum of Teucrium L. species (2014-2016)

	March			April			May			June			July			August			September		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
T. chamaedrys			▒	▒	▒	▒	▒	▒	▒	▒	▓	▓	▓	▓	◙	◙	◙	◙			
T. polium			▒	▒	▒	▒	▒	▒	▒	▒	▒	▓	▓	▓	▓	◙	◙	◙	◙		
T. flavum				▒	▒	▒	▒	▒	▒	▒	▓	▓	▓	◙	◙	◙					
T. hircanicum			▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▓	▓	▓	▓	◙	◙	◙		

Legend: ▒ - immature and virginal stages; ▒ - budding; ▓ - flowering stage; ◙ - seed maturation.

The phenologic observations and morphologic measurements demonstrate an intensive vegetative growth with abundant flowering for *T. chamaedrys* and *T. polium* species. It was revealed that under the conditions of cultivation, the rhythm of seasonal development of *T. polium* and *T. chamaedrys* is generally the same as in natural habitat. The difference is that compared with plants from spontaneous flora, plants from *ex situ* cultures are more vigorous and with higher values of morphometric parameters.

The rhythm of seasonal development of *T. flavum* differs from that of other investigated species. *T. flavum* plants instead showed depressed growth, the plants survived vegetatively, but did not flower during the first growing season. In the second vegetation period, *T. flavum* growth was excellent with high vegetative expansion and abundant flowering. After three vegetation seasons, they proved to be a very hardy plant tolerating summer drought periods. Perennial individuals start growing in the first decade of April. The budding stage of *T. flavum* occurs at the end of May and beginning of June. Total duration of budding stage is 12-14 days (figure 5).

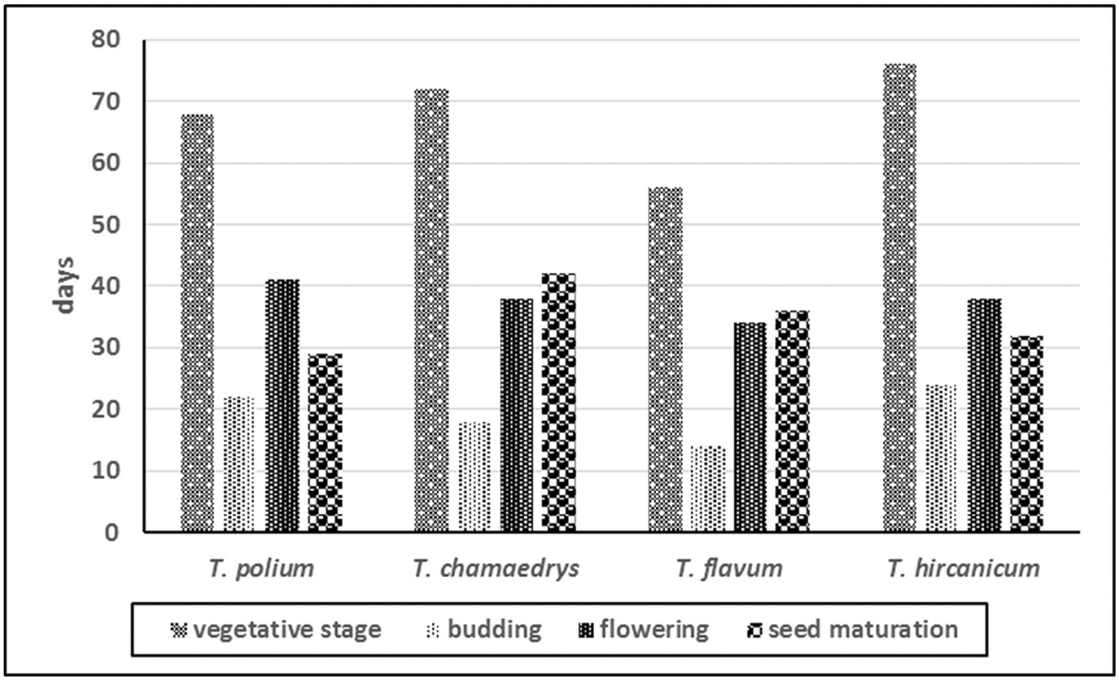

Figure 5. Duration of phenological phases

Flowering is observed from the middle of June, so that it becomes extensive during June till the middle of July. The flowering stage lasts for about 30-34 days. In the second decade of July, the plants simultaneously enter the fruitage stage. Complete ripening of seeds is observed within 26-35 days; in early and middle of August, the top part of the plants gets withered completely. The presence of generative phase, including full maturing of seeds indicates good adaptation of *T. flavum* plants to new climatic and edaphic conditions.

It was observed that *T. hircanicum* plants grew slowly in the first year of establishment and started to flower at the end of July. In the first vegetation period, plants have not reached the stage of seed maturity. The second year onwards the regrowth of *T. hircanicum* plants under introduction conditions was observed in the third decade of March and realized the entire ontogenetic cycle. The budding stage occurs in the second decade of June. Flowering period is observed from the beginning of July till the beginning of August and lasts for about 28 days. A specific feature of adaptation is increasing of the duration of vegetation phase and shortening of the duration of budding stage.

CONCLUSIONS

Phenology is one of the important phenomenon in the life cycle of the plant species. The investigation of phenological phases, their duration and character allows determining the adaptability of plants to the new climatic conditions. In our study, an intensive vegetative growth with abundant flowering under *ex situ* conditions was observed for *T. chamaedrys* and *T. polium* species. It was also revealed that, under the conditions of cultivation, the rhythm of seasonal development of spontaneous *T. polium* and *T. chamaedrys* is generally the same as in natural habitat. *T. flavum* and *T. hircanicum* species instead showed depressed growth during the first growing season in the next vegetation periods they proved an excellent growth with high vegetative expansion and abundant flowering. After three vegetation seasons, the plants proved to be very hardy, tolerating summer drought periods.

Including the studied *Teucrium* L. species in our collection, we will improve the assortment with new medicinal plants, which through their pharmacological activity would be appropriate not only for research but also for food and pharmaceutical industries.

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ADVANTAGES OF ECONOMIC CULTIVATION OF THE GENUS *RHEUM* PLANTS IN THE REPUBLIC OF MOLDOVA

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Abstract: Every third medical product in the world market is an herbal preparation. Preparations from the roots of medicinal genus *Rheum* species, *Polygonaceae* family, for the treatment of cardiovascular, gastrointestinal diseases, and as antitumor agents are used. The food-consumed petioles of the vegetable rhubarb are used in the food industry. Vegetable rhubarb petioles contain 1,0% organic acids, from 1,0 to 2,5% pectin substances and up to 3,2% of dietary fiber, as well as minerals and vitamins. The vegetable rhubarb petioles consumed fresh, in canned, dried, frozen form and are used for the preparation of a products variety with a high vitamin value. In our research, we used biologically active phenolic compounds (emodin, resveratrol, quercetin) of extracts from the roots and leaves of *R. raponticum* plants harvested in the period 2012-2016. In order to obtain practical data on the spectrum of action of *Rheum* extracts, the species belonging to different systematic groups were used as test objects: *Mycota - Fusarium moniliforme* Sheldon; *Fusarium graminearum* Shwabe, *Fusarium sporotrichiella* Bilai, *Sphaerotheca fuliginea* Poll. Extracts exhibit fungicidal and stimulatory properties. In view of the variety of possibilities for the economic use of *Rheum* plants, the simplicity of the agricultural techniques of cultivation and reproduction and the absence of significant losses from diseases and pests, we can recommend the creation of rhubarb plantations and its complex processing in the Republic of Moldova.

Keyworlds: *Rheum*, non-waste processing technology, medicinal and food plant, plant protection

INTRODUCTION

Every third medical product in the world market is an herbal preparation. Preparations from the roots of medicinal genus *Rheum* species, *Polygonaceae* family, for the treatment of cardiovascular, gastrointestinal diseases, and as antitumor agents are used. It should be noted that the plant of the genus *Rheum* is used not only in medicine, but also in the food industry. So, Katserikova N. V. (2003) found that the dye extract from rhubarb root has bacteriostatic, antioxidant and antiseptic properties that allow increasing the shelf life of food products and improving their quality in comparison with traditional ones (16). Vegetable rhubarb petioles contain 1,0% organic acids, from 1,0 to 2,5% pectin substances and up to 3,2% of dietary fiber, as well as minerals and vitamins. The vegetable rhubarb petioles consumed fresh, canned, dried, frozen form and are used for the preparation of a products variety with a high vitamin value. The yield of petioles of rhubarb for the first 3-5 years is 4-6 t/ha, for the 6-7th year it reaches to 25-30 t/ha and more (18).

Phenolic compounds are valuable chemotaxonomical markers of the entire P*olygonaceae* family, and the synthesis of these various low molecular weight substances is a characteristic feature of their metabolism (15). Samples of *R. rhaponticum* roots and petioles have recently been studied at the Institute of Pharmacology (Tartu, Estonia) on high-tech modern equipment. According to the results of the analysis, the rhubarb root contains anthracene derivatives (4.3-4.7%), tannins, resins. Blossoms and leaf blades can be a valuable source of flavonoid – quercetin (8).

In the composition of *R. rhaponticum* chemical raw materials, which we used in our experiments of 2013 - 2016, we should single out 3 key bioactive phenolic substances - emodin (pesticide, antifidant, bactericide, fungicide), resveratrol (phytoalexin, bactericide, fungicide) and quercetin (antifidant for flying insects, pesticide, bactericide, antiviral). The deterrent and insecticidal properties of these substances were used in their studies by M. Mehrabadi (2011), Xiao-Yun Wang (2005) (7, 14). The bactericidal and fungicidal properties of rhubarb extracts were successfully used by Magda M. Aly (2011), Babu K. S. (2003), Singh U. P. et al. (1992), Xiaojun Yang (2009), A. P. Keinath (2012), Chu, Y. L., (2006) (5, 1, 10, 13, 4, 2). We also found examples of *Rheum* extract biologically active substances applying as inducers of plant immunity (12, 3, 9). The least studied direction was the effect of *Rheum* extracts on seed germination, with pre-sowing treatment.

The plant of *R. rhaponticum* is a large, unpretentious plant, that can grow for about 15 years in one place. Seed and vegetative reproduction are used to obtain the planting material of rhubarb. The newest method of rhubarb reproduction is connected with the method of cell culture. Researchers from different countries develop and improve the cell culture media, elicitors and technologies for obtaining *Rheum* plants in vitro with given properties, as well as for biodiversity conservation of medicinal plant species belonging to this genus (11, 6).

The aim of our study was to determine the possibility of using an *R. raponticum* root extract in the plant protection.

MATERIAL AND METHOD

The subject of our research was the bioactive substances of *R. raponticum* plants. Anatomical and morphological analysis of the roots of *R. raponticum*, in order to identify the diagnostic features of this type of plant raw materials, was carried out according to standard methods (15). To identify the localization of anthracene derivatives, methods of microscopy and the following histochemical reactions were used: 1) with Lugol's solution for starch and starch grains; 2) with a 33% aqueous solution of sodium hydroxide on the cell cork shell.

We used ethanol extraction of rhubarb roots, to maximize the release of active substances (tannins, phenols and flavonoid) from plant raw materials. The component composition of ethanol extracts from the *R. rhaponticum* roots was investigated by liquid chromatography (HPLC): Agilent 1100 Diode Array Detector = HPLC, Agilent 1100, detector DAD. To determine the spectrum of the extract action, we used test objects: *Mycota - Fusarium moniliforme* Sheldon; *Fusarium graminearum* Shwabe, *Fusarium sporotrichiella* Bilai, *Sphaerotheca fuliginea* Poll.

Determination of the antimicrobial effect of the *R. rhaponticum* extract phenolic compounds against pathogenic fungi of the genus *Fusarium* was carried out by the method of paper discs on a nutrient medium. Diameters of pathogen inhibition zones were measured and calculated the average values.

The effect of presowing treatment with *Rheum* root extract in various concentrations on the germination of soybean seeds (*G. max*) «CLAVERA» variety, and maize (*Z. mays*) P 280 variety, was determined by standard analytical methods in rolls of filter paper.

The biological efficacy of the *R. raponticum* root extract for powdery mildew (*Sphaerotheca fuliginea*) control was determined by treating the seedlings (f. *Cucurbitaceae*) with *R. raponticum* root extract at concentrations of 0,2% and 0,5%. At the stage of 4 real leaves, the seedlings were treated, and then infected with a *Sph. fuliginea* conidia suspension. Infected plants were placed in randomized blocks of the greenhouse at a temperature of 25 to 28°C. The degree of damage (in points of areas covered by colonies) was assessed according to the standard method. Mathematical processing of the obtained data was carried out according to the method of ANOVA.

RESULTS AND DISCUSSIONS

Our anatomical and histological analysis of the *R. raponticum* roots revealed features of localization of anthracene derivatives - in the primary parenchyma of the secondary cortex and parenchyma of the core rays (Fig. 1, 2).

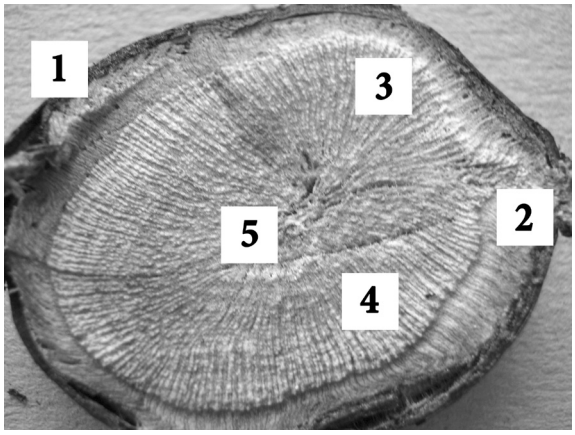

Fig. 1. Cross section of the root of R. raponticum
1 - cork;
2 - parenchyma of the cortex;
3 - cambium;
4 - vessels of xylem;
5 - primary xylem.

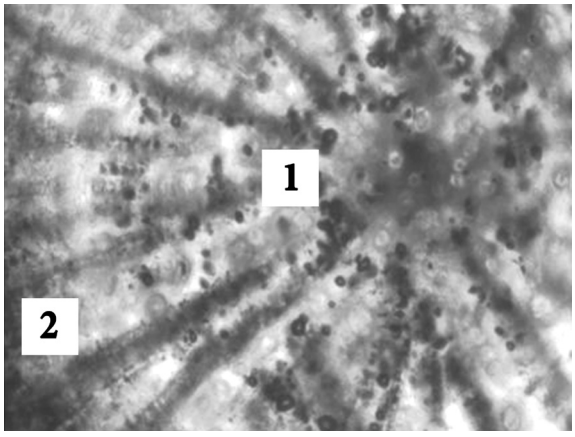

Fig. 2. Fragment of the *R. raponticum* root
1 - parenchyma of the core rays
2 - parenchyma of the crustal part of the root, individual cells of which are colored red, which is due to the presence of anthracene derivatives

Using a qualitative analysis of the extract from the *R. rhaponticum* root, we determined the lower limit of anthracene content of the derivatives, which was not less than 4.0%.

As a result of our studies to determine the antimicrobial effect of the root extract, the sensitivity of the genus *Fusarium* fungi to the extract in several concentrations (V1- 80%, V2- 50%, V3- 10%) was noted. We found that the formation of sterile zones of pathogens growth inhibition occurred, whereas in control and in V4 (1%), the pathogen growth was noted throughout the surface of the agar plate. The average diameter of the sterile zone for pathogens affecting maize - *F. moniliforme* in V1 was 6,5 mm, *F. graminearum* in V1 is 7,3 mm, *F. sporotrichiella* is 22,4 mm (Fig. 3, 4, 5).

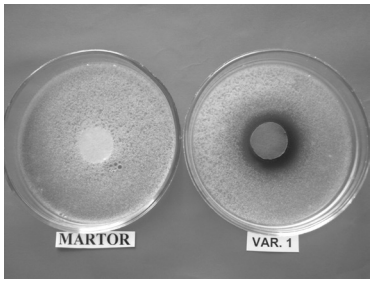

Fig.3. Fungicidal effect of rhubarb extract on the pathogen *Fusarium moniliforme*

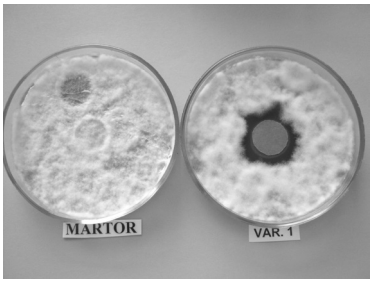

Fig. 4. Fungicidal action of rhubarb extract on the pathogen *Fusarium graminearum*

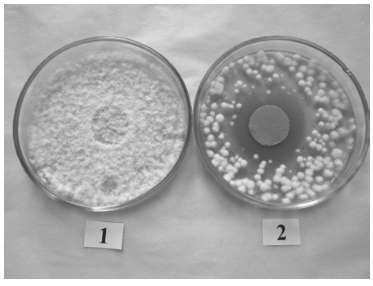

Fig. 5. Fungicidal action of the rhubarb extract on the pathogen *Fusarium sporotrichiella*: 1 - control, 2 - Var.1

Determination of the effect of the *Rheum* root extract on the germination of soybean seeds (Clavera) and maize (P 280), carried out by the "roll method" showed, in the experimental version, the *Rheum* root extract significantly increased the germination of soybean seeds in concentrations of 0,5% and 1,0% , and corn sugar seeds - in a concentration of 2% and 4%. Thus, we have proved that the *R. rhaponticum* extract can be used for

the presowing treatment of soybean seeds and corn, in order to increase germination and protection against the genus *Fusarium* root rot.

The biological effectiveness of the rhubarb root extract in the control of *S. fuliginea* under conditions of indoor soil was quite high. On the melon seedlings, the efficiency of the extract reached 79.5%, on the zucchini seedling - 57.5%, on the cucumber seedlings - 74.3%, on the pumpkin seedlings - 79.1%. Fungicidal action is due to the direct inhibition of fungus and its spores, using the main active substance of the extract - emodin. The preventive effect of the extract is related to the induced resistance to powdery mildew, based on an increase in the synthesis of phenolic acids and the activity of peroxidase in leaves treated with the extract.

Our phenological observations of the *R. rhaponticum* L. plants' life cycle, allow us to recommend plantations of these plants as economically viable, due to simple farming techniques and minimum costs for protection from diseases and pests. In order to increase the *R. rhaponticum* productivity, we recommend the use of Gliocladin-SC preparation for presowing seed treatment, created on the basis of suspension of the mushroom-antagonist *Trichoderma virens* (syn.*Gliocladium virens*) *Miller, Giddens* and *Foster*. We recommend planting *R. rhaponticum* with a 1-2 year old seedling, in order to protect against the main soil pest - larvae of *Melolontha* (f. *Scarabaeidae*). Since the hygrophilous plants of *R. rhaponticum* develop much better when watering, the rhubarb plantation is recommended to provide drip irrigation in the environmental conditions of Moldova.

CONCLUSIONS

All these opportunities of rhubarb economic use are the basis for its additional research and creation of a non-waste technology for its processing (bioconversion) by joint efforts of physicians, chemists, botanists and food processing technologists. We, having examined the properties of secondary metabolites of rhubarb, proved the prospects of using it in the creation of crop protection products. The use of such preparations will reduce the number of chemical treatments, and hence the residual amount of pesticides in agricultural products, thereby reducing the pollution of ecosystems. We believe that it is necessary to expand the species diversity of rhubarb in the Republic of Moldova through the introduction of medical species.

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METHODS OF THE INVESTIGATION OF BEE POLLEN AND BEE BREAD

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Abstract. The main purpose of this publication was to evaluate which types of studies are used with the purpose of the estimation of nutritional value of bee pollen and bee bread. Bee pollen and bee bread are important bee-products that contains large amounts of nutritional and essential active biological compounds. Among different bioactive compounds with antioxidant properties which could be found in bee pollen and bread are polyphenolic substances, flavonoids, unsaturated acids and carotenoids. Bee pollen and bee bread are investigated by plenty of scientists for different quality indexes over two last decades. However, the composition of bee pollen and bee bread are not yet standardized nor defined in pharmacopoeia as it primarily depends on their botanical and geographical origin, environmental conditions, time of collection of bee pollen by bees, location of used plants for collection of bee pollen, etc. For the purpose of the estimation of bee pollen and bee bread researchers the most often conduct palynological analysis and employ phytochemical studies for measuring total phenolic content using mainly the Folin-Ciocalteu colorimetric method, aluminum colorimetric method for sum of flavonoids determination and one or two methods for antioxidant activity. Large deviations of measuring the total antioxidant activity, total phenolic content, sum of flavonoids and other components are considerable. This fact and usage of different analytical procedures and reference standards interfere standardization of these products. Also investigators of bee keeping products utilize biological methods of study with the purpose of measuring antimicrobial and cytotoxic performances of extracts of bee pollen and bee bread. In general, phytochemical and biological studies show the prospects of usage of these bee keeping products in food and pharmaceutical industry as functional food ingredients and/or natural medicinal products.

Keywords: bee pollen, bee bread, antioxidant capacity, polyphenols, flavonoids, botanical origin.

INTRODUCTION

Bee keeping products (bee pollen, bee bread, honey, etc.) are considered to be the most promising sources of natural antioxidants (**Meda et al., 2005; Markievicz-Żukovska et al., 2013; Tohamy et al., 2014; Fatrcová-Šramková et al., 2015**). An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules and in such a way preventing oxidative stress **(Meda et al., 2005; Fatrcová-Šramková et al., 2015**). The latter is thought to contribute to the development of chronic and degenerative diseases. Among such diseases are cancer, autoimune disorders, aging, cataract, rheumatoid arthrithis, cardiovascular and neurodegenerative diseases, etc. **(Nagai et al., 2005; Silva et al., 2013; Tohamy et al., 2014; Bogdanov, 2016)**. Antioxidant ability has usually been attributed to the activity of antioxidant enzymes (mainly, superoxide dismutase, peroxidases glucose oxidase and catalases) and such low-molecular antioxidants as carotenoids, tocopherols, ascorbic acid, ubiquinol-10, gluthatione and phenolic substances. The same antioxidant classes occur in medical plants and bee keeping products **(Baltrušaityte et al., 2007; Čeksterytė et al., 2016; Markievicz-Żukovska et al., 2013; Silva et al., 2013; Aličić et al., 2014; Fatrcová-Šramková et al., 2015)**. Bee pollen and bee bread have been used for many years in traditional medicine, supplementary nutrition and in alternative diets, primary due to their nutritional properties and health benefits. Bee bread has a positive effect on the immune system of healthy people. It also has antibiotic and antioxidant properties, cytotoxic activity, hypolipidemic effect **(Markievicz-Żukovska et al., 2013; Aličić et al., 2014)**. However, the dependence of content of phenolic substances, flavonoids, antioxidant activity, antimicrobial action on botanical and geographical origin still are not defined considering very complicated content of biologically active substances in bee pollen or bee bread and usage of different methods and analytical procedures for the estimation of these bee keeping products. Therefore, different methods and analytical procedures for estimation of these bee keeping products are elaborated and

used.

The aim of this paper is an overview of publications dedicated to the study of bee pollen and bee bread phytochemical and biological properties with the purpose of generalization of the existing information on the chemical characterization and methods of the investigation of these bee keeping products.

Bee pollen and bread biological activity studies and composition determination

Bee pollen is rich in carbohydrates (13 – 55%), B complex vitamins (thiamine, niacin, riboflavin, pyridoxine, pantothenic acid, folic acid and biotin), carotenoids, flavonoids, phenolic acids, micro- and macroelements amino acids, lipids (1 – 10%), proteins (10 – 40%), sterols, terpens, vitamins, and etc. **(Nagai et al., 2005; Aličić et al., 2014**; **Barene et al., 2015**; **Rzepecka-Stojko et al., 2015)**. According to **Aličić et al. (2014)** there is no significant amount of vitamin C or lipid soluble vitamins in bee pollen. However, as stated by **Rzepecka-Stojko et al. (2015),** bee pollen collected from willow, pear and apple trees, and dandelion has the highest content of ascorbic acid.

Bee bread is a product of the hive obtained from pollen collected by bees to which they add flower nectar, honey and digestive enzymes and subsequently is stored in the combs where it undergoes lactic fermentation (**Markievicz-Żukovska et al., 2013; Aličić et al., 2014; Barene et al., 2015; Zuluaga et al., 2015**). The composition of the bee bread differs slightly from that of bee pollen. Lactic fermentation gives it greater power conservation and increases the content of lactic acid from 0.56% in bee pollen up to 3.06 – 3.20% in bee bread and, respectively, reduces pH of bee bread to 3.6 – 4.3 **(Nagai et al., 2005; Barene et al., 2015; Ivanišová et al., 2015**; **Zuluaga et al., 2015; Bogdanov, 2016)**. Bee bread has better availability of nutrients with respect to pollen because of partial destruction of pollen walls **(Čeksterytė et al., 2016; Aličić et al., 2014; Barene et al., 2015; Zuluaga et al., 2015)**. It contains many different compounds, such as lipids, proteins, aliphatic acids, mainly unsaturated ones (α-linolenic, linoleic, oleic and 11,14,17-eicosatrienoic acids), amino acids, carbohydrates (glucose, fructose, sucrose, arabinose), polyphenols (phenolic acids and flavonoids), alkanes (C21-C35), carotenoids, macro- and microelements (iron, calcium, phosphorus, potassium, copper, zinc, selenium, magnesium), vitamins E, C, K, and etc. **(Nagai et al., 2005;** Čeksterytė **et al., 2016, Markievicz-Żukovska et al., 2013; Silva et al., 2014; Barene et al., 2015; Ivanišová et al., 2015; Zuluaga et al., 2015)**.

The basic method of bee pollen studies is the microscopic analysis. This method determines botanical origin of bee pollen grains. Pollen analysis permits to make the identification of the major pollen sources used by bees, periods of pollen productions in the field (**Brovarskyi and Brindza, 2010; Grygorieva et al., 2010; Brindza et al., 2015**; **Redina et al., 2016; *Žiarovská* et al., 2015**; **Adamchuk and Akulonok, 2017).** Chemical composition of bee pollen varies depending on plant species, environmental conditions (different locations, seasons and years), age and status of the plant when the pollen is developing (Čeksterytė **et al., 2016; Brindza and Brovarskyi, 2013; Silva et al., 2014). Barene et al. (2015)** suppose that pollen of willow can be considered as an indicator of the origin of bee bread in Latvia.

The second method of the study of bee pollen and bee bread is the measure of antioxidant activity expressed by antioxidant capacity **(Baltrušaityte et al., 2007; Aličić et al., 2014)**. In the initial stages of the development of herbal preparations, especially from nonpharmacopeia plant material, preparations on the base of bee keeping products or food of plant origin the determination of antioxidant activity could generally predict the biological value of these products. **Baltrušaityte et al. (2007)** and **Aličić et al. (2014)** regard measuring antioxidant activity as a first step in characterization of honey and other bee products. There are some methods of measuring antioxidant activity. These methods are based on the removal or inhibition of free radicals or chelating metal ions. Among non-enzymatic indirect methods are the DPPH method, the TEAS assay, and the FRAP assay. Among direct methods used mostly is OPAC method **(Nagai et al., 2005; Oltica et al., 2007; Fatrcová-Šramková et al., 2008**; **Aličić et al., 2014;** Fatrcová-Šramková et al., 2012**;** Fatrcová-Šramková et al., 2015). As a rule, among antioxidants for positive control are used ascorbic acid, gallic acid, trolox and α-tocopherol (**Nagai et al., 2005; Ivanišová et al., 2015)**. The DPPH method is based on the acceptation of an electron or hydrogen radical by 2,2-diphenyl-1-picrylhydrazyl. The latter is reduced in the presence of an

antioxidant and becomes a stable molecule. The intensity of purple color of a reaction mixture of DPPH with a sample is diminished or even purple color is converted into yellow or pale color depending on ratio DPPH: antioxidants in a sample. Therefore, DPPH reduction is accompanied with change of the absorbance of reaction mixture of DPPH with a sample and monitored at 514 – 517 nm **(Baltrušaityte et al., 2007;** Čeksterytė **et al., 2016;** Fatrcová-Šramková et al., 2012**; Ivanišov**á **et al., 2015;** Fatrcová-Šramková et al., 2015). Our studies demonstrated that one of steps of the elaboration of analytical procedure of measuring antioxidant activity of bee bread extracts is the choice of a ratio of an extract: DPPH, solvent for DPPH, time of reaction. At incorrect ratio of an extract: DPPH, for example, antioxidant activity is approximately 80 – 85% at a high content of an extract and does not depend to certain extent on volume of the extract in reaction mixture **(Hudz et al., 2016)**.

The third type of studies of bee pollen and bee bread is the measure of total phenolic content. The phenolic compounds are considered as possible protection agents reducing oxidative damage of human body from oxygen reactive species (ROS) and retard the progress of many chronic diseases as well as lipid peroxidation **(Oltica et al., 2007)**. The Folin-Ciocalteu colorimetric method is widely used for measuring total phenolic content. The absorbance of a mixture of a sample, an appropriate volume of Folin-Ciocalteu reagent and sodium carbonate versus a prepared blank is measured at a wavelength from the range of 660 – 760 nm (**Markievicz-Żukovska et al., 2013**). Folin-Ciocalteau phenol reagent consists of a mixture of the heteropolyacids, phosphomolybdic and phosphotungstic acids in which the molybdenum and the tungsten are in the 6+ oxidation state. On reaction with a reductant, the molybdenum blue and the tungsten blue are formed and the mean oxidation state of the metals is between 5 and 6. **Agbor et al. (2014)** give the scheme of a chemical reaction of Folin-Ciocalteau reagent with phenolic compounds:

Na2WO4/Na2MO4 yellow⇒ (Phenol-MoW11O40)-4 blue

Mo+6 (yellow) + e-1 ⇒ Mo+5 (blue)

Mo+5 + e-1 ⇒ Mo+4 (blue).

Agbor et al. (2014) indicate that some conditions such as proper volume ratio, optimal reaction time, temperature for color development, standard optical density and use of a particular reference-standard polyphenol are required for the purpose of achieving meaningful, reliable and predictable results. In our studies, we employed gallic acid as reference standard, performed reading of an absorbance at a wavelength of 750±2 nm. This wavelength was established as a maximum of absorption of reaction mixture of a sample of bee bread with Folin-Ciocalteau reagent in the presence of sodium carbonate (Fig. 1, 2.

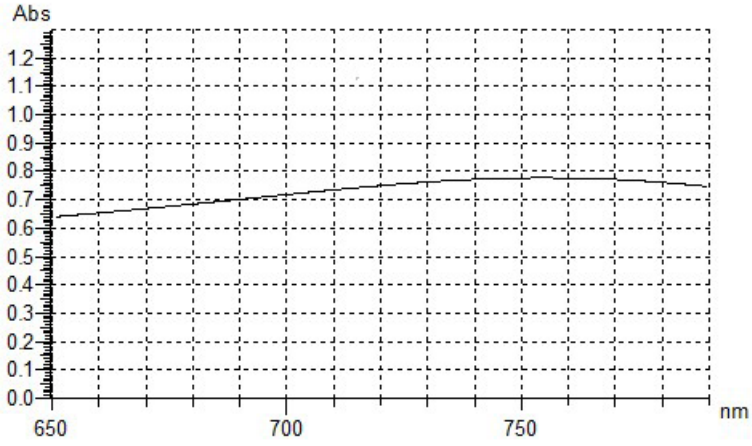

Fig. 1. The dependence of the absorbance of gallic acid in concentration of 120 µl/L with Folin-Ciocalteu reagent on wavelength (λmax=756.2 nm, A=0.777).

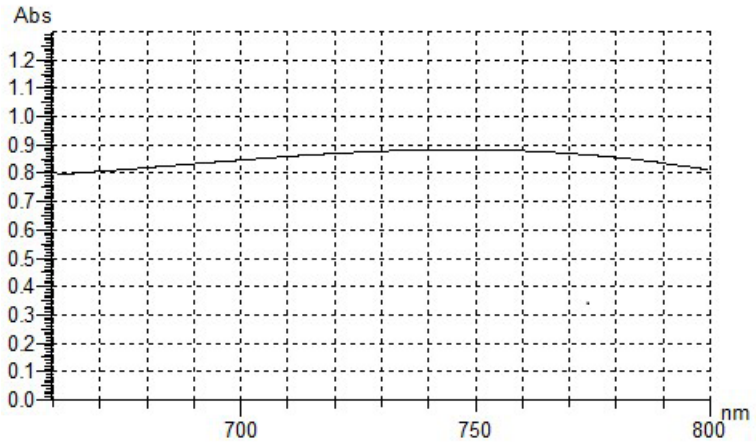

Fig. 2. The dependence of the absorbance of the extract of bee bread (batch 8) with Folin-Ciocalteu reagent on wavelength (λmax=748.0 nm, A=*0.883).*

Polyphenols are components of bee pollen and bee bread that determine its antioxidative activity as they scavenge free radicals and chelate metal ions. Among polyphenols in bee bread were identified *p*-coumaric acid, trace amounts of ferulic and caffeic acids and such flavonoids as kaemferol, quercetin, apigenin, chrysin, luteolin, and isorhamnetin **(Baltrušaityte et al., 2007;** Čeksterytė **et al., 2016; Markievicz-Żukovska et al., 2013;** Fatrcová-Šramková et al., 2015**)**. Čeksterytė **et al. (2016)** stated that flavonoids in the form of glycosides were not found in bee bread**.** The qualitive and quantative content of polyphenols and other components vary in bee pollen and, respectively, in bee bread depending on botanical origin, the year, time and location of collection of pollen and other factors such as climatic conditions, soil type and beekeepers activities and extraction condition (type of an solvent for extraction, time of extraction, type of extraction) **(Oltica et al., 2007;** Čeksterytė **et al., 2016; Markievicz-Żukovska et al., 2013; Aličić et al., 2014; Barene et al., 2015; Rzepecka-Stojko et al., 2015)**. In our opinion, this is the reason of different total phenolic contents in different publications. In most publications authors establish the dependence either between the total phenolic content and flavonoids, or the total phenolic content and antioxidant activity, or between different methods of measuring antioxidant activity **(Baltrušaityte et al., 2007)**. **Baltrušaityte et al. (2007)** established that there had not been correlation neither between two methods of measuring of antioxidant activity of bee bread extracts nor between their antioxidant activity and the content of *p*-coumaric acid, kaemferol, chrysin and apigenin in them. Total phenolics are found to be highest in methanol extracts (22.72 mg GAE/g) and lower in water extracts (8.32 mg GAE/g) **(Oltica et al., 2007)**.

The forth type of phytochemical study of bee pollen and bee bread is the measurement of sum of flavonoids mainly using aluminum colorimetric method and sometimes method of high pressure liquid chromatography. Flavonoids are the largest group of phenolic compounds, which have antioxidant, antimicrobial, antitumor, antiinflamatory activities, regulate free-radical reactions in the body. The pollen flavonoids quercetin, rutin and chrysin demonstrated a chemopreventive activity by increasing apoptosis. Flavonoids in plants constitute the antioxidant system, in which various antioxidants restore each other and exhibit a synergistic effect **(Baltrušaityte et al., 2007;** Čeksterytė **et al., 2007; Silva et al., 2014; Barene et al., 2015; Rzepecka-Stojko et al., 2015;** Fatrcová-Šramková et al., 2015). **Bogdanov et al., 2016)**. **Zuluaga et al.** (**2015**) state that the total content of flavonoids was in values of 3.2±1.0 mg quercetin/g bee bread and total phenolic content – 8,9±3.1

mg gallic acid/g in the investigated samples of bee bread. **Ivanišová et al. (2015)** make known that the total phenolic content varied in values of 12.36-18.25 mg gallic acid/g bee bread. **Markievicz-Żukovska et al. (2013)** state that the antioxidant effect of bee bread depends not only on phenolic antioxidants but also on non-phenolic antioxidant, for example, unsaturated fatty acids. According to our studies, differential spectra of bee bread extracts with aluminum chloride can serve as an important marker of determination of a dominating flavonoid group in bee bread.

Sometimes scientists determined the content of other compounds, such as lipids, carbohydrates, amino acids or carotene (carotenoids) and measure pH of bee bread (**Silva et al., 2014; Barene et al., 2015; Fatrcová-Šramková et al., 2015**). For example, **Silva et al. (2014)** established that proline and serine were the predominant amino acids, constituting approximately 56% of total free amino acids **in bee bread**. These authors also speculated that the *Melipona subnitida* bee converted glucose and fructose, the sugars commonly found in flowers, into mannitol and this process did not depend on the floral source (Silva et al., 2014). Some scientists perform PCR for pollen identification in parentage analysis, discrimination of self-pollen, or evaluation of the genetic diversity of pollen grains **(*Žiarovská* et al., 2015).**

Results of investigations conducted by different authors show different values of measuring total antioxidant activity, total phenolic content, sum of flavonoids and other components. This fact and usage of different analytical methods and procedures made standardization of these products very complicated.

One of the biological studies of bee pollen and bread extracts is the study of antimicrobial activity of their extracts. Extracts of these products show antimicrobial properties against pathogenic bacteria and fungi. **Ivanišová et al. (2015)** demonstrate antibacterial action of bee bread extracts against *Bacillus thuringiensis CCM19, Escherichia coli CCM 3898, Salmonella enterica subs. enterica CCM 3807*. **Abouda et al. (2011)** revealed that bee pollen and bee bread extracts inhibited some reference and clinical strains of *Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidus, Staphylococcus aureus, Streptococcus and Bacillus cereus.*

Usually disc diffusion and microbroth dilution methods are used to study antimicrobial action of bee pollen and bee bread extracts **(Ivanišová et al., 2015)**. However, it is difficult to compare results obtained by different authors because they employed extracts of different content of bee pollen or bee bread of sundry botanical and geographical origin, different solvents for extraction, various antimicrobial active pharmaceutical ingredients for positive control and different species of microorganisms**.**

The next type of the biological studies of bee bread extracts is cytotoxic assay (**Sobral at al., 2017**). **Markievicz-Żukovska et al. (2013)** demonstrated cytotoxic activity of some ethanolic extracts on the glioblastoma (U87MG) cell line. Cell viability was measured by quantitative colorimetric assay using MTT method, which is based on the conversation of MTT to formazan crystals by mitochondrial dehydrogenases.

CONCLUSIONS

Summing up the results of studies presented by different researchers, it is concluded that phytochemical and biological studies show the prospects of usage of bee bread and bee pollen in food and pharmaceutical industry as functional food ingredients and/or natural medicinal products with antioxidant properties. **Further studies of bee pollen and bee products are needed and should be directed on the** interrelation of botanical origin, total phenolic content, sum of flavonoids, vitamins, lipids and content of other biologically active compounds with their pharmacological properties.

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INTRODUCTION OF *AMPELOPSIS BREVIPEDUNCULATA* AND *RUTA GRAVEOLENS* AND THEIR ANTIMICROBIAL ACTIVITY

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Abstract. *Ruta graveolens and Ampelopsis brevipedunculata were introduced in the Botanical garden, and studied their ontogenesis, and requirements for environmental conditions in the Lviv region. For both of introduced species have been identified opportunities of cultivation with "the principles of good practice cultivation and collection" and obtaining sufficient for using medicinal raw material. It has been studied the antibacterial activity of plant extracts on a number of bacteria of collection strains and isolated from the oral cavity of patients with periodontitis. It was discovered the activity of water extracts on Enterobacter cloacae (Enterobacter spp.) only, while the 70% ethanol-water extracts were active against Escherichia coli, Staphylococcus aureus, Streptococcus salivarius, Enterobacter spp., and Neisseria sp.*

Keywords: *introduced Ruta graveolens and Ampelopsis brevipedunculata, antibacterial properties*

INTRODUCTION

Even though pharmacological industries have produced a number of new antibiotics in the last two decades, the treatment options available now for infected patients are severely limited. So, plant-derived traditional medicines continue to play an essential role in health care, as the introduction and study of plants that can be a source of biologically active compounds, including in the botanical gardens, for use in pharmacy and medicine is very important. Nowadays, it is very important to obtain medicinal herbs, which has a quality appropriate to the standards of EU [7, 8].

Ruta graveolens L., commonly known as Rue (*Rutaceae*), is an evergreen, perennial, semi-shrub with bluish-green leaves that emit a powerful odour and have a bitter taste, native to the Balkan Peninsula, and Mediterranean region. Leaves are bi- or tripinnatisected into oblong or spoon shaped segments, to 6 mm wide, inflorescence glabrous. The paniculate clusters of small yellow flowers (corolla with oblong, ciliate petals) appear in midsummer, held well above the foliage and often covering most of the plant. Fruit a globose capsule 6-9 mm. This medicinal plant used in traditional systems of European medicine for treatment of human diseases and in veterinary [9]. In the European Union, *Ruta graveolens* herb may be used in certain food commodities as for instance baked goods, frozen dairy products, soft candy or non-alcoholic beverages [3, 10]. It has been shown bactericidal properties of this species, and the most susceptible bacterium was *Bacillus cereus* and *Staphylococcus aureus* [1].

Ampelopsis brevipedunculata (Maxim.) Trautv. or Porcelainberry (Vitaceae) is deciduous, woody, perennial vine that resembles grape and climbs by non-adhesive tendrils at the base of each leaf; grows to 3-6m; its' leaves are alternate, simple, 3-5 lobed to highly dissected with heart-shaped base and coarsely toothed margins, shiny underneath with hairs on veins; the green flowers, arranged in cymes, are dense and small and appear in July-August; and the fruits appear in September are spherical and 6-8 mm in size. Porcelainberry grows well in most soils, especially forest edges, pond margins, stream banks, thickets and waste places, where there is full sunlight to partial shade, and where it is not permanently wet. It is less tolerant of heavily shaded areas, such as that found in mature forest interiors. The seeds germinate readily in the soil after natural or human disturbance. In nature, *A. brevipedunculata* grows in Japan, China, Korea and adjacent territories. Leaf buds, stem, leaves and fruits of *A. brevipedunculata* are edible (raw or cooked), but not very palatable [10]. The stem and root of *A. brevipedunculata* was traditionally used as an anti-inflammatory, diuretic and anti-hepatotoxic agent in folk medicine of these regions. There are no data on antimicrobial activity of *A. brevipedunculata*, except for

antiviral activity [4].

The aim of the study was to introduce these species into the Botanical Garden, and to assess the antimicrobial activity of the extracts of them.

MATERIALS AND METHODS

Ampelopsis brevipedunculata (leaves) and *Ruta graveolens* [≈ *Ruta hortensis* Miller.] (the above-ground part) introduced and cultivated in the Botanical Garden of Ivan Franko Lviv National University, were collected before flowering in June-July 2013-2015. Both species plant materials were dried in the shade at room temperature until achieved a constant weight and used for further investigation. Extracts of the plants were made like described previously [5]. Shredding of dry material was carried out mechanically (using a grinder). For extraction, we have used 20% and 70% ethanol, and distilled water, at a ratio of plant material 1:10 (m/v), and at 25˚C temperature. The extraction was performed by maceration for 14 days. The standard agar diffusion method was used to determine the sensitivity of microorganisms to the herbal extracts in modification by using glass cylinders [2, 6]. The tested Gram-positive and Gram-negative bacteria were *Staphylococcus aureus* (*ATCC 25923*), *Escherichia coli* (*ATCC 25922*), *Enterococcus faecalis* (ATCC 29212), and clinical strains of *Staphylococcus aureus, Enterococcus faecalis*, *Streptococcus salivarius*, *Enterobacter spp.* and *Neisseria spp.,* isolated from the oral cavity of patients with periodontitis from the Microbial Culture Collection of the Department of Microbiology of Uzhgorod National University. The diameter of zones of inhibition around the disks was measured after 24-h incubation. Water, 20% and 70% ethanol were used as controls. When the strain showed no activity, the value considered was equal to zero. Values ranging from 6 to 8 mm and less were considered as non-active. Bioassay was carried out in duplicate and later experiments were repeated twice.

RESULTS AND DISCUSSIONS

In Botanical garden of Ivan Franko National University of Lviv, *Ruta graveolens* L. was introduced in 1995, with seeds obtained from botanical garden of France. Plant height in culture is 50-90 cm. It propagated by seeds, which germinate without stratification and gives a massive self-seeding. It grows in sunny, sheltered from the wind sites; winter-, frost- and drought-resistant plant. The seeds germinate and the seedlings appear within 7-10 days. During the first year, seedlings reach up to 15cm in height, bloom in the second year, massive bloom occurs in the third year. Blooms are in June-July-August, the single shoots with flowers bloom in September and October. A comprehensive assessment of the success of the introduction is that *Ruta graveolens* is particularly promising for growing *ex situ* in Lviv region.

Ampelopsis brevipedunculata (Maxim.) Trautv. was introduced in botanical garden of Ivan Franko National University of Lviv in 2002, with seeds obtained from botanical garden of Estonia. In a culture it propagated by seed and vegetatively. *A.brevipedunculata* takes a complete cycle of seasonal development and produces complete, viable seeds, which are characterized by spread of germination period (4-5 months). The highest seed germination occurs after stratification for 90 days at -1°C. For a comprehensive assessment, the success of the introduction of *A.brevipedunculata* is promising for growing *ex situ* in Lviv region.

The results of antibacterial properties of *A. brevipedunculata* (leaves) and *R.graveolens* (the above-ground part) investigation show their dependence of group of bacteria (gram-positive or gram-negative), type of extractanct and its concentration (Table). The results indicated that aqueous extracts and 20% ethanol extracts of both investigated species did not inhibit the growth of all microbial strains which have been used, except *Enterobacter spp.* It has been shown moderate bacteriostatic properties of aqueous extracts of both investigated plant species against *Enterobacter spp.,* although 70% ethanol extract had the best ones.

Table.
Antimicrobial agent zone of inhibition (mm); Mean± SD

Test samples	Extractanct	*Str. faecalis* ATCC 29212	*E.coli* ATCC 25922	*S. aureus* ATCC 25923	*Str. salivarius,* clinical	*S. aureus,* clinical	*Enterobacter spp.,* clinical	*Neisseria* clinical
A. brvipedunculata.	Distilled water	-	0	0	0	0	12.3±2.5	0
	20% EtOH	0	0	0	0	0	0	0
	70% EtOH	19.0±1.0	15.7±0.3	18.0±1.7	21.0±07	18.0±0.0	13.0±1.7	13.0±1.0
R. graveolens	water	-	0	0	0	0	19.3±1.2	0
	20% EtOH	0	0	0	0	0	0	0
	70% EtOH	-	18.5	22.5	-	17.5	14	15.5±0.5
Controls:	water	0	0	0	0	0	0	0
	20% EtOH	0	0	0	0	0	0	0
	70% EtOH	0	12.5±1.4	10.8±0.6	16.5±0.6	10.0±0.6	13.3±0.3	10.4±0.6

70% ethanolic extracts of *A. brevipedunculata* and *R.graveolens* demonstrated antibiotic activity against *E.coli, S. aureus, Str. salivarius, Neisseria spp*., and *A. brevipedunculata* against *Str. faecalis.* The obtained results of influence of the 70% alcohol extracts of both plant species investigated against *S.aureus, E.coli* and *Neisseria spp* can be compared with the effect of tinctures of calendula and eucalyptus, which have long been used as antimicrobial agents [6].

As the most of these bacteria species are a normal commensals of the oral cavity and manifest in immunocompromised individuals, and are the cause of most hospital infections, *A. brevipedunculata* and *R.graveolens* should be examined in more detail for the purpose of using bacteriostatic action to create medical preparations. It is necessary to better understand their properties, their safety and effectiveness.

CONCLUSIONS

A. brevipedunculata and *R.graveolens* can be grown in Lviv region and in compliance with GMP and GACP [7, 8], and obtain good quality and quantity of raw material. This indicates that it is possible to get enough raw materials for use in pharmacy and medicine. The results show that the extracts of *A. brevipedunculata* and *R.graveolens* have a very potent activity against all the tested species and isolates of bacteria.

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Centaurea thirkei

Hieracium laevigatum
The Red Book of the Republic of Moldova, Third edition. 2015

IV. LANDSCAPE ARCHITECTURE, ENVIRONMENTAL PROTECTION, ECOLOGICAL EDUCATION

CHANGING INDIVIDUAL BEHAVIOR OF *LEPTINOTARSA DECEMLINEATA* ADULTS (*COLEOPTERA- CHRYSOMELIDAE*) ABOUT THE APPLICATION OF PLANT EXTRACTS AS TREATMENT TO COMBAT THEM

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Keywords*: pesticide; prolificacy; cannibalism; hyperexcitability.*

Abstract: Using medicinal plants to combat various insect pests is not a new method.

The first plants used for this purpose over time were nettles tobacco juice, basil, garlic, etc.

In experiments conducted for this paper, there were used two common plants namely savory *Satureja hortensis*, known as a natural antibiotic and common wormwood- *Artemisia vulgaris*.

On adults fed in growth cages were applied aqueous extracts and alcohol in these plants from time to time and in progressive concentrations then were monitored several aspects such as the social behavior of individuals, prolific generation of adults occurred after treatment, the potential of applied insecticide preparations, etc.

Insecticide potential of these solutions is the subject of another work. In the present study, there are addressed issues regarding the prolificacy post treatment and social behavior of individuals, because during treatment and after that time, there were interesting behavioral changes in adults receiving treatment, including hyperexcitability and cannibalism.

INTRODUCTION

Leptinotarsa decemlineata Say *(Coleoptera- Chrysomelidae*) is a multiple challenge for potato growers, for the fight for pesticide producers and researchers.

The great ecological plasticity and prolificacy, the ability to form resistant breeds to pesticides in an alarming rate, with which attacks a group of plants essential to feed the inhabitants of temperate regions (potato) and in Mediterranean climate regions (plant family *Solanaceae*) are each cycle of vegetation to raise issues on monitoring and combating it.

The control of the population of the Colorado potato beetle, the synthesis is performed with the pesticide and a series of microbiological preparations derived from plant metabolites or aromatic.

Aim of the study was to gather information on the behavior of the species *Leptinotarsa decemlineata* Say adults and larvae of Colorado beetle, by application of metabolites derived from two relatively common medicinal and aromatic plants in the flora of our country, or savory, *Satureja hortensis,* and *Artemisia vulgaris*.

MATERIAL AND METHOD

As treatment solutions were used two types of extracts, the aqueous and alcoholic extract obtained from the chemistry laboratory at a concentration of 20% active ingredients.

These extracts were applied in laboratory growth cages on a sample of 50 individuals of *Leptinotarsa decemlineata* adults- Colorado beetle (Ord. *Coleoptera*- family *Chrysomelidae*).

Adults use hibernating, so the first generation of 2016 and their installation in capture cages was made shortly after the onset of the eggs laying.

This resulted in 5 different experimental variants, namely: a control variant Vm over which there were applied treatments, two experimental variants for savory variant VC1 treated with alcoholic extract of thyme and variant VC2 treated with aqueous extracts of thyme, two variants of wormwood, variant Vp1 from the alcoholic extract of Artemisia applied and variant VP2 treated with aqueous extract .

Insects were fed daily with tomato and eggplant seedlings. Adults and evolution was followed oviposition period, and the hatching of larvae were applied to first spraying the plants used as food for adult individuals and the larvae.

From now checking cages and registration of changes were made for 30 days in the first 5 days twice a day, then daily up to 10 days and then every other day until the end.

There were times when the larvae feed administration was stopped for 24 hours to observe their reactions.

Besides prolificacy and mortality, there were monitored also behaviour flaws of individuals.

RESULTS AND DISCUSSION

Applying first treatment was done at 24 hours at the first larvae.

In the first 48 hours after application increased the amount of food consumed, both larvae and adults showing an increased agitation and hyperactivity .

Four days after the first spray, it was applied the second spray, the effect of the application of which is reported in a few hours when the first deaths were recorded in both adults and larvae hatched in the period monitored.

Also somewhat unnatural events were reported among which are found:

- Hyperactivity;
- Increased hunger;
- Cannibalism stressed shown by individuals on their own eggs and young larvae.

Once the second splash intensified manifestations such as paralysis, aggression, hyperactivity and cannibalism, manifestations of confusion, etc.

Then, there were almost immediate early larval mortality in the group treated with alcoholic extract of Artemisia and extract the aqueous Satureja treated.

Behavior manifestations deficient adults were reported heavily in variants treated with extracts of wormwood, irrespective of the manner of obtaining them, the conduct of adults and larvae after applying sprays and stopping them was like manifestations of withdrawal-induced drug abstinence.

If treated with wormwood variants have been reported repellent effect on individuals in growth cages.

In the variants in which have been applied treatments with extracts of garden thyme, reported events were slow reaction, lower fecundity, but there were no neuroleptic effects and the visible manifestations of aggression or cannibalism.

Adult behavior in the control version was one similar to that reported in conditions of freedom, in the field, the only differences being related to fecundity and viability of eggs, which were affected by life in captivity and controlled feeding.

CONCLUSIONS

The experiment showed that in captivity, to which is added a quantity and quality control feeding, the behavior of adults of the species *Leptinotarsa decemlineata* Say is relatively different than the behavior at large, when they eat the plants of the field.

Individuals in the control were subjected to reduced stress, strictly related to the lack of movement and amount of food, as such, it was mainly affected prolificacy and viability of the eggs.

In individuals in variants treated with thyme, it was reported a high sterility, number of eggs laid, viability of the eggs being visibly higher than the other variants.

Surprise variants that provided information were those treated with common wormwood. As a plant, wormwood has various effects, including effects on the neurovegetative system of man, so that it was expected to affect the nervous system of insects that undergo treatment. So, there have been events like effects of

ethnobotanical drugs, such as:

- Agitation, hyperactivity;
- Nervous hunger and cannibalism;
- disorientation and paralysis;
- Death of individuals.

Whereas the experience has occurred only during a biological cycle, it is necessary to repeat the experiment and its development over a longer period of time to validate the results about the effectiveness of using metabolites.

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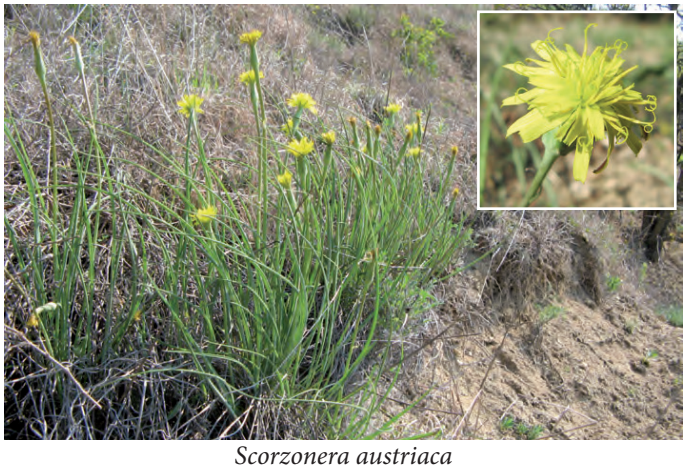
Jurinea stoechadifolia



Scorzonera austriaca

The Red Book of the Republic of Moldova, Third edition. 2015

THE USE OF MEDICINAL AND AROMATIC PLANT EXTRACTS AGAINST COLORADO BEETLE SPECIES- *LEPTINOTARSA DECEMLINEATA* (*COLEOPTERA - CHRYSOMELIDAE*)

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Abstract: *Colorado- beetle Leptinotarsa decemlineata (Coleoptera - Chrysomelidae) is the main pest of potato crops everywhere, and if near these cultures there are other solanaceous species, planted or from spontaneous flora, then it becomes as harmful for them.*

This paper is the result of research conducted on adults of the species of beetle Leptinotarsa decemlineata (Coleoptera - Chrysomelidae), carried out in laboratory conditions.

The I generation adults were collected from the field immediately after coming out of hibernation and brought to the lab where they were installed in growth cages. To have as much information aspossible and to confirm the results five experimental variants have been achieved:

First version - has not been treated; version 2 and 3 were those in which there were used alcoholic extracts of medicinal and aromatic plants, and version 4 and 5 were those in which the aqueous extracts were applied.

As plants used were chosen: Satureja hortensis and Artemisia vulgaris.

As a source of food for the monitored adults, young seedlings of tomatoes, peppers or eggplant were used.

It was monitored and recorded the possibility that the substances applied to be a natural and organic method of combating Colorado-beetle and determine the most effective ways of conditioning applied products.

INTRODUCTION

Leptinotarsa decemlineata Say is an alien, invasive pest, introduced accidentally from the Americas in Europe and due to the great adaptability and high prolificacy is currently one of the most dangerous pests of potato, both in our country and in most potato growing countries in Europe and worldwide.

Since the emergence of the insect in Romania, this created great difficulties in Plant protection specialists.

Colorado potato beetle population control is achieved preponderantly by synthetic chemicals that induce positive economic aspects in addition to a number of drawbacks, hazardous to human health and the environment.

As an alternative to these products, one of the counter-current concepts relates to the use of plant extracts as organic. The aim of the research was to analyze the paper biology and ecology of the *Leptinotarsa decemlineata* under laboratory conditions, and alternatives for the control test, the use of plant extracts.

MATERIAL AND METHOD

Observations and experiments have been carried out on a sample of the species *Leptinotarsa decemlineata* at adult stage (Ord. *Coleoptera* - family *Chrysomelidae*). Adult individuals were selected by first generation.

They were brought into the laboratory and placed in biological cages for growth.

Have been used for the treatment plants by wormwood common - *Artemisia vulgaris* - and savory - *Satureja hortensis*.

Solutions applied were made in two forms. Thus there were used aqueous and alcoholic extracts of the two plants as 20%.

For each of the two plants were mounted two experimental variants.

- Vm- control variant, of the fosat which have not been applied on any kind of treatment;
- Vp1 applied to the alcoholic extract of Artemisia;
- Vc1 solution was applied to the alcoholic extract from thyme;
- Vp2 and Vc2 variants are those that have applied the aqueous extracts of wormwood and thyme.

Each experimental embodiment was constituted by a group of 10 adult insects of the species *Leptinotarsa decemlineata* placed in a cage of controlled growth, as a source of food young tomato plants - *Licopersicum esculentum* and eggplant - *Solanum melongena*.

Adults were fed daily with fresh herbs for their biological behavior and reproductive choice is not affected.

RESULTS AND DISCUSSIONS

The application of the first treatment was done at 24 hours of the onset of the first larvae. Spraying was carried out both on food plants and the individuals in the adult and larval stages.

Concentrated substance was applied to 20%. In the first 48 hours after application, increased the amount of food consumed.

4 days after the first spray was applied to the second spray, the effect of the application of which was reported in a few hours, when the first deaths were recorded in both adults and larvae hatched in the period monitored.

The items were: adult behavior, the development of the period of oviposition (eggs group number, number of eggs per laying of eggs, egg viability), and development of larvae hatching from the eggs deposited occurred on the treated plants, death, etc.

The female adult Colorado potato beetle may lay, in a fertility period, 250-3000 eggs. An egg laying may comprise 50-160 eggs, and of these around 30% are infertile.

In this case, the individual monitoring lasted 30 days, considered installing adults in cages until death of all individuals.

During this time (in which splashing was performed) the total number of growth cages eggs group number was 12 in the control variant, with an average of 35 eggs in the laying of eggs.

In the variants treated with Artemisia there were recorded 21 eggs group, in the variant treated with alcoholic extract (VP 1), with an average of 38 eggs / eggs group 24, eggs group version aqueous extract (VP2), with an average of 31 eggs / Tip.(table nr. 1).

In the variants treated with solutions on the basis of thyme (VC1 and VC2) were recorded 22 eggs group variant treated with alcoholic extract (VC1) with an average of 22 eggs / laying of eggs and 6 eggs group in the version with aqueous extract (VC2) with an average of 20 eggs / laying of eggs (table no. 2).

Evolution of eggs group with treatments with extracts of Artemisia

Table nr. 1

Nr. Ord.	**Nr. eggs group VM**	**Nr. eggs group Vp1**	**Nr. eggs group Vp2**
-	12	21	24
Nr. of eggs	420	798	744
Nr. eggs/eggs group	35	38	31
Nr./ % eggs viable	300/ 71,42%	294/ 36,84	343/46,10

Evolution of eggs group with treatments with extracts of Satureja

Table nr. 2

Nr. Ord.	**Nr. eggs group VM**	**Nr. eggs group Vc1**	**Nr. eggs group Vc2**
-	12	22	6
Nr. of eggs	420	484	180
Nr. eggs/eggs group	35	22	20
Nr./ % eggs viable	300/ 71,42%	151/31,19	72/40,0

As can be seen in the tables above, the number of eggs group made by captive adults by the VM, untreated variant, was 12, with an average of 12 eggs group per individual (but are not known precisely how many females were found in each pot).

Average egg laying that was recorded constituted 35 pieces in average in the art but there is normally such a reduction in the number of eggs due to the controlled feeding and life in captivity.

The total number of eggs group in variants treated with Artemisia was significantly higher, namely 21 eggs group / 798 eggs, by alcoholic extract and 24/744 in variants treated by aqueous extract, but was greatly increased egg sterility, viability was maximum 46%.

The total number of variants of eggs group was treated with variable thyme, respectively eggs group 22/484 eggs and only 6 eggs group in alcoholic extract / extract the aqueous least 180, and viability of 30-40%.

Regarding the mortality of individuals from treatments applied, it was largely.

In the first 10 days, mortality has been reported, that after these days to record 2 adults' deaths (20%) in the case of aqueous solutions and 5 adult deaths where alcoholic solution of the Artemisia (50%) was used for 5 days after mass hatching of the larvae, the group treated with alcoholic extract to register a 64% mortality of the larvae. (table. 3)

The event process in adults and larvae mortality 10 days after the treatment with the extracts of wormwood

Table nr. 3

Days before treatment	**Mortality VM**		**Mortality Vp1**		**Mortality VP2**	
	adults	**larvae**	**adults**	**larvae**	**adults**	**larvae**
5	-	-	-	-	-	-
10	-	-	1	-	-	-
15	1	-	1	1	1	-
30	1	-	-	81	3	60

In the case of applied treatment with metabolites of thyme, in the first 10 days, mortality has been reported, that after these days to record 2 adults deaths (20%) in the case of alcoholic solutions and 4 where the aqueous solution of thyme (40%) was used for 5 days after hatching, the larvae mass, the group treated with alcoholic extract to register a 54% mortality of the larvae and 85% aqueous extract. (table nr. 4)

The event process in adult and larval mortality after 10 days after the treatment with the extracts of thyme

Table nr. 4

Days before treatment	**Mortality** VM		**Mortality** Vc1		**Mortality** Vc2	
	adults	larvae	adults	larvae	adults	larvae
5	-	-	-	-	-	-
10	-	-	2	-	4	-
15	1	-	2	2	2	-
30	1	-	-	35	3	56

CONCLUSIONS

The experiment showed that the metabolites of both plants, regardless of how to make the extract, had no lethal effect on adult mortality recorded in all five variants being within normal (age).

Obviously, the fact that the metabolites acted on the fertility of the eggs and the number of eggs in the laying of eggs deposited, fertility of the eggs treated with the extracts of Artemisia variants is between 36.84 and 46.10%, and in the variants treated with the extracts of Satureja, it is between 31.19 and 40.0%.

It can be seen that in both cases, the aqueous extract solutions obtained induced a higher percentage of sterility of the eggs.

With regard to the mortalities, there is 64% mortality in the group treated with alcoholic extract of Artemisia and 85% in the group treated with the aqueous extract of Satureja.

Whereas the experience has occurred only during a biological cycle, it is necessary to repeat the experiment and its development over a longer period of time to validate the results about the effectiveness of using metabolites.

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Scorzonera mollis

Serratula bulgarica
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REEVALUATION OF ETHOLOGY PARTICULARITIES OF IMAGO *HELIOTHIS* ARMIGERA DEPENDING ON PHENOLOGICAL PHASES OF DEVELOPMENT OF SOYBEAN CULTURE

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Abstract: The impacts of climatic factors are the differences in temperature, humidity and precipitations, especially in spring and autumn season. They are creating deviations in the preferable host species of *H. armigera*. These variations can modify a ethology of this pest, whose development is associated with choosing habitats for larval nutrition. Climatic factors of the vegetation period in 2016 in Moldova were characterized by a variety of precipitation and average temperature of the season. Exceeding the average air temperature by 1,5-2,4°C above the norm (20,8-23,0°C) is noted once in 10 years, in the whole period of observations. The revaluation of ethological peculiarities of pest *H. armigera* in the conditions of changing climate factors demonstrated that there is an extension of the period of activity. The beginning of the first generation activity started 15 days earlier and the third generation flight extended by 30 days.

Keywords: Climate conditions, *H.armigera*, Ethological particularities, Phenological phases, Pheromone traps

INTRODUCTION

Global warming and climate change will trigger major changes in geographical distribution and population dynamics of insect pests, insect – host plant interactions, activity and abundance of natural enemies, and efficacy of crop protection technologies [4]. Changes in geographical distribution and incidence will affect both crop production and food security. For all the insect species, higher temperatures, below the species' upper threshold limit, will result in faster development, resulting in rapid increase of pest populations as the time to reproductive maturity is reduced. In addition to the direct effects of temperature changes on development rates, increases in food quality due to plant stress may result in dramatic increases in growth of insect pest populations, while the growth of certain insect pests may be adversely affected. Global warming will lead to earlier infestation by *H. armigera*, resulting in increased crop loss [10]. Overwintering of insect pests will increase as a result of climate change, producing larger spring populations as a base for a build-up in numbers in the following season. Many insects such as *H. armigera* are migratory, and therefore, may be well adapted to exploit new opportunities by moving rapidly into new areas as a result of climate change. There will be an increased variability in insect damage as a result of climate change. Higher temperatures will make dry seasons drier, and conversely, may increase the amount and intensity of rainfall, making wet seasons wetter than at present [7].

Climate changes also will influence plant health and vigor, directly and indirectly through a modified reproductive performance of their associated herbivores. Climatic change will alter outbreak patterns and geographical ranges of insects, including those that vector diseases. The consequences are difficult to predict, especially in view of the complex interactions between crops, herbivores and pathogens, but climate-related changes most likely will combine to reduce yields [5]. Pheromones are increasingly efficient at low population densities, they do not adversely affect natural enemies and they can, therefore, bring about a long-term reduction in insect populations that cannot be accomplished with conventional insecticides [2, 9]. A changing climate with higher growing season temperatures and altered rainfall patterns makes control of native and invasive insects an increasingly urgent challenge [3]. Intensified insecticide use will not provide a solution, but pheromones and other semiochemicals instead can be implemented for sustainable area-wide management and will thus improve food security for a growing population [6, 8].

The impacts of climatic factors are the differences in temperature, humidity and precipitations,

especially in spring and autumn season. They are creating deviations in the preferable host species of *H. armigera*. These variations can modify a ethology of this pest, whose development is associated with choosing habitats for larval nutrition [6]. The total yield of plants depends on the accordance degree of growth and development factors of optimum value that varies depending on the phenological phase of the plants.

MATERIAL AND METHOD

On the experimental field of the IGFPP (S = 1.2 hectares), soybean "Nadezhda" was sown on April 29. During the vegetation period were noted phenological phases of soybean plants' growth.

Traps with sex pheromone were used for seasonal monitoring of *Heliothis armigera* sexual pest activity on soybean experimental lot. Traps were placed on April 29. The distance between the traps - 30 meters, the field size - 88 x 132 meters. We obtained weekly the evidence of males captured in the traps, pheromone capsules and glue liner were replaced with the same frequency (fig. 1).

Fig. 1. *Heliothis armigera* pest monitoring with pheromone traps on soybean culture (S = 1.2 ha)

It has also been pursued of the pest seasonal dynamics, the beginning and the top of flight of the cotton bollworm generations, depending on the values of temperature, was determined.

RESULTS AND DISCUSSIONS

Climatic factors of the vegetation period in 2016 in Moldova were characterized by a variety of precipitation and average temperature of the season. The summer was very hot and significantly rainfall deficient in July and August. Exceeding the average air temperature by 1,5-2,4°C above the norm (20,8-23,0°C) is noted once in 10, in years the whole period of observations. The temperature maximum in summer reached to 37,0°C, which is noted once in 5 years on average, and the minimum temperature was fixed to 3,5°C [1].

During the vegetation period were noted phenological phases of soybean plants' growth: germination (1-12 May), forming the first true leaves (18th), ramification (June 5th), butonization (June 20th), flowering (13 July) the appearance of the pods (26 July), beans maturation (4- 23 August).

Flight of the first *H. armigera* pest male was noted by pheromone traps on May 18. During this period, the maximum temperature reached 22,1°C, that is the threshold of pest population activity beginning. Due to heavy rainfall (80% higher than average) in the first ten days of June, the temperature values fell significantly (up to 9,1°C). It leads to a temporary suppression of the first generation of the pest population (1-3 males/pheromone trap) activity. However, July and August were characterized by a significant lack of precipitation.

The average temperature for this period was 20,8-23,0°C, which is more to 1,5-2,4°C from the norm. This factor led to the increase of *H. armigera* population activity and amounted to 15 males/pheromone trap. It was determined that the pest highest activity was manifested in the third generation, which flight lasted until the end of September. This is due to factors of high temperatures in this period.

Evaluation of agro-climatic and meteorological conditions of the year of research (2016) and the results of the impact of these conditions on soy and cotton bollworm is depicted on the chart below (fig. 2):

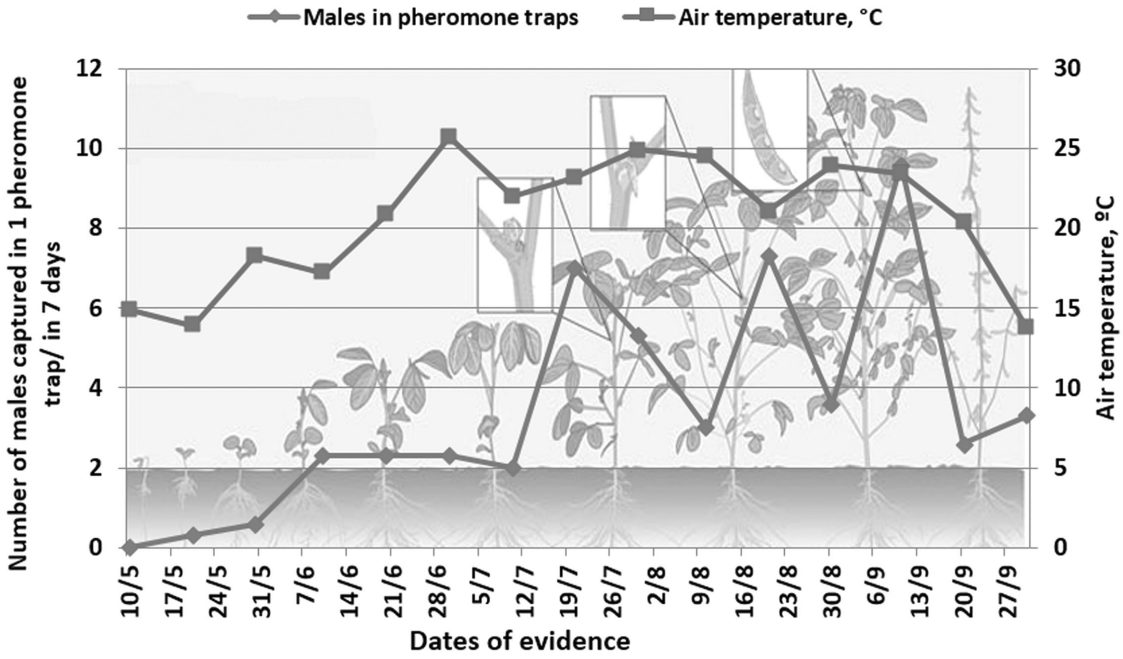

Fig. 2. Flight dynamics of *H.armigera* male in dependence on the temperature values and the phenological phases of soybean plant.

It was determined that the pest *Heliothis armigera* had three generations fixed with pheromone traps for soybean crop in the climatic conditions of this year. The development of the pest was extended from the first decade of May until the first decade of October. The population density is directly dependent on the air temperature and phenological phase of the crop. Intensive male flight on pheromone traps was the most significant (males 9.6 / 1 trap), when the air temperature was above 20°C and soybean plants were in the phase of bean maturation.

CONCLUSIONS

Thus, the revaluation of ethological peculiarities of *H. armigera* in the conditions of changing climate factors demonstrated that there is an extension of the period of pest activity. The beginning of the first generation activity started 15 days earlier and the third generation flight extended by 30 days. We need to use an integrated pest management system that takes into consideration the change in pest spectrum, cropping patterns and effectiveness of different components of pest management for sustainable crop production.

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Serratula coronata
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THE INFLUENCE OF SOIL POLLUTION WITH HEAVY METALS ON THE PRODUCTION QUALITY OF SOME PLANT TYPES GROWN IN THE IASI

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Abstract. Soil is a complex structure and contains mainly five major components i.e. mineral matter, water, air, organic matter and living organisms. The quantity of these components in the soil does not remain the same but varies with the locality. Soil possesses not only a nucleus position for existence of living beings but also ensures their future existence. Therefore, it is essential to make an adequate land management to maintain the quality of soil in both rural and urban soil. The presence of different kinds of heavy metals such as Cd, Cu, Mn, Hg and Zn etc. in trace or in minimum level is a natural phenomenon but their enhanced level is an indicator of the degree of pollution load in that specific area. In the modern ecologic conception, soil pollutions means any action that causes the degradation of the normal operation of soil as support and living environment within various natural or anthrop ecosystems, control expressed through soil physical, chemical or biologic degradation, or the occurrence of some characteristics in the soil that reflect the depreciation of its fertility, the decrease of bio-productive capacity both from qualitative point of view and/or quantitative point of view. The heavy metals have potent cumulative properties and toxicity due to which they have a potential hazardous effect not only on crop plants but also on human health This paper presents the study on the influence of soil pollution with heavy metals on the production quality of some plant types grown in the soil polluted with heavy metals in Holboca area-city Iasi.

Keywords: heavy metal, contamination, production quality, leaf vegetables

INTRODUCTION

With the development of economy and society, heavy metal contamination has become increasingly common in the world. It is almost a serious threat to every country. In the world's top ten environmental events, two events have related to heavy metal contamination (Yang et al., 2009).

The natural presence of metallic elements at the level of various parts of the environment, especially in the soil, is usually in accordance with the primary presence in the rocks and the needs required by the geochemical and biochemical processes in which these elements are involved. The existence of deposits in the subsoil is reflected in the occurrence of pedogeochemical anomalies. Depending on their amplitude, anomalies will influence the other parts of the environment differently, namely: from alterations of the chemical composition to morphological alterations (especially in plants) and even to the installation of diseases in plants and animals. In time, a natural selection of vegetal species has been made, some of them resisting and adapting to the high soil loading conditions, becoming indicator plants for areas that have abnormal concentrations of heavy metals.

The anthropic activity is an important source for heavy metal accumulation in the soil. There are several sources of heavy metals in the environment: 1) air which contains mining, smelting and refining of fossil fuels, production and use of metallic commercial products and vehicular exhaust, 2) water having domestic sewage, sewage and industrial effluents, thermal power plants and atmospheric fallout and 3) soil like – agricultural and animal wastes, municipal and industrial sewage, coal ashes, fertilizers, discarded manufacture goods and atmospheric fallout.

Heavy metals are elements that exhibit metallic properties such as ductility, malleability, conductivity, cation stability, and ligand specificity. They are characterized by relatively high density and high relative atomic weight with an atomic number greater than 20 (Raskin et al., 1994).

Some heavy metals such as Co, Cu, Fe, Cr, Mn, Mo, Ni and Zn are required in minute quantities by organisms. However, excessive amounts of these elements can become harmful to organisms. Other heavy metals such as Pb, Cd, Hg, and As (a metalloid but generally referred to as a heavy metal) do not have any beneficial effect on organisms and are thus regarded as the "main threats" since they are very harmful to both plants and animals.

The risk of soil and plant pollution depends on: plant species, chemical form of the chemical elements in the soil, the presence of other elements, especially of those that diminish the effect of metals and substances that decrease the absorption and desorption processes, the amount accessible in the soil as well as soil and climatic conditions. In fact, the harmful effects of heavy metals depend on their mobility that is on their solubility in the soil. Significant positive correlations have also been recorded between heavy metals and some soil physical properties such as moisture content and water holding capacity (Rakesh Sharma et al., 2013).

This is why, in the case of soils polluted with heavy metals, the first improving measures will refer to the creation of conditions that allow heavy metals to pass from soil solution to stable forms tied to various constituents (Neag et al., 2005).

This paper presents the influence of soil pollution with heavy metals on the production quality of some plant in Holboca area-Iasi city.

Here lies an important pollution source which exceeds the EU norms by over 3 times: CET (Electro-thermal power plant) Holboca which lies just 11.5 km away from the center of the city. In the vicinity, on a surface of 50 hectares, lies the slag and ash deposit of the power plant. The south of the area is bordering with another pollution source: the giant dump site of Tomesti.

MATERIAL AND METHODS

Sampling was made from the two surface horizon because it is considered that they are affected by pollution. Soil samples treatment collected for tests was made in accordance with the standard SR ISO 11464/1998 – Soil quality. Samples pre-treatment for physical-chemical tests. Therefore, samples were dried in the stove and were hashed with an electric soil mill. Heavy metals used were: Cd, Cu, Zn and Pb. Heavy metals determination was made in accordance with the standard SR ISO11047/1999- Soil quality, through atomic absorption spectrometry.

Metal extraction was made with concentrated sulphuric acid and oxygenated water 50%, with a mineralisator type Digestal HACH. (Standard SR ISO 11047/1999 – Soil quality).

The plant species chosen for this study are green salad (Lactuca sativa L. var. capitata) and spinach (Spinacia L. oleracea var. matador), as they are the most consumed vegetables because their leaves are very rich in nutrients. Green salad was chosen because it has the biggest capacity of accumulation of heavy metals, mainly of Cd without manifesting visible symptoms of phytotoxicity, which amplifies the risk over the human health.

The green salad and spinach crops were made in April 2015 and 2016 and the sampling of the plants was made in June 2015, 2016. In order to highlight the phenomenon of translocation of the heavy metal content determinations of Pb, Cd, Cu and Zn have been made from the roots and the leaves of these plants.

The used reagents for the effectuation of the analysis were of analytical purity: nitric acid 65%, purified water. For the effectuation of the calibration curve Dilutions of standard lead solutions (1 mg/ml) were used, respectively of cadmium (1 mg/ml) provided by Inorganic Ventures, Spain. For the determination of the heavy metal levels, the High Resolution Continuum Source Atomic Absorbtion Spectrometry method was used with the help of the Spectrometer ContrAA 300 HR-CS AAS (Analytic Jena, Germany). For the dry mineralization of the samples a calcination furnace was used (Nabertherm, Germany).

RESULTS AND DISCUSSIONS

The results regarding the content of heavy metals in the soil in Holboca area are presented in Table 1. They are the results of the measurements developed between 2015 and 2016.

Table No.1

The content of heavy metals in the soil in Holboca area in 2015-2016

Sampling point	Depth (cm)	Cd (mg/Kg dry s)		Cu(mg/Kg dry s)		Zn(mg/Kg dry s)		Pb(mg/Kg dry s)	
		2015	2016	2015	2016	2015	2016	2015	2016
N: 2100 m	0 – 10 cm	0.5	0.7	22.0	18.2	63.2	53.6	5.1	5.4
N: 2100 m	10 – 20 cm	0.7	1.3	32.6	19.8	42.8	51.4	4.2	4.3
V: 350 m	0 – 10 cm	0.3	2.1	152.8	32.7	115.2	93.8	18.2	9.2
V: 350 m	10 – 20 cm	0.5	1.5	28.6	30.2	88.2	75.4	16.1	7.8
SV: 1100m	0 – 10 cm	0.7	1.9	30.8	10.8	67.4	25.6	14.2	8.6
SV: 1100m	10 – 20 cm	0.3	1.6	24.8	31.6	78.8	51.8	12.6	11.4
S: 1600 m	0 – 10 cm	1.5	1.3	19.4	15.8	36.8	38.6	14.2	11.8
S: 1600 m	10 – 20 cm	1.3	1.5	22.8	10.9	64.2	24.4	3.1	9.8
SE: 700 m	0 – 10 cm	1.6	2.1	34.6	12.2	105.8	30.6	16.8	8.6
SE: 700 m	10 – 20 cm	1.3	2.0	23.6	13.2	69.8	30.2	6.4	5.8

The values of Cd, Cu, Zn, Pb concentrations in the soil from Holboca measured in 2015 and 2016 are presented in the chart from Figures: 1, 2, 3, 4.

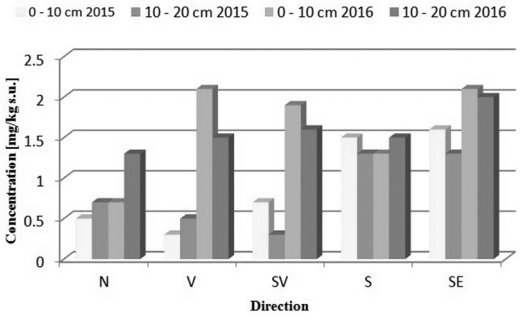
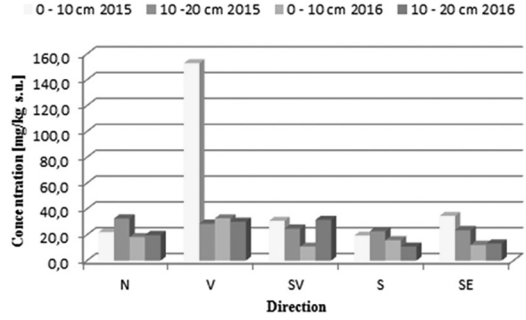


Figure 1. Variation of the Cd concentration in the soil



Fiure 2. Variation of the Cu concentration in the soil

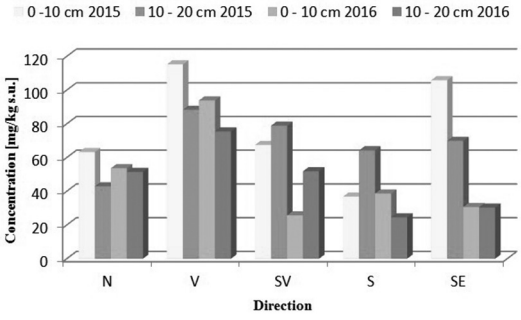


Figure 3. Variation of the Zn concentration in the soil

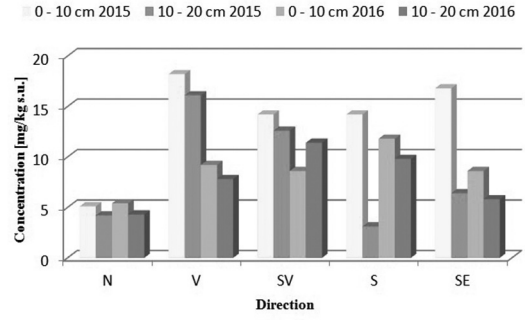


Figure 4. Variation of the Pb concentration in the soil

The normal content cadmium in soils is 1 mg/Kg dry substance, and the alert threshold for sensitive uses is 35 mg/Kg dry, and the intervention one is 5 mg/Kg dry substance. Analyzing the results for cadmium during the two years of study (Fig. 1), we notice excesses of the average values of 1 mg/ Kg dry the two sampling points, both in 2015 and in 2016.

Therefore, in 2015, the highest values for cadmium were recorded South and South-East from the steam power plant recording excesses of the average value on the two sampling depths. Values higher by 55% than the average value were recorded on the depth of 0-10 cm. On the other cardinal directions and sampling points, there were no records of excesses of the average value. The alert threshold and the intervention threshold were also not exceeded.

Copper has an average content in soils of 20 mg/Kg dry substance, an alert threshold for sensitive uses of 100 mg/kg and an intervention threshold of 200 mg/Kg dry substance. In 2015 (Fig. 2) excesses of both the average value and of the alert threshold were recorded.

Therefore, on all directions and sampling depths there were excesses of the average value. The highest concentration for copper was recorded West, on the depth of 0-10 cm, (152.8 mg/Kg dry substance), this being 7.64 times over the average value and 1.52 times over the alert threshold.

The lowest value of the copper concentration was recorded south, on the depth of 0-10 cm (19.4 mg/Kg dry substance), being little over the average value.

In 2016, the frequency of excesses as compared to the average value was only 30% in comparison with 2015, without records of any excess of the alert threshold or the intervention threshold. The highest concentrations for copper were recorded west and records of the average values were on the two sampling depths. Therefore, on the depth of 0-10 cm, the concentration for copper was (32.71 mg/Kg dry substance), 1.63 times higher than the average value and for the depth of 10-20 cm, it was (30.2 mg/Kg dry substance), 1.51 times over the average value. The lowest concentrations were recorded south-west and south, (10,8 mg/kg and 10,9 mg/Kg dry substance), this being almost half of the average value for copper in the soil.

Analyzing the results for zinc in 2015 (Fig. 3), we notice excesses of the average value, the frequency of excesses being of 20%. Therefore, the highest concentrations for zinc were recorded west, on the depth of 0-10 cm (115.2 mg/Kg dry substance) and south-east, the depth of 0-10 cm (105.80 mg/Kg dry substance), they being 1,18 times and respectively 0,7 times higher than the average value. The lowest concentration for zinc in 2015 was recorded south, on the depth of 0-10 cm (36.8mg/Kg dry substance), being in this case 2,6 times lower than the average value.

In 2016, the concentrations for zinc were below the average value on all directions and depths. The highest concentration below the average value was recorded this time west as well, on the depth of 0-10 cm (93.8 mg/Kg dry substance), being below the average value by 8,3%.

The lowest concentration of zinc was recorded south, on the depth of 0-10 cm (36.8mg/Kg dry substance), being only 36,8% of the average value.

In 2016, there were no records of any excess of the alert threshold and the intervention threshold for zinc. The concentrations for lead in 2015 and 2016, as well as the average value and the alert and intervention thresholds for sensitive uses are presented in (Fig. 4).

Analyzing the resulted concentrations, we notice that higher values for lead were recorded in 2015 than in 2016. Therefore, in 2015, the highest concentration for lead was recorded west, on the depth of 0-10 cm (18.2 mg/Kg dry substance), it being little over the average value.

The lowest concentration for lead in 2015 was recorded south, on the depth of 10-20 cm (3.1 mg/Kg dry substance), it being 6,23 times lower than the average value. Except for this value, the lowest concentrations for lead were recorded north. In 2015, all the resulted concentrations were below the average value for lead in the soil.

In 2016, the highest concentration was recorded south, on the depth of 10-20 cm (11,8 mg/Kg dry substance), it being approximately 1,8 times lower than the average value. The lowest concentrations for lead in 2016 were recorded north (4.3 mg/Kg dry substance), the biggest distance from the steam power plant.

For the assessing of the pollution/contamination of the soil-plant system, the obtained values for soils were compared to the normal reference values, alert threshold and intervention threshold for soils of sensible use provided by the Order no. 756/1997 of the Ministry of Waters Forests- Environmental Protection, and the

ones obtained for the vegetal samples were compared to the maximum values admitted in vegetables and leaves provided by FAO/WHO- Codex Alimentarius Commission, 2001.

It was considered the determination of heavy metals from the vegetal samples, from green salad and spinach crops and their interpretation in conjunction with the contamination level and the threatening of the food safety by reporting the obtained values to the maximum admitted values.

The assessment of the degree of contamination of study vegetables are in the Table no. 2.

Table No.2

The content of heavy metals in the green salad and spinach crops in Holboca area in 2015-2016

Heavy metal content of plants Minvalule		2015		2016		Maximum allowable values for leaf vegetables FAO/WHO- Codex Alimentarius Commission, 2001 (mg/Kg dry s)
		Max value	Min valule	Max value	Min valule	
Cd (mg/Kg dry s)	In the roots of green salad	0.095	0.276	0.107	0.788	0.2
	In the roots of spinach	0.064	0.234	0.071	0.679	
	In the leaves of green salad	0.073	0.215	0.091	0.573	
	In the leaves of spinach	0.076	0.244	0.097	0.653	
Cu (mg/Kg dry s)	In the roots of green salad	4.501	25.811	2.651	11.772	73.3
	In the roots of spinach	4.655	26.763	2.987	12.993	
	In the leaves of green salad	3.563	19.788	2.243	9.779	
	In the leaves of spinach	4.101	20.544	2.449	10.879	
Zn (mg/Kg dry s)	In the roots of green salad	3.217	10.545	2.989	9.547	99.4
	In the roots of spinach	5.122	11.989	3.508	11.133	
	In the leaves of green salad	2.561	9.855	2.369	8.985	
	In the leaves of spinach	3.988	11.023	3.543	9.882	
Pb (mg/Kg dry s)	In the roots of green salad	0.352	1.287	0.295	0.810	0.3
	In the roots of spinach	0.644	2.888	0.598	1.977	
	In the leaves of green salad	0.245	0.891	0.267	0.564	
	In the leaves of spinach	0.356	1.144	0.377	0.823	

CONCLUSIONS

1. The highest concentrations of cadmium were measured in the soil from Holboca area, this being also the area with records of excesses of the average value of 1mg/ mg/Kg dry s, provided by the relevant regulations in force. An excess of the average value was recorded south and south-east from the steam power plant and on the two sampling depths.
2. In the case of copper as well, the highest concentrations in the soil for this element were in Holboca area, recording excesses of the average value and of the alert threshold as well.
3. In 2015, all the values resulted for copper exceeded the average value and the concentration recorded west from the steam power plant, on the depth of 0-10 cm, were over the alert threshold, being the highest recorded in two.
4. Zinc had the highest concentrations in the soil from Holboca area, recording also the only excesses of the average value.
5. Spinach accumulates a bigger concentration of heavy metals compared to green salad.
6. The studied vegetables exceed the maximum admissible concentration of Cd and Pb.

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Serratula lycopifolia

Gymnospermium odessanum

The Red Book of the Republic of Moldova, Third edition. 2015

HOW CAN THE PROCESS OF EROSION CHANGE THE CHEMICAL STATUS OF THE QUALITY OF ORDINARY CHERNOZEMS

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Abstract. *The soil cover is the national wealth, natural resource and the principal means of production in the Republic of Moldova agriculture. Purpose and research tasks consist in setting up the changes made by erosion on the chemical characteristics of the ordinary chernozems. Were conducted the necessary investigations on the territory of the reception basin "Negrea". In this paper are assessed humus content, carbonates, pH, nitrogen, phosphorus, potassium and their distribution on the soil profile. From the data obtained on the changes of the chemical characteristics of investigated soils, it has been noted that appears significant quantitative and qualitative differentiation of these, depending on the degree of erosion. The research has shown that the chemical characteristics of soils with different degree of erosion are more strongly influenced by erosion. All this has led to the acceleration of erosion, including linear (Andrieș et al, 2003). Experiments were located within the perimeter of the village Negrea, Hincesti district, in the hydrographic basin of Lapușna river. We have studied four main profiles of ordinary chernozem with different degrees of erosion, characterized by different type of profile (Cojocaru, 2015). Soil samples collected in the laboratories of the Institute of Pedology, Agrochemistry and Soil Protection „Nicolae Dimo" were analyzed, and the results demonstrated the influence of the erosion process on the chemical characteristics of investigated soil (Arinushkina, 1970; Kauricev, 1980; Mineev, 2001; Sokolov 1965, 1975). In this article, we present data determining the humus content by the method Tiurin layers 0-30 cm for not eroded and weakly eroded soils, which are characterized as moderately humiferous with humus content of 3.1 - 3.7%. Soil reaction determined by potentiometric method of the not eroded soils, often the weakly eroded soils are neutral for the Ah and Bh1 horizons with values ranging from 6.9 to 7.2 and weak alkaline for the Bh2, BC and C horizons. According to calculations, by the investigated soils crops cultivated with hoes, nitrogen loss is 18 kg/ha. For poorly eroded soils lost 23 kg/ha of nitrogen, and the moderately eroded withto 28 kg/ha. Highly eroded soils lose - about 40 kg/ha of nitrogen (Cojocaru, 2015).*

Keywords: *erosion process, ordinary chernozem, status of the quality, reception basin, Republic of Moldova.*

INTRODUCTION

Soil is the main natural resource and national wealth of the Republic of Moldova, which is based on the use of 75% of the national economy (Cerbari et al., 2001). In recent decades, chernozems with high fertility are subjected to more intensive accelerated degradation due to inappropriate human activities, decreasing forest belts' surfaces and other factors, which led to a drop of essential productive capacity of agricultural crops. Within the limits the Central Moldova Plateau, the ordinary chernozem is widespread and soil quality on the majority of agricultural land is unsatisfactory (Andrieș et al., 2004). In the village of Negrea, continues to expand the area of lands affected by erosion, dehumification, damage and compaction, their share being higher. It is known that soil erosion presents a fairly widespread phenomenon in the country. Soil degradation processes are conditioned both by natural conditions and by inadequate human activity. Such degradation leads to the deterioration of physical, chemical and biological properties of soils (Andrieș et al., 2003). Today, the problem of the first order is in their rational use. Therefore, the knowledge of the chemical characteristics changes of the ordinary chernozem subjected of the erosion process is the important mainstay in determining the most effective anti-erosion measures. In order to estimate changes, the most characteristic of soil properties have been used as the most significant chemical indicators: humus content, pH, nitrogen, phosphorus, potassium and carbonates.

MATERIALS AND METHODS

Were highlighted forms of soil degradation and damage caused to the national economy as a result of

their extent? Instability of climate, especially precipitation regime, conditions such as the droughts and the floods and torrential character of rains in the warmer seasons present a decisive factor of soil erosion. Clearing forests, grubbing up steppes, land fund privatization led to excessive fragmentation and the division of quotas hill on valley. All this has led to the acceleration of erosion, including linear erosion (Andrieș et al., 2003). To accomplish the purpose of the research, it was conducted a field study by collecting soil samples and distributing, then, for detailed analysis in the laboratory. Harvesting of soil for analysis is a very important operation that depends largely on the accuracy of research results. The soil is collected in special containers.

The cylinders for the collected soil samples were rinsed with distilled water and then dried. The cylinders are normally used with a diameter of 5 cm and a height of 10 cm, which can comprise about 250 g of soil. The number of samples is fixed by the size of surface analyzed, evenness of ground, crop condition and the nature of analyzes performed. In laboratory, samples are made immediately, fresh or air-dried, put in cardboard boxes and stored in the laboratory evidence locker.

Among the methods used for the determination of total soil humus, best known are:

Calcination method - is simple, easy to execute, but imprecise. It is based on elimination by ignition of organic sample due to carbon oxidation with atmospheric oxygen. The weight loss by burning is determined by weighing (Аринушкина, 1970).

Schollenberger-Jackson method (wet oxidation method) that is based on the oxidation of carbon in the organic matter of the soil sample with a mixed oxidant potassium dichromate ($K_2Cr_2O_7$) and sulfuric acid (sulfochromic solution).

The principle of the *Tiurin method* (Аринушкина, 1970; Кауричев, 1980; Минеев, 2001; Соколов, 1965, 1975) to determine the content of humus in the soil consists in the oxidation of humus carbonate with a solution of chromic anhydride or potassium dichromate in presence of sulfuric acid.

Experiments were located within the perimeter of the village Negrea, Hincesti district, in the hydrographic basin of the Lapușna river. We have studied four main profiles of ordinary chernozem with different degrees of erosion, characterized by the following type of profile (Cojocaru, 2015):

- *not eroded* – (Ah+Bhk1)d→Bhk1→Bhk2→BCk→Ck;
- *poorly eroded* - (Ah+Bhk1)d→Bhk2→BCk→Ck;
- *moderately eroded* - (Bhk1+Bhk2)d→BCk→Ck;
- *strongly eroded* - (Bhk2+BCk)d→BCk→Ck.

From soil samples collected in the laboratories of the Institute of Pedology, Agrochemistry and Soil Protection "Nicolae Dimo", we examined the following chemical properties of investigated soil (Аринушкина, 1970; Кауричев, 1980; Минеев, 2001; Соколов, 1965, 1975):

a) the quantity of humus from the soil calculated by the *Tiurin method*;
b) pH determined by *potentiometric method*;
c) total nitrogen determined by *Kjeldahl method*;
d) mobile phosphorus, by *Macighin method*;
e) mobile potassium *Macighin method, flame photometry*;
f) carbonates by *volumetric-gaseous method*.

Based on this analysis, the amendment of chemical characteristics was pursued of ordinary chernozems depending on the degree of erosion.

RESULTS AND DISCUSSIONS

The research done in this direction has established the degree of change through erosion of the main chemical properties of the soil and the first modification of nutritive substance content.

Humus is the most important component of soil as it gives the health of soil and plants, ensures an excellent quality food, which increases their resistance to disease and pests. Humus content can be appreciated visually or can be determined exactly by chemical analysis in laboratory. The appreciation humus content is

performed according to the color of the soil. Soils rich in humus are darker, thus absorbing heat with greater ease than those lighter. This is an important factor which is largely dependent on the buffering capacity of the soil, its relationship with water, air, temperature and other properties of the soil. By determining the reserve of humus in the soil organic matter, it is highlighted the content of nutrients, especially nitrogen, the element most affected by erosion. Losses of humus in agricultural soils are related to several factors, of which the most important are practical crop rotation, soil tillage, the amount of organic matter returned with organic fertilizers etc. (Andrieş, 2004).

After the humus content determined by the *Tiurin method* (Кауричев, 1980; Минеев, 2001), in layers 0-30 cm (Fig. 1), not eroded and poorly eroded soils are characterized as moderately humiferous with a humus content of 3.1 - 3.7%. Moderately eroded soils have a humus content of 2.4 - 2.7%, considered humiferous submoderately and strongly eroded soils - poorly humiferous by 2 - 2.4% humus content (Cojocaru, 2015).

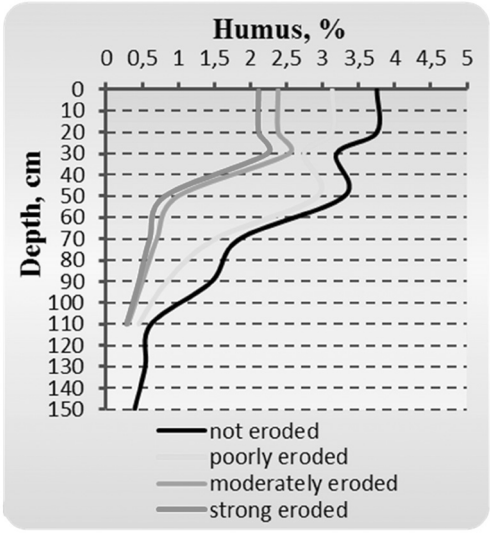

Figure 1. Humus content, % on ordinary chernozems

The created situation further favors the development of more prominent erosion phenomenon. The most important of humus losses have occurred in most cases in the depth of 30 cm. This variation is produced both by type of soil and the erosion process intensity (Аринушкина, 1970; Кауричев, 1980; Минеев, 2001). Based on the results, it was stated that the issue of increasing the flow of organic matter so, in eroded as well as in the not eroded soils is the main condition for maintaining their quality and production capacity (Cerbari, 2010). The ratio of C: N in the humus layer of arable soils investigated is fairly narrow - about 8-9. Thus (Table No. 1), the soils are poorly insured with mobile phosphorus and ensured optimally with mobile potassium (Cojocaru, 2015).

Regarding the changes in chemical characteristics depending on the degree of erosion, various researchers have found that humiferous soils are less exposed to the erosion process than poorly humiferous soils.

A particular influence on soil quality exercise the soil reaction (acidic or basic degree is given by the ratio between the concentration of ions H + and OH-). Soil reaction (pH) serves to the agroproductive characterization of a soil and constitutes an important criterion of how to use the land in choosing the assortment of plants. When the predominant cations - the reaction is acidic and when the predominant anion - the reaction is basic (alkaline). When these two are in approximately equal concentrations, the reaction is neutral. Thus, the acidic soils are poor or sometimes totally lacking calcium - important element for plant life and also deprived of some

micronutrients (boron, molybdenum, cobalt). Strongly alkaline reaction of the soil determines the blocking of micronutrients (Zn, Cu, Mn, Bo, etc.) and therefore, deficiencies in the supply of plants. The knowledge of the soil reaction helps to establish the form under which chemical fertilizers shall be used on different soils (Аринушкина, 1970; Соколов, 1965, 1975).

The value of pH determined in aqueous suspension of soil is an analytical index easily obtained, based on which, it is characterized the soil reaction and properties of acid - alkaline of the system of soil – water (Florea et al., 1987).

Table No.1

Modification of some chemical properties of ordinary chernozems spread on the territory of reception basin "Negrea"

Horizon and depth (cm)	C : N	Mobile forms (mg/100 g of soil)	
		P_2O_5	K_2O
Not eroded ordinary chernozem			
Ahp1 0-20	10,8	2,0	38
Ahp2 20-32	9,7	1,5	25
Ahd 32-52	10,7	1,2	20
Poorly eroded ordinary chernozem			
Ahp1 0-21	10,1	1,9	28
Ahp2 21-35	8,9	1,9	18
Ahd 35-53	9,1	1,7	16
Moderately eroded ordinary chernozem			
ABhp 0-21	10,6	1,9	17,0
ABhd1 21-48	10,7	1,7	13,0
BCk1 48-65	10,6	1,5	12,5
Strongly eroded ordinary chernozem			
ABhp 0-20	10,7	1,9	19,5
ABhd 20-40	11,0	1,6	13,0

The reaction of not eroded soils, and often the poorly eroded is neutral Ah and Bh1 horizons, is neutral for the Ah and Bh1 horizons with values ranging from 6.9 to 7.2 and weak alkaline Bh2, BC and C horizons. Moderately and strongly eroded soils are characterized by weak alkaline reaction on the surface (Fig. 2). This is explained by the fact that these layers are formed from inferior carbonate horizons, brought to the surface.

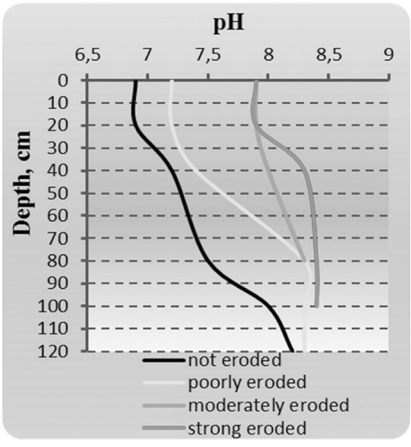

Figure 2. The values of pH on ordinary chernozems

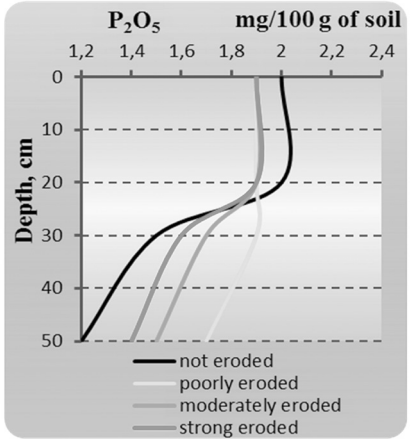

Figure 3. The phosphorus content, mg/100 g of soil on ordinary chernozems

The nitrogen in the soil is in organic and inorganic forms. In most of the nitrogen in the soil is located bound to organic matter (90%), namely: the dead organic residues, into the humic substances and microorganisms. In the upper soil levels, 20-40% of organically bound nitrogen is in the form of condensed amino acids in organic matter and released by acid hydrolysis, and, in addition, the soil contains very low amounts of free aminoacids that can be extracted in water, ethyl alcohol, ammonium acetate. Another portion of the organic nitrogen (5-10%) is in the amino-sugar composition, which, like amino acids, can be removed from the soil by acid hydrolysis.

Nitrogen plant available is formed of humus decomposition by microorganisms account. Nitrogen is represented as exchangeable form by ions of the NH_4^+ reversibly absorbed on the surface of soil colloids and in soluble form by ions of the NH_4^+ from the soil solution. These forms are readily available to plants. Determination of total nitrogen in the soil was carried out by the *Kjeldahl method*, which consists of the disaggregation (mineralization) of the soil with concentrated sulfuric acid, when nitrogen from the organic combinations is released and passed in the form of ammonium sulfate. This, by treatment with a strong base, releases the free ammonia that is removed from the solution by distillation and collected in a 0.1 N solution of sulfuric acid added into excess. The excess sulfuric acid by titration with 0.1 N sodium hydroxide and can be calculated from nitrogen content of the sample (Аринушкина, 1970; Соколов, 1965, 1975).

According to calculations made on the investigated soils cultivated with hoe crops, the nitrogen loss is 18 kg/ha. For weakly eroded soils is being lost to 23 kg/ha of nitrogen and the moderately eroded to 28 kg/ha. Highly eroded soils lose about 40 kg/ha (Cojocaru, 2015).

The phosphorus (P_2O_5) in the soil is in the form of inorganic and organic compounds (Аринушкина, 1970; Соколов, 1965, 1975). The majority of phosphates have low solubility and are difficult to reach plants. The phosphates in the soil are in the form of readily soluble primary phosphates and phosphate in the form of secondary, tertiary, octocalcic phosphates, phosphates adsorbed on the surface of iron and aluminum sesquioxide, or clay, which are poorly soluble. In neutral and alkaline soils, especially, phosphates are solubilized by the acetic acid used in the composition acetate lactate solution (AL). On measure to increase the degree of erosion, decreases the amount of phosphorus and more (Fig. 3).

The determination of assimilable potassium (mobile) was performed according to the *Machighin method*. Potassium (Аринушкина, 1970; Соколов, 1965, 1975) is one of the main elements of the earth's crust. Many agrochemical laboratories have adopted the method of determining of potassium in the (AL) extract using flame photometry. The extract obtained for the determination of phosphorus is used also to determine other considered elements accessible to plants. Soil extract containing potassium is converted into an aerosol by means of sprayer by the flame photometer. This one, reaching the flame, loses water by evaporation and forms a gaseous continuous flux composed of the atoms of different elements (Fig. 4). By means of a potassium interference filter and through a photoelectric cell, the intensity of this radiation emission permits the determination of extract concentration by potassium in the soil analyzed.

Carbonates are determined by titration with H_2SO_4 0.01N in the presence of phenolphthalein and bicarbonates by titration with H_2SO_4 0.01N in the presence of methyl orange. It was assessed first in the field using a solution by hydrochloric acid (1/3 concentration). In contact, the two elements make effervescence. Depending on the intensity of effervescence, it was possible to approximate the carbonate content in the soil. But a more accurate result was received by conducting analyzes in the laboratory (Cerbari, 2010; Соколов, 1965, 1975).

According to data obtained, carbonate profile of the investigated soils is derogated by reversing horizons (fig. 5), therefore not eroded soils are non-carbonated in the layer of 0-20 cm, and the poorly and moderately eroded the value of 2 - 3.9% g/g – carbonated weak. Strongly eroded soils from the surface are carbonated moderately and with the depth in the 30 cm layer - strong carbonate with values of 20% g/g.

Increasing the amount of carbonates and significant reduction of phosphorus, nitrogen and humus, directly influence the plant growth on eroded soils and level of productions achieved (Cojocaru, 2015).

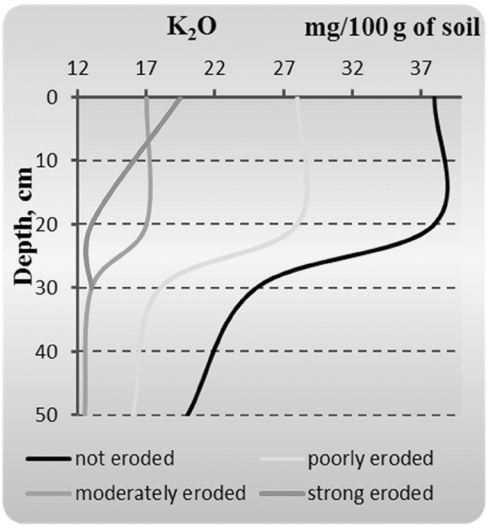

Figure 4. Potassium content, mg/100 g of soil on ordinary chernozem

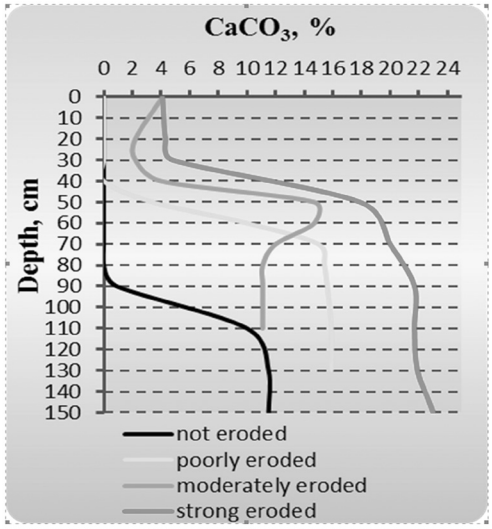

Figure 5. Carbonate content, % on ordinary chernozems

CONCLUSIONS

1. Experiments were located within the perimeter of the village Negrea, Hincesti district, in the hydrographic basin of Lapusna river. There have been considered for the study four main profiles of ordinary chernozems with different degree of erosion, characterized different type of profile.
2. After the humus content determined by the *Tiurin method*, in layers 0-30 cm not eroded and poorly eroded soils are characterized as moderately humiferous, with a humus content of 3.1 - 3.7%. Moderately eroded soils have a humus content of 2.4 - 2.7%, considered humiferous submoderatly and strongly eroded soils - poorly humiferous by 2 - 2.4% humus content.
3. The reaction of soils, not eroded often and the poorly eroded is neutral Ah and Bh1 horizons, is neutral for the Ah and Bh1 horizons with values ranging from 6.9 to 7.2 and weak alkaline Bh2, BC and C horizons. Moderately and strongly eroded soils are characterized by weak alkaline reaction on the surface (Fig. 2). This is explained by the fact that these layers are formed from inferior carbonate horizons, brought to the surface.
4. According to calculations made on the investigated soils cultivated with hoe crops, the nitrogen loss is 18 kg/ha. For weakly eroded soils is being lost to 23 kg/ha of nitrogen and the moderately eroded - to 28 kg/ha. Highly eroded soils lose about 40 kg/ha.
5. Increasing the amount of carbonates and significant reduction of phosphorus, nitrogen and humus, directly influence the plant growth on eroded soils and level of productions achieved.

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Alnus glutinosa

Alnus incana

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THE SITUATION OF PRINCIPLES OF DEVELOPMENT OF IRRIGATION IN THE REPUBLIC OF MOLDOVA

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Abstract: *As result of the theoretical and practical investigations of the 67 irrigation systems and sites, some important proposals were being necessary for the irrigation development in the Republic of Moldova in the actual conditions. It has been established that irrigation ensures an increase in the harvest of 30-80% on the gray soils, typical and levigated chernozems in the North and 180-250% on the ordinary and carbonate chernozems in the South (Andrieș et al., 1998; 2014). They regard at the equipment of the new irrigated lands at the area of 475 thou. hectares and reabilitation of lands equipped with the worst irrigation systems on the area of 125 thou. hectares. Principles are focused for an economic efficiency of the irrigated agricultural territories. The purpose of this paper is to analyze and generalize the situation on the field and determination of proposals to regulate basic principles of the development of irrigation in Republic of Moldova.*

Keywords*: irrigation system, specific capital investments, watering equipment, Republic of Moldova*

INTRODUCTION

Motto: "***Be ready to change your goals, but never change your values***."
The Dalai Lama

In the Republic of Moldova, productivity crop plants is largely determined by the pedologic and climatic conditions. During the vegetation period of plants, especially in the months from June to August, virtually every year there are droughts of soil and air. In such climatic conditions, irrigation is a radical measure to optimize water regime of the soil and of crop plants. It is about ensemble of land improvement works conducted guaranteeing supply with water to crops to increase productivity (Andrieș et al., 1998; 2014).

Peculiarities of natural processes on the territory of the Republic of Moldova are characterized by different hazards - in total 11 main groups of calamities (Mihailescu et al., 2005).

The more complex and dangerous natural phenomena here are considered drought, causing considerable loss of agricultural production, negatively affected the socio - economic development. In the last millennium this natural hazard, within the country, amplified from 35 droughts in the XI century up to 64 droughts in the twentieth century (Mihailescu et al., 2005).

In the years 1945-1996 there were various types of droughts that affected from 7% to 100% of Moldova's territory. In 60 years of this period of 42 years' droughts have affected the territory of between 40-100%. And during the last 20 years, from 1997 to 2016, there are repeated droughts practically once in two years (Mihailescu et al., 2005).

MATERIALS AND METHODS

The purpose of this paper is to analyze and generalize the situation in the field and determination of proposals to regulate basic principles of the development of irrigation in Republic of Moldova.

We performed theoretical and practical investigations in the fields of irrigation in the republic, analysis and generalization of results, examination of techno - functional indices of some systems of irrigation for internal household and management.

There are proposed the solution of regulation of major principles of founding of irrigation systems, taking

into account technical and scientific progress in the field of land improvements.

Restoring previously irrigated areas and new land irrigation planning must be conducted in accordance with some elaborations well argued scientifically and achievable under current conditions.

The aggregate data (Andrieș et al., 1998; 2014) by the Soils Improvement Laboratory of the Institute "Nicolae Dimo" have demonstrated that the efficacy of irrigation heads is different according to the country's agropedoclimatic area (Figure 1).

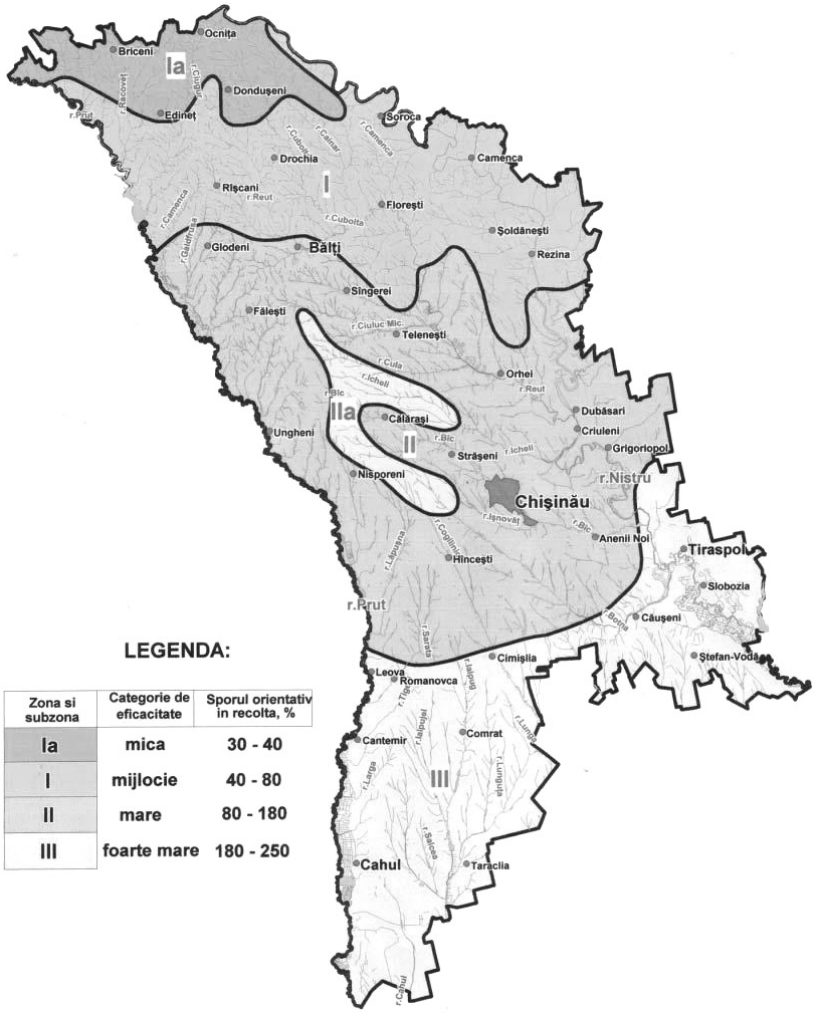

Figure 1. The effectiveness of irrigation in different climatic zones of Republic of Moldova (*dates: Iu. Rozloga, V. Filipciuc*)

RESULTS AND DISCUSSIONS

The Moldovan agriculture will develop with success only with an ample application of irrigation of land. Agricultural production, especially vegetables, fruits, under the conditions of market economy, through its

qualities, can`t be competitive without irrigation. Simultaneously, global harvest depends on irrigation too.

These circumstances have made the basis for the future of the agricultural sector, aiming at obtaining high yields and stable quality. Recently, in the mass media, it was broadcast and published information on the main problems of development of agricultural irrigation in the Republic of Moldova in the nearest future.

This program provides the increase of irrigation areas up to 600 thousand hectares, for which purpose is, needed 4.2 billion lei for capital construction, namely for the arrangement of new irrigation systems and 2.4 billion lei, for the rehabilitation, namely, reconstruction of existing irrigation systems.

Optimization of water regime is accomplished according to moisture reserves in the soil active layer and the critical stages of water used by plants (Andrieș et al., 1998; 2014).

Simultaneously, we mention that according to a strategic plan of action for rehabilitation of irrigation and development in the Republic of Moldova, elaborated in 2000 by the Republican Concern "Moldova's Waters" (***Ministry of Agriculture and Processing Industry, 2000) and approved by the Government Decision published in the Official Monitor of the Republic of Moldova No. 46 of 20 April 2001, were to be rehabilitated irrigation systems on a total area of 125 thousand hectares, which condition the special capital investments 19.200 lei per hectare.

Therefore, examining data from mass media and strategic development plan, in the years 2000 - 2016, we can generalize the following main indices as stated by the development strategies of irrigation in our country of 600 thousand hectares (Table No.1).

Table No. 1.

Main indices of development of irrigation in the Republic of Moldova (in the years 2000 – 2016)

Land development	Landscaping areas, thousand hectares	Total investments, billions MDL	Specific investment, MDL per hectare
The construction of new irrigation systems	475.0	4.2	**8842**
Rehabilitation of existing irrigation systems	125.0	2.4	**19200**
Total	**600.0**	**6.6**	**28.042**

Evidently, in the full developments of this strategic program, there are committed discrepancies and inconsistencies, which eloquently demonstrate the data from Table No.1. Really, specific capital investments to the rehabilitation of irrigation systems can`t be 2.2 times higher than those in the construction of new systems, being smaller in reality.

The analysis of information from the strategic plan of development and rehabilitation of irrigation (*** Ministry of Agriculture and Processing Industry, 2000), the general technical and economic data on 60 small systems and of irrigation sectors ranging in size from 7-100 hectares and the 7 largest irrigation systems ranging in size from 137-650 hectares (situation at 04.10.2002) and also the study on some recommendations for irrigating fruit and vegetables in Moldova (Coșuleanu, 1981; 1998) allow us to concretize to the possible extent that the irrigation situation in our country is characterized by technical-economic indexes (Table No.2).

Mention that capital investment indices generalized in Table No.2, expressed in MDL, they were obtained from dollars, recalculated cost of 60 small irrigation systems and of irrigation sectors and from EURO, of the 7 largest of irrigation systems. Specific investigations, 19,200 lei per hectare (situation of the 12.01.2016) are taken from the strategic plan of action. We realize that such recalculations are essential guidance and yet we use to

some conclusions.

We may remark that:

1. It is necessary to consider that for 16 years (2000-2016), there were more expensive building materials, the pipes used in irrigation equipment and sprinkler irrigation installations, pumping equipment and installations etc., that the construction and rehabilitation of irrigation systems have become more expensive, the specific value of investments increased. In this period, the expensive cost of electricity.
2. The generalization of information relating to the 67 systems and sectors of irrigation shows that investigations were provided, basically for the purchase of new techniques of irrigation (equipment sprinkler, pump systems and watering) without reconstruction of the existing network of irrigation or construction of new networks. So the investigation indicated in Table 2 refers absolute majority irrigation the endowment value by means of watering.
3. Agribusiness Development Project (ADP) that is implemented by CNFA American organization supported by the US Agency for International Development (USAID) so condition that the average specific of the small sectors sprinkler irrigation reaches 15751-26250 lei/hectare, which depends on the type of sprinkler installations and equipment (Gherciuc, 2007). According to the same information, specific capital investments to the drip irrigation constitute on average, for the cultivation of vegetables, 9450-15750 lei/hectare and for the cultivation of fruit and vines 14700-21000 lei/hectare. To emphasize that USAID ADP data are the latest and deserve greater truthfulness and these investments include only the cost of watering equipment and facilities without taking into account the cost of irrigation network with hydro technical constructions and related facilities.

Table No. 2.

Technical and economic indices of planning the systems and of irrigation sectors in the Republic of Moldova (situation to the 04.10.2002)

Rehabilitation of irrigation systems with spray irrigation with an area between 100-650 hectares		**Rehabilitation of irrigation systems with spray irrigation with an area between 15-100 hectares**		**Arrangement of drip irrigation sectors ranging in size from 7-100 hectares**	
Area, hectares	***Specific investment, MDL per hectare***	***Area, hectares***	***Specific investment, MDL per hectare***	***Area, hectares***	***Specific investment, MDL per hectare***
2432	**6314**	**1138**	**6342**	**491**	**15697**

4. Execution of the plan on development of irrigation will condition the major capital expenditures more like those provided (Table No.1). Approximately, the rehabilitation investment will be increased by 1.5-2 times, and the construction of new systems - by 4.5-5.0 times.

From intensive cultural practices, irrigation is the most contradictory practice. The irrigation can lead to erosion and salinisation, reduce the level of sequestered organic carbon and increases emission level, reduces productivity and can even lead to total loss of soil fertility. Salinization affecting at least 10% of irrigated areas worldwide. For these reasons, some researchers refuse irrigation within the sequestration carbon practices. In other cases, the negative effects of irrigation are due to faulty management of water (Moraru et al., 2010).

For further development of irrigation, the Republic of Moldova's natural need to reduce specific investments. We believe that guidance for this purpose is arranging irrigation systems with two categories of land: with guaranteed irrigation and mobile irrigation (Coşuleanu, 1978; 1998).

Lands with guaranteed irrigation will be serviced by a traditional network, of irrigation engineering, will achieve this regime the designed agricultural crops. The mobile irrigation lands, adjacent to guaranteed

irrigation sectors, will serve a network of rarer, simple or demonstrable irrigation that transports the water up to mobile watering installations. Such systems allow the growing of higher overall; a few extra watering areas increased, occupied with agricultural crops that are watered only 1-2 times.

Economic opportunity of these systems is higher than those with guaranteed irrigation, used on lower areas of agricultural crops. It is significant that pedologic action of irrigation on these lands is much slower as compared with guaranteed irrigation land.

Currently, it is designed the program to rehabilitate 11 irrigation systems with an area of 15 500 ha (Andrieș et al., 1998; 2014).

CONCLUSIONS

The development of irrigation problems in Republic of Moldova contains discrepancy and inconsistencies, their economic argument is difficult.

For the achievement of irrigation development programs, in the future, it is necessary to focus on specific investments relating to new systems of construction and rehabilitation of existing irrigation systems.

For the purpose of current social-economic scientific argumentation of irrigation, it is necessary to organize a laboratory, as soon as possible, in a research institution in this field. Such research will be focused mainly on optimizing the correct setting of the regime of irrigation and economic opportunity of agricultural crops' irrigation.

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THE COMPARATIVE ASSESSMENT OF INDICES OF SOIL IN DEPENDING OF AGROTECHNOLOGIES APPLIED TO GROWING MAIZE

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Summary. *An important component in the agricultural technology tillage system with a view to creating favorable conditions for plant growth and development, leading to modification of the physical condition of the soil and taking direct action on chemical and biological soil characteristics, regardless of the cultivated plant. Maize is a crop less demanding of the previous plant. The best results are obtained by annual grain legumes and fodder followed autumn cereal grains, flax, hemp, potatoes, beets and sunflowers. Wheat maize rotation is required due to the weight of approx. 60% of the two cultures. In this rotation is favored maize is grown after a run-up early (www.agricultura-romania.ro). From research data is mentioned that both in cenosis maize monoculture (34 years) and maize in the crop rotation (after maize) following application of tillage - No-till soil moisture preserves layer is 40-50 cm, which and other researchers argue, regardless of the cultivated plant (Andriucă V., 2015). Conservation tillage system is best for rooting crops, including maize. CA is a precision agriculture with the purpose of preserving soil fertility.*

Keywords: maize, soil properties, agrocenoses, soil tillage, crop rotation.

INTRODUCTION

Maize is a plant drought-resistant, heat-loving and light. It is an annual plant, which belongs to the grass family and originated in America. Maize was brought to Europe in the XVth century by Christopher Columbus and was adapted pretty quickly to new climatic conditions in Spain and Portugal so gradually spreading to all countries including the Republic of Moldova.

Maize occupies the third place between cultivated plants worldwide, totaling after the 2005 statistics, the surface of 147.0 mln. ha to 3587 kg/ha. The largest areas are in US maize 30.8 million. ha, followed by China (25.2 mln. ha), Brazil (11.4 mln. ha), Mexico (8.0 mln. ha), India (7.4 mln. ha). In the Moldova the maize is grown on large tracts of approx. 480 000 ha. High yields obtained in Italy (10063 kg/ha), USA (9315 kg/ha), France (8095 kg/ha).

Maize occupies third place in importance between cultivated plants worldwide. This position, in terms of agriculture, is motivated by a number of features, such as: has a large production capacity by about 50% higher than in other cereals; has great ecological plasticity, which allows a wide area of distribution, giving high yields and relatively constant, less influenced by climatic irregularities; is a plant hoes, good run for most crops; supports monoculture for several years; it has a high coefficient multiplier (150-400); having seeding later in the spring, enables better scheduling of agricultural operations; culture is mechanized 100%; harvesting is done without danger of shaking; well recovered organic and mineral fertilizers and irrigation water; their production possibilities are varied etc. (http://www.agrimedia.ro).

Tillage system influences many processes"chain" that directly or indirectly arise during the execution of the work. Soil works upon some organized structures through which you manifest effect on both the plant and the initial system. Organized system such as soil tillage and initially take their effect, amplify or shrink them action in relation to other systems (climate, plants and microorganisms).

Soil works through direct and indirect effects of soil layer influences both worked and Subari horizon. Changes are physical, chemical and then biological primarily variable characteristics (Pop A., 2008).

MATERIAL AND METHOD

The research was conducted in Didactic-Experimental Station UASM of being among long stay, founded in 1970, located in Anenii Noi district, about 26 km from Chisinau. Anenii Noi district is located at latitude 46.8827 29.2304 longitudes and altitude of 18 meters above sea level bud. Rayon composed of 45 municipalities,

including and Chetrosu.

As a subject was chosen agro maize that has both common features and significant differences. It is common place - Moldova's Central Area on the outskirts Codrilor regime weather, soil type, and the difference lies in the degree of anthropogenic action, expressed by tillage technology. The research aim in this paper is to evaluate some physical properties compared to growing maize under the influence of conservative technologies and conventional tillage and weather conditions.

In this work they were carried out research in the crop rotation of maize and maize monoculture, for 34 years. Some physical properties were evaluated: soil moisture thermostatic by the method of weighing and drying the samples in an oven at a temperature of 105°C and the bulk density effected by means of cylinders, also penetration resistance was determined in the field using penetrometer. Penetration resistance classification is according penetration resistance values shown in Table 1.

Table 1.

Classes' penetration resistance values (after Canarache A., 1990)

Name	The values (kgf/cm^2) (I.C.P.A., 1987, vol. 3)	Meaning	Increases resistance to plowing / Decreases permeability
Very small	Under 11	Normal growth of roots	
Small	11-25		
Medium	26-50	Partial limitation roots	
Very big	51-100		
Extremely big	101-150	Roots cannot penetrate	
	After 150		

RESULTS AND DISCUSSIONS

The research was conducted under agrocenoses maize and maize monoculture in crop rotation (34 years), applying technology conventional tillage and conservation.

Crop rotation within a properly drawn rotation provides a number of benefits for the agricultural ecosystem, helping to relieve the physical and chemical composition of the soil, the disappearance of pests, and weeds present in some crop fields weeding. (http://www.akademos.asm.md).

According to the researchers ICCC "Selection", especially the scientist B. Boincean rational use of crop rotation in growing and processing crops lead to a significant increase in productivity. Thus, sugar beet production may be increased by about 10-30%, and up to 40% maize and the sunflower 10-20% (Boincean B., 2003; 2004).

Monoculture maize was expanded in our country fertile soils, lower, shallow ground water, subjected to the wettest springs temporary excess moisture - land on which wheat not recovered as much as maize. It can be appreciated however that the prolonged monoculture reduces humus content, structure degrades, there is a progressive acidification of the soil, pests and diseases are proliferating (www.agricultura-romania.ro).

The research was focused on changing the physical properties of soil - moisture, bulk density and physico-mechanical - penetration resistance under maize agrocenoses applying conventional tillage system and conservative. Research conducted in this paper of on the 0-120 cm layer, soil moisture and bulk density in 0-50 cm layer and penetration resistance in the 0-50 cm layer in agrocenoses permanent crop maize are shown in Table 2, and the results agrocenoses maize in the crop rotation research (after maize) are shown below in Table 3.

Table 2

Agroindices and soil moisture based on the tillage system under agrocenoses maize monoculture, SDE Chetrosu, May 2016

Depth, cm	Mousture, %		Apparent density, g/cm^3		Soil resistance to penetration, kgf/cm^2	
	Plowing	No-till	Plowing	No-till	Plowing	No-till
0-10	13.55	12.75	1.09	1.42	8.5	14.5
10-20	15.62	15.78	1.10	1.35	9.4	23.7
20-30	18.63	19.22	1.19	1.32	10.2	20.9
30-40	20.68	19.57	1.20	1.29	16.3	22.6
40-50	20.16	19.97	1.13	1.25	19.6	21.0
50-60	19.39	20.12				
60-70	18.25	19.52				
60-70	18.25	19.52				
70-80	18.72	19.04				
80-90	18.58	17.93				
90-100	17.9	17.7				
100-110	17.05	17.44				
110-120	17.0	16.59				

Comparative evaluation of moisture in maize monoculture cenosis (34 years) and maize in rotation (after maize) with conservative system and conventional tillage is shown in Fig. 1 and observed that good soil moisture (not essential) is greater than the version of No-till research compared to maize variant in crop rotation and monoculture maize (34 years).

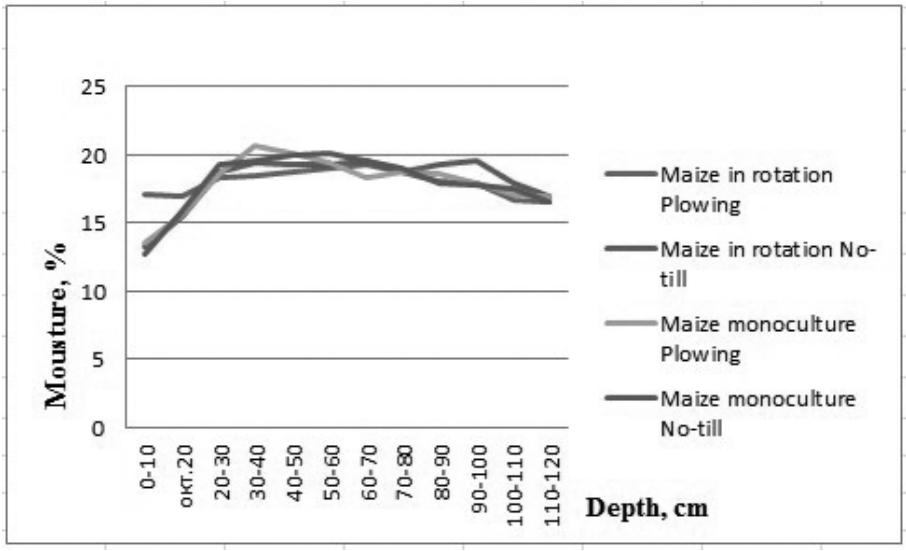

Fig. 1. Soil moisture depending on tillage system under agrocenoses maize and maize monoculture in crop rotation (34 years), SDE Chetrosu, May 2016

The apparent density is relatively independent ownership, knowledge of which provides physical characterization of soil elements independent, which can only be deducted in some cases from other properties. A soil belonging to a genetic type with a certain texture, with specific chemical properties, can have very different bulk density values, and vice versa more of the other physical properties of the soil depend, in addition to texture or some chemical characteristics and bulk density. For the characterization of the soil texture and bulk density are the basic characteristics (Canarache A., 1990).

From the data obtained on the apparent density No-till variant research in maize monoculture agrocenoses varies between 1.25 to 1.42 g/cm3, it is greater than plowing variant, ranging within 1.09 to 1.20 g/cm3, the same situation is observed in the maize crop rotation, plowing - density varies between 1.16 to 1.20 g/cm3, while the variant of maize research in crop rotation, No-till is the apparent density 1.19 - 1.35 g/cm3 (Fig. 2).

Table 3

Agroindices and soil moisture based tillage system under maize in the crop rotation agrocenoses, 34 years old (pre - maize), SDE Chetrosu, May 2016

Depth, cm	Mousture, %		Apparent density, g/cm^3		Soil resistance to penetration, kgf/cm^2	
	Plowing	No-till	Plowing	No-till	Plowing	No-till
0-10	17.06	13.27	1.19	1.27	8.4	7.8
10-20	16.88	15.41	1.20	1.35	10.2	11.1
20-30	18.34	18.71	1.16	1.29	15.2	14
30-40	18.5	19,34	1.18	1.21	20.6	19.3
40-50	18.68	19.31	1.20	1.19	20.9	22.5
50-60	18.96	19.23				
60-70	19.33	19.48				
70-80	18.73	18.73				
80-90	18.04	19.29				
90-100	17.85	19.52				
100-110	16.72	17.97				
110-120	16.5	16.9				

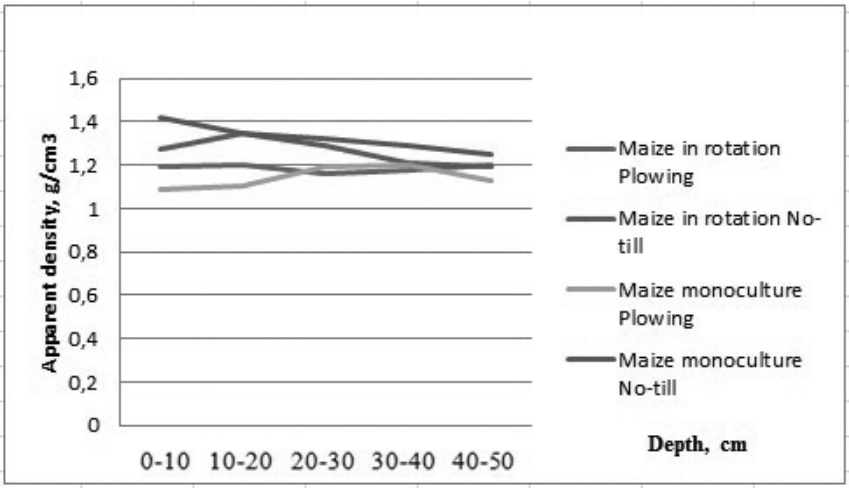

Fig. 2. The bulk density of soil by tillage system under agrocenoses maize and maize monoculture in crop rotation (34 years), SDE Chetrosu, May 2016

Penetration resistance presents special importance in relation to the study and development of the root system in the soil penetration. The similarity between the tip and root penetration is obvious (Greacen E., 1968). According to research by Taylor H. 1966 the penetration resistance values less than 10-15 kgf/cm2 is not bad for roots to penetrate the soil, while higher values of 35-40 kgf/cm2 root penetration is practically impossible.

Research in both versions of No-till plowing and so on maize monoculture and maize in rotation penetration resistance increases with depth, comparing maize crop in rotation with maize permanent soil tillage - No-till 0-50 cm layer resistance the penetration is higher in maize monoculture, ranging within 14.5 to 23.7 kgf/cm2, explained is a more rapid development of the plant and the roots, due to keeping water in the soil. Both variants research into crop rotation of corn and maize monoculture (34 years) soil moisture (not essential) is greater than the version of No-till research (Fig. 3).

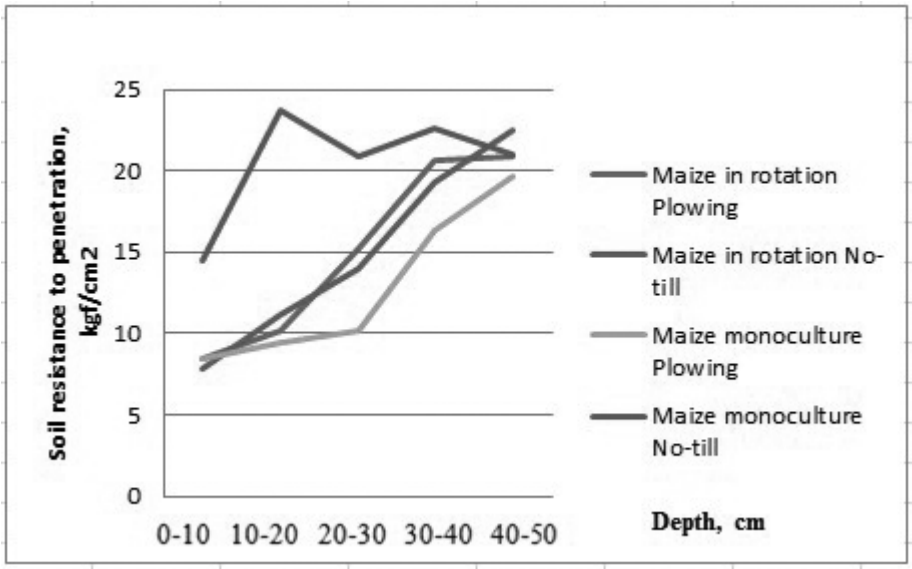

Fig. 3. Soil resistance to penetration by tillage system under agrocenoses maize and maize monoculture in crop rotation (34 years), SDE Chetrosu, May 2016

CONCLUSIONS

The conservative tillage systems choice of crop rotation is more needed than in conventional systems, having beneficial effects on both the soil by improving biological activity and the supply of nutrients and the mass development of root in better control of weeds, diseases, pests and plants grown in increasing the productivity. In the conservative system of tillage soil moisture is kept within 40-50 cm, giving the possibility to plants to grow better and faster, covering the soil with leaves, which results in slowing the growth of weeds.

The apparent density of the No-till variant research in maize monoculture agrocenoses varies between 1.25 to 1.42 g/cm3, is greater than plowing variant, ranging within 1.09 to 1.20 g/cm3, this observing and maize in the crop rotation, No-till bulk density ranging between 1.16 to 1.20 limits g/cm3, plowing - having bulk density limits from 1.19 to 1.35 g/cm3, while penetration resistance both versions of maize research different technologies of tillage increases with depth.

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Rindera umbellata

Alyssum gmelinii
The Red Book of the Republic of Moldova, Third edition. 2015

THE INFLUENCE ON THE SOIL OF PHYSICAL ACTIVITY INDEX CELLULOLYTIC AUTUMN WHEAT UNDER AGROCOENOSES

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Summary. *Microflora and microfauna in the soil is the most important living rhizosphere and consists of bacteria, cyanobacteria, fungi, algae and protozoa. Because of their mineralization of organic substances takes place and make the circuits of carbon, nitrogen, phosphorus or sulfur in nature. Scope this article consists in assessing the influence of soil physical indices on cellulolytic activity under agrocenoses autumn wheat. Research activities were conducted cellulolytic Didactic -experimental Chetrosu resort in Anenii Noi district on sandy loam carbonate chernozem in agrocenoses autumn wheat in rotation (after Bean) with different technologies of tillage - No-till plowing and (years 2016). Cellulolytic activity was determined by the Mişustin E., 1978 method, and evaluated according to Table 1 based on the principle of the method using the decomposition of cellulose under aerobic conditions blades in the 0-30 cm layer of soil. The research results cellulolytic activity in autumn wheat agrocenoses during earing depending on the technology applied to soil tillage is shown in Table 2 and 3 (G. Muller, 1968). According to research for the variant plowing cellulolytic activity varies between 48-56%, and the variant No-till varies between 27-33%.*

Keywords: cellulolytic activity, crop rotation, agrocenoses, autumn wheat, soil tillage

INTRODUCTION

Soil is a natural formation, the most superficial layer of the land is linked to changes in the world rocks under the influence of living organisms and non-living, solar heat and precipitation. Soil is the first development environment and resource base of the plant for the life of animals and humans (Demeter T., 2009).

It can`t be considered ground than crust altered the earth's surface, imbued with life. Although ground hosts many bodies as typical soil organisms are considered but only those to whom he serves as continuously living environment. Some creatures are driven into the ground, but could not find suitable living conditions must perish, others live only temporarily in the ground during a certain phase of their development. Some feed on the ground; others spend a short time in the soil without taking food. All these beings are not regarded as belonging to soil organisms themselves, can still play a role in certain cases and to influence soil properties (G. Muller, 1968).

It is evident that soil is a favorable environment for various microorganisms that convert plant residues are involved in the formation of soil structure, humus formation and mineralization.

Franz H. (1949) notes that independent of specific environmental conditions (biotope), the representatives of soil flora and fauna arose several forms of adaptation which development takes place the same in the different groups bodies. Report to community life in the soil have been identified 4 groups of which group bodies sessile (edafon sessile) lies first expressed by bacteria and fungi that can populate as a film even in the smallest particles ground. A special role in the transformation of organic material they have microorganisms represented by bacteria, fungi, actinomycetes, etc.

Bacteria are the most common group of organisms ranging from several hundreds of thousands to billions each soil cm3 and is about 40% of the microorganisms in the soil. The area most populated by bacteria is found in the vicinity of the roots of the plants in the soil layer of 2-5 mm, also called rhizosphere. By way of nutrition heterotrophic bacteria and autotrophic classified, and after the use of oxygen in aerobic and anaerobic.

Fungi are heterotrophic microorganisms predominantly aerobic, who prefer an acidic environment, live with bacteria and have a great importance in the process of humification and ammonification.

Actinomycetes is form of transition from bacteria to fungus develops in reaction from acidic to alkaline conditions, have a high capacity for decomposition of organic matter.

Microorganisms in the soil are two categories of biochemical processes specific multilateral order physico-chemical, biological and agriculture consequences. First are degradation processes (fermentations, Ammonification, etc.) that is issued in organic matter and minerals available to plants secondly synthesis processes (molecular nitrogen fixation and humification), which creates a reserve of substances in soil nutritious

organic form, which significantly the physico-chemical and soil fertility influences (www.determinarea existing microorganisms in soil html).

MATERIAL AND METHOD

Research cellulolytic activities were conducted Didactic-experimental Chetrosu resort in Anenii Noi district on sandy loam carbonate chernozem in agrocoenoses of autumn wheat in crop rotation (after Bean) with different technologies of tillage - No-till and plowing.

Autumn wheat, one of the most important grains occupying the largest areas on the globe, and the special importance of wheat is that its seeds can be stored for a long time. Products made from wheat is the basic food for a large proportion of the world population making up approx. 35-40% of research that has been chosen for this plant quite important. In the research they were placed bottles with cloth in soil at different depths in three repetitions, both rows and between rows of plants. Cellulolytic activity was evaluated depending on soil moisture, as determined by the drying of soil samples and conducted field penetration resistance with penetrometer.

The composition of the pulp falls more than 50% of organic carbon of the Biosphere. Cellulose is the most widespread polysaccharide from plant sources in nature. Higher plants contain cellulose in 40-70% of their body. In this connection, the microorganisms that break down cellulose play a very important role in the carbon cycle on Earth.

The diversity of soil microorganisms to break down cellulose allows different soil conditions, acid or alkaline pH, loose or compacted with different humidity and temperature. The microorganisms' breaks down the cellulose are specific: under aerobic decomposition running *actinomycetes* bacteria and fungi aerobic and anaerobic mesophilic bacteria and thermophilic. In good condition in terms of climatic conditions in the soil and creates aerobic cellulose decays slowly and is stopped completely if high temperatures and drought. The most common aerobic microorganisms that break down cellulose are of the genus *Cytophaga* and *Sparasytophaga*; of myxobacteria genres: *Mixococus, Sorangium* and *Polyangium*; fungi that break down cellulose are species of the class *Ascomycetes, Basidiomycetes*.

Fungi and bacteria found in the cellulolytic group of soil belong in mostly mesophilic, having an optimum temperature of 25-30°C activity. If water saturation exceeds 50% of the field capacity for water, the cellulose is carried out more and more bacteria, fungi while regressing activity (Gârla D., N. Cazmali, 2013).

Cellulolytic activity was determined by the Mişustin E., 1978 method and measured according to Table 1 based on the principle of the method using the decomposition of cellulose under aerobic conditions blades in the 0-30 cm layer of soil.

Table 1. Class's values cellulolytic activity on chernozioms (Mişustin E. method)

Index level	Cellulolytic activity
Very small	< 36
Small	36 - 52
Moderate	52 – 68
High	68 - 84
Very high	> 84

RESULTS AND DISCUSSIONS

The works carried out on soil physical properties resulting in a change in some cases of soil creating favorable conditions for the activity of microorganisms. The research results cellulolytic activity in autumn

wheat agrocoenoses during earing depending on the technology applied to soil tillage is shown in Table 2 and 3.

According to research for the variant plowing cellulolytic activity varies between 48-56%, and the variant No-till varies between 27-33%. Observe well that cellulolytic activity is reduced of No-till variant of autumn wheat under agrocoenoses (forerunner beans) compared with cellulolytic activity, plowing variant (Fig. 1). By plowing plow essential increase aerobic microflora (fungi, bacteria, actinomycetes) that have a role in decomposition of organic cellulose (resistant), for fresh organic substances decompose very intense, their transformation coefficient is very low in humus. Transformation (mineralization) fast fresh organic waste material leads to lack of energy in soil and bacteria begin to decompose the humus (G. Muller, 1968).

Table 2. Cellulolytic activity (%) in agrocenoses autumn wheat depending on soil tillage, May-June 2016, SDE Chetrosu

Depth, cm	Decomposition blade,% compared to the initial mass	Appreciation against the chernozioms cellulolytic activity (Mişustin E. method)
Variant, plowing		
0-10	55.67	Moderate
10-20	47.83	Small
20-30	50.26	Small
The variant No-till		
0-10	30.14	Very small
10-20	26.86	Very small
20-30	33.08	Very small

Table 3. Cellulolytic activity (%) in agrocenoses winter wheat depending on soil tillage, May-June 2016, SDE Chetrosu

Depth, cm	The location of blade	Decomposition blade,% compared to the initial mass	Findings on chernozioms cellulolytic activity (Mişustin E. method)	Mediate
The plowing variant				
0-10	At a time	59.85	Moderate	55.67
	Between rows	49.94	Small	
	At a time	57,23	?Medium	
10-20	At a time	51,44	Small	47,83
	Between rows	42,87	Small	
	At a time	49,18	Small	
20-30	At a time	53,39	Medium	50,26
	Between rows	47,87	Small	
	At a time	49,53	Small	
The No-till variant				
0-10	At a time	27,25	Very small	30,14
	Between rows	38,46	Small	
	At a time	24,72	Very small	
10-20	At a time	20,86	Very small	26,86
	Between rows	33,29	Very small	
	At a time	26,43	Very small	
20-30	At a time	33,59	Small	33,08
	Between rows	35,47	Small	
	At a time	30,18	Small	

The area most populated by microorganisms is in close proximity to the plant roots, it explains that the cellulolytic activity is higher compared to rows between rows cellulolytic activity and decomposition of the canvas to the original table is shown in Fig. 2. The analysis of data shows that the depth of sampling indicates the characteristic changes in the numerical distribution of microorganisms. Thus, most organisms have been identified in the surface layers of soil (0-10 cm depth harvest), which can be explained by the presence in the soil horizon of large quantities of organic substances as nutrients used by the microorganisms. The amount of microorganisms depends on soil moisture, weather conditions, soil tillage technology etc.

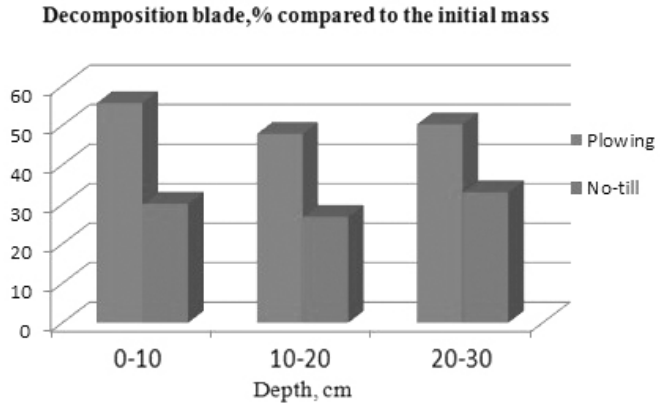

Fig. 1. Depending on the technology cellulolytic activity agrocenoses applied to soil tillage in autumn wheat, SDE Chetrosu, May 2016

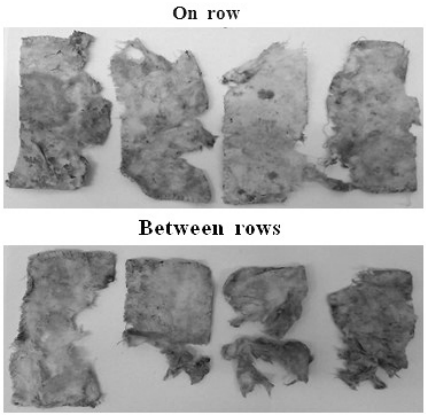

Fig. 2 - Decomposition blade, % compared to the initial mass of autumn wheat under agrocenoses

In agrocenoses autumn wheat (pre beans) on both variants No-till plowing and soil moisture was investigated in the 0-110 cm layer and soil penetration resistance are shown in Table 4.

Table 4.

Soil moisture, resistance to penetration agrocenoses autumn wheat under different tillage systems, May 2016, SDE Chetrosu

Depth, cm	Moisture,%		The soil resistance to the penetration, kgf/cm^2	
	Plowing	No-till	Plowing	No-till
0-10	10.11	11,27	8,6	19,5
10-20	11.97	11,88	11,8	21,6
20-30	12,54	13,02	15,9	20,5
30-40	13,22	14,38	19,2	22,1
40-50	13,99	15,18	19,6	22,7
50-60	14,22	14,81		
60-70	13,62	14,22		
70-80	12,7	13,97		
80-90	12,96	13,78		
90-100	14,14	14,69		
100-110	13,24	14,32		
110-120	13,58	13,01		

Agrocenoses of soil moisture in autumn wheat on plowed version is smaller compared with the No-till variant because the plants are well developed and fully covered surface. From the data obtained on the soil moisture, soil moisture is observed that maintaining the depth of the No-till variant autumn wheat agrocenoses.

If we evaluate soil resistance to penetration of alternative research notes that soil penetration resistance research No-till variant autumn wheat under agrocenoses is higher and varies between 20 to 23 kgf/cm2, so soil this variant research is compacted and penetration resistance research on alternative agrocenoses plowing under autumn wheat ranges between 9-20 kg/cm2.

The soil resistance (http://www.madr.ro/images/agricultura/agricultura-romaniei-aprilie 2012.pdf) is a measure of the capacity of soil to resist deformation and refers to the amount of energy that is required to break aggregates or implants penetration into the soil. The soil penetration resistance depends on certain properties thereof, such as the composition of the grain size, degree of compaction and structure, the content of the humus.

The soil penetration resistance is related to, among other things, the moisture content of the soil (http://www.recolta.eu/arhiva/harta-diferitelor-tipuri-de-soluri-din-romania-9508.html, Schothorst C., 1968). The higher the moisture contents of the soil, the lower the penetration resistance and, therefore, the load carrying capacity.

CONCLUSIONS

The obtained data from research on cellulolytic activity under agrocenoses autumn wheat with different tillage technologies, we can certainly mention that cellulolytic activity plant rows of wheat is higher by approx. 18-21% (decay blade to the initial mass) compared with cellulolytic activity between rows of plants. Both variants research cellulolytic activity under agrocenoses autumn wheat with different technologies tillage - No-till plowing and it is noted that cellulolytic activity is higher in the 0-10 cm layer, decomposition blade version research on plowing is 55.67% over the initial mass, and the No-till variant research decomposing blade to the 30.14% initial mass. Cellulolytic activity is directly influenced by soil moisture, soil tillage technology, weather conditions, resistance to penetration and other factors which act directly and indirectly, the activity of microorganisms in the soil. Soil moisture depends on the climate, nature and inclination of soil and vegetation. Along with temperature, soil moisture influences greatly the biological activity and the possibility of self-purification. Knowledge of soil moisture is important both in terms of agro-technical and ecological point of view.

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DECISION

International Scientific Symposium

"Conservation of Plant Diversity",

Fifth edition, 01-03 June 2017, Chisinau, Republic of Moldova

Biological diversity is the foundation on which human civilization has been built. Its conservation is a prerequisite for sustainable development and is one of the challenges of the modern era.

The Republic of Moldova devotes much attention to the protection of nature, evidenced by the approval of the National Biodiversity Strategy for 2015-2020, which highlights priority issues and defines strategic objectives.

Botanical Gardens, over time, have played an important role in the conservation of plant diversity, being involved mainly in the protection of phytodiversity, the accumulation of a rich gene pool of scientific interest, the processes of carrying out high-level research in the field, monitoring, the intensification of the activities concerning environmental education and raising awareness about environmental issues, the improvement of energy efficiency and the reduction of the impact of climate change and the cultural development of people.

Examining the reports presented at the International Scientific Symposium "*Conservation of plant diversity*", fifth edition, 01-03 June 2017, by the researchers of the Botanical Garden (Institute) of the Academy of Sciences of Moldova, Botanical Garden „A. Fătu" of the "Alexandru Ioan Cuza" University of Iasi (Romania), "M. M. Hryshko" National Botanical Garden (Ukraine), State Dendrological Park "Alexandria" (Ukraine), Nikitsky Botanical Garden, Yalta (Crimea), Institute of Genetics, Physiology and Plant Protection (Moldova), Forest Research and Management Institute (Moldova), "Codrii" Scientific Reserve (Moldova), Agricultural Research Center, Tbilisi (Georgia), Institute of Research and Development for Grasslands, Brasov (Romania), Mureş County Museum (Romania), "Stejarul" Biological Research Center, Piatra-Neamt (Romania), Institute for Agricultural and Forest Environment (IAFE) of the Polish Academy of Sciences (Poland), "M. G. Kholodny" Institute of botany of National Academy of Sciences (Ukraine), Institute of Botany, Yerevan (Armenia), Institute of Agricultural Biology and Biotechnology, Milan (Italy), State University (Moldova), State University of Medicine and Pharmacy "N. Testemitanu" (Moldova), State Agricultural University (Moldova), "Alexandru Ioan Cuza" University of Iasi (Romania), University of Agricultural Sciences and Veterinary Medicine Iaşi (Romania), University of Medicine and Pharmacy of Tîrgu Mureş (Romania), "V. Goldiş" University of Arad (Romania), University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca (Romania), Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timişoara (Romania), Bila Tserkva National Agrarian University (Ukraine), Zhytomyr National University of Agriculture and Ecology (Ukraine), "Danylo Halytsky" Lviv National Medical University (Ukraine), "Vasyl Stefanyk" Precarpathian National University (Ukraine), "V. G. Korolenko" Poltava State Pedagogical University (Ukraine), "Taras Shevchenko" National University of Kyiv (Ukraine), Uzhgorod National University (Ukraine), University of Opole (Poland) and Slovak University of Agriculture in Nitra (Slovakia), the participants at the Symposium mention that:

- the reports reflected the results of recent investigations, conducted in the respective countries, on the conservation of the plant world, plant introduction and sustainable use of plant resources, landscaping, urban and rural planning, environmental education etc.;
- the scientific reports of the participants, presented at the Symposium, marked an advanced stage in the elaboration and implementation of research methods, the evaluation of the results and the implementation of the achievements in the branches of the national economies and the promotion of knowledge in society;
- the botanical gardens, universities and institutes of the Republic of Moldova, Romania, Poland, Ukraine, Italy, the Russian Federation, Georgia, Slovakia etc., which participated in the Symposium, are scientific and cultural centres of national and international importance, in the field of conservation, introduction and acclimatization of scientifically, economically and aesthetically valuable, native and non-native

species of plants. The collections and exhibitions of plants serve as centres where the plant gene pool is stored and as genetic resources of reproductive material, and are meant to preserve it for the present and future generations.

- the botanical research in the Republic of Moldova has intensified after the accession of RM to the European Union's Seventh Framework Programme, which mobilized the creative potential of researchers, offered opportunities for the integration in the Horizon 2020 programme of the international scientific community and created conditions for the training and promotion of young researchers.
- Unfortunately, in the RM, there is:
- insufficient funding of scientific research and not enough high-quality laboratory equipment and reagents in the institutions, necessary for carrying out advanced research;
- a dangerous tendency regarding the increase in the number of threatened plant species and the continuous destruction of natural habitats, which leads to a decrease in the stability of the natural capital of the RM;
- an inadequate level of the institutional and functional capacities of the subjects of the fund of protected natural areas (Scientific Reserves, "Orhei" National Park etc.).

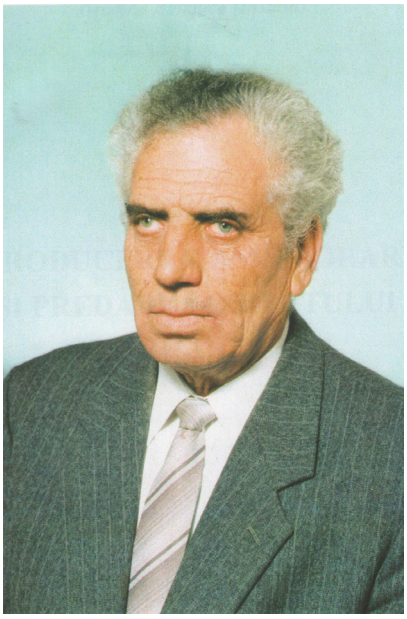
The International Scientific Symposium *"Conservation of Plant Diversity"*, fifth edition, held on 01-03 June 2017, in Chisinau, Republic of Moldova, RECOMMENDS:

1. the promotion of the scientific and technical themes regarding the study of plant diversity and the sustainable use of plant resources, the introduction of plant species, the enrichment of the existing collections and the creation of new collections with the help of the international exchange of seeds, the promotion of expeditions together with international specialized institutions;
2. the promotion of the draft law on assigning, to the Botanical Garden (I) of the ASM, the status of National Botanical Garden and the status of national heritage, due to the unique plant collections from the BG (I) ASM;
3. the alignment of the BG (I) ASM to the international standards, regarding its structure, management and organization, which reflects the mission of this institution in the social system.
4. the improvement of public awareness regarding biodiversity conservation and maintenance of ecological balance by organizing round tables, contests, seminars, centres of environmental education etc.
5. the integration of the aspects regarding the conservation of plant diversity and the sustainable use of plant resources in economic sectors, the wider use of tools related to ecosystem services;
6. the organization of an international scientific symposium on the protected natural areas in the Republic of Moldova;
7. the expansion of the fund of protected natural areas to ensure the optimal functionality of natural ecosystems and the adoption of measures to restore the populations of endangered species;
8. the intensification of the cooperation with scientific centres from Romania, Belarus, Russian Federation, Georgia, Poland, Slovakia etc., in plant research and taking measures on sustainable use of plant resources, going on joint expeditions to collect plant gene pool, the scientific research on the introduction and acclimatization of plants, the promotion of landscape architecture as an active tool in the conservation and sustainable use of the plant world and environmental education and awareness raising;
9. the intensification of the cooperation with higher education institutions in professional training of young scientific staff through various master, doctorate and post-doctorate specializations.
10. the implementation of scientific achievements in the field of conservation and sustainable use of plant resources in the branches of national economy, through technology transfer agencies;
11. the elaboration and the promotion of a program of restoration of the collections and exhibitions of the BG (I) ASM, severely damaged by natural disasters, construction and development of the Botanical Garden (Institute) of the ASM, regarding the Main Entrance, Exhibition Greenhouse, Botanical Museum,

Network of Paths and Ornamental Bridges.

12. the promotion of the draft law on the creation of the "Lower Prut" Biosphere Reserve;
13. the development of the institutional, human and operational capacities of the institutions dealing with scientific research in the forestry sector, namely ICAS (Forest Research and Management Institute), Scientific Reserves and "Orhei" National Park.

IN MEMORIAM
VICTOR SAVA
(15.05.1938 – 27.01.2017)



Fiecare om își are menirea sa pe Terra. Și fiecare dintre noi pe tot parcursul vieții, mai mult sau mai puțin, lăsăm generațiilor viitoare rodul muncii noastre, exprimat prin lucruri materiale sau spirituale. Așa rămâne în amintirea celor vii omul, care a muncit cu dedicație, depunând efort și suflet în tot ce a realizat.

Născut în satul Cojușna, județul Lăpușna, la 15 mai 1938, într-o familie de țărani, viitorul savant Victor Sava și-a contopit destinul cu natura și cu munca din fragedă copilărie. Fiind cel mai mare dintre cei 6 copii, a fost totodată și sprijinul părinților în tot ce ține de gospodărie, educația și îngrijirea fraților mai mici. Spiritul artistic și tendința de a cunoște l-au adus, imediat după absolvirea școlii medii din satul natal, pe băncile școlii de Cultură și Iluminare din orașul Soroca (aa. 1957-1959). Deși muzica și dansul l-au însoțit pe tot parcursul vieții, totuși nu ele aveau să-i pecetluiască destinul. Altceva a selectat spiritul său energic și iubitor de natură...

După cinci ani de facultate la Institutul Pedagogic din Tiraspol și trei ani în rol de învățător de biologie și geografie în școala medie din satul Recea, raionul Strășeni, vine în capitală, hotărât să ia cu asalt culmile științei. Astfel, în anul 1967 este acceptat în doctorantură în cadrul Grădinii Botanice (Institut) a AȘM. În 1972 susține cu succes teza de doctor în biologie, iar în 1991 – teza de doctor habilitat la aceiași specialitate – botanica. Astfel, timp de 47 ani a desfășurat o amplă activitate de cercetare în domeniul introducerii plantelor decorative și a ameliorării lor.

Energia nemărginită, perseverența și tenacitatea, punctualitatea, spiritul organizatoric ce la-u însoțit mereu, nu au rămas neobservate. Peste 33 ani doctorul habilitat Victor Sava a stat la cârma Laboratorului „Introducerea Plantelor Ornamentale" (actualmente – Laboratorul Floricultură). A fost un neobosit cercetător, dar și participant în construcția verde a grădinii botanice. A elaborat zeci de tehnologii de cultivare, a creat colecțiile de plante anuale și perene, a contribuit la întocmirea sortimentelor noi de plante ornamentale pentru spațiile verzi ale țării. Colecția de ruji de toamnă, ce însuma peste 600 soiuri, a fost mândria sa. Soiurile obținute erau apreciate de nenumărate ori în cadrul expozițiilor internaționale și naționale. Astfel, dragostea nemărginită față de lumea florilor a trecut și peste hotarele țării, devenind un nume recunoscut în Rusia, Ukraina, Belarusi etc.

Rezultatele muncii sunt impresionante: peste 300 lucrări științifice, inclusiv 10 monografii, 2 manuale, co-autor la 87 certificate și brevete pentru soiuri de plante ornamentale. A fost mentorul pentru 2 doctoranzi, care au susținut teza de doctor și a 30 de licențiați, ce au absolvit cu succes. Pentru meritele sale în cercetare și educație a fost apreciat cu un șir de diplome, mențiuni, medalii, titluri onorifice.

Prin acest mesaj ne exprimăm profunda recunoștință către cel care a fost profesor universitar, doctor habilitat și Om Emerit în știință - Victor Sava, și considerabilul regret, că nu-l mai avem alaturi.

Colectivul Grădinii Botanice exprimă sincere condoleanțe familiei, prietenilor în legătură cu trecerea la cele veșnice a doctorului habilitat Victor Sava. Dumnezeu să-l odihnească în pace.