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SOME BIOLOGICAL PECULIARITIES AND PRODUCTIVITY OF THE SPECIES *Anthyllis macrocephala* IN MOLDOVA

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Abstract

The results of the evaluation of the growth and development rates, the green mass yield, the biochemical composition and the content of amino acids, phosphorous and calcium, the nutritive and energy value of the forage, as well as the biomethane production potential of the local ecotype of the leguminous species Anthyllis macrocephala, maintained in monoculture in the Botanical Garden (Institute) of the Academy of Sciences of Moldova, are presented in this article. It has been determined that the green mass yield in the first mowing reached 24.1 t/ha; 100 kg green mass contain 27 nutritive units, 282 MJ metabolizable energy and 3.90 kg digestible protein, exceeding substantially Medicago sativa; the calculated gas forming potential of the fermentable organic matter reached 507 litre/kg volatile solid, content of methane - 52.5 %.

Key words*: anthyllis macrocephala*, *biological peculiarities, biochemical composition, biogas yield, fodder value.*

INTRODUCTION

In the conditions of the biologization for a sustainable development of agriculture, the legumes are of great ecological importance due to their capacity to fix biological nitrogen by symbiotic associations, improve the physical properties of soil, form a large amount of organic raw material for various industries and, besides, they are an important source of food and feed protein. Considering the limited material and technical resources in the Republic of Moldova, the efficient use of the biological potential of the local wild leguminous plants that are adapted to the specific climatic conditions becomes more and more relevant (Teleuţă and Ţîţei, 2016).

Temperate herbaceous legumes constitute a major proportion of plants. The genus *Anthyllis* L. belongs to the tribe *Loteae* DC., family *Fabaceae* Lindl., is taxonomically complex, with many intergrading variants which are to some extent ecologically and geographically separated. The genus is considered to range from 25 (Cullen, 1986) to 60 (Minjaev and Akulova, 1987) species, distributed in Europe, Middle East and North Africa.

The species of the genus *Anthyllis* L. possess valuable biological properties, are not demanding to soil and can grow on poor sandy, marly, loamy and calcareous soils where such legumes as genus *Trifolium* L. and *Medicago* L. do not grow. That is why *Anthyllis* is called "clover of sandy soils". These species are frost hardy and can withstand temperatures of -8°C, are drought tolerant and propagate easily by self-seeding. The species of the genus *Anthyllis* are used as fodder, melliferous, medicinal plants and to produce dyes (Bailey, 1949; Peterson, 1967; Medvedev and Smetannikova, 1981; Akulova, 1985). In folk medicine, they are used as plants with soothing, astringent, diuretic, wound healing and tonic properties.

Many species of genus *Anthyllis* are pasture plants for livestock (eaten mostly by sheep), are also used as hay and have been known since the XIX century as forage plants, are able to regenerate rapidly after trampling and grazing and can be used for livestock grazing for many years. Werner (1907) described in details the biological features and the advantages of forage species of genus *Anthyllis* in the Central Europe.

In the wild flora of the Republic of Moldova, there is only one species - *Anthyllis macrocephala* Wend., which has insufficiently researched (Gheideman et al., 1962).

The synonyms of *Anthyllis macrocephala* Wend.: *Anthyllis grossheimii* Chinth., *Anthyllis polyphylla* (Ser.) Kit. ex Loudon, *Anthyllis*

vulnearia L. subsp. *polyphylla* (Ser.) Nyman, *Anthyllis arenaria* (Rupr.) Juz., *Anthyllis schiwereckii* (DC.) Blocki. Common names: macrocephalous kidney-vetch, Kidney Vetch, ladyfinger. Romanian names: vătămătoare.

It is a biennial or perennial plant. Stems (1-6 in number) are erect and tall, 25-50 (90) cm in height, thick, robust and straight, branchy in the upper part. The lower part of the stems is covered with long and stiff squarrose hairs. Root leaves have 1-2 pairs of small lateral leaflets and a large ovoid or elliptic terminal leaflet ending with a rounded tip or a small sharp point. Stem leaves are 3-6 in number; they are uniformly distributed along the stem or may be absent in the upper third. Usually there are 5-6 pairs of leaflets on a leaf, elliptic or ovate on the lower leaves and linear-lanceolate in the upper ones. The terminal leaflet is large, obtuse in a lower leaf, and sharpened in an upper one. The leaflets of the uppermost leaf are often drawn together, so this leaf looks like a digitate one. All leaflets are naked or covered with sparsely set, small, rough hairs from above, and rather thickly villous from below, owing to slightly squarrose, long, rough hairs. Leafstalks are covered with small squarrose hairs. The inflorescences may be single, set on the top of a stem, or they are several (2-4), connivent enough, large (3-5 cm in diameter), compact and polyanthous. Bract leaves are equal in length to the calyx, dissected deeper than by one-half. Calyx is long and narrow, 12 mm in length and 3-5 mm in width, a little swollen under fruit, covered with long squarrose hairs, pale coloured. Corolla is usually yellow, often with a reddish-tipped carina, less frequently reddish all over. Banner blade is short, about 6 mm long, with an unguis – 6-7 mm long. Blossoms in June; bears fruit in August. Pods are ovoid monospermous, indehiscent. Seeds are ellipsoid, biconvex, lateral compressed. Hilum in the middle of the ventral side – 2.3-2.5 x 1.6-1.8 mm. The surface is glabrous, faint lustrous, the upper 2/5 – green, the bottom 3/5 – yellowish. Chromosome number 2n=12. Entomophilous (Bojnansky and Fargasova, 2007; Smekalova, 2008). *Anthyllis macrocephala* grows in dry meadows, steppes, river valleys, in dry open forests, forest edges, clearings and open slopes. (Abushaeva, 2013). It can abundantly grow in soils containing more than 3 % of $CaCO_3$. *Anthyllis macrocephala* has been included in the list of protected plants of the Republic Moldova, status: 4R - a rare species.

This research was aimed at evaluating the biological peculiarities, the productivity and the biochemical composition of the local ecotype of *Anthyllis macrocephala* and the possibility to use it as forage for ruminant animals or as biogas substrate.

MATERIALS AND METHODS

The local ecotype of the species *Anthyllis macrocephala*, maintained in monoculture, served as subject of study. The traditional leguminous fodder crops *Medicago sativa* and *Onobrychis viciifolia* served as control variants. The experiments were performed on non-irrigated experimental land in the Botanical Garden (Institute) of the Academy of Sciences of Moldova, latitude 46°58′25.7″ and longitude N28°52′57.8″E. The experimental design was a randomised complete block design with four replications, and the experimental plots measured 10 m^2. The seeds were sown at a depth of 2.0-3.0 cm with soil compaction before and after sowing. The scientific researches on growth and development, yield and biochemical composition of the plants were carried out according to the methodical indications (Novosiolov et al., 1983; Petukhov et al., 1989). The carbon content of the substrates was determined from data on volatile solids (organic dry matter), using an empirical equation reported by Badger et al. (1979). The biogas production potential and specific methane yields were evaluated by the parameter "content of fermentable organic matter", according to Weissbach (2008).

RESULTS AND DISCUSSIONS

After the phenological observations, it was found that, in the first growing season, the growth and development rates of the species *Anthyllis macrocephala* differed from those of the traditional leguminous forage crops. Thus, the seedlings emerged uniformly on the soil surface 12 days after sowing, or, 5 days earlier than the control *Medicago sativa* and 11 days earlier than *Onobrychis viciifolia*.

The type of germination – epigeal (aboveground). The cotyledons of plants were better developed, larger, with thicker leaf blade. The true leaves appeared 2-4 days earlier, in comparison with the control, had more developed leaf blades, were brightly coloured. At the stage of the emergence of the 3rd-4th true leaves, the seedlings had well-developed system of the main root with lateral roots. The development of the root system in *Anthyllis macrocephala* was considerably faster than the growth of the aerial organs. When the cotyledons emerged at the soil surface, the taproot of plants reached a depth of 3.8-5.7 cm. This characteristic makes possible to obtain large amounts of nutrients from soil and provides high life potential of plants at the earliest stages of development. At the stage when the first true leaves started developing, the size of the root system reached 4.9-8.3 cm, it later influenced positively the formation and development of the rosette, the capacity of the plants to grow shoots, the number of leaves on shoots and the further development of the root system, which reached 1.8-2.1 m depth. *Anthyllis macrocephala* was distinguished by slower growth and development of the aerial part and, by the end of the growing season, it reached the budding-flowering initiation stage, but *Medicago sativa* and *Onobrychis viciifolia* went through all the ontogenetic stages. Alfalfa was harvested twice, common sainfoin – once. The fresh mass yield of *Anthyllis macrocephala* was 0.99 kg/m^2 with high content of leaves (78 %) and dry matter (39 %), suitable for grazing.

In the following years, *Anthyllis macrocephala* plants resumed their development in spring when the average temperatures were above 5°C, but they needed higher temperatures than the control, so, they started developing more rapidly about 2 weeks later than *Medicago sativa* and *Onobrychis viciifolia.*

In the second year, *Anthyllis macrocephala* plants were characterised by the formation of shoots from the shortened internodes of rudimentary stems of the rosette from the first year, the leaves mostly retained their structure and provided protection to the meristem of the shoots. The juvenile period of development determines the presence of rosette leaves with an enlarged terminal leaf, and the virginile period is associated with several generations of compound odd-pinnate cauline leaves and the beginning of the generative period is accompanied by the formation of bracts – leaves surrounding the inflorescences, simple, palmate-dissected, and the formation of inflorescences. Thus, the leaf morphology is a diagnostic feature for establishing the age and the period of development of *Anthyllis macrocephala* plants.

The root system developed intensively, long ramifications of the root system were observed and a decrease in the mass of roots, as they grew deep in the soil, occurred gradually, in comparison with the control species. The research carried out by Abushaeva, 2015 has shown that when sowing in wide rows (45 cm), *Anthyllis macrocephala* had the most developed root system – 6.53 t/ha, but *Medicago falcata, Medicago varia, Lotus corniculatus* – 4.20 - 4.81 t/ha. This characteristic is of great importance due to the anti-erosion effect it can have on grass-covered slopes and any areas with loose soil, where it is necessary to secure the mechanical particles and to maintain the structure of soil.

Analyzing the 3-year-old plants (Table 1), we noted that the *Anthyllis macrocephala* plants had resumed the development 7-9 days later, their growth and development rates being very slow in comparison with *Medicago sativa* and *Onobrychis viciifolia.* Therefore, by the end of April, the *Anthyllis macrocephala* plants reached 16.60 cm high, while the control species – about 35.90-38.10 cm. It was determined that the period of time from the resumption of development till the formation of flower buds was shorter for *Anthyllis macrocephala* and constituted 64 days, but for the control species – from 70 to 75 days. In the budding period a faster growth rate of thick hairy stems was observed, probably due to the weather conditions (warmer weather), this tendency was maintained during the flowering stage, when *Anthyllis macrocephala* plants reached 74.40 cm, while the traditional leguminous forage crops reached 83.20-85.50 cm.

In other studies, it was mentioned that *Anthyllis macrocephala* plants in the budding-flowering period might reach development rates of 1.4-4.4 cm/days (Abushaeva, 2013). The flowering period of *Anthyllis macrocephala* started in June with 5 days earlier than *Onobrychis viciifolia.*

Table 1. Some biological peculiarities and productivity of *Anthyllis macrocephala*

Indicators	*Medicago sativa*	*Onobrychis viciifolia*	*Anthyllis macrocephala*
Resumed development up to:			
- budding, days	70	75	64
- flowering, days	82	99	78
- seed ripening, days	143	133	127
Plant height, cm			
- at the end of April	39.10	35.90	16.60
- at flowering	83.20	85.50	74.40
The yield at the first mowing:			
- fresh mass, kg/m^2	3.11	3.95	2.41
- dry matter, kg/m^2	0.82	1.03	0.82

Table 2. Biochemical composition, nutritive and energy value of *Anthyllis macrocephala*

Indicators	*Medicago sativa*	*Onobrychis viciifolia*	*Anthyllis macrocephala*
Raw protein, % dry matter	17.03	17.44	15.16
Raw fats, % dry matter	2.30	3.39	2.73
Raw cellulose, % dry matter	33.31	33.50	35.47
Nitrogen free extract, % dry matter	39.41	39.43	40.11
Minerals,% dry matter	8.01	6.24	6.53
Nutritive units/ kg green mass	0.21	0.23	0.27
Metabolizable energy, MJ/kg green mass	2.28	2.86	2.82
Dry matter, g/kg green mass	263.70	274.00	338.30
Digestible protein, g/kg green mass	34.50	35.87	39.00
Calcium, g/kg green mass	4.46	3.53	2.32
Phosphorus, g/kg green mass	1.16	1.55	0.81
Carotene, mg/kg green mass	19.05	30.50	13.00
Vitamin C, mg/%	45.58	41.21	53.00
Digestible protein, g/ nutritive unit	164.29	156.00	143.74

Table 3. The content of amino acids in fodder of *Anthyllis macrocephala* (mg/100 mg dry matter)

Amino acids	*Medicago sativa*	*Onobrychis viciifolia*	*Anthyllis macrocephala*
asparagine	1.711	1.751	2.480
threonine	0.564	0.565	0.549
serine	0.687	0.685	0.646
glutamine	1.360	1.398	1.316
proline	0.922	1.154	1.009
glycine	0.550	0.557	0.505
alanine	0.674	0.672	0.590
valine	0.559	0.654	0.570
methionine	0.139	0.091	0.130
isoleucine	0.459	0.459	0.443
leucine	0.913	0.920	0.924
tyrosine	0.458	0.491	0.329
phenylalanine	0.850	0.937	0.490
histidine	0.326	0.371	0.338
lysine	0.619	0.706	0.541
arginine	0.655	0.587	0.473

The studied *Fabaceae* species needed a different period from the beginning of flowering until the full ripening of seeds. Thus, *Anthyllis macrocephala* needed 49 days, while *Onobrychis viciifolia* needed 34 days and *Medicago sativa* - 61 days.

It is well known that yield is the product of a complex interrelationship among climatic, genetic, and agronomic variables. The green mass yield of *Anthyllis macrocephala*, at the first mowing, reached 24.1 t/ha. The harvested fodder was poorer in leaves (33 %), but richer in dry matter (34 %).

The knowledge of constituents of animal feed is of primary importance to animal production and productivity. Forages are a major source of

nutrients for herbivores. Sometimes the balance of nutrients or the presence of some constituents in the forage will have positive or negative effects on animal health and productivity. The biochemical composition of green mass *Anthyllis macrocephala*, is presented in Table 2, obtained values are expressed as percentage of dry matter. The dry matter of *Anthyllis macrocephala* was characterized by optimal protein content (15.16 %), which was lower in comparison with traditional forage leguminous crops (17.03-17.44 %), about the same nitrogen free extractive substances (40.11 %) and high level of raw cellulose (35.47 %). As compared with traditional forage leguminous crops, the dry matter of *Anthyllis macrocephala* was characterised by lower fat content (2.73 %) than *Onobrychis viciifolia,* but higher – in comparison with *Medicago sativa.*

Some authors mention similar findings about the quality of *Anthyllis macrocephala* fodder. So, as a result of a research conducted in Penza region, Russia, it was found that the dry matter content of green fodder was 39.66 %, including 4.75 % raw protein, 0.32 % raw fats, 16.07 % raw cellulose, 3.43 % minerals, 15.09 % nitrogen free extracts (Kshnikatkina et al., 2005); in the Kabardino-Balkar Republic, Russia – 15.9 % raw protein in dry matter (Galushko, 1964)

An essential component of the characteristics of protein is its amino acid composition, which is its main structural characteristic, irrespective of the kind, origin and physiological function.

Analyzing the results on the amino acid content in the fodder (Table 3), it was found that the species *Anthyllis macrocephala* was distinguished by an optimal content of both essential and nonessential amino acids. Comparing each amino acid separately, we could mention that the content varied in comparison with traditional forage crops. We could mention that the methionine content, the first deficient essential amino acid limiting the nutritive value of protein of the species *Anthyllis macrocephala* reached 0.130 mg/100 mg dry matter, thus, it was higher than in *Onobrychis viciifolia*, but lower as compared with *Medicago sativa.* The second limiting amino acid for protein biosynthesis – lysine (0.541 mg/100 mg) was much lower as compared with *Onobrychis viciifolia* and *Medicago sativa.* We found that *Anthyllis macrocephala* fodder was very rich in asparagine, contained about the same amount of threonine, serine, glutamine, isoleucine, leucine, but had lower content of phenylalanine, alanine and arginine in comparison with traditional forage crops.

Table 4. Gas forming potential of the fermentable organic matter from the studied species of the family *Fabaceae*

Indicators	*Medicago sativa*	*Onobrychis viciifolia*	*Anthyllis macrocephala*
Ratio of carbon and nitrogen (C/N)	19	19	21
Fermentable organic matter, g/kg VS	642	658	633
Biogas, litre/kg VS	514	526	507
Methane, litre/kg VS	270	276	266
Methane productivity, m^3/ha	2214	2843	2181

The organic matter content, its biochemical composition and digestibility determine the nutritional and energy value of the green mass (Table 2). It was determined that 100 kg green mass of *Anthyllis macrocephala* contained 27 nutritive units, 282 MJ metabolizable energy and 3.90 kg digestible protein, exceeding significantly *Medicago sativa*. The fodder of *Anthyllis macrocephala* was very poor in calcium (2.32 g/kg), phosphorus (0.81 g/kg), carotene (13 mg/kg), but richer in ascorbic acid (53.00 mg/%). A high content of vitamin C in the leaves (191 mg/%) was also mentioned in other studies (Gheideman et al., 1962). Biogas production from energy crops mainly depends on their biodegradability and biochemical composition.

The ratio of carbon and nitrogen (C/N) in the raw material is essential in the production of biogas. The optimal C/N ratio for the methane-producing bacteria is 20:1-30:1. High C/N ratio favours ammonia production, while low C/N ratio limits the growth of bacteria (Dobre et al., 2014).

The C/N ratio of the studied species varied from 19 in the biomass of *Medicago sativa* and *Onobrychis viciifolia* to 21 in the biomass of *Anthyllis macrocephala* (Table 4). The calculated gas forming potential of the fermentable

organic matter of local ecotype of the species *Anthyllis macrocephala* reached 507 litre/kg VS, being lower than in the control species (514-526 litre/kg VS), but it had similar content of methane (52.5 %).

The highest methane yield per ha was achieved by *Onobrychis viciifolia*, the lowest – by the biomass of *Anthyllis macrocephala*.

CONCLUSIONS

In the conditions of the Republic of Moldova, it was observed a slower growth and development of the aerial part of *Anthyllis macrocephala* during the first growing season, but it developed stronger root system in comparison with the control.

The 3-year-old *Anthyllis macrocephala* plants had moderate growth and development rates that allowed mowing them in June, when the green mass yield reached 2.41 kg/m^2, but they had higher content of dry matter, in comparison with the traditional crops.

The biochemical composition of the dry matter of *Anthyllis macrocephala*: 15.16 % raw protein, 2.73 % raw fat, 35.47 % raw cellulose, 40.11 % nitrogen-free extractive substances, 6.53 % minerals and a high amount of vitamin C.

The 100 kg of green mass of *Anthyllis macrocephala* contained 27 nutritive units, 282 MJ metabolizable energy and 3.90 kg digestible protein, exceeding essentially *Medicago sativa*.

The gas forming potential of *Anthyllis macrocephala* reached 507 litre/kg VS with 52.5 % methane. The calculated methane yield of the green mass of *Anthyllis macrocephala* harvested at the first mowing reached 2181 m^3/ha.

The local ecotype of the species *Anthyllis macrocephala* is a perennial plant and has xerophytic features (poor foliage, thick hairy stems, well developed roots that grow deep into the soil). It is an excellent plant to prevent erosion on grass-covered slopes and any areas with loose soil, where it is necessary to secure its mechanical particles and to maintain its structure. Besides, *Anthyllis macrocephala* is useful for restoring degraded, polluted and eroded land, and for reseeding and increasing the economic value of grasslands.

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