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Cultivation of high antioxidant activity *Alchemilla* spp. (Rosaceae) for sustainable use

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Abstract

Guidelines for introduction and *ex situ* cultivation of species from genus *Alchemilla*, known by its curative properties for a variety of health disorders are presented for the first time. Subject of the study are the rare and protected Bulgarian species *Alchemilla achtarowii* Pawl., *A. jumrukczalica* Pawl. and *A. mollis* (Buser.) Rothm., which showed high antioxidant activity in our recent research. Transplant material from natural populations grown in two live collections in the regions of Vitosha Mt. (1404 masl) and West Rhodopes Mt. (1500 masl) (Bulgaria) was used. The growth and development rate of the new plants was assessed according to the method of phenological observations. Nine morphometrical indices were studied and the biological productivity of the species was determined in *ex situ* conditions. The quantities of flavonoids (calculated as % quercetin) and tannins (calculated as % pyrogallol) during the different phenological stages of *ex situ* plant development were assessed via spectrophotometric methods. Several differences between the species in the two experimental stations were summarized as dependent on the ecological conditions. All results of the study were used to elaborate methodological instruction for successful cultivation of the species in field conditions.

Key words: *Alchemilla achtarowii* Pawl., *A. jumrukczalica* Pawl., *A. mollis* (Buser.) Rothm., medicinal plants, endemics, *ex situ*, biological productivity, flavonoids, tannins

Introduction

Many species of the genus *Alchemilla* L. are valuable medicinal plants referred to under the collective name *Alchemilla vulgaris* complex. In the phytotherapy, the aboveground parts and the rhizomes of the plants (Herba et rhizoma *Alchemillae*) are used for curing different conditions related to skin and gynaecological diseases, initial stages of diabetes, accelerated recovery of wounded tissues *etc*. The herbal drug contains mainly tannins, flavonoids, leucoanthocyanidins and mucus substances, and expresses styptic and regenerating epitelium action (Nikolov, 2007). Dried herbal extract of *A. vulgaris* complex is used in various natural beauty products and food supplements. Moreover, many cultivars of *Alchemilla* spp. mostly those of *A. mollis* (cv. Auslese, Thriller) are very popular ornamental plants which are used in parks and rock gardens.

Usually the raw materials from *A. vulgaris* complex are harvested from the nature (the genus *Alchemilla* is endemic for Europe) and the species differences in the quantity of biologically active compounds, phenological phase at the time of harvest and timing of the harvest bring many difficulties for standardisation process of the herbal drug. In the reviewed literature we found no information on cultivation of *Alchemilla* species which can prove useful both for lowering the producing cost of the raw materials and increasing the quality of the processed herbal drug.

The main objective of this study was to test the possibility of introduction of the three species *viz., Alchemilla mollis* (critically endangered species in Bulgaria according to the IUCN criteria) and the Bulgarian endemics – *A. jumrukczalica* (critically endangered) and *A. achtarowii* (endangered) (Biological Diversity Act, 2002; Vitkova, 2009) in conditions close to their natural habitat in Bulgaria (mountain climate and altitude in the range of the beech forest belt). The specific goals were to study the growth and development of the species *ex situ*; to assess the aboveground yield and to compare tannin and flavonoid content in the plant parts collected from the natural habitats and from the field collections and to present methodological guidelines for introduction for cultivation, based on the results of this survey.

The choice of the species resulted from preceding studies of nine *Alchemilla* species in Bulgaria – *A. glabra* Neigenf., *A. flabellata* (Buser), *A. mollis* (Buser.) Rothm., *A. jumrukczalica* Pawl., *A. gracillis* Opiz, *A. crinita* Buser, *A. connivens* Buser, *A. subcrenata* Buser, *A. monticola* Opiz. (Vitkova, 1996). All of these species except *A. mollis* and *A. jumrukczalica* are widely distributed in Europe but susceptible to fungal pathogen attack (*Erysiphe* spp. and *Trachyspora alchemillae* J. Schröt) both in natural and field conditions (Vitkova, 1996; Vitkova, 1997). The susceptibility to powdery mildew and rust diseases causes severe withering of the plants. Our recent unpublished observations on *A. achtarowii in situ* evidenced its resistance to these fungal pathogens. In addition, recent phytochemical studies on phenolic compounds from the three *Alchemilla* species proved that they express high free radical scavenging activity (Nikolova *et al.*, 2012; Trendafilova *et al.*, 2011; Trendafilova *et al.*, 2012).

Materials and methods

The study was conducted in the period 2010-2012. Explants derived from rhizomes and with intact aerial parts were collected in August 2010 from the natural clone-populations of the three species (*A. achtarowii* Pawl., *A. jumrukczalica* Pawl., *A. mollis* (Buser.) Rothm.) of interest in the region of Central Balkan Mountain. The plants' rhizomes were cut into pieces 4-5 cm long with 2-3 regenerative buds. These explants were planted at two experimental stations of the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences – station "Beglika" (Rhodopes Mt.) on 28.08.2010, and station "Zlatni mostove" (Vitosha Mt.) on 09.05.2010. Both experimental stations are situated under the conditions of mountain version of the temperate climate. Station "Zlatni mostove" is located at 1404 m altitude and station "Beglika" at 1500 m. The experimental plots in both stations differed in slope, exposure and composition of the soil (Table 1). The temperature and precipitations data were obtained from the Hydrological and Meteorological Institute at the Bulgarian Academy of Sciences (Table 2). In both experimental plots 12 rhizome cuttings of each species were planted with distance between the rows of 50 cm and distance between the cuttings of 40 cm. Phenological observations were performed according to the methodological guidelines of Beydeman (1976) evaluating growth and development rate of the plants. Comparative morphometrical study included nine features: height of the plants, diameters of the projection of the plants measured in north/south and east/west direction, number of flowering stems, number of basal leaves, fresh and dry weight of the flowering stems and fresh and dry weight of the basal leaves. The average value of each feature, the error of the mean, the standard deviation, and the minimum and maximum values were calculated (Zaitsev, 1984). The yield of the aboveground mass of the plants per ha as well as the content of flavonoids and tannins in air dried aboveground parts of the plants was determined. The plant materials for phytochemical experiments were collected from the natural clone-populations and experimental fields in phase of full blossoming of the plants. The content of the flavonoids was determined spectrophotometrically at 425 nm (absorption maximum of the complex of flavonoids with $AlCl_3$) and was expressed as g quercetin per 100 g dry plant material (Kitanov, 1987). All determinations were performed in triplicate (n = 3). Determination of tannins was performed according to the Anon. (2005) using Folin-Chiocalteu reagent and pyrogallol as standard. The absorbance was read at 760 nm and the percentage content of tannins (expressed as g pyrogallol) was calculated from the following equation: (%) = $[3.125.(A_1-A_2)]/[A_3.m]$ where A_1 and A_2 were the absorbances of the sample solutions measured before and after hide powder treatment, respectively, A_3 - the absorbance of the test solution containing 0.05 g of pyrogallol, and m - the mass of the dry plant material (g). All determinations were performed in triplicate (n = 3).

Table 1. Ecological characterization of the stations Beglika (Western Rhodopes Mt.) and Zlatni mostove (Vitosha Mt.)

Ecological characteristics	Station Beglika	Station Zlatni mostove
GPS coordinates	N 41.849332°; E 24.117781°	N 42.614948°; E 23.234493°
Altitude (masl)	1500	1404
Exposition	NE	SW
Slope (°)	3	7
Soil	Brown forest	Brown forest
N%	0.15	0.35
P_2O_5 mg/100 g	19.83	5.64
K_2O mg/100 g	16.60	11.90
pH	6.50	5.90
Humus (%)	2.84	6.98

Results and discussion

Growth and development: All of the three studied species – the Bulgarian local endemics *A. achtarowii, A. jumrukczalica* and *A. mollis* occur in Central Balkan mountain on the territory of National park "Central Balkan" at an altitude range between 1150-2000 masl. *A. achtarowii* and *A. jumrukczalica* can be found along mountain streams and gullies in the subalpine mountain belt. The only small clone-population of *A. mollis* in Bulgaria is situated in the range of the beech forest belt. In nature, all three *Alchemilla* species grow as perennial herbaceous plants with creeping rhizomes and 10-50 cm long erect to ascending foliated stems with racemose inflorescenses, the basal leaves form a tuft. The plants have prolonged vegetation period with winter dormancy.

At both stations, by the end of the growing season 2010, 100% transplants formed leafy tufts (6-7 cm in height and 8-10 cm in diameter). After the first winter the survival rate of the young plants was 70-100%. The highest percentage of surviving plants (100%) was recorded for *A. mollis* and lowest (70%) for *A. jumrukczalica*. The phenolocigal observations showed that the plants cultivated at station "Zlatni mostove" started their vegetative development in the mid of April and formed leafy tufts on 5-10th of May. At station "Beglika" the vegetative development of the plants started later (on 5-10th of May). Leafy tufts were completely formed till 15-25th of May. In the first year, at both stations the plants of *A. mollis* reached a height of 30 cm with 39 cm average diameter of the tufts. *Alchemilla jumrukczalica* reached 24 cm of height with 34 cm in average diameter of the tufts, and *A. achtarowii* – 24 cm of height and 20 cm in diameter. In 2011, again at both stations 50% of the one-year old plants of *A. mollis* and *A. jumrukczalica* and 30% of *A. achtarowii* entered the generative development stage by forming 1-2 (3) flowering stems.

The measurements of nine metric attributes of the two-year-old plants showed some differences in the rate of their *ex situ* growth and development between the three species. At both experimental stations, the plants of *A. mollis* (56-57 cm) were tallest, followed by *A. jumrukczalica* (39-50 cm) and *A. achtarowii* (33-41 cm) (Fig. 1). *A. mollis* plants were distinguished by the largest mean diameter of the leafy tufts followed by *A. achtarowii* and *A. jumrukczalica*. The highest number of flowering stems per plant was observed in *A. mollis* (27-35) and highest number of basal leaves per plant was observed in *A. achtarowii* (42-67). The plants of all three species were larger in the station "Beglika" where the humus and nitrogen content of the soil was lower while the phosphorus and potassium contents were higher in comparison with station "Zlatni mostove" (Table 1). The meteorological data (Table 2) showed some differences for the areas of the experimental stations "Beglika" and "Zlatni mostove". For the period May-October during the three years of study the average monthly temperatures in station "Beglika" were slightly lower than those in station "Zlatni mostove". The precipitations at

Table 2. Temperature and precipitations in the period 2010-2012 at the stations Beglika and Zlatni mostove

Station	Beglika						Zlatni mostove					
Index	Temperature (°C)			Precipitations (mm)			Temperature (°C)			Precipitations (mm)		
Years	2010	2011	2012	2010	2011	2012	2010	2011	2012	2010	2011	2012
January	-5.7	-3.2	-7.8	72	35	115	-4.8	-3.6	-6.4	73	62	93
February	-3.6	-3.0	-7.7	163	28	125	-3.2	-2.7	-6.9	99	65	79
March	-0.7	-1.3	-0.7	86	38	27	-0.6	-0.1	0.3	115	74	63
April	3.3	1.5	4.5	43	55	88	4.3	3.5	5.6	97	61	103
May	9.3	7.1	7.5	68	73	251	9.4	8.4	9.2	108	75	150
June	11.3	10.9	14.4	169	85	42	12.5	12.7	15.5	118	59	55
July	12.7	14.3	17.4	134	45	9	15.0	15.6	18.6	96	149	85
August	16.2	13.4	15.4	32	124	45	17.4	15.9	16.9	85	88	89
September	10.3	12.7	13.1	84	56	38	10.9	13.7	13.3	90	69	91
October	4.2	3.6	-	136	99	-	4.4	4.5	-	130	104	-
November	6.0	-0.4	-	94	1	-	6.8	0.3	-	73	48	-
December	-1.8	-1.3	-	77	59	-	-2.7	-2.1	-	144	82	-
Mean temperature / precipitation sum	5.13	4.53	6.23	1158	698	740	5.78	5.51	7.34	1228	936	808

"Zlatni mostove" (Vitosha Mt.) were comparatively constant, the average monthly level through the entire period exceeding 48 mm. It was found that the trend was more unstable in the region of station "Beglka" (Western Rhodopes Mt.) where the average monthly value of precipitations repeatedly dropped below 48 mm (Table 2). This trend continued in May, June and July, when plants formed the main part of the aboveground mass and bloomed. A comparison of climate data with the results of phenological observations and morphometrical measurements showed that the local microclimatic and ecological conditions in the two experimental plots were more significant for the development of the plants than average climatic parameters for the respective stations. The experimental plot at the station "Zlatni mostove" has south-west exposure and inclination of 7-8° which results in fast draining of rainfall and low soil and air humidity throughout the year and especially during the warm months. During May, June and July, when the plant vegetation was most active the sunshine in the afternoon was particularly strong due to the southern exposure of the terrain. This resulted in drought soil, intense transpiration and withering of the plants. On the contrary, the experimental plot of station "Beglika" has north-east exposure with very slight inclination (3-4°), being situated in the immediate vicinity of spruce forest close to dam lake. Here the average annual temperature and precipitations are slightly lower compared to the station "Zlatni mostove", but it was obvious that plants grew better. This is also supported by the fact that although the annual vegetation of the plants in station "Beglika" started 15-20 days later than those in station "Zlatni mostove" their development was faster taking only 10-15 days for complete formation of leafy tufts vs. 20-25 days in station "Zlatni mostove". The morning mists which are characteristic for "Beglika" region and provide high humidity lasting through big part of the day were particularly favourable for plant development. It is known that soil and air humidity are very important for the development of the studied species which naturally grow in mesophyte habitats (Vitkova *et al.,* 2012). In the conditions of natural habitats *A. mollis* reached a height up to 30-32 cm (Vitkova *et al.,* 2011), *A. achtarowii* – 40-45 cm, and *A. jumrukczalica* – 10-25 cm (Vitkova, 2012). Cultivated plants of *A. mollis* and *A. jumrukczalica* were larger than those in their natural habitats while in the cultivated plants of *A. achtarowii* the reversed trend was observed. In natural conditions, *A. achtarowii* is hygromesophyte (Vitkova *et al.,* 2008) while in *ex situ* field conditions, the plants experienced water stress regardless of the artificial irrigation during the summer months. The phenological studies showed no significant differences in the occurrence of full flowering stage in all three species. In station "Zlatni mostove" this phase started on 15-20 June and in station "Beglika" on 25-30 June, when 75% of the plants were in bloom. In this period the aboveground biomass was harvested and the resulting values were extrapolated in order to obtain the potential yield per ha. The highest yield of fresh aboveground biomass in both stations measured in 2012 was determined for *A. mollis* (26.2-36.1 t/ha). The yield from the other two species was significantly lower: for *A. achtarowii* – 6.6-23.6 t/ha and *A. jumrukczalica* – 10.0-18.8 t/ha (Table 3). It was found that dry matter was 32-34% of the fresh mass. The comparative analysis showed that the yield of aboveground biomass varied significantly between the plants grown in different conditions in the experimental stations. In "Beglika" the yield was 1.4 (*A. mollis*) to 3.6 (*A. achtarowii*) times higher than that in station "Zlatni mostove". It was found that in station "Beglika" the aboveground biomass of *A. jumrukczalica* and *A. mollis* consisted of 70% flowering stems and 30% basal leaves while in *A. achtarowii* it consisted of 60% flowering stems and 40% basal leaves. In station "Zlatni mostove", *A. achtarowii* maintained the same ratio while a significant change was recorded in *A. mollis* (79% / 21%) and *A. jumrukczalica* (54% / 46%). These results confirmed that the local environmental conditions significantly affected both the yield of aboveground phytomass

Table 3. Yield of fresh plant materials of *Alchemilla* spp. grown at the experimental stations measured in 2012

Species	Yield of plant materials (t/ha)					
	Station Beglika			Station Zlatni mostove		
	Above ground mass	Flowering stems	Leaves	Above ground mass	Flowering stems	Leaves
A. mollis	36.1	24.4	11.7	26.2	20.7	5.5
A. achtarowii	23.6	14.6	9.0	6.6	4.0	2.6
A. jumrukczalica	18.8	13.1	5.7	10.0	5.4	4.6

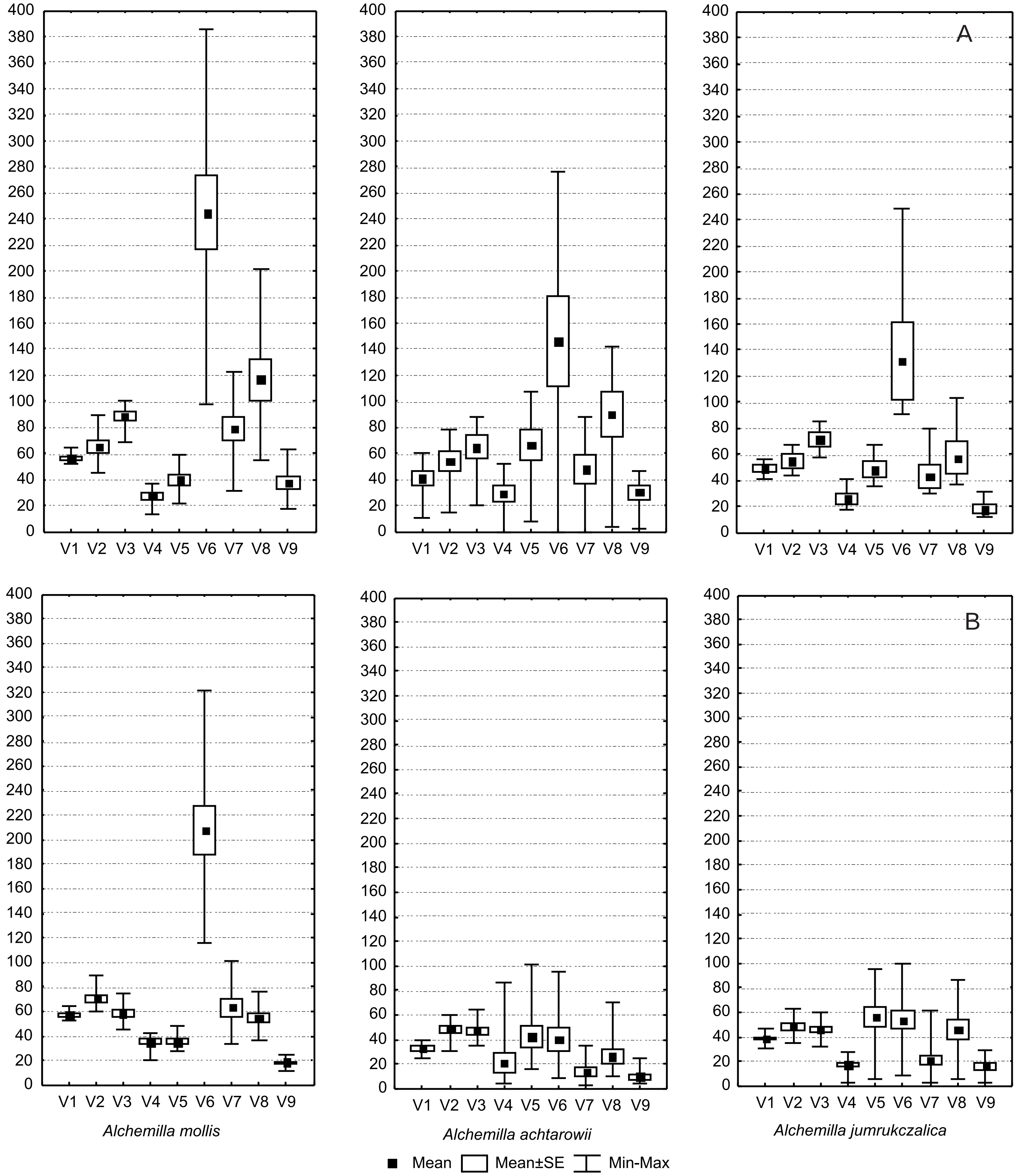

Fig. 1. Metric data of two year old plants of *A. mollis, A. achtarowii* and *A. jumrukczalica* grown at two experimental stations A: Station Beglika; B: Station Zlatni mostove. Legend: V1 – plant height (cm); V2 – length (cm) of the tuft north/south; V3 – length (cm) of the tuft east/west; V4 – number of flowering stems per plant; V5 – number of basal leaves per plant; V6 – fresh weight (g) of flowering stems per plant; V7 – dry weight (g) of flowering stems per plant; V8 – fresh weight (g) of basal leaves per plant; V9 – dry weight (g) of basal leaves per plant

and its structure. In both stations the plants entered the phase of fruit formation in the period of 10th to 15th of August. In late August the seeds ripened and the flowering stems faded. 60 to 70 days after harvesting of the aboveground phytomass, the plants formed new aboveground phytomass and once again bloomed. This allowed two harvests in one growing season. No attack by any diseases and pests, including the typical for the *Alchemilla* species powdery mildew was found in both experimental fields. The resistance to fungal pathogens could be explained by the high content of tannins and by the favourable climatic conditions in both stations. It is reported (Tuka and Popescu, 1979) that the high levels of tannins in the leaves of *A. mollis* limit the infection of plants with the fungus *Trachyspora alchemillae*. It is known that tannins have a protective role in plants against fungal diseases (Baraboy, 1984).

Phytochemical studies: In the process of introduction of medicinal plants in field conditions, one of the most important requirements is the content of biologically active compounds to remain unchanged. In this connection, a comparative phytochemical investigation of the content of tannins and flavonoids in aboveground parts of *A. mollis*, *A. achtarowii* and *A. jumrukczalica* collected from both naturally growing and *ex situ* grown plants was conducted (Fig. 2). It can be seen that among the native populations, *A. mollis* was richest in flavonoids (0.902%) and *A. jumrukczalica* in tannins (6.494%). The content of flavonoids and tannins of *ex situ* grown plants at both experimental stations was lower in comparison with that in the natural habitats with few exceptions. Thus, one-year-old *A. achtarowii* and *A. jumrukczalica ex situ* grown in "Beglika" station contained higher amounts of flavonoids (0.844%) and tannins (7.386%), respectively. A significant difference in the amount of the target compounds between plants of different age groups of each species grown in the station "Beglika" was also observed. Thus, in *A. mollis* the content of flavonoids and tannins of two-year-old plants was higher than that of one-year-old plants. In *A. achtarowii* the amount of tannins was slightly enhanced at the second year of development, while that of the flavonoids diminished. In opposite, the content of tannins in *A. jumrukczalica* decreased, whereas that of flavonoids remained almost unchanged. This is probably due to the change in the ratio of flowering stems and basal leaves in the different age groups. It is worth noting that the two-year-old *ex situ* grown plants in both experimental stations "Beglika" and "Zlatni mostove" did not differ substantially in the content of the studied types of compounds.

In order to characterize the elements forming the structure of the aboveground biomass – flowering stems and basal leaves, their flavonoid and tannin contents were separately examined in two-year-old *ex situ* grown *A. mollis*, *A. achtarowii* and *A. jumrukczalica* (Fig. 3). As it can be seen, the flavonoids accumulated mainly in the basal leaves of the three species in concentrations 0.871% (in *A. achtarowii* "Zlatni mostove") and 1.006% (in *A. mollis* "Zlatni mostove"), while their content in the flowering stems varied from 0.702% (in *A. achtarowii* "Beglika") to 0.875% (in *A. mollis* "Zlatni mostove"). The highest amounts of tannins were detected in flowering stems of *A. mollis* (8.334%) and *A. jumrukczalica* (7.007%) as well as in *A. achtarowii* basal leaves (6.574%) from the experimental station "Zlatni mostove". Furthermore, *A. achtarowii* was the only species in which the tannin content in basal leaves was higher than that in flowering stems. It was found that *A. mollis* flowering stems produced more flavonoids and tannins in comparison with *A. achtarowii* and *A. jumrukczalica* flowering stems.

In addition, flavonoid and tannin contents in the basal leaves of the target species from station "Beglika" gathered in different developmental phases were also studied. The results showed a significant increase in the flavonoid content at the phase of ripening of seeds for *A. mollis* (1.116%) and *A. achtarowii*

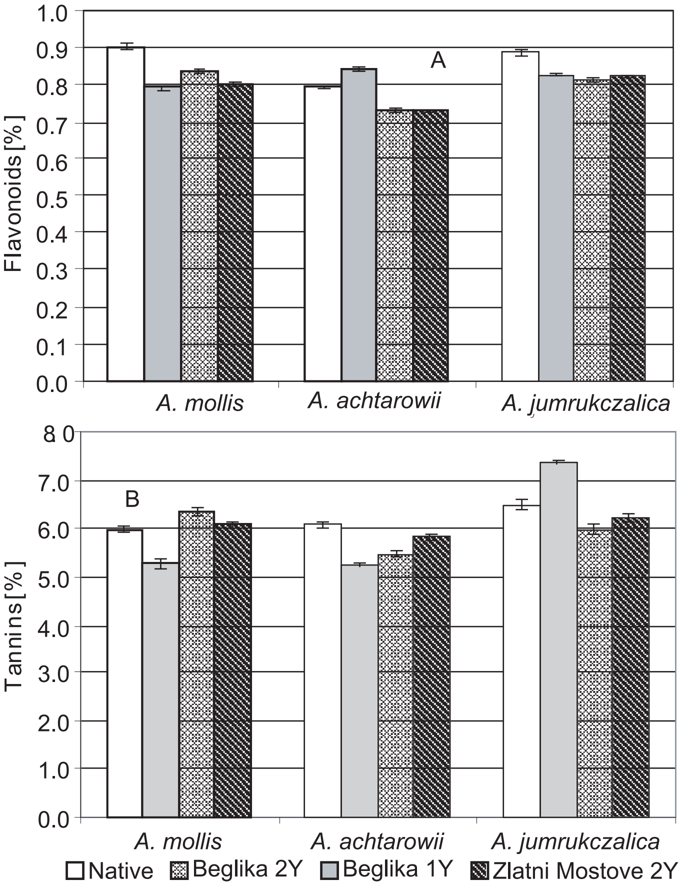

Fig. 2. Content of flavonoids (expressed as % quercetin) [A] and tannins (expressed as % pyrogallol) [B] in the aerial parts from natural clone-populations and *ex situ* grown of plants from the experimental bases Beglika and Zlatni mostove; 1Y and 2Y –age of plants (one-year- and two-year-old, respectively).

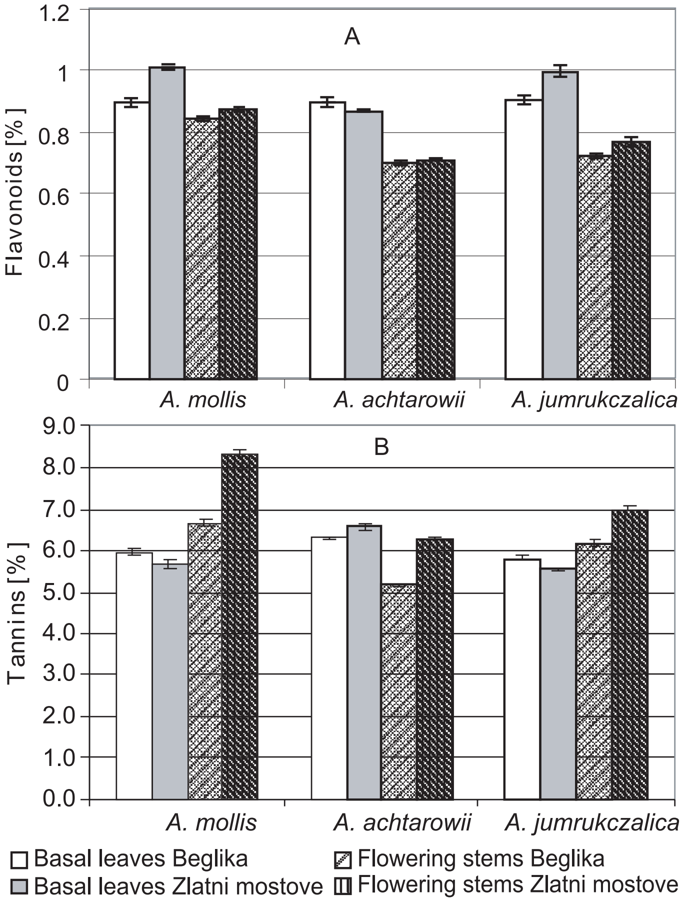

Fig. 3. Content of flavonoids (expressed as % quercetin) [A] and tannins (expressed as % pyrogallol) [B] in the basal leaves and flowering stems from two-year-old *ex situ* grown plants from the experimental bases Beglika and Zlatni mostove

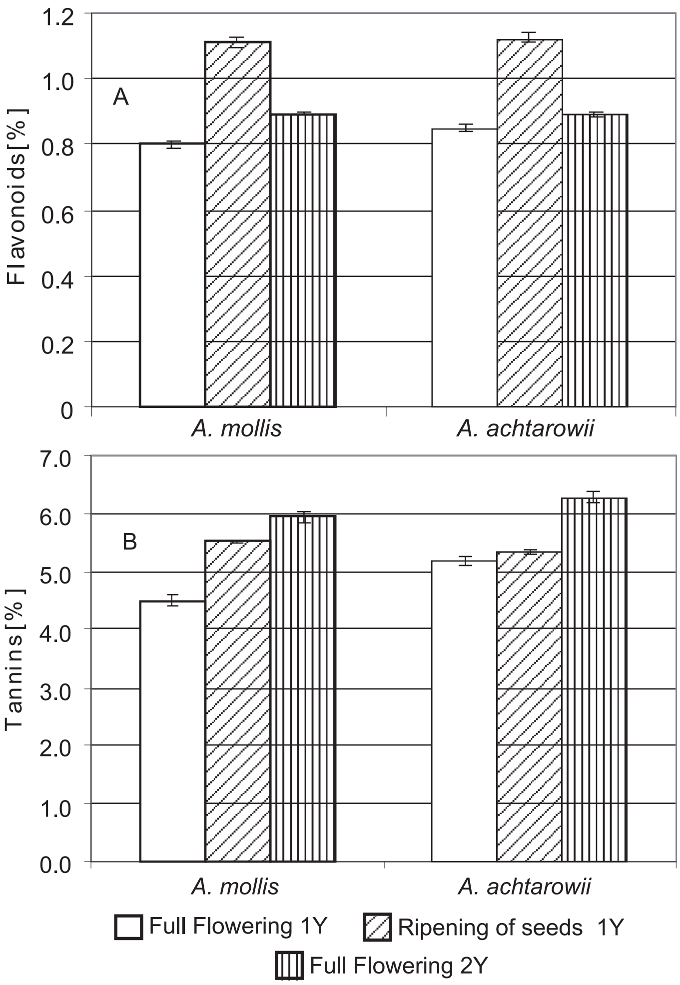

Fig. 4. Content of flavonoids (expressed as % quercetin) [A] and tannins (expressed as % pyrogallol) [B] in the basal leaves from *ex situ* grown plants from base Beglika at different development phase; 1Y and 2Y – age of plants (one-year- and two-year old,. respectively).

(1.122%) as well as a slight enhancement between that of the first and second year at full flowering phase (Figure 4). As it can be seen, there was a tendency of increasing accumulation of tannins during the different developmental phases for *A. mollis* and *A. achtarowii*.

In the literature there is evidence that the cultivation of *A. mollis* at different altitudes (363-1100 m) does not affect the contents of flavonoids in the leaves and flowering stems which insignificantly vary (Tuka and Tomas, 1977). Our research is in line with this study showing that the cultivation of *A. mollis*, *A. achtarowii* and *A. jumrukczalica ex situ* at an altitude similar to their natural distribution did not cause significant change in the quantity of flavonoids and tannins in the aboveground biomass.

Cultivation: A new contribution of this study is the compilation of guiding list of activities when introducing *Alchemilla* species in field conditions for cultivation. The sequence of activities should be as follows:

Field site selection: For cultivation of the three species, the field sites should be situated in mountain regions with altitude above 1000 m, preferably in the vicinity of rivers, lakes and with availability of irrigation system. The terrain chosen for planting of the perennial plants should be flat or near flat which will ensure the retention of moisture from precipitations. The favourable exposure of plants is north or with north component. The soil has to be light, slightly acidic, with low content of the soil humus (<7%) and nitrogen (<0.35%) and high content of the phosphorus (>10%) and potassium (>12%).

Soil preparation: To achieve a favourable granular soil structure and to allow rapid infiltration and good retention of water the soil should be prepared by ploughing 20-30 cm in depth in combination with disk harrowing. Immediately before planting the soil should be cultivated 12-15 cm in depth. These activities cause loosening of the soil and also provide good air exchange regime within the soil and make easier the root penetration.

Propagation material: The best planting material for making new plantations are the rhizomes. The production of seedlings from seeds is rather slow and *in vitro* propagation is complicated and expensive process. For the preparation of rhizome cuttings, 3-4 year old plants are most suitable. The rhizomes have to be divided into 4-5 cm long cuttings having 2-3 propagation buds. Our experiments showed that the average propagation coefficient of a single plant is 21-25 cuttings.

Creation of perennial plantations: The rhizome cuttings have to be planted in the early autumn or early spring in rows with distance between the cuttings within the row of 40 and 60 cm between the rows. If the rows are east-west positioned the shadows that the plants will cast on one another could improve the overall temperature conditions in the plantation. In that case the distance between the plants within the rows will be about the height of the fully grown plants. Thus they could partly protect each other from the direct sun as well as save some of the soil humidity. About 100000 cuttings per ha should be provided. They have to be planted in furrows 8-10 cm deep. The plantation should be maintained free of weeds and regularly irrigated especially in the dry summer period. When properly maintained the plantation can serve its purpose for 4-5 years.

Harvesting: The harvesting of the aboveground biomass should be done at the phenological phase of full blossoming and in dry weather by using mower machine. The average yield from a three-year old plantation was 20 and 7 t /ha dry material. The mowed fresh plant biomass has to be quickly transported to a suitable place for drying otherwise the yield will be compromised. The drying process should take place in ventilated rooms. The fresh biomass should be spread in 5-6 cm layers. The plant material may also be dried in a drying-room at 40-45° C.

The presented results show that the three species can be easily cultivated at various places with mountain climate, with suitable conditions. Rhizome cuttings are the most appropriate propagation material for establishment of plantations. The plants of *A. mollis* and *A. jumrukczalica* grown *ex situ* were larger and more robust than those from natural populations while the plants of *A. achtarowii* were smaller. *A. mollis* possesses the best adaptive capacity to *ex situ* conditions followed by *A. jumrukczalica*. The highest yield of aboveground phytomass is reported for *A. mollis*. The local ecological conditions in the experimental plots significantly influence the yield of the phytomass and its structure; this highlights the importnace of site selection. The benefits of correct selection of field site can alter partially, possible unfavourable climate preconditions. No considerable differences were found in the timing of the phenological phase of full blossoming in all three species. During the vegetation period the plants could be harvested twice. No attack by diseases or pests were detected in the *ex situ* collections which can be explained by the high tannin content of plants. Based on the results of the study, a technical guidance for growing of the studied species in field conditions is proposed. Perspective clones for breeding experiments are distinguished. With regard to phytochemical studies, some important findings have to be underlined. The cultivation of *A. mollis*, *A. achtarowii* and *A. jumrukczalica ex situ* at an altitude similar to their natural distribution does not cause significant changes in the content of flavonoids and tannins in the aboveground mass. Moreover, negligible variation in flavonoid and tannin content was observed between plants grown *ex situ* in the two experimental stations. A more significant variation in the content of flavonoids and tannins was observed between the different age groups within a single species than between the plants grown in two distinctive physical - geographical regions (station “Zlatni mostove” in Vitosha Mt. and station “Beglika” in Western Rhodopes Mt.). The highest average content of tannins and flavonoids in aboveground phytomass from both experimental stations was found in *A. jumrukczalica* followed by *A. mollis*. The study of separate plant parts constituting the aboveground phytomass showed that flavonoids are predominately accumulated in the basal leaves, and tannins in flowering stems of the plants. *A. mollis* flowering stems produced more flavonoids and tannins in comparison with *A. achtarowii* and *A. jumrukczalica*.

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