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Flower Yield of *Arnica* sp. Cultivated in Two Floristic Regions in Bulgaria

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Authors' contributions

This work was carried out in collaboration between both authors. Author VB designed the study, wrote the protocol and wrote the first draft of the manuscript. Author AV managed the literature searches, analyses of the study performed the structural equation modeling and discuss the conclusion. Both authors read and approved the final manuscript.

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ABSTRACT

An experiment was conducted to evaluate the impact of climate and soil conditions on arnica plant development with different origin in two floristic regions at Vitosha Mt. and Rhodopi Mts. in Bulgaria and to calculate the yield of *Arnicae* flos from the studied collections. The measurements were done at Faculty of Pharmacy, Medical University, Sofia, Bulgaria, between May 2010 and June 2013. The altitude of the experimental fields was determined by General Positioning System and the exposition was established by compass. Climatic areas and regions were classified and the soil type was defined. The meteorological data for monthly average temperatures and rain falls for the experimental areas during the investigated period were given by National Institute of Meteorology and Hydrology, Bulgarian Academy of Science. The flower head diameter has been measured during the full flowering stage on 50 model plants. The data were statistically processed by

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Medcalc Program. Harvesting of plant substance *Arnicae* flos of 3-year old *A. montana* and *A. chamissonis* was performed by hand picking at full flowering stage. The results showed a significant difference in the origin of the herbal drug in the carried out study. Regarding to *A. montana*, advantageously greater yield was obtained from plants originating from Ukraine at both fields. Depending on the methods of propagation, higher values were obtained by *in vivo* seedlings, up to 268 kg/ha. With regards to the experimental area, on Vitosha Mt. the yield was approximately the same for both origins regardless of the method of propagation while at Rhodopi Mts. significantly greater was the yield of *in vivo* seedlings compared to *in vitro* seedlings. Concerning *A. chamissonis,* there was a predominantly higher yield at Vitosha Mt. compared to Rodopi Mts. (58 kg/ha and 35 kg/ha, respectively) and showed values up to four times less compared to *A. montana*. Both experimental areas had similar ecological characteristics, but the presence of much air humidity and soil moisture at Rhodopi Mts. implied a better plant development and bigger yield. The cultivation of *Arnica* sp. in mountainous regions in Bulgaria has been proved to be perspective and the country appears to be a promising source of plant substance *Arnicae* flos.

Keywords: Arnica chamissonis; Arnica montana; Arnicae flos yield; cultivation; Bulgaria.

1. INTRODUCTION

Arnica montana L. (Asteraceae) is a high-valued medicinal plant since it has been used for centuries in the treatment of sprains, bruises, muscle pains etc. [1]. The plant substance *Arnicae* flos contains sesquiterpene lactones which are responsible for its anti-inflammatory action and properties [2]. During the last decades *A. montana* (mountain arnica) natural populations are rapidly declining [3]. At the same time a large quantity of plant substance *Arnicae* flos is used every year and the need for it is greater [4]. Many countries have conducted trials on its introduction and cultivation, but there are some problems concerning with its acclimatization and adaptation [5-8]. Recently, *Arnica chamissonis* Less. (leaf arnica) appears to be an alternative and a substitute for *A. montana* [9].

For better introduction and cultivation of both the species, it is necessary to know the characteristic and their habitats. *A. montana* occurs in mountain meadows, pastures and fields, up to 2850 m asl. The presence of air humidity, soil moisture and providing conditions closest to the natural habitats are of great importance for the development of these species. As regards to the soil composition, arnica can develop only on poor and acid soil (pH 3.5 – 6.5), which has not been nourished and fertilized [10]. The authors also examined the characteristics of mountain arnica populations growing at different altitudes. Temperature and air humidity appeared to be the main abiotic factors that distinguished the habitats. Development of arnica on poor soil is explained by its coexistence with mycorrhizal fungi [11].

The alternative species *A. chamissonis* is found on moist meadows and pine forests, in the alpine and subalpine zone, from 0 – 3500 m asl [12]. Leaf arnica does not differ in the environmental requirements even has better adaptive potential as compared to mountain arnica. Sugier [13] compared the yield of both arnica, which are characterized by increasing yield till the end of the third year of cultivation, while in the fourth year has been recorded apparently reduction. [14] examined morphometrically arnica natural populations in different regions in Italy, taking into account fresh/dry weight of arnica flowers.

The objective of this study was to evaluate the impact of climate and soil conditions on arnica plant development in two floristic regions in Bulgaria and to calculate the yield of *Arnicae* flos from the studied collections.

2. MATERIALS AND METHODS

2.1 Plant Material

A. montana seedlings with different origin of seeds (Ukraine - Carpathians natural population and Germany - cultivar “Arbo”) and methods of propagation (*in vivo*, *in vitro*) were obtained. In addition, *A. chamissonis* seedlings bred by seeds originating from Poland and *in vivo* propagated were also bred as a referent species (Table 1).

A. montana seeds collected from Carpathians (Ukraine) were provided by Prof. A. Tashev PhD (Forestry University, Sofia, Bulgaria); *A. chamissonis* seeds were provided by D. Suiger PhD (University of Natural Sciences, Lublin, Poland). The *in vitro* seedlings were bred at the Institute Plant Physiology and Genetics, Bulgarian Academy of Science, Sofia, Bulgaria by Prof. E. Zayova PhD and M. Petrova PhD.

The seedlings were grown in plastic pots (diameter 9-11 cm) with enriched soil mixture with composition: black and white peat, nitrogen, phosphorous, potassium fertilizer, trace elements. Then the plants were placed under greenhouse conditions. When the plants attended the juvenile stage (May 2010) they were transplanted to the experimental fields at Vitosha Mt. and Rhodopi Mts. [15].

2.2 Experimental Plots and Ecological Conditions

Two experimental fields were established in two different floristic regions (May 2010) and the study was carried out during the third vegetation season (June 2013) (Table 2).

The influence of the following factors was monitored: climate region, monthly average temperatures and rain falls, altitude, slope, exposition and soil type. The altitude was determined by General Positioning System (GPS) and the exposition was established by compass. Climatic areas and regions were given according to the classification done by Velev [16] and soil type was defined by Ninov [17]. The meteorological data for monthly average temperatures, t (℃) and rain falls, r (mm) for the experimental areas during the investigated period were given by National Institute of Meteorology and Hydrology, Bulgarian Academy of Science (Table 3).

2.3 Arnica Flower Head Characteristic and Yield

In 2013, arnica flower heads diameter has been measured. It was done during the full flowering stage (50% of disk flowers were fully opened) on 50 model plants. The flower heads diameter was measured at the most distant points and the number of arnica flower per plant was counted. Then the data were statistically processed by MEDCALC Program. Harvesting of plant substance *Arnicae* flos of 3-year old *A. montana* and *A. chamissonis* were performed by hand picking at full flowering stage in the second half of June (16-23.06.2013). Anthodium's drying was conducted in a laboratory and the temperature did not exceed 30℃. Initially the herbal drug was measured fresh and then dried on an analytical scale Kern ABT 120-5DM. The weight of arnica flower was measured and the yield was calculated per unit area [kg/ha] (Table 4).

Table 1. The origin of seedlings planted at the experimental plots

Sample	Seedling, method of propagation	Origin
AMU1	*Arnica montana*, *in vivo*	Ukraine, Carpathians
AMU2	*Arnica montana*, *in vitro*	Ukraine, Carpathians
AMG1	*Arnica montana*, *in vivo*	Germany, cultivar "Arbo"
AMG2	*Arnica montana*, *in vitro*	Germany, cultivar "Arbo"
AChP	*Arnica chamissonis*, *in vivo*	Poland

Table 2. Ecological characteristic of the experimental fields

Ecological characteristic	Experimental field	
	Vitosha Mt., "Zlatni mostove" locality	Rhodopi Mts., "Beglika" locality
GPS coordinates	N 42.61°E 23.23°	N 41.84° E 24.11°
Altitude [m]	1 404	1 500
Exposition	SW	NW
Slope [°]	4-7	3
Soil type	Brown mountain-forest	Brown mountain-forest
Area [m^2]	100	100
N [%]	0.459	0.147
P_2O [mg/100 g]	4.10	19.83
K_2O[mg/100 g]	7.8	16.6
pH	6.1	6.5
Humus %	7.02	2.84

Table 3. The meteorological data for monthly average temperatures and rain falls for Vitosha Mt. and Rhododpi Mts

Experimental plot	Month 2013/factor	January	February	March	April	May	June	July	August	September	October
Vitosha Mt.	t (℃)	-3.4	-1.9	0.2	6.0	11.1	13.0	14.4	16.0	10.3	7.6
	r (mm)	82	97	84	77	76	169	102	59	66	85
Rhodopi Mts.	t (℃)	-4.3	-3.3	-0.2	4.8	10.5	11.7	12.6	14.5	10.6	8.0
	r (mm)	124	126	118	59	60	153	35	1	45	85

Table 4. *Arnicae* flos characteristic and yield

Sample	Flower head diameter [cm]	Flower head number	Fresh weight [kg]	Dry weight [kg]	Fresh flower head yield [kg]	Dry flower head yield [kg]
AMVU1	6.77±0.59	7.94±4.29	7.52	1.41	752	141
AMVU2	5.41±0.48	16.63±12.32	12.36	2.43	1 236	242
AMVG1	6.28±0.64	8.09±5.57	6.41	1.22	641	122
AMVG2	5.36±0.31	12.40±8.36	8.10	1.44	810	144
AMRU1	6.57±0.79	23.00±11.87	14.04	2.68	1 404	268
AMRU2	6.12±0.80	20.60±10.53	4.25	0.70	425	70
AMRG1	6.56±0.80	24.02±17.93	6.94	1.35	694	135
AMRG2	5.88±0.25	13.80±3.49	2.30	0.43	230	43
AChVP	3.34±0.42	18.00±12.48	2.22	0.58	222	58
AChRP	2.84±0.40	17.59±16.13	1.58	0.35	158	35

AMVU1 - A. montana, Ukraine, in vivo seedlings, Vitosha Mt.
AMVU2 - A. montana, Ukraine, in vitro seedlings, Vitosha Mt.
AMVG1 - A. montana, Germany, in vivo seedlings, Vitosha Mt.
AMVG2 - A. montana, Germany, in vitro seedlings, Vitosha Mt.
AMRU1 - A. montana, Ukraine, in vivo seedlings, Rodopi Mts.
AMRU2 - A. montana, Ukraine, in vitro seedlings, Rodopi Mts.
AMRG1 - A. montana, Germany, in vivo seedlings, Rodopi Mts.
AMRG2 - A. montana, Germany, in vitro seedlings, Rodopi Mts.
AChVP - A. chamissonis, Poland, in vivo seedlings, Vitosha Mt.
AChRP - A. chamissonis, Poland, in vivo seedlings, Rodopi Mts.

The yield of *Arnicae* flos from both experimental fields was compared and the ratio fresh:dry flower heads was calculated.

3. RESULTS AND DISCUSSION

3.1 Experimental Fields and Ecological Conditions

Both experimental fields were located in the mountainous area: Vitosha Mt., "Zlatni mostove" locality is in the climatic region of Vitosha Mt. and Rhodopi Mts., "Beglika" locality - in Western Rhodopi Mts. The experimental areas are within the Mediterranean soil area and they are included in mountainous soil zone (Table 2).

Vitosha Mt. experimental field soil analysis results showed that the soil is humus (7.02%), very rich in nitrogen (0.459%) and acidity value is between average and lightly acid (pH 6.1). Phosphorus and potassium were reported as very poor and poor (4.10 mg/100 g and 7.8 mg/100 g, respectively) (Table. 2).

Rodopi Mts. experimental field soil analysis results revealed that the soil is deficient in nitrogen (0.147%), light acidic to neutral (pH 6.5) and has a low humus content (2.84%). As regards to the content of phosphorus and potassium, the soil is medium rich (19.83 mg/100 g and 16.6 mg/100 g, respectively) (Table. 2).

The average temperatures for Vitosha Mt. experimental field during the investigated period were close to or slightly above the monthly norms (average perennial). 2013 year was warm and there was not regular rain. The winter was wet, with temperatures at norm (-3.4℃ – 0.2℃). Spring was reported as a warm and dry. Late summer was very hot and dry (August 16℃). During autumn, October was the warmest month (7.6℃) (Table 3).

According to data obtained on rain falls, 2013 year was excessive from January to March (82 mm, 97 mm and 84 mm, respectively) and also in June and July (162 mm and 102 mm, respectively) and below the norm in April and May (77 mm and 76 mm, respectively). During the rest of the period, the data were close to the norms (Table 3).

Regarding to Rhodopi Mts. experimental field, the data show that the average monthly air temperatures were generally similar to those perennial averages. The season (2013) was characterized by temperatures close to the norms and slightly above the perennial average, as it was during April, May and October (4.8℃, 10.5℃ and 8.0℃, respectively).

The rainfall conditions in the area were more diverse than the perennial average. There were excessive rain falls in October and December (144 mm and 124 mm, respectively), and then during 2013 – from January to March and in June (124 mm, 126 mm, 118 mm and 153 mm, respectively) and below the norms in May, July, August and September (60 mm, 35 mm, 1 mm and 45 mm, respectively) (Table 3).

3.2 Arnica Flower Head Characteristic and Yield

The collected flower heads were about 5 – 7 cm in diameter (Table 4). Involucres consisted of 18 – 24 numbers elongated lanceolate bracts, with acute apices in 2 rows. The receptacle was about 6 mm in diameter, covered with hairs. Its periphery had 15-20 ligulate flowers, 20-30 mm in length, and the disc beared numerous tubular flowers, 15 mm in length. Achenes were brown in color, and pappus was presented. The herbal drug had an aroma and a bitter taste.

The diameter of *A. montana* flower head with origin Ukraine and Germany, for both types of propagations and collections, ranged from 5.36 cm (AMVG2) to 6.77 cm (AMVU1). Regarding to *A. chamissonis*, inflorescences diameter was bigger in the collection at Vitosha Mt. than Rhodopi Mts. (3.34 cm and 2.84 cm, respectively).

The number of *A. montana* flower heads per plant counted for samples of both origin and types of seedlings and experimental fields was from 7.94 (AMVU1) up to 24.02 (AMRG1). Concerning *A. chamissonis*, the inflorescence number on both areas was similar (18.00 and 17.59, respectively).

Data received from Vitosha Mt. experimental field show advantageously greater yield of mountain arnica, Ukrainian origin, *in vitro* propagated. It is almost double compared to yield received from *in vivo* propagated plants (141 kg/ha and 242 kg/ha, respectively) (Table. 4). Samples with German origin, *in vivo* and *in vitro* propagated, have very similar values (122 kg/ha and 144 kg/ha, respectively). All compared samples are very similar, except sample AMVU2.

Data obtained from Rhodopi Mt. experimental field show predominantly higher yield of arnica with origin Ukraine, *in vivo* seedlings (268 kg/ha dry weight). The plants obtained by *in vivo* propagation from both origin (268 kg/ha and 135 kg/ha, respectively), have three times higher yield compared to *in vitro* seedlings (70 kg/ha and 43 kg/ha, respectively) (Table 4).

In the studied samples, the biggest *Arnicae* flos yield, derived from *A. montana*, is recorded for the sample from "Beglika" locality, origin Ukraine, *in vivo* propagated (AMRU1) – 268 kg/ha, and the smallest is the yield from Rodopi Mt. again, but with German origin, *in vitro* propagated (AMRG2) - 43 kg/ha (Table 3). The results on *A. chamissonis* revealed predominantly higher yield on Vitosha Mt. compared with Rodopi Mt. (58 kg/ha and 35 kg/ha, respectively) (Table. 4). The ratio fresh:dry mountain and leaf arnica flower heads is 1:5.5 and 1:3.4, respectively. At both experimental fields the soil is acidic in reaction (pH 6.1 – 6.5), similar as it is in the natural habitats [18].

The altitude is also an important factor in establishment and cultivation of arnica [10]. As a plant with natural distribution in the Northern regions of Europe [18], arnica can be successfully cultivated in the mountainous areas, although Bulgaria is the southern boundary of the species distribution.

The characteristic of plant substance *Arnicae* flos, cultivated in Bulgaria, is in accordance with Ph. Eur. 7.0. [19]. The reported mountain arnica yield ratio fresh:dry flower heads is up to 6, which coincides with literature data [20]. The results for the studied mountain and leaf arnica morphological parameters on both experimental plots are in the dimensions given by the other authors [6,8,13,14]. The diameter of leaf arnica inflorescence is smaller but their number is bigger compared to mountain arnica and it is as well reported by Sugier [13].

The yield of *A. chamissonis* (AChVP and AChRP) is the lowest and show values up to 4 times less compared to *A. montana*. Despite the rapid adaptation of leaf arnica to the environmental conditions and the entry at generative stage in the first vegetative year, it must be consider the great difference in the yield of both plants. Therefore, the yield of leaf arnica was smaller in comparison with mountain arnica and the work involved for the collection of its herbal drug was greater as well.

Our results on arnica flower yield are average and very closely relative to the data reported by Pljevljakušić et al. [8]. Concerning *Arnicae* flos yield should be considered the influence of the environmental conditions at the experimental area. Both established fields have very similar characteristics, but the presence of more moisture at "Beglika" locality (presence of a water reservoir, forests, etc.), implied a better development and bigger yield.

In the carried out study there was a difference in the origin of the plant substance. Advantageously greater yield was obtained from plants with Ukrainian origin at both experimental fields. Depending on the propagation, higher values were obtained by *in vivo* seedlings, except for sample AMVU2. With regards to the experimental area, at Vitosha Mt. the yield was approximately the same for both origins regardless of the method of propagation. At “Beglika” locality significantly greater was the yield of *in vivo* seedlings compared to *in vitro* ones.

4. CONCLUSION

For the first time in Bulgaria arnica flowers yield was obtained from cultivated plants with different origin (Ukraine, Germany and Poland) and different method of propagation (*in vivo* and *in vitro*), grown under different environmental conditions in two floristic regions of the country. The results showed that Arnica species can be grown successfully in Bulgaria, over 1400 m altitude, with high air and soil humidity, soil poor and acid in reaction. *A. chamissonis* was more adaptive compared to *A. montana*. Bulgaria, known worldwide for its traditions in medicinal plants marketing, is a promising source of plant substance *Arnicae* flos.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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