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Resource assessment of *Leucojum aestivum* L. (*Amaryllidaceae*) populations in Bulgaria

Abstract

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Monitoring of 22 populations of *Leucojum aestivum* L. (*Amaryllidaceae*) was performed in the period 2001-2004. Analysis of management practices and resource assessment of 16 economically significant localities were made in 2003 and 2004. Population density varied from 7.38±4.10 to 46.37±2.67 ind/m^2. Exploitation reserves (from 483 to 52 903 kg) and possible annual harvest were estimated by means of model individuals. Comparison between populations of fresh-mass productivity of individual (7.47±0.40 – 25.43±2.08 g) and galanthamine content (0.9 – 2.6 mg/g average) showed great variability.

Introduction

Leucojum aestivum L. (*Amaryllidaceae*) is a resource species for the pharmaceutical industry in Bulgaria, as Galanthamine-containing natural populations have been of economic importance since 1960. We published an earlier detailed account of the problems related to the protection and sustainable use of this species with regard to the great demand in crude drug in the last 3 years (Gussev & al. 2003). Several assessments have been carried out to quantitatively and qualitatively characterize the resources of *L. aestivum* in Bulgaria (Stoyanov & Savchev 1964; Astadjov 1969; Stefanov 1990; Mitrev 1995). However, contemporary understanding of the sustainable use of medicinal plants requires the analysis of management practices, annual monitoring of populations and quantitative assessment of reserves, and the monitoring of Galanthamine content and requirements for sustainable management.

Commonly known as summer snowflake, *Leucojum aestivum* is categorized as endangered in the Red Data Book of Bulgaria (Velchev 1984). A regime of protection and regulated use was prescribed according to the Protection of Biological Diversity Act (2002) in Bulgaria and Order # RD-521 of the Ministry of the Environment and Waters, pursuant to which:

Access to economically important localities is controlled, and only 16 Galanthamine populations are permitted for use (Tab. 1).

Table 1. Localities of *L. aestivum* in Bulgaria permitted for use.

No	Locality	District	Coordinates	Area (ha)
1	Gradina	Plovdiv	42°08'N 25 12'E	20.0
2	Vinitza	Plovdiv	42°08'N 25°08'E	18.6
3	Debelata Koria	Plovdiv	42°22'N 24°47'E	0.4
4	Ormana	Yambol	42°32'N 26°31'E	30.0
5	Palauzovo	Yambol	42°32'N 26°44'E	16.0
6	Blatetz	Sliven	42°38'N 26°32'E	15.0
7	Blatoto	Burgas	42°24'N 27°40'E	29.0
8	Kalinata	Burgas	42°42'N 27°40'E	20.0
9	Chairite	Burgas	42°48'N 27°32'E	2.0
10	Karaagach	Burgas	42°13'N 27°45'E	0.5
11	Zidarovo	Burgas	42°20'N 27°24'E	30.0
12	Lozenski Pat	Haskovo	41°46'N 26°10'E	48.0
13	Biser	Haskovo	41°52'N 25°60'E	35.8
14	Dolnata ova	Haskovo	41°49'N 26°08'E	20.0
15	Kotchovo	Shumen	43°14'N 26°48'E	12.0
16	Osmar	Shumen	43°13'N 26°51'E	19.0

The defined terms, methods and regulations of use include plant-friendly exploitation methods, capacity of the ecological reserves of individuals, and floating herbage yields.

The regulations and exploitation quotas were set. The amount of herbage to be collected annually is regulated by an explicit Order of the Minister of the Environment and Waters after assessment of population status. The owners of the localities then issue permits to buyers and herb-gatherers and control use.

Methods

The main methodologies were based on the guidelines established in the design of plant conservation or recovery plans (Jakson & Akeroyd 1994), The Handbook of Ecological Monitoring (Clarke ed. 1986), and Methodology for determination of medicinal plant resources (Anonymus 1986).

The population monitoring and assessment from 2001-2004 were carried out according to a standard protocol which included the following basic parameters: total and effective productive area, density (total density as well as that of generative and vegetative individuals), anthropogenic influence (habitat destruction, grazing, irregular harvesting), yield, exploitation reserves and possible annual harvest.

The method of concrete localities was applied to measure quantitative reserves, as yield was calculated by the following parameters: area of locality (ha) and yield (g/m^2). The yield was calculated as a product of the average fresh mass of model individuals (g) and

density (ind/m^2) (M – average, m – standard error). The model individuals (30-40 from locality) were harvested at the beginning of flowering and their fresh mass was measured by electronic balances. The density was measured by plot units of 0.025 m^2, representative of the locality ($P<0.1$). The exploitation reserves (kg) were calculated as the product of yield (its lower limit M-2m) and effective productive area. The possible annual harvest (kg) was calculated by dividing the number of exploitation reserves by 4 (herbage yield, i.e. one year of harvesting plus a 3-year recovery period).

Galanthamine content of the model individuals was presented as an average for the population. The standard protocol of analyses included the following main procedures: model individuals were dried at 60ºC; methanol extraction (3 times 30 min sonification for 24 h), centrifugation, filtration, evaporation, dissolution, filtration) and HPLC analysis (Waters quaternary system, PDA detector) (Pandova & al. in press). ANOVA were applied to prove the significance of variations between the populations according to Gal-content ($P<0.05$).

A Bulgarian *Leucojum* Data Base (BLDB) was developed for the purposes of preservation and management of data relating to the monitoring of populations and resource assessment (Georgiev & al. 2003).

Results and Discussion

The analysis of management practices showed that existing legislation is adequate for the sustainable management of *L. aestivum* populations as a source of *Herba Leucoji aestivi* (Galanthamine source). The management of most localities was facilitated by their proclamation as protected areas, where the regime ensures ecologically-friendly utilization.

The assessment of population status and exploitation reserves has been carried out using an unsuitable protocol and the control of herb-gathering is poor and ineffective. As there is no audit of the permitted quotas, these quotas are often exceeded. Illegal gathering also occurs and business is not integrated in the protection of populations or efforts for the sustainable use of resources.

Twenty-two populations were subjected to monitoring (density assessment of the 16 localities permitted for gathering were presented in Table 2, data concerning the rest of populations is contained in BLDB). In four localities (Vinitza, Debelata Koria, Chairite, Lozenski Pat) we observed permanent habitat degradation (forestation with poplar cultivars, drainage and intensive grazing), as well as the destruction of populations (low density, lack of flowering and fruit-bearing). These populations could not be used for industrial purposes and urgent restoration measures need to be undertaken.

The remaining 12 localities are of interest for use under the legal regime prescribed. The total area of these localities is 247.3 ha (varying from 0.5 to 35.8 ha) representing 80% of the total national population of Galanthamine-containing plant species. The effective production area where *Leucojum* populations occur is 74.7 ha. It was found that herb-gatherers cut all individuals regardless of their development (young and generative).

Population density (ind/m^2) varied within a wide range from 7.38±4.10 to 46.37±2.67 ind/m^2. The annual fluctuations in density are mainly due to soil moisture content (flooding in spring and autumn) and gathering methods (height above ground at which herbage is cut). Cutting close-to-the ground negatively influences the preparation of the bulb for

Table 2. Density (ind/m^2) assessment of the *Leucojum aestivum* localities permitted for use. M - average, m - standard error.

Locality	2001						2002					
	vegetative		generative		total		vegetative		generative		total	
	M	m	M	m	M	m	M	m	M	m	M	m
Gradina	29.60	5.72	8.09	4.47	37.69	6.37	39.53	2.51	6.84	0.67	46.37	2.67
Ormana	15.38	4.25	5.78	4.00	21.17	4.44						
Palauzovo	3.52	4.00	3.86	3.97	7.38	4.10						
Blatez	32.00	5.90	3.16	4.01	35.16	5.93						
Blatoto							27.63	2.16	8.16	0.96	35.79	2.27
Kalinata	13.40	4.30	3.93	4.04	17.33	4.57						
Karaagach							19.15	1.58	5.28	0.53	24.43	1.70
Zidarovo												
Lozenski Pat	20.63	4.54	1.88	3.96	22.51	4.50						
Biser	8.34	4.24	1.24	3.97	9.58	4.31						
Dolnata Ova	9.52	5.10	6.29	4.09	15.81	5.32						
Kotchovo												
Osmar												

Locality	2003						2004					
	vegetative		generative		total		vegetative		generative		total	
	M	m	M	m	M	m	M	m	M	m	M	m
Gradina	27.20	4.99	16.19	4.01	43.39	5.13						
Ormana	11.79	4.05	10.45	4.05	22.24	4.20	19.36	1.90	12.00	1.46	29.98	2.57
Palauzovo	9.18	4.02	4.58	4.00	13.77	4.03	8.29	0.89	2.92	0.36	11.21	1.06
Blatez					27.15	4.59	17.58	1.37	5.23	0.35	17.58	1.37
Blatoto	15.94	4.12	8.16	4.01	24.03	4.15	13.17	1.42	6.00	0.60	17.58	1.37
Kalinata	16.45	4.14	2.86	4.00	19.31	4.16	9.09	1.04	5.52	0.84	14.61	1.45
Karaagach	14.34	4.03	9.04	3.98	23.39	4.06						
Zidarovo	20.36	4.29	11.37	4.09	31.74	4.48						
Lozenski Pat												
Biser												
Dolnata Ova												
Kotchovo	5.00	4.00	7.15	4.00	12.15	4.04	4.17	0.61	3.83	0.44	8.00	0.85
Osmar	10.23	4.15	5.66	4.01	15.89	4.26	3.82	0.70	6.62	0.82	10.44	1.37

the next vegetation period and often leads to temporary dormancy or poor vegetation. *L. aestivum* maintains a high soil reserve of conserved bulbs and seeds. Density could be increased through artificial flooding. For example, total density at the Gradina locality increased from 37.69±6.37 ind/m^2 in 2001 to 46.37±2.67 in 2002 and 43.39±5.13 for 2004. Population density is an initial parameter for yield calculation, which is why reserve assessment must be carried out annually at the beginning of flowering.

Mean fresh mass of model individuals varied between populations (7.47±0.40 – 25.43±2.08 g) due to differences in ecological conditions and hydrological regime. In case of low variability of soil moisture in 2003 and 2004 (Gradina, Ormana, Dolnata Ova), the

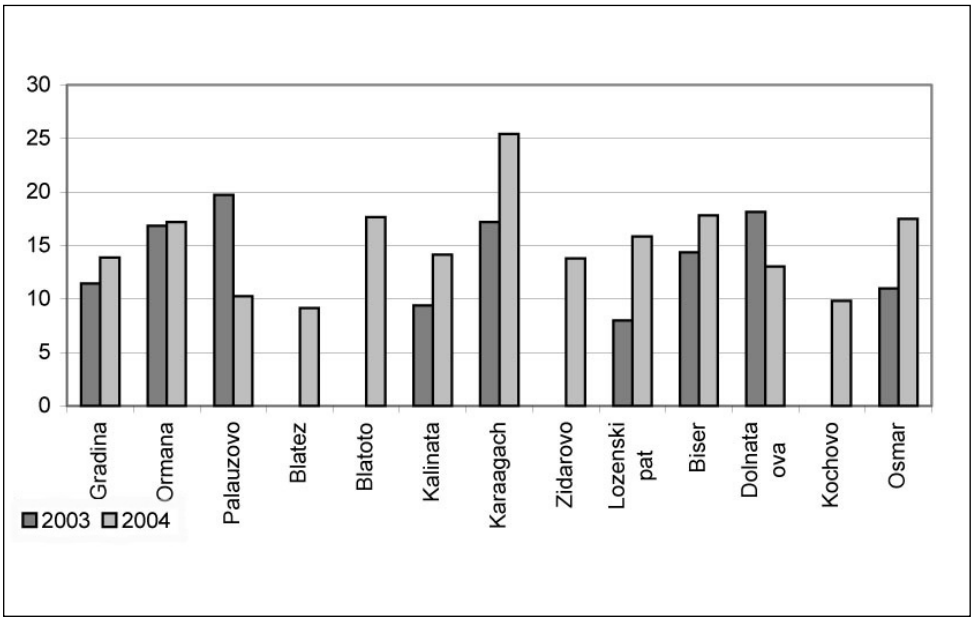

Fig. 1. Average fresh mass of model individuals (g).

differences in the average mass were insignificant. Excessive drought and accidental flooding drastically influence herbage mass and that is why annual assessment of the mass is obligatory for the resource assessment (Fig. 1).

The exploitation reserves and possible annual harvest were calculated for localities with a good status at the time of assessment (well-moisturized, normal plant phenology and development without extreme influences): 6 localities in 2003 and 9 in 2004 (Tab. 3).

Galanthamine content in 2003 varied between and within populations (Fig. 2, Tab. 4). All populations were profitable according to Galanthamine content (0.9-2.6 mg/g average). The same regularity in Galanthamine content by populations was detected by Stefanov in 1990, which confirms the stability of this parameter.

Conclusions and Recommendations

Only 12 of the monitored populations of *L. aestivum* are economically important. The developed methodology in this study was effective and comparatively easy to apply.

An Action Plan should be developed for the localities permitted for usage, with emphasis on habitat reclamation. Management Plans need to be developed for all populations.

The basic condition for the effectiveness of adaptive management for sustainable use are: precise and well-timed monitoring of population status according to preset population parameters; annual assessment of the reserves immediately prior to harvesting time of the locality; control of gathering and observing permitted quotas.

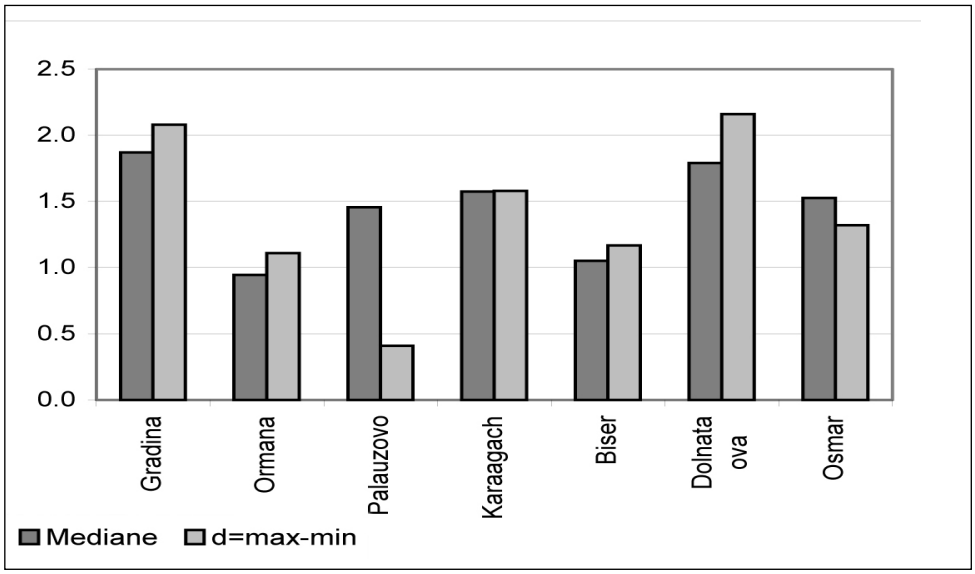

Fig. 2. Galanthamine content (mg/g) – 2003.

Table 3. Assessment of exploitation reserves and possible annual harvest of *Leucojum aestivum* in Bulgaria in 2003 and 2004.

Locality	Density (ind/m^2)				Fresh mass (g)				Yield (g/m^2)				Effec. area (ha)	Exploit. Reserv.(kg)		Possib. Ann. Harvest (kg)	
	2003		2004		2003		2004		2003		2004						
	M	m	M	m	M	m	M	m	M	m	M	m		2003	2004	2003	2004
Gradina	43.39	5.13			11.46	0.97	13.86	0.95	497.29	72.30			15	52903		13226	
Ormana	22.24	4.20	29.98	2.57	16.84	0.73	17.18	0.92	374.47	72.57	515.01	52.05	5	11467	20545	2867	5136
Palauzovo	13.77	4.03	11.21	1.06	19.71	2.35	10.25	0.52	271.31	85.77	114.90	12.30	4	3991	3612	998	903
Blatetz			17.58	1.37			9.14	0.35			160.64	13.93	4		5311		1328
Blatoto			17.58	1.37			17.67	1.00			310.56	29.87	10		25081		6270
Kalinata	19.31	4.16	14.61	1.45	9.38	0.44	14.13	0.87	181.13	39.94	206.45	24.05	4	4050	6334	1013	1583
Karaagach	23.39	4.06			17.18	1.69	25.43	2.08	401.84	80.17			0.2	483		121	
Kotchovo			8.00	0.85			9.81	0.52			78.48	9.33	1.5		897		224
Osmar	15.89	4.26	10.44	1.37	12.00	1.03	17.5	0.91	190.68	53.68	182.72	25.83	2	1667	2621	417	655

Table 4. ANOVA analysis of Galanthamine content of model individuals (mg/g) – 2003.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Populations	62.436378	9	6.9373753	36.940379	5.304E-42	1.9153106
Within Population	49.766801	265	0.1877992			
Total	112.203180	274				

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