ECOLOGIA BALKANICA

2017, Vol. 9, Issue 1 June 2017 pp. 39-51

Distribution and Resources of the Medicinal Plant Colchicum autumnale L. in Bulgaria

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Abstract. *Colchicum autumnale* (Colchicaceae) is a perennial geophyte and a medicinal plant. Its biomass is collected for industrial uses for obtaining the alkaloids colchicine and demecolcine. The objective of the present study was to estimate the distribution and potential resources of *C. autumnale* populations in Bulgaria in terms of their sustainable use. Monitoring of habitats was carried out in concrete harvesting areas. The distribution of the populations and the amount of drug production in specific sites and conditions were studied. In 2014-2015, eleven localities were established in seven floristic regions, spread on an area of 498000 m². Seed resources obtained from the different populations ranged from 3.57 g to 12225 g. The seed yield depends on the environmental conditions, the number of plants per m², the number of fruit capsules per plant and the weight of the seeds contained in them. Changes in the management approach to habitats occupied by *C. autumnale* caused degradation of the areas, resulting in the decrease of the population density of the species.

Key words: *Colchicum autumnale*, meadow saffron, autumn crocus, resources, medicinal plant, Bulgaria.

Introduction

Colchicum autumnale (meadow saffron, autumn crocus) is a valuable medicinal plant containing over 20 alkaloids, such as colchicine, demecolcine, colchicoside, etc. Colchicine is among the major alkaloids and it is an acid amide derivative of tropolone (BOICHINOV & AHTARDZHIEV, 1969). At lower doses (0.0001 g) it is used for treatment of gout and it was proven to have an antitumor effect (ASENOV et al., 1998). In the world literature it has been announced that the drugs obtained from the plant species are used in veterinary medicine for arthritis and as a diuretic (JAEGER & FLESCH, 1990).

In world literature studies of the species were most often directed to: 1) investigating the species as a raw material for pharmaceutical industry and 2) investigating the species as poisonous for livestock after grazing on pastures, in which there are populations of *C. autumnale*.

A significant share of the research studies in world literature were aimed at determining

the chemical composition of the species. Some of them refer to the territory of Turkey where representatives of *Colchicum* genus are spread, including endemic species (KAYA *et al.*, 2013). Colchicine dynamics at different periods of collecting specimens of the genus were studied in a series of research experiments, including species not found in our flora (NOGHONDAR *et al.*, 2012; AKRAM *et al.*, 2012; MORTEZA *et al.*, 2013; METIN *et al.*, 2014). POUTARAUD & GIRARDIN (2003) estimated seed alkaloid yield of the species in natural and cultivated conditions. The authors reported an increase of the seed yield under cultivation conditions, due to the increase in the number of capsules per plant. They established that the alkaloid content was stable over the years. MROZ (2008) carried out a study on the effect of the mineral soil content on the population density of the species. The author established differences in the reproductive and vegetative performance of plants in 25 populations as a result of the influence of the different chemical composition of soil.

In different studies on the population density of the species in Central Europe, a number of authors recommended measures for grassland management with the aim of protecting animals against poisoning (JUNG *et al.*, 2011; WINTER *et al.*, 2011, 2014; PERATONER *et al.*, 2014). The authors found out that the early cut, before the formation of the fruit capsules of *C. automnale*, reduced poisoning in animals, regulated the population density of the studied plant species, but also reduced hay yield.

Developing a strategy for limiting the species distribution in Germany, SEITHER & ELSÄSSER (2014) recommended a number of measures. The authors established that mulching treatment (in April-May), mowing or herbicide application reduced the population density of the species.

Scientific literature review shows that there are no current studies on the species in Bulgaria, both on its phytochemistry and on its resources and distribution. The earliest data about the distribution of *C. automnale* in our country were presented in the publications of VELENOVSKY (1892), who mentioned that the species was found in the regions of Lovech, Troyan, Yablanitsa, Knyazhevo, etc.

URUMOV (1906; 1908; 1917; 1929) announced that the species was found in the region of Krapetz and between Sevlievo and Lovech, in grassy habitats. The same author noted that the species prefers the meadow communities on the slopes of the village of Shtraklyuvo and the dry pastures in Gorna Klisura, Bratsigovo, etc.

During the same period TOSHEV (1895; 1902; 1903) expanded the knowledge about the distribution of *Colchicum* in the Thracian lowland – Haskovo district, Tatarevo, around Stara Zagora, Kobilin dol, etc.

STRANSKY (1921) and YORDANOV (1923-1924) identified *C. automnale* in dry habitats in the areas of Batak, Beglika, Seminska Kula in the Rhodope Mountains. They also emphasized that the species prefers the grassy communities in Lozen Mountain, Kocherinovo, Govedarnika, around Dupnitsa, Yakorouda, etc.

In "Flora of the Republic of Bulgaria" KOUZMANOV & KOZHUHAROV (1964) mentioned that the species required moist, grassy habitats and it was spread all over the lowland and mountainous parts of the country.

DIMITROV *et al.* (1967) recorded that populations of the species in the region of Kardzhali, the village of Razdel, Knyazhevo and Elhovo.

The revised herbarium specimens and literature showed that the information on the distribution of the species is outdated, it has not been updated over the last 50-60 years and data about the resources were not found. That determined the aim of the present study, namely to investigate the distribution, as well as to establish the resources of *C. autumnale* L. in Bulgaria.

Material and Methods

Colchicum autumnale L. (Meadow Saffron, Autumn Crocus) of Liliaceae/Colchicaceae family was the object of the present study. The investigation was carried out in the period 2014-2015 at mass flowering and fruit-bearing of the species.

The starting point of the study on the species distribution was a review of herbarium specimens in the herbarium of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (SOM), the herbarium of Sofia University "St. Kliment Ohridski" (SO) and the herbarium of the Agricultural University, Plovdiv (SOA). The

available horological information, the inspected herbarium specimens, personal collections and field inspection were the basis for studying the distribution and current status of *C. autumnale* populations.

Three hundred and thirty two herbarium specimens were studied. Seventy six specimens from the herbarium of the Institute of Biodiversity and Ecosystem Research (SOM), 39 from the herbarium of Sofia University "St. Kliment Ohridski" (SO) and 117 from the herbarium of the Agricultural University, Plovdiv (SOA).

Mapping was conducted following the methodology of KOZHUHAROV *et al.* (1983) by using the route and the point (stationary) methods. The climatic characteristics of the habitats were described according to KOPRALEV *et al.* (2002). The soil types were identified according to "Soil Atlas of Bulgaria" of KOYNOV *et al.* (1998) and according to "Soil Map of Bulgaria" (1968) in scale 1:400 000.

The exact location, the geographical coordinates and altitude of the habitats were determined by GPS (Garmin Dakota 20) and the data are presented in Table 1 and Fig. 1.

The following populations were selected in the study for evaluating the diversity of climatic factors and the relief of the different floristic regions:

Floristic region of Black Sea Coast (South) – Arkutino, climate region of Burgas Lowlands, continental and Mediterranean Climatic Zone.

Floristic region of Thracian lowland (southernmost point) – Mezek, climate region of East-Rhodope river valleys, continental and Mediterranean Climatic Zone.

Floristic region of Struma valley – M. Pole, Kulata, Kresna gorge, located in Petrich-and-Sandanski continental and Mediterranean Climatic Zone.

Floristic region of the Balkan mountain range (Western part) – Balyuvitsa, Balkan foothill climate region of the European Continental Climatic Zone.

Fig. 1. Indicative map of the studied populations.

Table 1. Coordinates and resource of *C. autumnale* in the studied populations (2014-2015).

No./Location/ coordinates	Altitude in m	Net area in %	Total area in m²	Average number of plants per m² X±SE	Weight in g/m²	Resource in g
1.Chirpanskata koriya N42⁰13'579", E25⁰15'444"	170	1.5	1000	5.3±0.49	2.96	27.03
2. Kresna Gorge N41046'765", E23009'231"	142	2	20 000	7±0.90	4.76	952
3. Marino pole/Kulata N41024'963", E23021'336"	135	1.5	50 000	3.6±0.37	1.94	729
4. Observatory Rozhen N41041'747" E24044'134"	1774	0.5	1000	2.6±0.30	1.43	3.57
5. Rozhen grasslands N41040'331", E24043'867"	1423	4	100 000	10.4±0.13	5.51	11024
6. Chokmanovo N41031'887", E24043'793"	1090	50	5000	9.8±0.41	5.48	6850
7. Smilyan N41030'650" E24045'291"	799	50	10 000	8.9±0.52	4.89	12225
8. Mezek N41043'875" E26006'020"	132	1	100 000	2.8±0.90	1.56	784
9. Arkutino N42019'931" E27043'263"	29	1	1000	4.0±0.25	2.20	11
10. Elhovo/Balabana N42084'830'' E26032'207''	108	2	200 000	10.4±0.68	5.40	10800
11.Balyuvitsa N43029'897'' E23020'117''	270	40	10 000	10.8±0.40	5.61	11232

Floristic region of Thracian lowland – Chirpanska Koria, climate region of East Central Bulgaria of the European Continental Climatic Zone.

Floristic region of the Rhodope Mountains (Central part) – Rozhen, in the high mountainous part of the Mountain climate region of the European Continental Climatic Zone.

Floristic region of the Rhodope Mountains (Middle part) – for evaluating the effect of the altitude on the distribution of the species, specimens were collected from the middle mountainous and high mountainous parts of the Mountain climate region of the European Continental Climatic Zone. The resource was developed at four different altitudes: 799 m a.s.l. (Smilyan), 1090 m a.s.l (Chokmanovo), 1423 m a.s.l (the low part of Rozhen) and 1744 m a.s.l (Rozhen Observatory).

Floristic region of Tundzha hilly valley – Elhovo, Balabana locality, in the climate region of East Central Bulgaria of the European Continental Climatic Zone.

The present research study on *C. autumnale* resources was carried out following the methodology of the Russian School by the

methods of SHRËTER *et al.* (1986) in concrete habitats (harvesting areas). This methodology has been officially adopted in Bulgaria. It is applied in order to determine the resource potential stock of the medicinal plants in the available populations.

The total area of all the habitats was measured in m² by longitudinal transect routes.

Determining the yield harvested from the habitats was performed by setting control sites in accordance with the requirements of the methodology. As the studied species is spread in spots (colonies), the area of the entire territory was determined first and then the percentage of the net area occupied by the species was calculated. The yield obtained was measured by collecting the capsules with seeds, following the instructions for gathering and drying of herbs. The dried seeds were weighed in grams with a precision of ±5%. The results obtained were processed by the methods of variation statistics. Mean arithmetic values (M) of the studied indicators and standard errors of the mean arithmetic (SE) were calculated.

The possible annual resource was estimated, as follows: the net area occupied by the species was multiplied by the lowest seed yield (M-2SE) and then the obtained exploitation stock was divided by the number of years necessary for the population to be restored according to the requirements of the methodology. When estimating the resources of the studied medicinal species, the plants found in settlements, along railways and highroads and in contaminated areas, were not taken into consideration, following the methodology used.

Phytocenological description of the habitats was made when evaluating the resource, as the plant species are an indicator of the conditions existing in the biotope and an evidence of the balance in the environment. Descriptions were made following the principles of the Western phytocenological school of BRAUN-BLANQUET (1964). The projective cover of the vegetation from each species was visually assessed. Five-point cover-abundance scale was used in the fieldwork, which is of a mixed type – numeric and symbolic. Natural habitats were determined according to phytocenological descriptions, following the "Guidelines for determining the habitats of European importance in Bulgaria" (KAVRAKOVA *et al.*, 2009).

Results and Discussion

According to the distribution of the deposited herbarium specimens, the largest amount of material of *C. autumnale* was collected from the Floristic Region of the Rhodopes (Western, Central and Eastern parts) – 26.58%. The species was found in grassy habitats in the region of Batak dam, the villages of Startsevo, Sivino, Smilyan, Byala Cherkva, etc.

Second came the Floristic Region of the Balkan Mountain Range (Eastern, Central, Western) – 22.78%. The species is distributed in Kalofer Dzhendem, above Karlovo, above the town of Berkovitsa, etc.

According to the deposited herbaria, the next group comprised the Floristic Region of Sofia (13.29%) and the Floristic Region of the Upper Thracian lowland (10.12%). The presented herbaria were collected from Mezek, Elin Pelin, the district of Stara Zagora, Panagyurishte, etc.

Single deposited specimens had been collected in the Floristic Regions of the Danube, Black Sea Coast, North-East Bulgaria, Rila Mountain, Slavyanka Mountain, Struma valley. It should be mentioned that all the deposited specimens were collected in the period 1921-1967. In the first year of the study, 11 populations of the species were established, distributed in 7 Floristic Regions of Bulgaria. *C. autumnale* was found at the Black Sea Coast, in the lowlands of the Thracian, Struma and Tundzha valleys, as well as in the highlands of the Rhodopes and the Balkan Mountains. The altitude varies from 29 to 1744 meters above sea level (Table 1). The soil types, on which the populations were mapped, also varies – leached cinnamon forest (Cromic Cambisols/FAO), gray forest, alluvial-meadow (Fluvisols/FAO), forest brown (Cambisols/FAO).

The first studied population was established in the protected area Chirpanska Koria in Thracian Floristic Region. The area is located on the border between Pazardzhik and Plovdiv districts and Stara Zagora district. It is characterized by transitory-continental climate and the influence of the

Mediterranean Sea is also felt (KOPRALEV *et al.*, 2002). Soils are typical Pellic Vertisols/FAO and Fluvisols/FAO, characterized by varying content of humus and total major elements (KOINOV *et al.*, 1998). The total area of the habitat is 1000 m² and the net area of distribution is 1.5%. The average number of plants of the studied species is 5.3 per square meter. The possible resource potential per sq. m. is insignificant – 3.60 g (Table 1). The total population resource is scarce (27.3 g) and it has no practical value.

The phytocenological description (Table 2) shows that the dominating species are *Cynodon dactylon*, *Potentilla erecta* and *Dactylis glomerata*, the *Poaceae* family being most represented.

The second studied population occupies Kresna gorge in the region of "Kresna Hanche" in the Floristic Region of the Struma valley. The gorge connects Simitly valley to the north and Petrich-Sandanski valley to the south (KOPRALEV *et al.*, 2002) and the climate is characterized by mild winters and dry summers. The soil types, on which the species grow in that region, are shallow leached Cromic Cambisols/FAO (Soil Map of Bulgaria 1968; KOINOV *et al.*, 1998). The total area occupied by the species is 20000 m². The mean number of plants per sq. m. is 7 with a yield of 4.76 g/m². The net area occupied by the species is 2%. The total population resource is 952 g. The other species found are mainly *Juniperus oxycedrus*, *Paliurus spina-christi* and *Jasminum fruticans* (Table 2). Those plants are diagnostic of habitat 5210 "Scrubs with *Juniperus sp.*" (KAVRAKOVA *et al.*, 2009).

The third population was established on the territory of the village of Marino Pole (Petrich municipality). It is located in Sandanski-Petrich valley along the left bank of the Struma river, also in the Floristic Region of the Struma valley. The climate is continental Mediterranean, with hot and dry summers. The soil types, on which the *C. autumnale* grows, are eroded Cambisols/FAO and Fluvisols/FAO (KOINOV *et al.*, 1998). The population consists of single plants, 3.6 per m². in average. The species is scattered among light-permitting shrubs, mainly *Paliurus spina-christi*, *Carpinus orientalis*, *Crataegus monogina* (Table 2). The habitat could be defined as 5210 "scrubs with *Juniperus sp.*" (KAVRAKOVA *et al.*, 2009), which is the final stage of degradation of xerothermic oak forests in South Bulgaria. Total area of the habitat is 50000 m². The net area occupied by the species is 1.5%. The estimated yield is 1.94 g/m² and the resource potential is 729 g.

Four of the studied populations were found in the Floristic Region of the Rhodope Mountains. It includes the central mountainous and the high mountainous parts of the Mountain Climate Region of the European Continental Climatic Zone. The resources of the species were assessed at four different altitudes: 799 m a.s.l. (Smilyan), 1090 ma.s.l. (Chokmanovo), 1423 m a.s.l. (the low part of Rozhen) and 1744 m a.s.l. (Rozhen Observatory).

The population growing at the highest altitude (1744 m a.s.l) occupies an area of 1000 m² around the Rozhen Observatory. The soil types are mountain meadow, secondary grassed brown forest (Cambisols/FAO) (KOINOV *et al.*, 1998). The mean number of plants is 2.6 pcs./m² with a yield of 1.43 g/m². The established total resource is 3.57 g, the net area being 0.5 %. Observations on the development of the species in that population showed that a large number of the fruit capsules were underdeveloped and they had 3-4 seeds. Probably the reason is the higher altitude, cooler climate and smaller number of insects for pollination. The phytocenological description (Table 2) shows that the vegetation is typical of habitat 6520 "Mountain hay meadows" (KAVRAKOVA *et al.*, 2009), with a large number of representatives from *Fabaceae* family.

The next habitat is located at 1423 m a.s.l. in the locality Rozhen Meadows, situated in Rozhen saddle. It is the climatic border of the Mediterranean influence. The area is characterized by the rapid drop of temperatures and a precipitation increase with height. The soil types are mountain meadow, secondary grassed brown forest (Cambisols/FAO). They are characterized by low natural fertility due to the processes of acidification and erosion (KOINOV *et al.*, 1998). Comparatively large number of plants per m² (10.4) was registered in that population,

with a yield of 5.51 g. Our observations revealed that the fruit capsules per plant were 3-4 in average. Weight and number of seeds in the capsules varied significantly.

The area occupied by the species is 100000 m² and the net area is 4%. The total resource is 11024 g. The species is concentrated at the edges of the meadows along the border with the forest. The population of the species (Table 2) is found in a typical habitat 6520 "Mountain hay meadows" (KAVRAKOVA *et al.*, 2009). Grasslands are dominated by *Agrostis capillaris*, *Calamagrostis arundinaceae* and *Dactylis glomerata*.

The next habitat of the species in the Floristic Region of the Rhodopes is distributed at the outskirts of the village of Chokmanovo at 1090 m a.s.l. Soil types are brown forest (Cambisols/FAO), with a light mechanical composition, acid in nature, with a low content of mineral substances (KOINOV *et al.*, 1998). The established population density is comparatively high – 9.8 pcs./m² and the yield is 5.48 g. The total area of the habitat is 5000 m², the net area being 50%. The possible resource of the habitat is 6850 g. The phytocenological evaluation of the population (Table 2) showed that the vegetation in the habitat was strongly influenced by the anthropogenic activity and it has lost its natural appearance. A significant number of ruderal species are found, typical of abandoned agricultural ecosystems (*Daucus carota*, *Cynodon dactylon*).

The last population of *C. autumnale* in the Floristic region of the Rhodopes was found on the territory of the village of Smilyan at 799 m a.s.l. The species is spread on mountain meadows used for hay production. The soil types are brown forest (Cambisols/FAO), (KOINOV *et al.*, 1998). The average number of plants per sq. m. is 8.9, the yield is 4.89 g. The total area occupied by the population is 10000 m², the net area being 50%. The possible annual resource of that population is 12225 g. The phyto-cenological evaluation of the population (Table 2) showed that vegetation in the habitat was strongly influenced by the anthropogenic activity.

The next studied population of *C. autumnale* was established at the southernmost point of the Floristic region of Thracian lowland (Mezek). The species was found in a plant community typical of 5210 "scrubs with *Juniperus sp*." (KAVRAKOVA *et al.*, 2009). It is characterized by shrubs and grasses dominated by *Festuca sp.*, *Clinopodium vulgare* and *Dactylis glomerata* (Table 2). The area is influenced by Continental Mediterranean climate and the soil types are leached cinnamon (Chromic Cambisols/FAO). They are characterized by a high content of clay, a low content of mineral substances and comparatively high humus content (KOINOV *et al.*, 1998). The total area occupied by the plant is 100000 m² and the net area of the studied species is 1%. The average number of plants per m² is 2.8, but due to the large area of the habitat the obtained resource is 784 g.

The ninth population was found in the Floristic region of the Black Sea Coast (South) close to the state hunting area 'Ropotamo' – Arkutino. The population is located at the edges of meadows bordered by dense longos forests. The soil types in that region are alluvial-meadow, delluvial (Fluvisols/FAO). They are characterized by shallow ground waters and a low content of nitrogen and phosphorus and well supplied with potassium (KOINOV *et al.*, 1998). The total area of the habitat is 1000 m² and the net area occupied by the species is 1%. The established yield is 2.2 g/m² and the possible resource of the population is 11 g. The vegetation is described in Table 2.

The tenth studied population of *C. autumnale* is located in lowland meadows close to the maintained reserve "Balabana" in the Floristic Region of Tundzha hilly valley. The area of its distribution in that Floristic Region is influenced by transitional continental climate, characterized by hot summers and mild winters, but strong north-east winds (KOPRALEV *et al.*, 2002). The species is distributed in groups or as single plants. The total area occupied by the *C. autumnale* population is 200000 m² and the net area is 2%. The established resource is 10800 g and the yield is 5.4 g/m² (Table 1). Plant vegetation is dominated by wheat grass species and it is characterized by rich diversity of species (Table 2). The habitat is

attributed to 6510 "Lowland hay meadows" (KAVRAKOVA *et al*., 2009).

Balyuvitsa is the eleventh studied population (Table 2), found in the Floristic region of the West Balkan mountain range of the Balkan foothill climate region. The climate is characterized by comparatively cool summers, increased amounts of summer rainfalls and the influence of the Balkan mountain range. The population is spread on an area of 10000 m². The soil types are typical of the region – gray forest to light gray forest soils (Ortic luvisols/FAO). They are characterized by a low content of humus, low contents of total nitrogen and phosphorus (KOINOV *et al*., 1998). The net area occupied by *C. autumnale* is 40%, which is a comparatively high population density. The average number of plants is 10.8 pcs./m² and the yield is 5.61 g. The resource potential is 11232 g.

From the literature review it becomes clear that the species was widely distributed in Bulgaria in the period of 1923-1964 (KOUZMANOV & KOZHUHAROV, 1964). High plant populations were established. As a result of the present research and the conducted field observations, a number of widespread localities of the species were not found in some floristic regions (Northeast Bulgaria, Eastern Balkan Mountain, etc.). Most often single plants were found in herbaceous communities, which do not constitute an industrial interest. The observations showed that the anthropogenic impact, namely the urbanization of some of the habitats, had a negative effect on the population density of the species (construction, drainage, etc.). We assume that the individual plants, found in the habitats, remained from the larger populations occupied by the species in previous years. That suggestion confirmed the results obtained in the study of ADRIAENS *et al.* (2009). Those authors noted that environmental changes decreased the survival rate of the plants. They responded by reduced reproductive capacity (generative and vegetative).

During the study carried out on the distribution of *C. autumnale* in seven Floristic Regions of Bulgaria, it was found out that the species form small (2.6-5.3 plants per m² in average) to large groups (7-10.8 plants per m² in average). It was established that the number of fruit capsules varied from 1-2 to 4-5 in the separate plants in the different habitats. Probably the reason for the variation is complex (the mineral composition of soil, climatic characteristics of the region, etc.). Often some of the fruits were underdeveloped and seedless. Seed amount and weight per plant also showed variable values (0.35-0.56 g). The analysis of the results shows that the population showing the highest resource capacity (12225 g) is the one growing in the territory of the village of Smilyan. That resource is a result of the high percentage of net area occupied by the species and the number of plants per m². Next in the ranking come the populations located in Balyuvitsa (11232 g). The population in Elhovo region, which is close to 'Balabana' maintained reserve, also has a high resource capacity (10800 g). That could be explained by the fact that it is the largest area, on which the species is found. The average number of plants is high (10.4 pcs./m²), and hence, the harvested yield. Similar resource potential was also established in the populations spreading in Rozhen meadows (11024 g) and Chokmanovo (6.850 g).

The lowest resource was established in the habitats of the species in the vicinity of Arkutino (11 g), Rozhen observatory (3.57 g) and Chirpanska Koria (27.3 g) and those habitats have no practical value. From the field study it became clear that the distribution of the species in some populations (Chirpan, M. pole, Arkutino) is on abandoned areas, heavily affected by anthropogenic activities in the past. Usually plant species diversity is confined in those areas and it is rich in ruderal species (*Prunus spinosa*, *Cynodon dactylon*, etc.). Processes of secondary succession are going on there, which have an adverse effect on the development and distribution of the species. Changes in the regime of pasture management are likely to cause degradation of the areas and a reduction of the population density, which determines the limited resource.

In highland populations (Rozhen Meadows), traditionally used as meadows for hay production, *C. autumnale* appears as a species causing poisoning of the animals and the farmers destroy the species in that area. Therefore it is found at the edges of the meadows along the border with the forest. Evaluating the effect of the altitude on the formation of the resource, it was established that out of the four studied populations in the Floristic Region of the Rhodopes, the resource of the population located at the highest altitude (Rozhen Observatory 1744 m a.s.l.) is insignificant, as only single plants were found. Similar resource was established for the other three populations.

Table 2. Cover-abundance in the studied areas with *C. autumnale* L. populations (2014-2015).

Studied area	Species	Assessment Braun-Blanquet	Species	Assessment Braun-Blanquet
Chirpanskata koriya	*Poa bulbosa*	1	*Centaurea sp.*	+
	Cynodon dactylon	2	*Inula conyza*	+
	Potentilla erecta	+	*Plantago lanceolata*	1
	Dactylis glomerata	1	*Polygonum aviculare*	1
	Potentilla argentea	1	*Epilobium parviflorum*	+
	Convolvulus arvensis	+	*Colchicum autumnale*	1
	Achillea millefolium		*Ulmus minor*	r
	Pyrus pyraster	r	*Rosa canina*	r
	Inula conyza	+		
Kresna Gorge	*Jasminum fruticans*	1	*Plumbago europea*	+
	Juniperus oxycedrus	2	*Achilea millefolium*	+
	Fraxinus ornus	2	*Verbascum sp.*	r
	Clematis vitalba	+	*Gypsofila sp.*	r
	Quercus polycarpa	+	*Teucrium polium*	+
	Colchicum autumnale	1	*Asparagus sp.*	r
	Paliurus spina-christi	3	*Chrysopogon gryllus*	+
Marino pole/Kulata	*Carpinus orientalis*	2	*Brachypodium sylvaticum*	1
	Juniperus oxycedrus	+	*Poa bulbosa*	r
	Fraxinus ornus	1	*Quercus pubescens*	r
	Crataegus monogina	2	*Colchicum autumnale*	+
	Cornus mas	r	*Asparagus sp.*	r
Observatory Rozhen	*Festuca rubra*	3	*Trifolium pratense*	1
	Trifilium repens	2	*Nepeta sp.*	r
	Lathyrus sp.	2	*Mentha sp.*	r
	Heracleum sibiricum	1	*Silene bupleroides*	+
	Trifolium latinum	1	*Ranunculus sp.*	r
	Erodium cicutarium	+	*Myosotis sp.*	r
	Vicia sp.	r	*Fragaria vesca*	+
	Alchemilla sp.	r	*Pastinaca sativa*	r
	Stachys sp.	+	*Colchicum autumnale*	+
	Taraxacum sp.	+	*Viola sp.*	r
Rozhen grasslands	*Calamagrostis arundinaceae*	2	*Euphorbia helioscopia*	1
	Agrostis capillaris	2	*Carduus sp.*	r
	Dactylis glomerata	2	*Thlaspi arvense*	+
	Festuca rubra	1	*Sanguisorba officinalis*	+
	Teucrium chamaedrys	1	*Galium sp.*	+
	Potentilla reptans	+	*Fragaria vesca*	+
	Trifolium repens	1	*Jniperus communis*	r
	Verbascum thapsiforme	+	*Picea abies*	r
	Lotus corniculatus	1	*Achillea sp.*	r
	Plantago lanceolata	+	*Thamus sp.*	+
	Potentilla ternata	+	*Salvia pratensis*	+
	Colchicum autumnale	2	*Rumex sp.*	r

	Bromus mollis	1		
	Festuca rubra	1	*Daucus carota*	2
	Prunella vulgaris	1	*Agremonia eupatoria*	+
	Lotus corniculatus	1	*Cynodon dactylon*	2
	Potentilla reptans	+	*Colchicum autumnale*	1
Chokmanovo	*Plantago lanceolata*	1	*Asparagus sp.*	r
	Clinopodium vulgare	+	*Viola odorata*	r
	Teucrium chamaedrys	1	*Trifolium repens*	1
	Centaurium erythraea	r	*Thamus sp.*	+
	Veronica officinalis	r		
	Festuca sp.	3	*Colchicum autumnale*	1
	Poa sp.	2	*Prunus spinosa*	r
	Plantago lanceolata	1	*Potentilla argentea*	1
Smilyan	*Teucrium chamaedris*	1	*Inula sp.*	+
	Achillea millefolium	r	*Cynodon dactylon*	1
	Daucus carota	+	*Sonchus sp.*	r
	Ononis sp.	r	*Setaria pumila*	r
	Juniperus oxycedrus	3	*Plantago lanceolata*	1
	Rosa canina	1	*Cirsium sp.*	+
Mezek	*Paliurus spina-christ*	2	*Centaurea stoebe*	+
	Pyrus amygdaloides	+	*Eringium campestre*	r
	Rubus sp.	1	*Dianthus campestris*	r
	Scabiosa sicula	1	*Sanguisorba minor*	1
	Festuca sp.	3	*Cichorium intybus*	+
	Dactylis glomerata	2	*Echium italicum*	+
	Clinopodium vulgare	2	*Prunus cerasifera*	r
	Potentilla reptans	2	*Prunus spinosa*	r
	Hieracium hoppeanum	3	*Pyrus bulgarica*	r
	Campanula sparsa	r	*Colchicum autumnale*	1
	Linaria sp.	+	*Agrimonia eupatoria*	1
	Galium verum	+	*Hypericum perforatum*	1
	Taraxacum sp.	2	*Cichorium intybus*	+
Arkutino	*Cynodon dactylon*	3	*Trifolium repens*	2
	Alliaria petiolata	+	*Daucus carota*	1
	Inula sp.	+	*Plantago lanceolata*	1
	Rumex sp.	r	*Colchicum autumnale*	+
	Agrostis stolonifera	2	*Ranunculus acris*	2
	Festuca arundinacea	3	*Convolvulus arvensis*	+
	Trifolium ptatense	1	*Sanguisorba minor*	r
	Alopecurus pratensis	2	*Cynodon dactylon*	1
Balabana	*Carex caryophyllea*	+	*Tragopogon pratensis*	r
	Dactylis glomerata	2	*Lysimachia nummularia*	+
	Cirsium conium	1	*Prunella vulgaris*	1
	Cynosurus cristatus	1	*Daucus carota*	r
	Stellaria graminea	+	*Colchicum autumnale*	1
	Dactylis glomerata	2	*Thlaspi arvense*	+
	Festuca rubra	1	*Sanguisorba officinalis*	+
Balyuvitsa	*Teucrium chamaedrys*	1	*Galium sp.*	+
	Potentilla reptans	+	*Fragaria vesca*	+
	Trifolium repens	1	*Jniperus communis*	r

Lotus corniculatus	1	*Achillea sp.*	r
Plantago lanceolata	+	*Thamus sp.*	+
Potentilla ternata	+	*Salvia pratensis*	+
Colchicum autumnale	2	*Rumex sp.*	r
Bromus mollis	1	*Cynodon dactylon*	r

Despite of the relatively high resource obtained in some habitats, it should be emphasized that the changes in land management, drainage of many territories and changes in climatic environmental factors are the most likely reason for the disappearance of the species or for the reduction of the population density.

Conclusions

Depending on the amount of the obtained resource, the populations of *C. autumnale* can be grouped, as follows:

Areas with a large resource potential – from 6850 to 12225 g. That group includes 5 populations (around Smilyan, Balyuvitsa, Rozhen Meadows, Balabana, Chokmanovo), representing 45.45% of all the populations. The total exploitation resource of that group is 50131 g.

Areas with a medium resource potential – from 729 to 952 g. That group includes three populations (Kresna, M. pole, Mezek), representing 27.27%. The total exploitation resource of that group is 2465 g.

Areas with a low resource potential – from 0.01 to 27.3 g. That group includes three populations (Chirpanska Koria, Arkutino, Rozhen Observatory), representing 27.27%. The total exploitation resource of that group is 41.6 g.

We recommend collecting plant resources of the species from populations with a large resource potential – around Balabana, Smilyan, Rozhen Meadows, Balyuvitsa. Collecting the fruit capsules will free the meadows from seeds and prevent poisoning of animals.

Acknowledgements. The present study was supported by the Scientific Research Centre of the Agricultural University Plovdiv: Project №12-15: "Biological and phytochemical study of some species of the genus *Colchicum* L. (Liliaceae Hall.) and genus *Galanthus* L. (Amaryllidaceae Lindl.). The authors are thankful to Prof. PhD Malgorzata Berova Agricultural University-Plovdiv for the support of the activities of this research group.

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Received: 28.11.2016
Accepted: 20.02.2017