

Batumi Shota Rustaveli State University  
Faculty of Natural Sciences and Health Care  
Department of Biology

Elza Makaradze

“Bioecological characteristics of *Cyclamen* L. and *Galanthus* L. populations in  
Ajara“

Presented to receive Academic degree of Doctor in Biology  
Speciality: Biodiversity

Annotation

Batumi

2020





## Introduction

**The actuality of the Research:** There are different reasons of destroying herbal species, for example: cultivation of agriculture, herb collection for decorative and medical purposes, grazing and infrastructure arrangement. *Galanthus L* and *Cyclamen L* represent endangering species of herbaceous plants characterized by medical and decorative qualities owning the ephemeral nature. *Galanthus L* and *Cyclamen L* species with GPS coordinates of distribution, growth-development characteristics related to environmental conditions, modern state of populations and developmental dynamics, the chemical content of the soil is still unknown, as well as the IUCN conservative status. According to the enclosure of *Convention on International Trade in Endangered Species of Wild Fauna and Flora*, there are 5 species in the flora of Ajara endangering other plants restricted only to small, local geographic areas: 1. *Galanthus woronowii* Losink, 2. *G. rizehensis* Stern, 3. *G. krasnovii* Khokh, 4. *G. caucasicus*, 5. *Cyclamen adzharicum* Pobed. *Cyclamen adzharicum* Pobed is the local endemic plant (N. Memiadze, 2010) having various status in various literature. According to A. Dmitrieva (1990), *Cyclamen adzharicum* Pobed is the synonym for *C. coum* Kuhn, *C. Coum* var. *ibericum* Kuhn. Exact status of the genus has to be defined through RAPD-PCR method and studied as the endangered species. It is worth mentioning that A. Dmitrieva represents *G. cilicicus* as the synonym for *G. rizehensis*, but based on the flora of Turkey (Devis, 1982) they represent different species. Taxonomic status of these species has been confirmed in the aftermath of their detection by the molecular method. *Cyclamen adzharicum* Pobed is considered as the endemic species confined to small areas in Ajara, it has no precise taxonomic status, moreover, different authors refer to it in different ways, for instance: According to The plantlist.org *Cyclamen adzharicum* Pobed is synonym for *C. coum* Kuhn, *C. coum* var. *ibericum* Kuhn. According to S. Czerepanov (1995) *Cyclamen adzharicum* Pobed is synonym for *C. coum* Mill susp *C. caucasicum* etc. According <http://www.theplantlist.org>, and <https://www.ipni.org/> *C. adzharicum* Pobed is synonym of *Cyclamen coum* subsp. *Caucasicum* K. Koch (O. Schwarz) In Ajara, Genus *Galanthus L* has distributed four species: *Galanthus alpinus* Sosn, *G. Krasnovii* Khokh, *G. rizehensis* Stern, *Galanthus woronowii* Losink. According to the old list of Ajara (Дмитриева 1990), *Galanthus rizehensis* Stern, *Galanthus cilicicus* and *Galanthus glaucescens* Khokhr, are

synonyms . according to <http://www.theplantlist.org>, and <http://www.ipni.org> *Galanthus glaucescens* Khokh. is Synonymous with *Galanthus rizehensis* Stern, *Galanthus cilicicus* Baker is an independent species according to S. Cherepanov (Czerepanov 1995), *Galanthus cilicicus* Baker is synonymous with *Galanthus glaucescens* Khokh.

So, The determination the status of the species *Cyclamen adzharicum* Pobed and *Galanthus rizehensis* Stern by the method of RAPD-PCR is one of the most actual problems in plant systematics.

**Research objectives:** Our aim is to study following endangered species: *Cyclamen adzharicum* pobed, *Galanthus woronowii* Losinsk., *G.rizehensis* Stern, *G.krasnovii khokh.*, *G. Alpinus* Sosn determine their status, spreading area and mapping (Arc.Gis). Presumed IUCN conservation status, Plant description was carried out by the following field research methods: Braun-Blanquet, Transect and Quadratic net methods. In order to identify species, we have applied morphological and molecular method (RAPD-PCR) and managed to define the dynamic status of the genus.

We were tasked to carry out the following things:

- Determination of the presumed IUCN conservation status of the species under study
- Determine GPS coordinates of target species.
- Study the populaton dynamics of *Galanthus woronowi* Losinsk, *G.rizehensis* stern, *G. krasnovii khokh*, *G. Alpinus* sosn and *Cyclamen adzharicum* pobed.
- Carry out ecomorphological analysis of target genus
- Study the amount of target species resources
- Detect target species based on RAPD-PCR molecular method
- Elaborate the target genus protection measurements
- Study the target genus phytochemical consistence

**Research material and methodology-** Following species represent the object of the study: *Galanthus woronowii* Losinsk, *G.rizehensis* stern, *G. Krasnovii khokh*, *G. alpinus* Sosn, *Cyclamen adzharicum* pobed and also *Cyclamen coum* Mill and *Galanthus rizehensis* Stern, which are spread in Turkey, Artvini. Field research has been conducted in the gorge of Chakvistskali and Ajaristskali, on adjacent hills of Kobuleti, Keda, Erge, Sarfi and Gonio and

in different areas within the height of 10-700 metres above the sea. We created a special field form for fieldwork. The form indicates: Surface and Specific Coverage (%), Estimation by Braun-Blanquetia Scale, Height, Tier.

The basic methodologies of the research were traditional route, expedition-excursions, also the methodology of herbarium collection and its biological treatment. Separate types of herbarium were collected in the different phases of development according to the Skvortsov ( 1977 ) methodology. We used to identify plants by the means of Adjara (Дмитриева, 1990) and Georgia plant identifiers (1964; 1969) and “ Georgian Flora “(1971-2016 volume .I-XVI), We used to grant systematic status to plant by using Systematic nomenclature and comparison of Cherepanov (Черепанов, 1995) and [www.theplant list](http://www.theplantlist.org/). We performed phenological observations on the species studied by Beideman (Бейдеман 1974) method. We observed population dynamics by Broun-Blanque, transect and quadratic methods. (<http://serc.fiu.edu/seagrass/!CDreport/methodsbb.htm>) We used the method proposed by Mace and Land (Mase 1991) to determine the presumed IUCN conservation status of the species under study. We used the method of Borisov and Schreiter to determine the resources of the species. In order to carry out genetic study on target species, we have applied RAPD-PCR method (Göğmen, 2012) using 18 primers with ten stems (Operon Technology). It has been chosen based on the literature source (Göğmen, 2012) . Soil study has been carried out according to: GOST 26483-85, GOST 26213-91, GOST 26107-84, GOST 26206-91. In order to detect Alkaloids belonging to Galantamine group compounds, we have used Waters Acquity UPLC-PDA, MS method.

**Basic results of the research:** We have examined hypsometric dissemination regularities of following endangered target species: *Galanthus woronowii* Losinsk, *G. rizehensis*, *G. Krasnovii* khokh, *G. alpinus* Sosn, *Cyclamen adzharicum* pobed; We have studied population dynamics and singled out target genus DNA by means of RAPD-PCR method and determined the precise status of target genus; elaborated target genus protection measurements and we have determined the raw material of target genus in Ajara floral region; We have studied phytochemical consistence, population dynamics and soil composition of the target species.

**Material technical base:** Dissertation thesis is performed in the Department of Biology, Faculty of Natural Sciences and Health Batumi Shota Rustaveli State University, Department of Plant Disease Monitoring, Diagnostics and Molecular Biology Batumi Shota Rustaveli State University, Regional Chromatographic Center of Western Georgia, Ministry of Agriculture of Autonomous Republic of Adjara Laboratory Research Center.

**Thesis approbation** - was carried out at the meeting of the faculty of Natural Sciences and Healthcare / Department of Biology, Batumi Shota Rustaveli State University. (**Report N16, July 15, 2019**).

Research results represent the basis of the work and were successfully introduced to the audience on different scientific conferences and symposiums:

- Students international scientific conference Papers honoring to the 80<sup>th</sup> anniversary of Batumi Shota Rustaveli State University. Batumi 2015.
- Instruments for Modelling Black Sea River Basins: Research Proceedings for Guria Region of Georgia. ILMM-BSE Project ENPI Partner Georgia. International Association CIVITAS GEORGICA. Batumi 2015
- The 2<sup>nd</sup> International Symposium on Eurasian Biodiversity, Antalya 2016
- The 3<sup>rd</sup> International Symposium on Eurasian Biodiversity. Belarus, Minsk 2017
- International Scientific Conference Future Technologies and Quality of Life. Batumi 2017
- International Conference Innovation in Science and Education, Prague, Czech, 2019

**Publication:** The results of the research are presented in 6 scientific articles and 6 international scientific conferences. 3 of them are found by google scholar, one of them by Web of Science

**Scientific Novelty of the Research :** Population dynamics, mapping of spreading areas of endangered species such as: *Cyclamen adzharicum* Pobed, *Galanthus woronowii* Losinsk, *G. rizehensis* Stern, *G. krasnovii* Khokh, *G. alpinus* Sosn has been done for the first time. Also identify approximate IUCN conservation status, By means of RAPD-PCR method we managed to differentiate between common and distinguished characteristics of target species on molecular level in line with their population area and population dynamics. What is more, application of the molecular biology newest methods has greatly contributed to the development of country's biodiversity.

**Structure and content of the thesis:** Thesis consists of 135 printed pages, deals with introduction, 9 chapters, 7 sub-chapters, conclusions, 115 references. Also, 11 tables, 30 photos, 3 maps and 2 diagrams are attached to the thesis.

**Review of the literature-** Thesis deals with the brief physical-geographical characterization of Ajara floral region, Ajara flora review, botanical description of target species, economic significance of spreading areas, challenges related to target species.

## **Experimental part**

### **Chapter I. Bioecology, population development dynamics and spreading of target species**

#### **1.1 Bioecology and population development dynamics of *Cyclamen adzharicum* pobedin Ajara.**

*Cyclamen adzharicum* pobedis local endemic, perennial, herbaceous genus in Ajara, used for decorative and medical purposes.

Its study is of extreme importance in order to define fluorogenesis ways in Ajara. Due to its excessive picking, spreading area of the genus significantly decreases. Spreading ecotypes are woods covered with beech, chestnut, oak and pine trees. It grows at the borders of the wood, in shrubs, on humid slopes, rocks, gritstones. As for location, they are found on 40-728m above the sea: Chaisubani, Bobokvati, Dagva, Erge, Gonio, Sarfi, Makho, Khelvachauri, Kapandibi, Acharistskali, Kibe Makhuntseti, Mtsvane Kontskhi, Tsikhisdziri, Chakvi, Keda, Shuakhevi, Khulo village Almemde. GPS coordinates: Chaisubani 44m above the sea N 41°42'26.38E 41°46'53.6, Bobokvati 46 m above the sea N 41°45'894' E 041°48'125', Erge 61m above the sea, N 41°34'9.49E 41°40'39.88, Dagva 64m above the sea. N 41°45'691' E 041°48'457, Tsikhisdziri 65m above the sea, N 41°46'2.98 E 41°45'13.22, Khala 100m above the sea N 41°42'24.13E 41°47'44.69., Makhuntseti 174m above the sea N 41°34'20.88E 41°52'2.58., Khokhna 174m above the sea N 41°35'11.18E 41°53'25.18, Daba Shuakhevi 228m above the sea N 41°37'2 E 41°58'19.79., Chakvistavi 308m above the sea N 41°40'40.75 E 41°52'8.57., Kuchula 361m above the sea N 41°35'18.64 E 41°57'12.29., Merisi 489m above the sea N 41°34'45.75 E 41°59'31.43., Alme 728 m above the sea N 41°37.695'E 042°17.838.

In order to study environmental influences on seasonal development rhythm of *Cyclamen adzharicum* pobed in 2016-2018 for phenological observation, we opted



populations in village Khala on the north exposition slope 100m above the sea N 41°42'24.13 E 41°47'44.69 and in village Erge on the south-west exposition slope 61m above the sea N 41°34'9.49 E 41°40'39.88. According to Table#1, *Cyclamen* L starts its vegetation 5-10 days earlier in village Erge on 61m above the sea than in village Khala.

Winter and Spring of 2016 was relatively cold and humid and winter and spring of 2018 was relatively warm without any snow. Absolute minimum temperature in the first decade of January was -4-7° C, snow cover 50-6 °C, average temperature in January-February 2017-2018 + 6 + 8 °C, March-April + 12 + 15 °C, May-June +16+ 20 °C, precipitation 60-80mm, Exactly in this period *Cyclamen* alters its phenological phases, starts vegetation in the last decade of November and finishes in the third decade of January. As for flowering, it takes place from the second decade to the end of March, at the beginning of April genus yields its fruits and is in the phase of fertility during the whole month. In the first decade of May fruits start seed dissemination, in the third decade till June plant dries out and goes into relaxation period.

Table 1 . Phenological observation results of *Cyclamen adzharicum* Pobed in 2016-2018

Genus	The year of observation	Hight from sea level	Observation place	Vegetation		Flowering		Fertility		Rapidity		The end of vegetation	
				Beginning	Ending	Beginning	Beginning	Ending	Beginning	Beginning	Ending	Beginning	Beginning
<i>Cyclamen adzharicum</i> Pobed	2016	100	Khala	25.11	5.01	10.01	20.03	5.04	31.04	1.05	15.05	25.05	15.06
		61	Erge	20.11	25.12	5..01	25.03	1.04	25.04	30.04	15.05	30.05	10.06
	2017	100	Khala	25.11	2.01	17.01	20.03	10.04	25.04	5.05	20.05	25.05.04	10.06
		61	Erge	20.11	25.12	17.01	25.03	15.04	15.04	25.04	20.05	30.05	15.06
	2018	10	Khala	25.11	27.0	5.01	29.03	1.04	5.05	5.05	20.05	30.05	10.06

		0			1								
		61	Erge	10.11 5.11	17.0 1	23.12	28.03	30.03	1.05	10.05	15.05	20.05	5.06

## I.II Characteristics of *Cyclamen adzharicum* pobed population dynamics

We have conducted research on quantity, density and dynamics of different species in *Cyclamen adzharicum* pobed population in 2016-2018 by means of transect, quadratic net and Braun-Blanquet methods. Target objects were located in village Khala 100m above the sea N 41°42'24.13 E 41°47'44.69, in village Erge 61m above the sea N 41°34'9.49 E 41°40'39.88, in village Sarfi 61m above the sea N 41°31'18.03 E 41°32'59.14, all in all, 10m<sup>2</sup> (1mX 1m) were designated to each object. *Cyclamen* genus quantity in each square meter on both objects was 43-47. There was negligible change of *Cyclamen* genus quantity in 2016-2018.

Also, it is worth mentioning that one and the same species in *Cyclamen* population on both objects increase in different quantities, this peculiarity can be taken into consideration in case of their implementation in the culture. Results are given in Table #2.

Table #2. Coexisting species of *Cyclamen adzharicum* pobed population

Genus	Khala	Erge	Sarpi
<i>Cyclamen adzharicum</i> pobed	+	+	+
<i>Primula sibtorfii</i>	+	+	-
<i>Duchesnea indica</i> (Andr.) Focke,	+	-	-
<i>Vinca minor</i>	+	+	+
<i>Poa bulbosa</i> L	+	-	-
<i>Artemisia vulgaris</i>	+	-	-
<i>Hedera helix</i> L	-	-	+
<i>Hedera colchica</i> L	-	+	-
<i>Symphatum ibericum</i> Stev	+	+	+
<i>Microstegium vimineum</i>	+	-	+
<i>Urtica dioica</i>	+	-	+
<i>Ornitogalum woronowii</i> Krasch	+	+	+
<i>Marshantia polymorpha</i> L	+	-	+
<i>Convolvulus arvensis</i> L	+	+	-
<i>Senecio loterii</i>	+	+	+
<i>Phyllitisscolopendrium</i>	+	+	+

<i>Ficcaria popovii</i> A. Khokhr	+	+	+
<i>Cicerbitapontica</i>	+	-	-
<i>Helleborus caucasicus</i>			+
<i>Microstegium imberbe</i> (Nees.ex Steud) Tzvel	+	+	+
<i>Comellinacomunis</i> L	+	+	+
<i>Stelaria media</i> (L) Vill	+	+	+

**Determination of approximate IUCN conservation status of *Cyclamen adzharicum* poded in Adjara:** *Cyclamen adzharicum* poded individuals in the study populations in 2016-2018 were 45-50 in each quadrat net. At the end of each year of observation, the number of individuals in each quadrant increased by  $5 \pm 0.1$ , that makes IUCN recommendations in line with Lr's low risk-dependent conservation-CD.

**I.III Reproduction of *Cyclamen adzharicum* poded :** Reproduction of *Cyclamen adzharicum* poded takes place through seeds in Ajara. Seeds taken from fruits after dissemination in June, were seeded in Petri Dish, soil and containers. Seed cropping up amounted 50%. Seeds were dried and kept for 7 months.. In the aftermath of 3-4 years, germinated seed morph into generative phase.

**I.IV Soil Study:** We have studied soil consistence of *Cyclamen adzharicum* poded spreading areas, particularly: organic peculiarities of PH, consistence of organic compounds, nitrogen, phosphorus and potassium. In all target habitats of species PH amounted organic substance general consistence 4,07- 3,02, nitrogen 0,22-0,18, existence of  $P_2O_5$  - 119-321,  $K_2O$  was not detected. 5,94-5. (GOST 26483-1985, GOST 26213-1991, GOST 26107-1984, GOST 26206-1991)

**I.V. *ex situ* conservation of *Cyclamen adzharicum* poded :** *Cyclamen adzharicum* poded is spread on every protected area of Ajara, in order to carry out extra protection of the genus it should be implemented in the culture. We sowed its seeds in open soil in partially shadowed, dry slope (in tangerine plantation), soil was cultivated, loosened, cleaned out of weeds and we put 2cm depth seed bed and tubers there. Experiment was carried out in 2 ways: First, we planted tubers taken from natural habitats distanced from each other by 5cm, as for the rows,

they were distanced by 10 cm. In spring of the first year we got 90% of plants, in the second year we got 60% and in the third year we got 50% of plant. Part of tubers became wrinkled and rotten. As for the second way, tubers were processed by “B 58” solution. In the second way of the experiment, result was improved by 10%. Pests such as Aphids, Thrips and Acari were detected on tubers.

#### I.IV. Genetic study results of *Cyclamen adzharicum* pobed and *Cyclamen coum* Mill

Due to the purpose of our dissertation, one of the main objectives of our study was to compare the *Cyclamen adzharicum* pobed and the *Cyclamen coum* Mill genome ,spread across the Turkish territory by RAPD-PCR method. As a result of our observation, *Cyclamen adzharicum* pobed and *C. coum* Mill differ morphologically. By means of RAPD-PCR decanucleotide primers we have tried to find this difference on the genetic level (Williams, 1990). (Tab. 3)

**Table 3.** : Information about test samples

#	Test species	Samples collected area	Samples data
1	<i>Cyclamen adjaricum</i>	v. Tsikhisdziri, Adjara	March 2018
2	<i>Cyclamen adjaricum</i>	v. Khala, Adjara	March 2018
3	<i>Cyclamen adjaricum</i>	v. Chaisubani, Adjara	March 2018
4	<i>C. coum</i>	Artvin, Turkey	April 2018

The RAPD-PCR method was performed with 18 ten-base primers (Operon Technology) selected on the basis of literature data (Göğmen, 2012). The PCR reaction used was already mixed (Pure Taq Ready – To – Go PCR Beads) in a Thermo 412 Thermo cycler. Each reaction was performed in a 25 µl volume containing: 20 - 40 ng/µl DNA, 2.5 mM MgCl<sub>2</sub>, and 0.2 mM each dNTP, 1 µM primer, 0.2 U Taq DNA polymerase, PCR buffer and sterile distilled water.

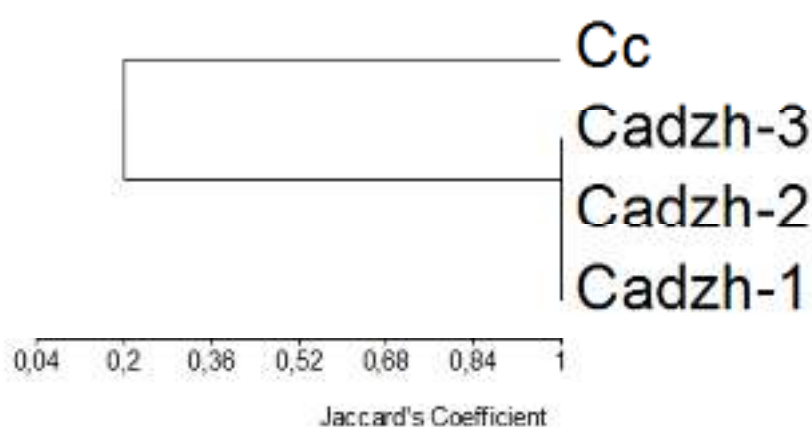
**Table 4.** The names of Primers used in this study

#	primers	sequence 5'...3'	#	Primers	sequence 5'... 3'
---	---------	---------------------	---	---------	----------------------

Data	1	OPA-2	TGCCGAGCTG	10	OPI-2	GGAGGAGAGG
	2	OPB-4	GGA CTGGAGT	11	OPI-7	CAGCGACAAG
	3	OPC-9	CTCACCGTCC	12	OPJ-2	CCCGTTGGGA
	4	OPE-2	GGTGCGGGAA	13	OPK-6	CACCTTTCCC
	5	OPF-1	ACGGATCCTG	14	OPK-7	AGCGAGCAAG
	6	OPF-10	GGAAGCTTGG	15	OPL-6	GAGGGAAGAG
	7	OPG-3	GAGCCCTCCA	16	OPM-7	CCGTGACTCA
	8	OPG-10	AGGGCCGTCT	17	OPP-8	ACATCGCCCA
	9	OPH-3	AGACGTCCAC	18	OPQ-1	GGGACGATGG

**analysis:** Cluster analysis was based on similarity matrices using the unweighted pair group method analysis (UPGMA) program in the software package MVSP (Version 3.1). The Jaccard coefficient was used for dendrogram construction.

As a result of the conducted researches it was revealed that 65 RAPD-markers were selected from 150 to 1500 BP in length. The number of the amplified fragments DNA varied depending on the primer from 6 (OPA-2) to 11 (OPB-4). The obtained data were presented in the form of a binary matrix to study the quantification of the polymorphism degree of the species, which the presence of the component was designated as 1, the absence - as 0. Based on this matrix, a similarity matrix was calculated of the species using the Jaccard coefficient. We carried out a hierarchical cluster analysis (UPGMA) and have constructed a dendrogram (Pic.3)



Pic. 3 . UPGMA diagram based on Jaccard's Coefficient of

An analysis of the dendrogram showed that the analyzed genotypes were divided into two classes. In the first case were the three were of *C. adzharicum*, whereas *C. coum* - belonged to the second cluster. From the dendrogram, it is evident that there is the law of the variability between the types of *C. adzharicum* Pobed which means that there are no genetic polymorphism between these three populations. However, there is the law similarity between *C. adzharicum* Pobed and *C. Coum* Mill . However, the variability between *C. adzharicum* Pobed and *C. coum* Mill species is 33.3% .

#### I.V. Phytochemical study results of *Cyclamen adzharicum* and *Cyclamen coum*

Phytochemical research on leaves, flowers and tubers of *Cyclamen adzharicum* pobed and *Cyclamen coum* Mill has been conducted in Western Georgian Regional Center of Chromatography close to Batumi Shota Rustaveli State University, which was led by professor A. Kalandia. In order to study saponin consistence dynamics in leaves, flowers and tubers, we have applied the weight method in the following way: 20-20 separated raw materials are put in flask, 40ml methanol is added to it and is kept in this condition during one day. Afterwards, it is pressed in backflow condenser in hot water bath by threefold constant stirring. Pressed mass is dried on porcelain cup (weighed beforehand) till constant weight. By means of gained results we can calculate extract productivity from raw tubers as well as by dry material calculation.

**Substance 1**  $m/z = 1243.22$   $[M + Na + H]^+$  retention time on chromatogram is 6.821-6.835 minute, absorbance maximum on ultraviolet ray 221 nm is found in every species. According to chromatographic characteristics, literature and mass basis showings, we have identified it as Mirabilinlactone (Positive FABMS:  $m/z = 1243$   $[M + Na]$ ).

**Substance 2**  $m/z = 1099.20$   $[M + Na + H]^+$  retention time on chromatogram is 7.596-7.607 minute, absorbance maximum on ultraviolet ray 214.9 nm is found in every species. According to chromatographic characteristics, literature and mass basis showings, we have identified it as Cyclacoumin, FABMS:  $m/z = 1099$   $[M + Na]$ .

As a result of biochemical study conducted on 2 *Cyclamen* species, we can conclude that Miribilin lactone is largest in amount in *C. coum* Mill 24.51 mg/kg than in *C. adzharicum* poded, however cyclacoumin *C. coum* Mill -40.04 mg/kg.

## Chapter II. Spread of *Galanthus* L. genus species, Bioecology, Population development dynamics, Reproduction

II.I. *Galanthus* L. genus in Ajara floral region is represented in 4 species: *Galanthus alpinus* Sosn, *Galanthus woronovii* Losinsk, *Galanthus krasnovii* Khokh, *Galanthus rizehensis* Stern. *Galanthus alpinus* Sosn is synonym for *Galanthus caucasicus* (Baker) Grossh. (the plant list.), target population was located in Keda municipality village-Pirveli Maisi on 187m above the sea GPS N 41°35'12.33. declination 132°, roadside south exposition slope, gritstone ecotype. (Pic.6).



Pic. 2 . *Galanthus alpinus* Sosn's preading area

Observation results are given in Table#4

Table 5. *Galanthus alpinus* Sosn phenological observation results in 2016-2018

Genus	The year of observation	Vegetation		Flowering		Fertility		Scattering of seeds		The end of vegetation	
		beginning	ending	beginning	ending	beginning	ending	beginning	ending	beginning	ending
	2016	25.01	20.02	30.02	20.03	5.04	30.05	7.06	10.06	25.06	5.07

<i>Galanthus alpinus</i> Sosn	2017	25.12	20.01	25.01	20.03	10.04	25.05	1.06	15.06	20.06	30.06
	2018	25.12	27.01	5.01	29.03	1.04	5.05	5.06	10.06	12.06	30.06

Researches took place from 2016 to 2018, winter and spring of 2016 was characterized by cold snowy weather, as for 2017 and 2018 winter and spring, it was characterized by warm and snowless weather, The average temperature in November-January varied between  $+7$  to  $+10^{\circ}\text{C}$ , precipitation 30-50 mm, without snow cover change of *Galanthus alpinus* Sosn phenological phases in these conditions takes place in the following way: In 2018, it starts vegetation in the last decade of winter and finishes in the third decade of January. Flowering takes place in the end of January and lasts till the end of March. At the beginning of April, genus starts fruit yielding, during the whole April and May plant remains in fruitfulness phase, seed dissemination of fruits start from the first decade of January and take place till the end of June. From the end of June plant dries up and morphs into relaxation condition. Second half of June is optimal time for *Galanthus alpinus* Sosn seed collection from natural habitat.

**II.II *Galanthus alpinus* Sosn features of Population dynamics** :Also, by means of Transect, Quadratic net and Braun-Blanquet methods we conducted percentage research on number and coverage of different species in *Galanthus alpinus* Sosn population in 2016-2018. In order to conduct the experiment we took 10m<sup>2</sup> (1mx1m). In the period of observation there were 30-35 individuals of species in each square meter. In 2016-2018 number of individuals increased for 5-8 times. Coexisting species and interaction frequency coefficient in population is indicated based on Braun-Blanquet abundance-coverage scale (Table.6).

Table 6. Coexisting species of *Galanthus alpinus* Sosn population

Genus	Types of popularity are covered by the Brown-Blanket coefficient.
<i>Galanthus alpinus</i> Sosn	3



<i>Cyclamen adzharicum</i> pobed	1
<i>Poa bulbosa</i>	1
<i>Sympatum ibericum</i>	1
<i>Helleborus caucasicus</i>	+
<i>Doronicum orientalis</i>	+
<i>Dentaria quinquefolia</i> Bieb	1
<i>Dushesnea indica</i>	1
<i>Vinca minor</i>	+
<i>Stellaria media</i>	+
<i>Primula sibthorpii</i> Hoffm.	+
<i>Aristrochia pontica</i>	1
<i>Viola arvensis</i>	+
<i>Calistegia sepium</i>	+
<i>Ranunculus bulbosus</i>	+
<i>Smilax excellsa</i>	+
<i>Hedera helix</i>	+
<i>Melandrium balance</i>	+
<i>Rubus fruticosus</i>	Γ
<i>Hedera colchica</i>	Γ
<i>Sambucus ebulus</i> L	Γ
<i>Sedum caucasicum</i>	Γ
<i>Pteris cretica</i>	Γ
<i>Carpinus caucasicus</i>	3

As Table #5 demonsrates, species of *Galanthus alpinus* Sosmpopulation are characterized by shallow spreading type. Four species are characterized by 25-50% coverage: *Galanthus alpines* Sosn , *Cyclamen adzharicum* Pobed , *Poabulbosa*, *Symphitum ibericum*. Seven

species are characterized by 5-25% coverage: *Helleborus caucasicus*, *Doronicum orientalis*, *Dentaria quinquefolia* Bieb., *Dushesnea indica*, *Vinca minor*, *Stellaria media*, *Primula sibthorpii* Hoffm., as for the rest species, their coverage is only 1-01%.

**II.III *Galanthus alpinus* Sosn reproduction peculiarities:** Vegetative reproduction of *Galanthus alpinus* Sosn in nature takes place by means of bulbs and seeds. Seeds were taken from fruits in the aftermath of dissemination at the beginning of June and were seeded in Petri Dish, soil and containers. Germinating process was begun but terminated later on. Afterwards, their stratification was carried out in the condenser and germination number reached 30-40%. Seeds were dried and kept during 7 months. 40% of the sown seeds sprouted. As a result of storage, seed germination decreases by 20%. 2 years later it will enter the generative phase.

**II.IV Determination of approximate IUCN conservation status of *Galanthus alpinus* Sosn :** In the 2016-2018 study population, the number of *Galanthus alpinus* Sosn individuals in each quadrant was  $30 \pm 5$  units. At the end of each year of observation, the number of individuals in each quadrant was increased by  $5 \pm 1$  individuals, consistent with IUCN recommendations for Lr low risk, depend on conservation-CD status.

**II.V *Galanthus alpinus* sosn Soil study:** We have studied soil consistence of *Galanthus alpinus* Sosn spreading areas, particularly: organic peculiarities of PH, consistence of organic compounds, nitrogen, phosphorus and potassium. In all target habitats of species PH amounted organic substance general consistence 3.26%, PH - 4,72- 3,02, nitrogen 0.16

**of *G. alpinus* Sosn:** We found only one habitat of *G. alpinus* Sosn in the village of 1<sup>st</sup> May, at the bottom of the Khokhna ridge on the left slope of the motorway. The species does not grow in any of the Adjara Protected Areas, based on our research, we recommend to carry out the conservation of those natural habitats to prevent the reduction of their spreading areas.

## **Chapter II.2 Spreading of *Galanthus woronowii* Losinsk species, Bioecology, Population development dynamics, Reproduction.**

II.2. *Galanthus woronowii* Losinsk target population grows in lower and middle zone of mountain, broadleaf forests, humid, shadowed places, river gorges. We have studied populations in village Chaisubani N 41°41'20.61, E 41°46'33.67; Erge N 41°34'9.49, E 41°40'39.88; Tsikhisdziri N 41°45'52.79, E 41°45'16.68; Makhinjauri N 41°41'21.56, E 41°42'54.93; Kvartiati N 41°33'8.16, E 41°33'52.49; Sarfi N 41°31'18.03, E 41°32'59.14; Bəjdgo N 41°42'35.86, E 41°43'40.54; Bobokvati N 41°45.894, E 41°48'125 Chakhati N 41°48'8.65, E 41°56'26.13 Sakhalvasho N 41°41'21.41, E 41°43'33.17; Chakvistavi N 41°40'29.99, E 41°52'32.13. Results are given in the table -7

Table- 7 *Galanthus woronowii* Losinsk phenological observation results in 2016-2018

Genus	The year of observation	Vegetation beginning	flowering ending	Fertility		Scattering of seeds		The end of vegetation		Genus		The year of observation	
				beginning	ending	beginning			beginning	ending	beginning	ending	beginning
<i>Galanthus woronowii</i> Losinsk	2016	305	Chaisubani	30.12.01-15.01	10.05-15.05	5.02-15.02	20.02-27.02	9.03-14.03	16.03-25.03	15.05-23.05	30.12.01-15.01	10.05-15.05	15.05-23.05
		61	Sarpi	27.12-5.01	10.05-15.05	25.01-31.01	29.01-5.02	22.04-29.04	5.03-12.03	12.05-20.05	27.12-5.01	10.05-15.05	10.05-21.05
	2017	305	Chaisubani	10-0117-01	20-0415_05	25-0131_01	5.02-28.02	7.03-14.03	14.03-30.03	1.04-20.04	10-0117-01	20-0415_05	3.0515.05
		61	Sarpi	27.12-5.01	1.05-15.05	12.01-20.01	20.0110.02	1.03-7.03	9.03-25.03	28.03-25.04	27.12-5.01	1.05-15.05	2.0520.05
	2018	305	Chaisubani	5-10.01	15.04-10.0	12-18.01	20-25.01	27.01-15.0	20.02-25.0	1.04-25.04	5-10.01	15.04-10.0	28.04-14.0

					5			02	03			5	5
		61	Sarpi	30.12- 11.01	20- 04- 30.0 4	12.0 1- 10.0 2	15.01- 25.01	10. 02- 15. 02	28. 02- 20. 03	25.03- 5.04	30.1 2- 11.0 1	20- 04- 30.0 4	29.0 4- 15.0 5

Researches were conducted in 2016-2018, winter and spring of 2016 was characterized by cold and snowy weather, as for winter and spring of 2017/2018, it was characterized by warm, snowless weather. In these conditions, phenological phase change of *Galanthus woronowii* Losinsk takes place in the following way: In 2018, genus starts vegetation in the last decade of December and finishes in the third decade of January. Flowering starts at the beginning of January and lasts till the end of March. Fruit yielding takes place at the beginning of April and the plant remains in fruitfulness condition during the whole month, seed dissemination starts in the first decade of May and takes place till the end of month. From the first decade of June plant dries up and morphs into relaxation period. First part of May is the optimal time for *Galanthus woronowii* Losinsk seed collection from natural habitat. Target genus phenophase change in 2016-2017 took place in approximately similar periods.

**III.II Characteristics of population dynamics of *Galanthus woronowii* Losinsk:** Percentage research on number and coverage of different species in *Galanthus woronowii* Losinsk population was conducted in 2016-2018 by means of Transect, Quadratic Net and Braun-Blanquet methods. We took 10m<sup>2</sup> (1mx1m) for experiment on each object. In observation period there were 55-58 genus individuals in each square. Number of genus individuals increased for 5-8 times in 2016-2018. Coexisting species in population and their interaction frequency is indicated based on Braun-blanquet abundance-coverage scale (Table.8).

Table 8. *Galanthus woronowii* Losinsk coexisting species according to Braun-Blanquet method

Genus	Scale			
	vegetation	flowering	Rapidity	The end of

				vegetati on
<i>Galanthus woronowii</i> Losinsk	5	5	-	-
<i>Pteridium tauricum</i> (Presl.) Krecz.	-	-	3	5
<i>Ornitogalum woronowii</i> Krasch.	-	-	3	-
<i>Convolvulus arvensis</i> L	-	-	1	-
<i>Dentaria quinquefolia</i> M.Bieb	-	-	2	-
<i>Ficaria popovii</i> A. khokhr.	-	-	+	-
<i>Poa bulbosa</i> L. ssp.vivipara (Koel.)Arcang.	-	-	1	-
<i>Sambucus ebulus</i>	-	-	2	-
<i>Lysimachia japonica</i>	-	-	Γ	-
<i>Mnium stellar</i>	-	-	+	-
<i>Duchesnea indica</i> (Jacks.) Focke	-	-	1	-
<i>Corydalis caucasica</i> DC.	-	--	1	-
<i>Stellaria media</i> (L.) Vill.		-	1	-

As the table #7 demonstrates, *Galanthus woronowii* Losinsk population is characterized by tight spreading fashion. During the fertility period 30-40% of the study area covers following species: *Ornitogalum woronowii* Krasch, *Dentaria quinquefolia* Bieb, *Pteridium tauricum* . 25-50% coverage is typical for 2 species: *Sambucus ebulus*, *Dentaria quinquefolia* Bieb, 5-25% coverage is typical for 4 species: *Duchesnea ia indica*(Andr.) Focke, *Corydalis solida*, *Stellaria media* (L) Vill. *Poa bulbosa* L. ssp.vivipara (Koel.)Arcang. *Convolvulus arvensis* L and the rest genus coverage amounts only 1-01%.

**III.III *Galanthus woronowii* Losinsk reproduction peculiarities:** Vegetative reproduction of *Galanthus woronowii* Losinsk in nature takes place by bulbs and seeds.

Seeds were taken from fruits in June and were seeded on Petri Dish, soil and containers. Seed germination was started but terminated later on. Afterwards, we carried out stratification in

the condenser, number of cropping up reached 52%. 4 years later, the plant has entered the generation phase. Seeds lose their ability to emerge after 3 months of storage.

### **III.IV Determination of approximate IUCN conservation status of *Galanthus woronowii***

**Losinsk :** The number of individuals in each quadrant in the study population for 2016-2018 was  $90 \pm 5$  in each quadrant. At the end of each year of observation, the number of idioms in each quadrant was increased by  $10 \pm 2$ , which is in accordance with IUCN recommendations Lr Low Risk-Dependent on Conservation-CD

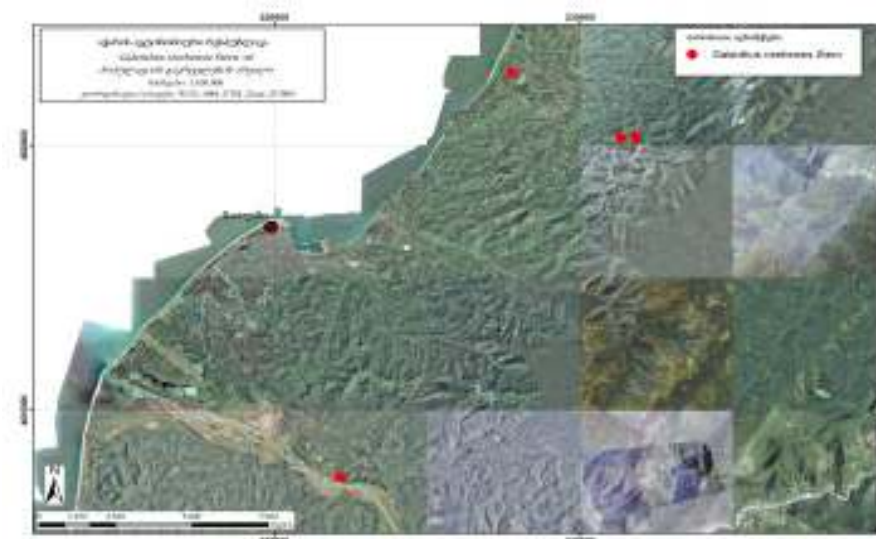
**III.V. We have studied Chemical consistence of *Galanthus woronowii* Losinsk soil in spreading habitats,** percentage consistence of PH, Nitrogen, Phosphorus and Potassium. In target habitat PH amounted 4,06 PH, organic sunstances amounted 2.73 %, Nitrogen 0,15 %,  $P_2O_5 - 100 \text{mln}^{-1}$ ,  $K_2O - 25 \text{ mln.}$  (GOST 26483-1985, GOST 26213-1991, GOST 26107-1984, GOST 26206-1991)

**III.VI *Galanthus woronowii* Losinsk protection measurements:** *Galanthus woronowii* Losinsk populations are preserved in Mtirala National Park, Machakhela National Park and Kintrich State Reserve. Georgia annually purchases *Galanthus woronowii* Losinsk bulbs and exports them through Turkey to Europe by state-licensed organizations. Therefore, new plant protection measures need to be developed. One way to protect the plant is to introduce it into culture and cultivate a plantation. By us in the village Chisuban, we have prepared 20-25 cm depth in mandarin plantations and planted bulbs at 5 cm depth, 20 cm between rows. 90% of the bulbs sprouted in the spring and vegetation began.

## **Chapter IV. Spreading of *Galanthus rizehensis* Stern, bioecology, Population development dynamics, Reproduction.**

**IV.I *Galanthus rizehensis* Stern. Dissemination, bioecology** *Galanthus rizehensis* Sternis spread in damp coast woods with high humidity, bright, woodside grit-stone habitats. *Galanthus rizehensis* Stern population is found with *Galanthus woronowii* Losinsk population and is restricted to small area. We found its populations in village Chaisubani, (GPS N 41°41'20.99 E 41°46'37.94 H 295), Kabandibi GPS N 41°42'35.86 E 41°43'40.54 H 57, Daba Chakvi (GPS N 41°35'12.33 E 41°53'26.33 H 169). Daba Chakvi is already well-known as the habitat

for *Galanthus rizehensis* Sternbut Kabandibi and Chaisubani were identified by us for the first time. ( Map1)



Map 1. *Galanthus rizehensis* Stern spreading in Ajara

Table- 9 *Galanthus rizehensis* Stern phenological observation results in2016-2018

Genus	The year of observation	Veg etati on begi nnin g	flowerin g ending	Fertility		Scattering of seeds		The end of vegetatio n		Genus		The year of observation	
				beginnin g	ending	beginnin g			beginn g	ending	beginn g	ending	beginn g
<i>Galanthus rizehensis</i> Stern	2016	251	Chaisubani	18.01- 27.01	10.0 5- 16.0 5	12.0 2- 19.0 2	9.03- 15.03	15. 03- 25. 03	— — —	— — —	18.0 1- 27.0 1	10.0 5- 16.0 5	12.0 2- 19.0 2
		40	Chakvi	5.01- 11.01	20.0 4- 29.0	5.02 - 10.0	13.02 20.02	12. 03- 20.	— — —	— — —	5.01 - 11.0	20.0 4- 29.0	5.02 - 10.0

					4	2		03			1	4	2
	2017	251	Chaisubani	7.01 13.01	10.0 4- 15.0 4	5.01 - 20.0 1	20.01 5.02	15. 02- 21. 02	— —  	—  	7.01 13.0 1	10.0 4- 15.0 4	5.01 - 20.0 1
		40	Chakvi	2.01- 10.01	15.0 4- 25.0 4	5.01 - 20.0 1	20.01 5.02	15. 02- 21. 02	— — — 	—  	2.01 - 10.0 1	15.0 4- 25.0 4	5.01 - 20.0 1
		251	Chaisu bani	5.01 12.01	25.0 4- 1.05	15.0 1- 31.0 1	20.01 -25.01	10. 02- 25. 02	— —  	—  	5.01 12.0 1	25.0 4- 1.05	15.0 1- 31.0 1
		40	Chakvi	3.01- 12.01	15.0 4- 27.0 4	15.0 1- 31.0 1	18.01- 25.01	6.0 2- 20. 02	— — — 	—  	3.01 - 12.0 1	15.0 4- 27.0 4	15.0 1- 31.0 1

Researches were conducted in 2016-2018, winter and spring of 2016 was characterized by cold and snowy weather, as for winter and spring of 2017-2018, it was characterized by snowless and warm weather, in these conditions, phenological phase change of *Galanthus rizehensis* Sterntakes place in the following way: In 2018, genus starts vegetation in the first decade of January and finishes in April. Flowering takes place in the third decade of January and lasts till the end of February. Fruit yielding starts at the beginning of March and plant remains in fruitfulness condition during the whole month, in the first part of April plant fruits become rotten and do not develop seeds. Plant finishes vegetation in May and bulbs morph into relaxation period. Phenophase change of target genus in 2016-17 took place in approximately one and the same periods.



IV.II Developmental dynamics of *Galanthus rizehensis* Stern populations :We have conducted percentage research on number and coverage of different species of *Galanthus rizehensis* Stern population in 2016-2018 by means of Transect, Quadratic Net and Braun-Blanquet methods. We took 10m<sup>2</sup> (1mx1m) for experiment on each object. In the period of observation there were 45-52 genus individuals in each square. Genus individual number increased for 1-2 times in 2016-2018, so, no population growth took place at this time. Coexisting species in population and interaction frequency coefficient is indicated based on Braun-Blanquet abundance-coverage scale.(Table.-10).

Table 10. Coexisting species in population of *G. rizehensis* Stern 2016-2018

Genus	Scale			
	vegetation	Flowering	fertility	The end of vegetation
<i>Galanthus rizehensis</i> stern.	3	3	3	-
<i>Galanthus woronowii</i> Losinsk	1	1	1	+
<i>Ficaria popovii</i> A. khokhr.	1	1	1	-
<i>Artemisia vulgaris</i> L.	1	1	1	5
<i>Dentaria quinquefolia</i> M. Bieb	-	+	+	-
<i>Sambucus ebulus</i> L.	-	-	+	+
<i>Stellaria media</i> (L.) Vill.	+	+	+	-
<i>Urtica dioica</i> L.	-	+	+	+
<i>Symphytum tuberosum</i> Stev.	-	+	+	+
<i>Duchesnea indica</i> (Jacks.) Focke	-	+	+	+
<i>Corydalis caucasica</i> DC.	-	+	+	-
<i>Arum albispathum</i> Steven ex Ledeb	-	+	+	+
<i>Ornithogalum woronowii</i> Krasch.	+	+	+	—
<i>Convolvulus arvensis</i> L	-	+	+	+
<i>Poa trivialis</i> L.	-	+	+	+

As table #10 demonstrates, *Galanthus rizehensis* Stern is characterized by shallow spreading fashion. It covers 30-40 % of spreading area. Which corresponds to the Brown-Blanket scale coefficient 3, *Galanthus woronowii* Losinsk and *Ficaria popovii* A. Khokhr covers 23%, which corresponds to the Brown-Blanket scale coefficient 2, *Artemisia vulgaris* L. covers 2-4% during vegetation, flowering and fertility, after the vegetation covers 85-90% of the population. The other species : *Dentaria quinquefolia* M.Bieb., *Sambucus ebulus* L., *Ornithogalum woronowii* Krasch., *Dentaria quinquefolia* Bieb., *Duchesnea indica* (Jacks.) Focke., *Stellaria media* (L.) Vill., *Corydalis caucasica* DC., *Arum albispalum* Steven ex Ledeb., *Poa trivialis* L., *Convolvulus arvensis* L cover 0,5-0,7% .

#### IV.III Determination of approximate IUCN conservation status of *Galanthus rizehensis* Stern:

*Galanthus rizehensis* Stern number of individuals in each quadrant for 2016-2018 was  $30 \pm 5$  units. At the end of each year of observation, the number of individuals in each quadrant increased by  $5 \pm 2$  individuals, which is consistent with the IUCN recommendation on the status Lr low risk, depending on conservation-CD.

IV.IV *Galanthus rizehensis* Stern reproduction peculiarities: Vegetative reproduction of *Galanthus rizehensis* Stern in nature takes place by bulbs and seeds. Due to Ajara climate, plant develops seedless fruits.

IV.V .We have studied Soil chemical consistence of *Galanthus rizehensis* Stern in its spreading habitat, in particular, percentage value of PH, organic substances, nitrogen, phosphorus and potassium. In target habitat PH amounted 3.83PH, organic substances amounted 4.45%, nitrogen 0,23 %,  $P_2O_5$  - 490mln<sup>-1</sup>,  $K_2O$  - 12. 25mln.(GOST 26483-1985, GOST 26213-1991, GOST 26107-1984, GOST 26206-1991)

IV. VI *Galanthus rizehensis* Stern protection measurements: We have studied 3 habitatsof *Galanthus rizehensis* Stern 40m above the sea reaching the wood zone. Due to its excessive bulb collection and export, this population is under anthropogenic pressure. As *Galanthus rizehensis* Stern is found with *Galanthus woronowii* Losinskits bulbs are collected in parallel

with *Galanthus woronowii* Losinskbulbs, resulting in extreme reduction of *Galanthus rizehensis* Stern spreading area.

#### Chapter V . 4 Spreading of *Galanthus krasnovii* khokh species, Bioecology and Reproduction.

*Galanthus krasnovii* khokhr population grows in lower and middle zone of mountain, target population grows in lower and middle zone of mountain, grows, humus-rich ,shadowed places, river gorges. We have studied one populations in village Chalati , E 42°0'51.499, N 41°33'38, 740 m from sea level .(map-2)



map.2 distribution map of *G. krasnovii* khokh. In Chalati

The distribution habitat of *Galanthus krasnovii* khokh is the highest among the studied species- 740 m. Absolute minimum temperature at the study site in the first decade of January 2016 was -4 -6, precipitation is 120 mm, The habitat of the study populations was covered with snow in January-February, Accordingly, the vegetation began only in early March and continued until the third decade of March, Flowering began in late March and continued for 1 month until the end of April, By the end of May, the study species have moved to the fertility phase, which continued for 1 month, From early June the fruits of *H. G.Krasnovii* Khokh are opened and the process of seed dispersal begins, which lasts for 2 weeks, From the second half of June the leaves turn yellow and dry, the plant shifts to a state of dryness. The temperature and precipitation patterns of 2017-18 did not differ significantly, Consequently the periods of the phenological phases of the *G. Krasnovii* khokh whale coincided. The species began vegetation in the second decade of February and continued until the third decade of March. From the beginning of April the species begins to fertilize,

throughout April and May the plant is in the fertility phase, From the first decade of June the fruits begin to scatter the seeds, which last until the end of June, From June to the third decade the leaves turn yellow and dry. The plant enters the flowering phase. Optimal time to collect *G.krasnovii* khokh seeds from Natural location is first and second decade of June.

**V.II. *Galanthus krasnovii* Khokhr Population development dynamics** : Percentage research on number and coverage of different species in *Galanthus krasnovii* khokh population was conducted in 2016-2018 by means of Transect, Quadratic Net and Braun-Blanquet methods. We took 10m<sup>2</sup> (1mx1m) for experiment on each object. In observation period there were 30-34 genus individuals in each square. Number of genus individuals increased for 4-6 times in 2016-2018. Coexisting species in population and their interaction frequency is indicated based on Braun-blanket abundance-coverage scale (Table.11).  
Table 11. *Galanthus krasnovii khokhr* coexisting species according to Braun-Blanquet method

Genus	Types of popularity are covered by the Brown-Blanket coefficient.
<i>Galanthus Krasnovii</i> Khokhr	3
<i>Galanthus woronowii</i> Losinsk	3
<i>Pteridium tauricum</i>	3
<i>Coridalis caucasica</i>	+
<i>Poa bulbosa</i> L. ssp.vivipara (Koel.)Arcang.	2
<i>Stelaria media</i>	3
<i>cardamine hirsuta</i>	1
<i>alnus barbata</i>	2
<i>Rubus fruticosus</i>	3
<i>Asplenium adiatum-nigrum</i>	+
<i>melandrium balance</i>	+
<i>Symphatum ibericum</i>	1
<i>hedera chelix</i>	1

As the table # 10 demonstrates, *Galanthus krasnovii khokhr* population is not characterized by tight spreading fashion. 75% coverage is typical for 4 species: *Galanthus woronowii* Losinsk, *Pteridium tauricum*, *Stelaria media*, *Rubus fruticosus*. 25-50% coverage is typical for 2 species: *alnus barbata*, *Poa bulbosa* L. ssp. *vivipara* (Koel.) Arcang. the rest genus coverage amounts only 1-01%.

**V.III *Galanthus krasnovii khokhr* eproduction peculiarities:** *Galanthus krasnovii khokhr* vegetative reproduction of nature takes place by bulbs and seeds. Seed is easily dispersed and allows the plant to colonise new suitable habitat.

**V.IV Chemical consistence of *Galanthus krasnovii khokh* in spreading habitats :** We have studied Chemical consistence of *Galanthus krasnovii khokh* soil in spreading habitats, percentage consistence of PH, Nitrogen, Phosphorus and Potassium. In target habitat PH amounted 4,15 PH, organic sunstances amounted 3.75 %, Nitrogen 0,20 %,  $P_2O_5$  -  $100\text{mln}^{-1}$ ,  $K_2O$  was not detected .(GOST 26483-1985, GOST 26213-1991, GOST 26107-1984, GOST 26206-1991)

**V.V Determination of approximate IUCN conservation status of *Galanthus krasnovii khokh*:** In 2016-2018, the number of *Galanthus krasnovii khokh* individuals in each quadrant was  $34 \pm 5$  units. At the end of each year of observation, the number of individuals in each quadrant was increased by  $7 \pm 2$  individuals, which is in line with IUCN recommendations for low-risk status Lr, depending on conservation-CD

**V.VI *Galanthus krasnovii khokh* protection measurements :** We have studied one habitats of *Galanthus krasnovii khokh* 900 m above the sea reaching upper zone of the wood. Due to excessive production of genus bulbs they are under risk of extinction on natural habitat. We recommend to carry out conservation of natural habitat of above-named species to prevent reduction of their spreading areas.

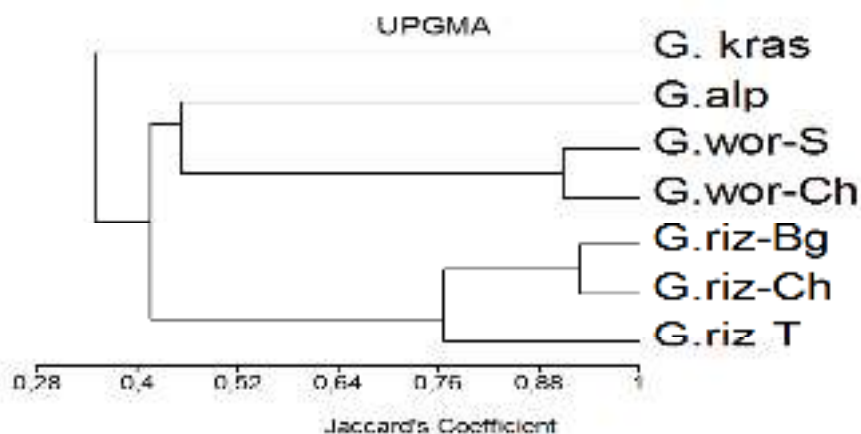
**V. VII . Genetic study results of target *Galanthus* L species :** Due to the purpose of our dissertation, one of the main objectives of our study was to compare the four *Galanthus* species.: *G. rizehensis* Stern (spread across the Turkish territory) *G. krasnovii* Khokh , *G. alpinus* Sosn and *G. woronowii* Losinsk genome , by RAPD-PCR method.

A set of 10 RAPD- primers (Operon Technology) (OPB-1; 2; 4; 11; 12;;15; 16;17;18; 20) was tested on the DNA samples of four *Galanthus* species.: *G. rizehensis* (3 samples), *G.*

*krasnovii* Khokh (1 sample), *G. alpinus* Sosn (1 sample) and *G. woronowii* Losinsk (2 samples). The different primers produce a number of bands ranging from 1 to 12. The size of all the amplified fragments is between 350 and 1500 bp.

### Molecular phylogeny of *Galanthus* spp.

The dendrogram conducted for the 4 *Galanthus* L species which studied via cluster analysis allowed us to visualize the relative position of each species within the species in the ecoregions studied (pic.7) The hierarchical classification of the RAPD molecular markers makes it possible to distinguish two large groups with a similarity index higher than 35 %



**Pic.7** Dendrogram of RAPD markers based on the Jaccard similarity index of *Galanthus* sp.

Each cluster groups are genetically close populations. The first cluster is formed by the population of *Galanthus krasnovii* khokhr, while the second cluster comprises two subgroups; one is constituted by the populations *G. rizehensis* Stern (3 samples) with a similarity index of 73% whereas the other subgroup is represented by the populations *G. woronowii* Losinsk (2 samples) and *G. alpinus* Sosn (1 sample) with a similarity index of 44 %.

RAPD markers also revealed a 78% genetic similarity between the Georgian and Turkish populations of *Galanthus rizehensis* Stern, while *G. krasnovii* khokh had a low genetic similarity (35%) with other *Galanthus* L species.

## Chapter IV . Phytochemical study of target *Galanthus* L species

Phytochemical study of target species led by Professor A. Kalandia has been carried out at Biochemistry laboratory of Batumi Shota Rustaveli State University. Two sub-groups of alkaloids (Graciline and Plicamine) have been found in *Galanthus* genus (Ünver, 2007). Alkaloid existence in *Galanthus* is still being studied by means of classical phytochemical approaches (G.I. Kaya, 2019:107). *Galanthus* genus is famous for alkaloid compounds and is the main examination object for Pharmacological Research. Substance identification was carried out by substance mass free basis <https://metlin.scripps.edu>, in comparison with reviewed literature data. (L. Stanojevic, 2018:6). Chemical study of plant diversity allows us to find relevant compound for species and apply it as one of the objective indicators of species detection.

**Substance 1**  $m/z = 288.03$   $[M + H]^+$  retention time on chromatogram is 2.188 minute (Picture 10), absorbance maximum on ultraviolet ray 214 and 288 nm is found in every species and as the basic component is in GW and GK. According to chromatographic characteristics, literature and mass basis showings, we have identified it as Epigalantamine ( $C_{17}H_{21}NO_3$ , MW = 287.36 g/mol)  $C_{17}H_{21}NO_3$ ).

**Substance 2**  $m/z = 287.97$   $[M + H]^+$  retention time on chromatogram is 3.549 minute, absorbance maximum on ultraviolet ray 214 and 288 nm is found in every species (Pic.11), as the basic component is in GW and GK. According to chromatographic characteristics, literature and mass basis showings, we have identified it as Galantamine ( $C_{17}H_{21}NO_3$ , MW = 287.36 g/mol)  $C_{17}H_{21}NO_3$ ).

**Substance 3**  $m/z = 274.06$   $[M + H]^+$  retention time on chromatogram is 2.681 minute, absorbance maximum on ultraviolet ray 211.3 and 288.4 nm is found as the basic component in every species apart from GR. According to chromatographic characteristics, literature and mass basis showings, we have identified it as O-Desmethyl-galantamine ( $C_{16}H_{19}NO_3$ , MW = 273.33 g/mol).

**Substance 4**  $m/z = 332.03$   $[M + 2Na - H]^+$  retention time on chromatogram is 5.8-6.3 minute, absorbance maximum on ultraviolet ray 211.3 and 289.7 nm is found in every species as the dominant component. As a result of charging, starting mass of Substance 4 attaches 2 cations of sodium and therefore, increases in weight. According to chromatographic characteristics,

literature and mass basis showings, we have identified it as Lycorine (C<sub>16</sub>H<sub>17</sub>NO<sub>4</sub>, MW = 287.36 g/mol)

As a result of conducted biochemical studies, we can conclude that alkaloid galantamine and epigalantamine in 4 species of *Galanthus* have been found in relatively large amount *G. woronowii* Losinsk-19,2/137,60g/kg; O-Desmethyl-galantamine-*G. krasnovii* Khokh-78,12mg/kg, Lycorine-*G. alpinus* Sosn.- 548,05mg/kg .

### Conclusions

1. The target species: *Cyclamen adzharicum* is distributed from the level of the sea of 40-728 m, *Galanthus woronowii* Losinsk 10-1800 m, *G. alpinus* Sosn 187-1470 m, *G. rizhensis* Stern 40-251 m, *G. krasnovii* Khokh 740-1200 m.
2. In January-February 120-180 mm of atmospheric precipitation, with a rise of -4<sup>0</sup>-6<sup>0</sup> C and a height of 40 m above sea level, causes a late start of vegetation and flowering of all species for 5-15 days.
3. The target species: *Cyclamen adzharicum* is distributed from the level of the sea of 40-728 m, *Galanthus woronowii* 10-1800 m, *G. alpinus* 187-1470 m, *G. rizhensis* 40-251 m, *G. krasnovii* 740-1200 m.
4. In January-February 120-180 mm of atmospheric precipitation, with a rise of -4<sup>0</sup>-6<sup>0</sup> C and a height of 40 m above sea level, causes a late start of vegetation and flowering of all species for 5-15 days.
5. The study 2 species: *Cyclamen adzharicum* and *Galanthus woronowii* populations are preserved in Kintrich State Reserve, Mtirala and Machakhela National Parks, Species: *Galanthus alpinus*, *G. krasnovii* and *G. rizhensis* populations are not distributed within any protected area. We recommend to carry out conservation of natural habitat of above-named species to prevent reduction of their spreading areas.
6. The study species: *Cyclamen adzharicum*, *Galanthus woronowii*, *Galanthus alpinus*, *G. krasnovii*, multiplies vegetationally underground and by seeds. The exception is *G. rizhensis*,



which develops rotten fruits and do not develop seeds. In vitro (17-18 °C per petrium) *Cyclamen adzhagicum* seed germination begins 35 days after sowing, 80% + 0.2% open. *Galanthus woronowii* *Galanthus alpinus*, *G.krasnovii* seeds germinate after 2 months, 70% germinate.

7. The highest pH (5.94-5) in the soil samples of the study species habitats was observed in the soil samples of *Cyclamen adzhagicum*, *Galanthus rizehensis* Stern is typical for soils rich in high organic compounds, organic matter - 4.45%. Nitrogen content in soil habitat distribution of all species is almost the same (0.15-0.23%), no K<sub>2</sub>O content was observed in soil samples of *Galanthus alpinus*, *G. Krasnovii*, *G. Woronowii*. The highest content of P<sub>2</sub>O<sub>5</sub> 490 ml<sup>-1</sup> is due to the soil sample of *Galanthus rizehensis* Stern populations.
8. RAPD markers allowed us to identify a low genetic similarity (33%) between *Cyclamen azharicum* Pobed and *C. coum* Mill, while the three *Cyclamen adzhagicum* populations had a 99% similarity.
9. RAPD markers also revealed a 78% genetic similarity between the Georgian and Turkish populations of *Galanthus rizehensis* Stern, while *G.krasnowii* khokh had a low genetic similarity (35%) with other *Galanthus* L species.
10. From the *Galanthus woronowii* resources survey, we found that this species is spread over 75 hectares in the territory of Kobuleti municipality, 195 hectares in Khelvachauri municipality and 197 hectares in Keda municipality. The whole territory of Adjara covers 467 ha, which corresponds to 210150000 copies
11. As a result of *Cyclamen* bulb chemical study, we have identified 3 glycosides: Miribilin lactone-m/z =1243.22 [M +Na+H], Cyclacoumin-m/z =1099.20[M+Na+H], Desglucocyclamin- m/z =1083.28[M + H]
12. We have studied phytochemical consistence of *Galanthus* L species. By means of HPLC-UV, IR and UPLC-PDA, MS methods we have identified 4 alkaloids: Epigalantaminem/z =288.03 [M + H], Galantamine-m/z =287.97 [M + H+], O-Desmethyl Galantamine -m/z =274.06 [M + H]+, Lycorine-m/z =332.03 [M+2Na-H].

#### Works published in relation to the Dissertation Thesis:

- **Makaradze.E**, Varshanidze N. Early spring blooming relict herbaceous plants diversity of hilly places of Adjara. Students international scientific conference

Papers honoring to the 80<sup>th</sup> anniversary of Batumi Shota Rustaveli State University. Batumi 2015.

1. **Makaradze E**, Varshanidze N. Adjara-Turkey transboundary area rare and endangered species. Instruments for Modelling Black Sea River Basins: Research Proceedings for Guria Region of Georgia. ILMM-BSE Project ENPI Partner Georgia. International Association CIVITAS GEORGICA. Georgia 2015. Pp. 131-133.
2. Dolidze K, Diasamidze I, **Makaradze E**, Chitanava J, Varshanidze N, Turmanidze N, Bolkvadze G. A preliminary review of endemic plants *in situ* conservation of Adjara protected areas in Georgia. International Journal of Environmental Sciences. Vol.6 No 1.2017. Pp 13-16
3. **Makaradze E**, Mepharishvili G, Varshanidze N, Dolidze K, Diasamidze I, Jakeli E, Zarnadze N. RAPD-ANALYSIS OF CYCLAMEN SPP. GENOME POLYMORPHISM. International Conference Innovation in Science and Education, Prague, Czech, 2019
4. **Makaradze E**, Varshanidze N, Mepharishvili G, Diasamidze I, Shainidze G. Species bioecology and growth development of genus *Galanthus* in the South Colchis. International Journal of Life Sciences Vol 8 Issue 3. India 2019
5. **Makaradze E**, Varshanidze N, Diasamidze I, Dolidze K, Turmanidze N, Jakeli E. Species bioecology and growth development of *Cyclamen adzharicum* Pobed in the South Colchis. International Journal of Life Sciences. India 2019

#### Participation in International Scientific Conferences:

- **Makaradze E**, Varshanidze N. Early spring blooming relict herbaceous plants diversity of hilly places of Adjara. Students international scientific conference Papers honoring to the 80<sup>th</sup> anniversary of Batumi Shota Rustaveli State University. Batumi 2015.
- **Makaradze E**, Varshanidze N. Adjara-Turkey transboundary area rare and endangered species. Instruments for Modelling Black Sea River Basins: Research Proceedings for Guria Region of Georgia. ILMM-BSE Project ENPI Partner Georgia. International Association CIVITAS GEORGICA. Batumi 2015
- **Makaradze E**. Diversity of genus *Galanthus* in Georgia. The 2<sup>nd</sup> International Symposium on Eurasian Biodiversity, Antalya 2016
- **Makaradze E**. Diversity of genus *Cyclamen* in Georgia. The 3<sup>rd</sup> International Symposium on Eurasian Biodiversity. Belarus, Minsk 2017

- **Makaradze E**, Varshanidze N. The healing properties of genus *Galanthus L.* Interntional Scientific Conference Future Technologies and Quality of Life.Batumi 2017
- **Makaradze E.** ,Mepharishvili G, Varshanidze N, Dolidze K, Diasamidze I, Jakeli E, Zarnadze N. RAPD-ANALYSIS OF CYCLAMEN SPP. GENOME POLYMORPHISM. International Conference Innovation in Science and Education, Prague, Czech,2019

### Bibliography

1. **Бейдеман И. Н.** Методика фенологических наблюдений при геоботанических исследованиях.Изд. АН СССР.Москва-Ленинград. 1954. 157с.
2. **Борисова Н. А.**, Шретер А. И. К методике учета и картирования ресурсов лекарственных растений. Растительные ресурсы. Т. II. вып. 2. 1966. Наука. М.- Л. С. 271-277.
3. **Гроссгейм А.А.** О новом геоботаническом районировании Кавказа. Ботанический журнал, том 33, №6, 1948-а. стр. 619-621.
4. **Дорохов Д.Б., Клоке Э.**, „ Быстрая и экономичная технология RAPD анализа растительных геномов „ Генетика“ Т.3. №4. Издательство „Наука,“ Москва. **1997**
5. **Долуханов... 1942: Долуханов А., Сахокиа М., Харадзе А.,** К вопросу о высоко-горных растительных поясах Кавказа, Тр. Тбил. бот. ин-та., т. VIII. 1942.
6. **Долуханов А.Г** Колхидский подлесок. „Мецниереба“, 1980. 261 стр
7. **Дмитриева А.А 1959:** Дмитриева А., определить растения Аджарии,Тиф., 1959.
8. **Дмитриева А.А.** Определитель растений Аджарии. Тбилиси, „Мецниереба, т.1,II 1990.
9. **Колаковский...1958: Колаковский А.,** „Ботанико-географическое районирование Колхиды“, Тр. Сухумского бот. Сада“, вып. XI., 1958.
10. **Манджavidze Д.,** Реликтовые леса Аджарии и их народно - хозяйствен-ное значение, Тбилиси, „Мецниереба“, 1982. 262 стр.

11. **Сихарулидзе З.В., Цецхладзе Ц.М.,** "Мучнистая роса ячменя в Грузии". Информационно-рекламный научно-практический журнал по сельскому-хозяйству „Агромеридиан“ 4 (10). Издательство „Полиграфсервис“, Алматы .2008
12. **Хохряков 1991: Хохряков А.,** „Эволюция Биоморф Растений“ Академия Наук СССР., Издательство Наука москва., 1991.
13. **Цховребашвили Н.** Геоморфология Аджаро-Триалетского крутогорья. Тбилиси. часть№1. 1978. 294 стр.
14. **Постнова Е. Л.,** „Исследование внутривидового поли-морфизма штаммов *Ganoderma lucidum* „ Автореферат. Москва 2009
15. **Akita Y, Kitamura S, Hase Y, Narumi I, Ishizaka H, Kondo E, et al.** Isolation and characterization of the fragrant cyclamen O-methyltransferase involved in flower coloration. *Planta*. 2011;234(6):1127-36.
16. **Assyov B., Petrova, A. (eds),** Conspectus of the Bulgarian vascular flora. Distribution maps and floristic elements, 4 (In Bulgarian). BBF, Sofia 2012.
17. **Bastida, J.; Lavilla, R.; Viladomat, F..** Chemical and biological aspects of Narcissus alkaloids. In *The Alkaloids*, Vol. 63, Cordell, G.A. (Ed.), Elsevier Scientific, Amsterdam, pp. 87–179. 2006
18. **Berkov, S.; Georgieva, L.; Kondakova, V.; Atanassov, A.; Viladomat, F.; Bastida, J.; Codina, C..** Plant sources of galanthamine: phytochemical and biotechnological aspects. *Biotechnology and Biotechnological Equipment*. 23, 1170-1176, .2009
19. **Bokov D.O, Samylina I.A, and Nikolov S.D.** Amaryllidaceae alkaloids GC/MS analysis in *Galanthus woronowii* and *Galanthus nivalis* of Russian origin. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* · ISSN: 0975-8585, pp.1625-1629, January 2016
20. **Bokov DO, Krasikova MK, Sergunova EV, Bobkova NV, Kovaleva TY, Bondar AA, Marakhova AI, Morokhina SL , Krasnyuk I , Moiseev DV** Pharmacognostic, Phytochemical and Ethnopharmacological Potential of Cyclamen coum Mill. *Pharmacogn J*. 2020; 12(1):204-212, DOI : 10.5530/pj.2020.12.31
21. **Briggs, F., Stanford E.,** „Linkage relations to coldfoil factors for resistance to mildew in barley”. *Genetics*. Res. 66. № 4.1943 **Davis P.H.** *Flora of Turkey*. vol I-IX. Edinburg. 1965-1982.

22. **Brown, J. K. M., Jørgensen J. H.**, „ A catalogue of mildew resistance genes in European barley varieties“. IIntegrated Control of Cereal Mildews: Virulence Patterns and Their Change. Risø National Laboratory, Roskilde, Denmark. 1991.
23. **Curuk P, Sogut Z, Bozdogan E, Izgu I, Sevindik B,Tagipur EM, *et al***. Morphological characterization of *Cyclamen sp.* grown naturally in Turkey: Part I. S Afr J Bot. 2015;100:7-15.
24. **Curuk, P., Sogut, Z., Izgu, T., Sevindik, B., Tagipur, E.M., Teixeira da Silva, J.A., Serce, S., Solmaz, I., Kacar, Y.A., Mendi, N.Y.Y.** (2016). Morphological characterization of *Cyclamen sp.* grown naturally in Turkey: Part II. Acta Sci. Pol. HortorumCultus, 15(5), 205–224.
25. **Catană, R., Mitoi, M., Ion, R.** (2013). The RAPD techniques used to assess the genetic diversity in *Drabadorneri*, a critically endangered plants species. Advances in Bioscience and Biotechnology, 4, 164-169.doi: 10.4236/abb.2013.42024
26. **Compton, J.A., Clennett, J.C.B., Culham, A.** (2004). Nomenclature in the dock. Verclassification leads to instability: a case study in the horticulturally important genus *Cyclamen* (Myrsinaceae). Bot. J. Linn. Soc., 146, 339–349.
27. **Czerepanov S.K.** Vascular plants of Russia and Adjacent stases (the former USSR) Cambridge University press. 1995.
28. CYCLAMEN (*Cyclamen purpurascens* Mill.) TUBERS,University of Niš, Faculty of Technology, Leskovac, Serbia ((ORIGINAL SCIENTIFIC PAPER) UDC 582.689.1:66.061.34:543.5 doi:10.5937/savteh1801005S),2018
29. **David B. Neale Claire G Williams.** Conifer wood quality and marker-aided selection: a case study. Canadian Journal of Forest Research, 1990
30. **Davis P.H.** Flora of Turkey. vol I-IX. Edinburg. 1965-1982.
31. **Davis, A.P., Grimshaw, J.A.** (Eds.), Monograph of Cultivated *Galanthus*. The Griffin Press, Maidenhead, pp. 9–63.
32. **Davis, A.P.** The Genus *Galanthus*. A Botanical Magazine Monograph. Timber Press, Portland published in association with the Royal Botanic Gardens, Kew 1999
33. **Davis, A.P.** The genus *Galanthus* – snowdrops in the wild. In: Bishop, M, 2001
34. **DOLUCHANOV...1971: DOLUCHANOV A., MIKELADZE I.,** Flora von Georgien 1: 34. – Tbilisi, Metsniereba., Ingeorgischer Sprache., 1971.

35. **Deragon, J-M., Landry, B.** (1992). RAPD and other PCR-based analyses of plant genomes using DNA extracted from small leaf disks. *PCR Methods and Applications*
36. **Dugar, Yu., Popov, V.** (2011). RAPD analysis of Ukrainian red clover (*Trifolium pratense* L.) cultivars of different ecology-geographical origin. *Vestnik Khar'kovskogo natsional'nogo universiteta imeni V.N. Karazina. Seriya: biologiya.* 13 (947), 81-86.
37. **Ewoud De Gussem, Kourosch Abbaspour Tehrani, Wouter A. Herrebout, Patrick Bultinck, and Johannessen C.** Comparative Study of the Vibrational Optical Activity Techniques in Structure Elucidation: The Case of Galantamine. *ACS Omega* ,14133-14139 Antwerp, Belgium, 2019 .
38. **Flora of Georgia**, Families Pyrolaceae -Verbenaceae (1985) [in Russian], 2nd Ed., Nauka, Tbilisi, 90–96.
39. **GÖÇMEN TAŞKIN, VARDARELİ, N., DOĞAÇ, E., MAMMADOV, R., TAŞKIN, V.** (2013). Genetic diversity of natural *Cyclamen alpinum* populations. *Turk J Biol*, 36 (2012) 413-422. © TÜBİTAK. doi:10.3906/biy-1111-9
40. **Ghezala Mihci-Gaidi, Suheyra Ozbey, Ilkay Orhan, Bilge Sener, Tomofumi Miyamoto, Jean-François Mirjolet, Olivier Duchamp, Anne-Claire Mitaine-Offer, Marie-Aleth Lacaille-Dubois-** **Triterpene** Saponins from *Cyclamen trocopteranthum*, *Planta Medica* · May 2010 DOI: 10.1055/s-0029-1240727 .
41. **Grey-Wilson, C.** (2002). *Cyclamen: A guide for gardeners, horticulturists and botanists.* Batsford, London, UK.
42. **Grozeva N., Todorova M., Gerdzhikova M., Panayotova G., Dohchev D, Tsutsov K.,** Studies On *Cyclamen Coum* In *Sinite Kamani Natural Park*, Bulgaria. 2nd International Symposium for Agriculture and Food, 7 - 9 October 2015, Ohrid, Macedonia. 2015
43. **GROZEVA N, GERDZHIKOVA M , PANAYOTOVA G, TODOROVA M .** OPPORTUNITIES FOR EX-SITU CONSERVATION OF *CYCLAMEN COUM* MILL. IN *SINITE KAMANI NATURAL PARK, EASTERN BALKAN RANGE*, Scientific Papers. Series B, Horticulture. Vol. LX, BULGARIA 2016
44. **Heinrich, M.; Teoh, H.L.** (2004). Galanthamine from snowdrop-the development of a modern drug against Alzheimer's disease from local Caucasian knowledge. *Journal of Ethnopharmacology* 92, 147–162

45. Images for ISOLATION AND CHARACTERIZATION OF SAPONINS FROM CYCLAMEN hederifolium ALTUNKEY!K, Hilal M. Sc. Thesis, Chemistry Department Supervisor: Assoc.Ast. Prof. Dr. Tamer KARAYILDIRIM July 2010, 45 pages
46. IUCN. (2006). Guidelines for using the IUCN Red List categories and criteria. Version 6.2. [www.redlist.org/info/categories\\_criteria.html](http://www.redlist.org/info/categories_criteria.html)
47. Ivanka Semerdjieva , Boryana Sidjimova , Elina Yankova-Tsvetkova , Milena Kostova , Valtcho D. Zheljazkov d- Agricultural University, Plovdiv, Bulgaria,2019
48. Jaradat N.A. , Al-Masri M , Hussien F , Naser Zaid A , Iyad Ali , Ala Tammam ,Mostafa Odeh D, Hussein Shakarneh O, Areej Rajabi . Preliminary Phytochemical and Biological Screening of Cyclamen coum a Member of Palestinian Flora (Pharmaceutical Sciences,, 231-237)An-Najah National University, Nablus,2017
49. KACHARAVA...1982: KACHARAVA W., KETSKHOVELI N., KURASHVILI B., MARUASHVILI L., Red Data Book of the Georgian SSR: 77. – Tbilisi., 1982.
50. Kaffke 200: Kaffke et al., Ispanii the world first percolation bog. 2000.
51. KAYA Gulen Irem, CICEK POLAT Derya, EMIR Ahmet, BOZKURT SARIKAYA Buket, Mustafa Ali ONUR, Nehir UNVER SOMER, Quantitative Determination of Galanthamine and Lycorine in Galanthus elwesii by HPLC-DAD Ege University, Faculty of Pharmacy, Department of Pharmacognosy,107-112,2014, 35100 Bornova-Izmir, TURKEY
52. Kurkin VA. Pharmacognosy as a methodological basis of evidence-based herbal medicine. Izvestia of Samara Scientific Center of the Russian Academy of Sciences. 2015;5(2):592-7.
53. Luchkina MA. Comparative analysis ontogenesis peculiarities *Cyclamen coum* Mill. And *Cyclamen kuznetzovii* Kotov et Czernov. Vestnik Moskovskogo universiteta. Biologiya 2010;16(2):46-51.
54. Mace. G.M. , and Lande R., 1991. Assessing extinction threats: toward re-evaluation of IUCN threatened species categories. Conserv.Bio. 5.2:148-157.
55. Makaradze E. (2017). Diversity of Genus Cyclamen in Georgia. The 3rd International Symposium on EuroAsian Biodiversity, Minsk,Belarus, 55. <http://aves.istanbul.edu.tr/YayinGoster.aspx?ID=42057&NO=28>

56. **Makaradze Elza**, Natela Varshanidze, Inga Diasamidze, Ketevan Dolidze, Nazi Turmanidze, Eteri Jakeli · Species bioecology and growth development of genus *Cyclamen adzharicum* in the South Colchis. India.2019
57. **Maláková Jana**, **Nobilis Milan**, **Zbyněk Svoboda**, **Miroslav Lída**, **Michal Holčápek**, **Jaroslav Kvetina**, **Jiří Klimeš**, **Vladimír Palička**, High-performance liquid chromatographic method with UV photodiode-array, fluorescence and mass spectrometric detection for simultaneous determination of galantamine and its phase I metabolites in biological samples , Journal of chromatography. B, Analytical technologies in the biomedical and life sciences, DOI:[10.1016/j.jchromb.2007.03.025](https://doi.org/10.1016/j.jchromb.2007.03.025). Czech Republic
58. **Manvelidze, Z.**, **Memiadze, N.**, **Kharazishvili, D.**, **Varshanidze N.** (2010). Diversity of floral area of Adjara. (List of wild grown plants species). Annalis of Agrarian science, 8 (2), 93-164.
59. **Mazouz W**, **Djeddi S.** A Biological Overview on the Genus *Cyclamen*. European Journal of Scientific Research. 2013;110(1):7-22.
60. **Maznev N.** Highly effective medicinal plants. Great Encyclopedia. - Litres, 2018.
61. **Memiadze, N.** (2004). Botanical and geographical survey of the endemics of Ajara-Lazeti flora. Bull. Georg. Acad. Sci. 169 (2), 341-343.
62. **Mucina, L.**, **Grabherr, G.** & **Ellmauer, T.** (eds) Die Pflanzengesellschaften Österreichs. 1993.
63. **Nigmatullina N.V.**, **Kuluev A.R.**, **Kuluev B.R.** (2018). Molecular markers used to determine the genetic diversity and species identification of wild plants. Biomics. 10(3). P. 290-318. DOI: [10.31301/2221-6197.bmcs.2018-39](https://doi.org/10.31301/2221-6197.bmcs.2018-39)
64. **Ozbucak TB**, **Polat G**, **Akcın OE**, **Kutbay HG**. The Effects of Elevation on the Morpho-Anatomical and Ecological Traits in *Cyclamen coum subsp. coum* Mill. Populations in the Central Black Sea Region of Turkey in Contrasting Habitats. Pol J Ecol. 2017;65(2):211-26.
65. **Pauli, GF**, **Godecke T**, **Jaki BU**, **Lankin DC**. Quantitative 1H NMR. Development and potential of an analytical method: an update. J Nat Prod. 2012;75(4):834-51.
66. **Piyoosh Sharma**, **Manish Kumar Tripathi**, **Sushant Kumar Shrivastava**- Cholinesterase as a Target for Drug Development in Alzheimer's Disease pp 257-286, New Delhi, India 2019
67. **Proskurina, N.**; **Ordzhonikidze, S.** (1953). Alkaloids of *Galanthus woronovii*. Structure of galanthine. Dokladi Akademii Nauk SSSR. 90, 565-567



68. **Proskurina, N.; Yakovleva, A.; Ordzhonikidze, S.** (1955). Alkaloids of *Galanthus woronovii* III. Structure of galanthamine. *Zhurnal Obshchei Khimii* 25, 1035-1039
69. **Ramazan ERENLERa , Nusret GENÇa , Mahfuz ELMASTAŞb , Özgür EMİNAĞAOĞLU-** Turkish Journal of Biodiversity, Botanical Garden Application and Research Center of Artvin Coruh University. e-ISSN:2667-4386,2019
70. **Ramakrishna V.S. Nirogi, Vishwottam N. Kandikere, Koteshwara Mudigonda, Santosh Maurya-**Quantitative Determination of Galantamine in Human Plasma by Sensitive Liquid Chromatography–Tandem Mass Spectrometry Using Loratadine as an Internal Standard . Biopharmaceutical Research, Suven Life Sciences Ltd, Serene Chambers, Road # 7, Banjara Hills, Hyderabad 500034, *Journal of Chromatographic Science*, Vol. 45, February 2007,pp 97-103 India
71. **Ridley A.M.,** „ Genomic Fingerprinting by Application of rep-PCR. *Molecular Bacteriology*”. Protocols and Clinical Applications. Totowa: Humana Press.1998
72. **Savelkoul P.H., Aarts H.J., De Haas J., Dijkshoorn L., Duim B., Otsen M., et al.,** „Amplified-Fragment Length Polymorphism Analysis: the State of an Art. *J Clin Microbiol* 1999.
73. **Saboora A, Seyedeh-Toktam Sajjadi,Parisa Mohammadi,Zahra Fallahi.** Antibacterial activity of different composition of aglycone and glycosidic saponins from tuber of *Cyclamen coum* Mille. Jun 2016 DOI: 10.18869/acadpub.cmm.2.2.7ISBN: 2423-3439
74. **ST Sajjadi A Saboora and P Mohammadi** Comparison of aglycon and glycosidic saponin extracts of *Cyclamen coum* tuber against *Candida* spp (Department of Plant Sciences, Faculty of Biological Sciences, Alzahra University, Tehran, Iran) Published by Mazandaran University of Medical Sciences on behalf of Iranian Society of Medical Mycology and Invasive Fungi Research Center,2016
75. **Sefa Gün,Burhan Öztürk-** Some Phenological and Morphological Properties of Snowdrop (*Galanthus woronowii*) Grown Naturally in Piraziz District of Giresun Province.International congress of theTurkish journal of Agriculture ,Food science and Technology, Turkey 286-289,2019
76. **Serdar Dusen, Cigdem Aydin , Hesna Yaka Gul, Cennet Ozay, Olcay Dusen, Ramazan Mammadov-** IN VITRO CYTOTOXIC ACTIVITIES OF CYCLAMEN L. (PRIMULACEAE) ETHANOL EXTRACTS FROM TURKEY Volume 25 – No. 12a/2016, pages 6224-6228

77. **Spiridonova GYa.** Alphabet of flowers or German-Russian etymological dictionary of names of flowers. Izhevsk: Udmurt University; 2015.
78. **Stanojević Ljiljana, Dragan Cvetković, Saša Savić, Sanja Petrović, Milorad Cakić,** BIOACTIVE COMPOUNDS AND MINERAL COMPOSITON OF THE AQUEOUS EXTRACT FROM WILD. Jan 2018 DOI: 10.5937/savteh 1801005S ISBN: 2406-2979
79. **Swaminathan B., Matar G.M.,m „** Molecular Typing Methods. Diagnostic Molecular Microbiology. Principles and Applications”. Washington: ASM Press. 1993 .
80. **Tabatadze B .** "Study of Cyclamen adzharicum pobed triterpene glycosides" Tbilisi 2006
81. **Teil I.** Gustav Fischer Verlag, Jena. 578 pp
82. **Thomsen, T.; Bickel, U.; Fischer, J.; Kewitz, H.** (1998). Stereoselectivity of cholinesterase inhibition by galanthamine and tolerance in humans. European Journal of Clinical Pharmacology 39, 603-605
83. **Tyuvetskaya MA.** The rhythm of seasonal development (growth) of *Cyclamen L.* (*Primulaceae*) species in conditions of green-house culture. Bulletin of Moscow Society of Naturalists. Biological Series. 2013;118(1):61-72.
84. **Turan Z,** Eylem Tokler, Mehtap Omac Sonmez, Fatma Kutla- Plant use as a traditional method by women against vaginal discharge in western Anatolia, Turkey: A qualitative research. Journal of Herbal Medicine Volumes 17–18. Turkey 2019
85. **Ünver, N.** (2007). New skeletons and new concepts in Amaryllidaceae alkaloids. Phytochemical Reviews 6, 125–135
86. **Vakhrusheva LP, Yena AV, Boldyrev EV.** *Cyclamen coum* in the Crimea: evaluation of species morphological criteria and age stages. Optimization and Protection of Ecosystems. 2009;20:74-81.
87. **Varshanidze N. Dolidze K, Turmanidze N, zarnadze N. Chitanava J.** (2015). Protected species of adjarian flora. Proceedings ICAE International Conference "Applied Ecology: Problems, Innovations" . Tbilisi, ISBN 978-9941-0-7644-2; 250-254.
88. **Varshanidze, N., Jakeli, E., Turmanidze, N.** (2013). Wild medical plants diversity in Adjara National Parks. International Caucasian forestry Symposium. Artvin. Turkey. <https://ekonferans.artvin.edu.tr/index.php/ICFS/ICFS/paper/view/343>

89. **Varshanidze, N., Turmanidze, N., Dolidze, K., Zarnadze, N., Diasamidze, I., Epitashvili, T., Katcharava, T.** (2018). Biodiversity of Medicinal Plants Containing Essential Oil and Their Spreading in Adjara. *Universal Journal of Agricultural Research* 6(3), 99-104. DOI: 10.13189/ujar.2018.060301
90. **Wang and Fujiwara** 2003, 2005; Wang et al. 2006a, b; Zhi-Rong et al. Warm-Temperate Deciduous Forests around the Northern Hemisphere
91. **Weising, K., Nybom, H., Wolff, K., Kahl, G.** (2005). *Fingerprinting in Plants: Principle, Methods, and Applications*. CRC Press. Boca Raton, FL, USA.
92. **Willis, J.C.** (1988). Amaryllidaceae. In: Shaw, A.H.K. (Ed.), *A Dictionary of the Flowering Plants & Ferns*, 8th edn. Cambridge University Press, Cambridge
93. **William, s J., Kubelik, A.R., Livak, K.J.** (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acid Res.* 18: 6531-6535.
94. **World Cheklist of Selected Plant Families**, 2018. <sup>¼</sup> Kew Garden. [http://apps.kew.org/wcsp/qsearch.do;jsessionid=56A58C50E62834507137259ECD\\_D7B0E0](http://apps.kew.org/wcsp/qsearch.do;jsessionid=56A58C50E62834507137259ECD_D7B0E0). (Accessed 15 December 2018).
95. **Yesson, C., Culham, A.** (2006). A phyloclimatic study of Cyclamen. *BMC Evol. Biol.*, 6, 72.
96. **Yoo-Sin Park**, Shin-Hee Kim, Sang-Yeon Kim, Youn-Hee Kim, Min-Ho Lee, Seok-Chul Yang, Leslie M. Shaw, Ju-Seop Kang- Quantification of Galantamine in Human Plasma by Validated Liquid Chromatography– Tandem Mass Spectrometry using Glimepride as an Internal Standard: Application to Bioavailability Studies in 32 Healthy Korean Subjects. *Journal of Chromatographic Science* 2012;50:803–809 doi:10.1093/chromsci/bms074, Published by Oxford University Press, 2011
97. **Zazanashvili N., Gagnidze R., Nakhutsrishvili G.**, High Mountain Vegetation on the new vegetation map of Georgia, *Journ. of vegetation Science*, 1995.
98. **Zazanashvili N., Gagnidze R., Nakhutsrishvili G.**, Main types of vegetation zonation on the mountain of the Caucasus, *Proceedings IAYS Symposium*, Uppsala, 2000.
99. **Zernov, A., Tyuvetskaya M.** (2013). Taxonomy and nomenclature of the caucasian Cyclamen L. species. *Byulleten' Moskovskogo obshchestva ispytateley prirody. Otdel biologicheskii, izdatel'stvo Izd-vo Mosk. un-ta (M.)*. 118 (3), 28-36.

100. **Zhong, J.** (2005). Amaryllidaceae and Sceletium alkaloids. Natural Products Reports 22, 111-126
101. <https://www.cites.org>
102. <https://www.iucnredlist.org>
103. <http://fitopedia.com.ua/lechenie-paporotniki.html>
104. <https://www.iucnredlist.org/>
105. <https://metlin.scripps.edu>
106. <https://www.gbif.org>
107. <https://flowermeanings.org/snowdrop-flower-meaning/>
108. <https://www.redlandsdailyfacts.com/2015/12/14/use-cyclamen-blooms-for-holiday-color/>
109. <http://dogalhayat.org/property/cyclamen-coum/>
110. <https://www.ipni.org/>