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Bioecological Peculiarities of Introduced and Local Species
of the Genus *Rhododendron* (*Rhododendron* L.) in the Conditions of
Ajara Littoral

Submitted for the degree of Doctor of Biology

Specialty: Plant Biodiversity

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A B S T R A C T

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Introduction

Theme topicality:

The genus *Rhododendron* (*Rhododendron* L.) is distinguished by its diversity over the world, which can be explained by its ancient origins. Six species of the genus *Rhododendron* L. grow wild in Georgia, they are *Rhododendron ponticum* L., *Rhododendron smirnowii* Trautv. ex Regel., *Rhododendron unguernii* Trautv. ex Regel., *Rhododendron caucasicum* Pall., *Rhododendron luteum* Sweet., *Rhododendron* × *sochadzeae* Kharadze & Davlian., except the last one, all these species are significant representatives of Ajarian flora.

Rhododendron ponticum L. and *Rhododendron luteum* Sweet are widely spread in foothills, slopes and gorges under the conditions of humid subtropical climate of Ajara littoral. Representing the species for the creation of the sub-forest of Colchian forest, till today, they are massively spread not only in the conditions of highlands but also coastal areas. Foreign species of rhododendron are available only in the collection of the Batumi Botanical garden (BBG). Up to 20 introduced species plus four others from local flora: *Rhododendron ponticum*, *Rhododendron smirnowii*, *Rhododendron unguernii*, *Rhododendron luteum* grow in the BBG. Among the said local species, *Rhododendron Unguernii* Trautw. and *Rh. Smirnovii* Trautw., are endemic to Ajara-Lazeti, included in the Red List of Georgia with the status VU (Vulnerable).

The representatives of the genus *Rhododendron* L. are hardwood evergreen, semi-evergreen, deciduous species, breeds and forms distinguished by being highly decorative with original flowers and habitus. They are important cultures not only for open soil but also rooms, interior design and containers. Some species of rhododendron are characterized by containing high content of bioactive substances in aboveground organs. Bearing medicinal qualities, they are often used for healing cardiovascular systems, rheumatic diseases, vegetative neurosis, epilepsy, chronic colitis, etc.

In Ajara littoral, the biggest diversity of the species of the genus *Rhododendron* L. is available in the hardwood plant collection of the Batumi Botanical Garden and their complex studying has not been conducted yet. Here one can find rare, single introduced species, which have not been studied in their natural places of origin. Studying bioecological and other peculiarities of unexplored species of the genus *Rhododendron* L. in the soil and climatic conditions of the Batumi Botanical Garden is topical and essential for their further propagation and rational consumption.

Research aims and objectives:

The research objectives are to study the bioecological peculiarities of the species of the genus rhododendron (*Rhododendron* L.) growing in Ajara littoral, in particular, under the soil and climatic conditions of the Batumi Botanical Garden, propagate single and threatened species in the garden collection and identify antimicrobial qualities and other characteristics.

The following tasks were set to accomplish for achieving the said objectives:

- Clarifying the species of the genus *Rhododendron* L. existing in the collection of the Batumi Botanical Garden.
- Studying introduction history and areas of their natural coverage of the rhododendron species growing in the Batumi Botanical Garden.
- Comparative description of the areas of natural coverage and soil and climatic conditions of Ajara littoral of the introduced species of rhododendrons.
- Analyzing practical values of the species of rhododendron.

- Identifying adaptation possibilities and qualities of introduced species in new environmental conditions.
- Studying growth and development peculiarities of the research object.
- Biomorphological and anatomical studying of the research objects.
- Propagation of research objects.
- Identifying the age of *Rhododendron* sp.
- Studying antimicrobial qualities of the species of the genus *Rhododendron*.
- Working on newly introduced species of the genus *Rhododendron* L. and carrying out activities for creating a new collection plot.

Scientific novelty and practical values

Based on the implemented researches, bioecological peculiarities of the genus *Rhododendron* L. growing in Ajara littoral, in particular, under the soil-climatic conditions of the Batumi Botanical Garden was studied for the first time, including the following introduced single examples: *Rhododendron dalavayi* Franch., *Rhododendron brachycarpum* D.Don ex G.Don., *Rhododendron japonicum* (A.Gray) Suringer, *Rhododendron arborescens* (Pursh.) Torr., *Rhododendron macrosepalum* Maxim., *Rhododendron arboreum* smith f. *Roseum*. and six species of *Rhododendron* sp.

Growth and development rhythm, pheno-phases, pheno-intervals and adaptation qualities were identified.

Moreover, certain results were achieved in propagation activities of single examples for the first time.

Based on the studies of the microstructural peculiarities of *Rhododendron brachycarpum* D.Don. ex G.Don, the taxonomic-nomenclature status of the said species was defined as an independent species and not a synonym because the preciousness of critical taxa are proved by anatomical data

The species with high antimicrobial activities are detected for their further deep biochemical research and pharmacognostic studies.

The age of the examples of *Rhododendron* sp. was identified.

Significant activities for the introduction of new species are carried out, which will be the basis for the creation of the collection of *rhododendron* species in the BBG in the future. It will be an innovation for Ajara littoral.

The paper represents the basis for further deep researches and practical, rational consumption of the species of the genus *Rhododendron* L. growing in Ajara littoral, in particular, the Batumi Botanical Garden.

Thesis approbation:

Research objectives as the basis for the thesis were presented in international scientific conferences:

- II Scientific-Practical Conference -Biodiversity and Georgia (Tbilisi, 2016).
- International Scientific Conference – Future Technologies and Life Quality (Batumi, 2017).

Publications - Six scientific works about the said thesis theme have been published. Two of them can be found in reviewed magazine and one in the impact-factor magazine.

Thesis volume and structure – The paper includes 179 printed pages, comprising of an introduction, 9 chapters, 5 sub-chapters, conclusions and annex. The paper also covers 7 tables, 90 photos and a bibliography, including 139 titles among them are 11 foreign ones.

Literature Survey

The first chapters of the dissertation analyze the results of the literature survey: General description of the species of the genus *Rhododendron* L.; specific diversity, introduction history and natural distribution areas of the *Rhododendron* species (*Rhododendron* L.) in the collection of the Batumi Botanical Garden; characterization of the habitat of the species of the introduced *Rhododendron* L. genus and the soil-climatic conditions of the Ajara littoral; practical value of *Rhododendron* L. genus species; general meteorological data of the Batumi sea coast for 2015-2020.

The results of the study are presented in the
Experimental part, Chapter IV and the following chapters:

Chapter IV. Research objects, place and methodology

IV.1. Research objects

The objects of the research were the following introduced and local species growing in Ajara Black Sea littoral, namely, at the Batumi Botanical Garden: *Rhododendron dalavayi* Franch., *Rhododendron brachycarpum* D.Don ex G.Don., *Rhododendron japonicum* (A.Gray) Suringer, *Rhododendron arborescens* (Pursh.) Torr., *Rhododendron macrosepalum* Maxim., *Rhododendron arboreum* smith f. Roseum, *Rhododendron ponticum* L., *Rhododendron luteum* Sweet., *Rhododendron smirnowii* Trautv.ex Regel, *Rhododendron ungernii* Trautv.ex Regel., as well as six specimens of *Rhododendron* sp.

IV 2. Research methods

The following methods have been used for the study of the bio-morphological and growth-developmental peculiarities of the species under study: in accordance with phenological observations Beideman (Beideman, 1974); ; Serebryakov method was applied (Serebriakov, 1974); ; a Guidebook of plant phenological phases by Yelagin and Lobanov (Elagin and Lobanov, 1979); ; biometric indices have been determined according to Tsitsvidze's "Dendrology (Cicvidze, 2004); "; Kolesnikov's "Decorative Dendrology" and "Decorative Dendrology" (1974). the propagation peculiarities have been studied with the method developed at the Minsk Central Botanical Garden (Володько...2015).

For the purpose of determining the age of the six sp. species or forms growing in the Garden, a 500 mm long and 4,3 diameter "Haglöf" Pressler drill and an AmScope zoom stereoscopic microscope were used (see Annex).

Modern systematic-nomenclature and other information about the species under study was found in the plant databases: <http://powo.science.kew.org/>; <http://www.theplantlist.org> ; <https://www.iucnredlist.org>, <http://biodiversity-georgia.net/>

Existing data of the experimental material were processed with the help of the Excel sub-program automatically built-in the computer program office, as well as with the support of the staff of the Ajara Division of Environmental Protection and Natural Resources.

The study of the antimicrobial action of plant raw materials was carried out in the laboratory of the Department of Biodiversity Monitoring and Conservation of the Institute of Phytopathology and Biodiversity at the Batumi Shota Rustaveli State University and the laboratory of microbiology. For the study of the antimicrobial properties, the Golyshin method (Голышин, 1970: 44) was used, which is based on determination of fungicidal activity on agarized food areas, for the identification of

antimicrobial susceptibility of extracts in in vitro conditions. For the study we regularly prepared various diluted extracts of water (tincture) and ethanol (40%) made from the leaves of the plants, which implies identification of the minimum fungistatic and minimum fungicidal concentrations. Sowing fungi and their sequential cultivation were carried out on agarized food areas that contained extracts of the plants under study. Analysis of the obtained results was performed according to the degree of fungal development. Phytopathogenic fungi were involved in the experiment. The works are done at the Institute of Phytopathology and Biodiversity of the Batumi Shota Rustaveli State University.

The research object –leaves of *Rh. brachycarpum* was collected in 2019 from plant introduced in the Batumi Botanical Garden in 1960 (the floristic region of Adjara). Transversal, longitudinal and surface slices cuticles of preparatory samples were done by a sharp razor from a live unfixed material, collected from a medial part of a leaf plate and midrib. Slices were kept in safranin solution for 24 hours and placed in glycerin on the slide. Observation of specimens was done using Carl Zeiss, Jeneval light microscope; digital images were taken by a camera Canon Digital IXUS75 and post-processed using Adobe Photoshop CS5 software.

Explant transfer into in vitro culture was carried out according to the methodology developed at the Minsk Central Botanical Garden (Володько...2015: 40).

Termination of oil acidity, humus and basic nutrient content at the *Rhododendron* distribution sites of the Batumi Botanical Garden was carried out with the Oniani Method (see Annex).

Chapter V

Results of the Bio-morphological and Anatomic Study of the *Rhododendron* L. Genus Species under Study

The life form of the species of genus *Rhododendron* growing at the Batumi Botanical Garden, history of their introduction to the Garden, natural habitat and distribution areas are given in Table №1.

Table №1

Life form, Introduction History, Habitats of the Species of
genus *Rhododendron* growing at Batumi Botanical Garden

№	Species	Life Form	Year of Introduction	Habitat
1	<i>Rhododendron delavayi</i> Franch.	Evergreen shrub	1960	China, the Himalayas
2	<i>Rhododendron brachycarpum</i> D.Don ex G.Don,	Evergreen shrub	1960	Japan, Honshu, Hokkaido
3	<i>Rhododendron japonicum</i> (A.Gray) Suringer	Deciduous shrub	1913	Japan, Hokkaido

4	Rhododendron arborescens (Pursh.) Torr.	Deciduous shrub	1974	North America
5	Rhododendron macrosepalum Maxim.	Deciduous shrub	1913/2008	Japan, Honshu-Southern part
6	Rhododendron arboreum var. roseum	Evergreen shrub	1913	The Himalayas
7	Rhododendron ponticum L.	Evergreen shrub	Natural	The Caucasus, The Balkans, Asia Minor
8	Rhododendron luteum Sweet.	Deciduous shrub	1934	The Caucasus, Asia Minor
9	Rhododendron smirnowii Trautv. ex Regel.,	Evergreen shrub	1962/2009	Ajara-Shavsheti Range, Artvin
10	Rhododendron ungernii Trautv. ex Regel.,	Evergreen shrub	1942/2008	Transcaucasia, Lazica

V.1. Bio-morphological Description of Rhododendron L. Genus Species under Study.

Based on our observations as well as checking with the reference literature data, the species of the genus Rhododendron L. growing at the Batumi Botanical Garden can be characterized as follows:

Rhododendron dalavayi Franch. – is a 1-7 m tall evergreen shrub or tree. The bark is greyish, with scales (lepidote). Young sprouts are of greenish-whitish color. Leaves are hairy (indumentum), 7-15 cm long and 1-5 cm wide, dark green at the top and light green at the bottom, stalk is 7-20 cm long. 10-20 flowers are gathered and form an inflorescence, of purple-red in color, the crown is mostly five-petalled, the number of stamens is 10, of unequal length, with elongated anther stalks. The fruit is a capsule, 8 cm long, blackish, ripens in autumn.

Rhododendron brachycarpum D.Don ex G.Don. – evergreen, erect tree 2-4 m tall. Young greyish-brown piliferous shoots. Leaves are mostly lanceolate, 8-10 cm long and 3-5 cm wide, smooth, with slightly pointed terminations; narrowed at the stalk, light green on top, glossy, with greyish furs; stalk 1-3 cm long; 2-3 cm long and 4-5 cm diameter 10-20 whitish-pinkish flowers are gathered in 10-12 cm diameter roundish inflorescence. Calyx foliole – 5, number of stamens – 10.

Rhododendron japonicum (A. Gray) Suringer. – 1-2 m tall heavily branched deciduous shrub; bark is grey. Young shoots are covered with colorless or silver furs. Buds are ovate, pointed, greyish-brown, scales are covered with white furs at the edges. Leaves are narrow longish-lanceolate, 4-10 cm long and 2-4 cm wide, with pointed terminations. Mature leaves are green on both sides; covered with small furs in the venation places below. The length of the stalk – 0.5-1.0 cm.

Flowers are gathered in 6-12 inflorescences and develop either before or after frondescence. Pistil is wide infundibular, with broad snout and usually short compared to lateral parts. Color – orange-reddish, velvety; has large orange spot, 6-7 diameter. Calyx foliole are small, greyish, covered

with furs. Stamens – 5, shorter than pistils. The lower part of anther stalks is furry, dark brown. Blooms for a month. Fruit is a capsule.

Rhododendron arborescens (Pursh.) Torr. – 2-3 m tall deciduous shrub. Young shoots are glossy and covered with scurf. Leaves are narrow, obovate, oblong-lanceolate, 4-8 long and 1.5-3 cm wide, slightly pointed, mostly with smooth terminations. Sharp green color on top whereas light green at the bottom; violet or light green in autumn, with orange spots. Sometimes in venation places furs can be noticed. Stalk – 5-7 cm long.

Flowers are gathered in 3-6-flower inflorescences. Crown petals are whitish-pink, very aromatic. Heavily furred from outside. The length of cylindrical-shaped pipe is 2.5-3.0 cm, stamen – 5-6, larger than pistils; anther stalks are purple in the upper part. Ovary is reddish, glandular. Column is of stamen length, mostly glossy, only reddish in the upper part. Fruit is a capsule.

Rhododendron macrosepalum Maxim. – deciduous evergreen 1-3 m tall shrub. Young shoots and stalks are slightly greyish-furred. Leaves are narrow, ovate-elliptic, 2-5 cm in length, slightly pointed, mostly with roundish terminations. Vividly furred on top, greenish and in autumn – reddish leaves of various colors are vividly visible.

Flowers 2-10, fragrant; calyx with lanceolate sides, 1-3 cm long glandular furs, sometimes exceeds pistil, pinkish-purple from above, irrorate, 3-5 diameter, wide infundibular, stamen 5, with short pistil, snout is glandular, column is longer than stamens.

Rhododendron ponticum L. – evergreen, 3-4 m tall shrub, leaves are coriaceous, completely glabrous, oblong or oblong-lanceolate shape, obtuse, narrowed at the bottom; stalk is short – 1.3 cm long, leaf blade is folded at the edge, dark green on top, lighter colored in underside.

Inflorescence of a large size, 10-18 cm long, 4-7 cm wide, multiflowered, thyroid cluster in shape, flower stalks are completely bare or more or less covered with glands, crown is bell-shaped, 4.5-6 cm diameter, purple colored, with 5 petals. The upper ovary of the crown is spotted in pharynx area. stamen – 10, anther stalks are bent, about 1/3 of the lower part is covered in furs. Pistil is arched, slightly detached from the crown, ovary is glabrous. The capsule is cylindrical, glabrous, 1.5-1.8 cm long.

Rhododendron smirnowii Trautv. ex Regel. – evergreen shrub, 1-1.5 m tall, shoots and stalks are thickly covered with white pannose furs. Leaves are coriaceous, glabrous on the upper part, glossy, thickly furred on the lower part, later furs become brownish. Leaves are of large size, 10-18 cm long, 4-5 cm wide, with short stalk, 1-3 cm long, oblong oval shaped, narrowly arched towards the top, obtuse, cuneate towards the stalk, new leaves are pipe-like bent, comparatively later the grown leaves are slightly edge-folded. Leaf stalk is 1-2.5 cm long.

Inflorescence is an apical, short-stalked thyroid cluster (raceme). Flower stalks are 2.8-3.5 cm long during flowering and after flowering – 3-5.5 cm long, with pannose furs. Calyx is of small size, with pannose or glandular indumentum, with slightly depicted 5 ovaries. Ovaries are very short – 0.5 mm in length and 2-2.5 mm in width, of large triangular shape more or less obtuse. The crown is bell-shaped (campanulate), reddish-pink, slightly furred, with a 3.5-4 cm long pipe and 5 obtuse ovaries. Stamens – 10, shorter than crown. Anther stalks are thickly furred from the very bottom, about 1/3 long, glabrous at the upper part, ovary thickly furred with white pannose; snout is glabrous, slightly bent. Capsule is cylindrical, 1.5-2 cm long, with thick white pannose fur from the start, subsequently weakening.

Rhododendron unguernii Trautv. ex Regel. – evergreen, 3.5-6 m tall shrub, in nature reaches 10 m height as well. Branches and shoots are with white pannose. Shoots are greyish, traces of fallen leaves are well visible in the form of grey leaf scar. Leaf stalks are furry (puberulent), 1.8-2.5 cm long. Leaf blade is large in size, coriaceous, glabrous in the upper part, thickly puberulent in the lower part; in youth with white and later with reddish pannose, oblong-ovate, narrowed or cuneate towards base, archly narrowed towards the top and terminated in a 1-3 mm long spike; seldom roundish, edge-folded, 13-20 cm long, 3.5 cm wide.

Inflorescence is an apical, multiflowered thyroid cluster, with oblong stalk. Flower stalks are pannose furred and covered with glands. 2.5-3.5 cm long during flowering, whereas up to 6 cm long during fruiting. Calyx is of small size, with five leaflets, with oval or linear-lanceolate shaped 5-6 mm long leaflets; calyx is covered with glands from outside. The crown petals are white colored, campanulate. Petals are reddish on the dorsal part and green in the upper part. The crown pipe is 3-3.5 cm long and puberulent from inside. Stamens – 10, equal size to crown petals, 2-3 among them are slightly protruded above the crown petals. Anther stalks are glabrous for 4-5 mm at the bottom and at the top, slightly thickened far from the bottom and covered with furs; ovary of silver color, puberulent with short close furs. Column is bent, glabrous. 1.2-1.5 cm long capsule is covered with glands or white-reddish pannose.

Rhododendron luteum Sweet. – a 1-2 m tall deciduous shrub, stalk is straight, branchy. Leaves are thin, covered with soft furs on both sides, mostly in the lower part. Leaves develop after flowering. Leaf blade is oblong-ovate or oblong-lanceolate, pointed or almost obovate-roundish, narrowed at the bottom, 6-12 cm long, 3-4 cm wide, leaf stalk is short, 0.3-1 cm long with adhesive glands. Inflorescence is an apical multiflowered cluster; co-inflorescences are oblong shaped, heavily adhesive, 1- 1.5 cm long and 0.5-0.7 cm wide. They fall down during flowering period. The lower stalk, like calyx, is heavily glandular, 2-4 cm in length. Calyx is of small size, linear-lanceolate shape, with glandular obtuse 1 cm long leaflets. Crown is yellowish-orange in color., 3-4.5 cm diameter, with glandular furs from outside, together with flower stalk and calyx is heavily adhesive or rarely with glabrous, infundibular crown pipe broadened in pharynx. Stamens – 5, semi-puberulent, bent, with anther stalk protruded from the pipe; pistil column is bent – protruded from the pipe. Fruit is a 1.5-2.2 cm long capsule, grooved, with 5 nests, short scattered furs and glands.

Rhododendron sp. 1. – an evergreen shrub of ericaceous family, 3 m tall, heavily branched. Young shoots are glabrous and greenish. Leaves are coriaceous, elliptic, 9.2 cm long and 3.2 cm wide. Both surfaces of grown-up leaves are glossy – without furs, light green below and dark green above. Inflorescence is collected out of 8-14 flowers. Flower stalk is 2-4 cm. crown petal – 5, of 2.5-4 cm length. Flowers are of pinkish-purple color with yellow tint expressed on one of the crown petals. Pistils – 8-12. Starts flowering in April, fruit ripens in October.

Rhododendron sp.2 – evergreen 2.7 m tall shrub, heavily branched from the height of 0.5 m. Leaves are hairy, wide with elliptic shape. Dark green on top and lighter from underside., 12 cm long and 5 cm wide.

Inflorescence is apical, gathered with 8-12 flowers. Crown is of 4.5-6 cm in diameter, light purple-pink, rarely whitish, with 5 petals. The upper ovary of the crown is with spotted tints in the pharynx area. stamen – 8-10, pistil is bent.

Rhododendron sp.3 – evergreen 3.5 m tall shrub, leaves are coriaceous, completely glabrous, of narrow elliptic shape, obtuse, cuneate towards the base, 21 cm long, 5 cm wide. Dark green on the

upper side and slightly whitish in the lower part. Large size – 10-18 cm long, 4-7 cm wide. Inflorescence is an apical, multiflowered thyroid cluster. Inflorescence is gathered with 8-14 flowers; crown is light purple – 5-petaled. Stamen – 6-10. Anther stalks are bent. Pistil is archlike bent and twice the stamen size.

Rhododendron sp. 4 – evergreen, 4.5 m tall shrub. Young shoots are glabrous. Leaf blades are coriaceous, glabrous, obovate shape, narrowed towards the base, arciform roundish towards the top, 16 cm long and 8 cm wide. Inflorescence is an apical, multiflowered thyroid cluster. Inflorescence is gathered with 8-10 flowers; crown is 7-petaled, large size – 4-6 cm, white, pinkish until opens. Stamen – 12-14, of crown size. Pistil is infundibular and twice exceeds stamens in size. Starts flowering in early March and ends in late May. Fruit ripens at the end of November.

Rhododendron sp. 5. – an evergreen shrub of ericaceous family, 4 m tall. Young shoots are glabrous and greenish. Leaf is coriaceous, elliptic, 18 cm long and 6.5 cm wide. Stalk junction place is heart-shaped. Termination is obtuse, roundish or pointed. Grown-up leaves are glossy, light green at the bottom and dark green at the top.

Inflorescence is gathered with 8-10 large-size flowers. Calyx leaflet is 4-7 cm long, whitish. Pistil is wide infundibular and twice protruded from the crown petals. Stamens – 12-15. Starts flowering in early May and lasts until the end of June. Fruit ripens at the end of December.

Rhododendron sp.6 – an evergreen 2.5 m tall shrub. Young shoots are glabrous. Leaf blade is coriaceous, glabrous, lanceolate, narrowed towards the base. Arciform roundish towards the spike, 14 cm long and 6.5 cm wide. Inflorescence is an apical, multiflowered thyroid cluster. Inflorescence is gathered with 8-10 flowers. Crown is 7-petaled, large size – 4-6 cm, white. Calyx is of small size, dissected in five parts. Stamens – 12-14, of crown size. Pistil is infundibular and twice exceeds stamens in size. Starts flowering at the end of May, fruit ends ripening in mid-December.

Rhododendron sp. 6 – an evergreen 2.5 m tall shrub. Young shoots are glabrous. Leaf blade is coriaceous, glabrous, lanceolate, narrowed towards the base. Arciform roundish towards the spike, 14 cm long and 6.5 cm wide. Inflorescence is an apical, multiflowered thyroid cluster. Inflorescence is gathered with 8-10 flowers. Crown is 7-petaled, large size – 4-6 cm, white. Calyx is of small size, dissected in five parts. Stamens – 12-14, of crown size. Pistil is infundibular and twice exceeds stamens in size. Starts flowering at the end of May, fruit ends ripening in mid-December.

V.2. MICROSTRUCTURAL CHARACTERISTICS OF THE LEAF OF RHODODENDRON BRACHYCARPUM D. DON.

Among the introduced species growing in the collection of the Batumi Botanical Garden: *Rhododendron dalavayi* Franch., *Rhododendron japonicum* (A. Gray.) Suringer, *Rhododendron brachycarpum* D.Don. ex G.Don., *Rhododendron arborescens* (Pursh.) Torr., *Rhododendron macrosepalum* Maxim., special attention is drawn by *Rhododendron brachycarpum* D.Don. ex G.Don., which is known for its rich content of biologically active substances. At the same time, it is interesting to note that there were some inaccuracies about its taxonomic-nomenclature status (Вриш, 2011:41).

At this stage, we aimed at studying microstructural peculiarities of above-ground vegetative organs, leaves of short-fruit *Rhododendron* – *Rhododendron brachycarpum* D.Don and identifying its diagnostic characteristics, because without knowing the inner structure of vegetative and generative parts of plants, it is impossible to understand the whole life of a plant and set the goal to obtain it thoroughly; Moreover, preciseness of critical taxa is strengthened by anatomic data, which is a reliable method for plant diagnostics together with the other parameters.

Rhododendron brachycarpum D.Don. ex G.Don. deserves attention due to its bioactive content being studied for the first time by our research team. The sum of bioactive phenolic compounds was obtained by extraction with 70% ethyl alcohol from dry and powdered leaves of *Rh. brachycarpum*. With the help of qualitative reactions and HPLC-MS spectrometry analysis the presence of flavonoids and catechins was detected in the said phenolic compounds. Research in this direction is underway.

The research object –leaves of *Rh. brachycarpum* was collected in 2019 from plant introduced in the Batumi Botanical Garden in 1960 (the floristic region of Adjara). Transversal, longitudinal and surface slices cuticles of preparatory samples were done by a sharp razor from a live unfixed material, collected from a medial part of a leaf plate and midrib. Slices were kept in safranin solution for 24 hours and placed in glycerin on the slide. Observation of specimens was done using Carl Zeiss, Jeneval light microscope; digital images were taken by a camera Canon Digital IXUS75 and post-processed using Adobe Photoshop CS5 software.



Pic.№1. Leaf of *Rh. brachycarpum* D.Don ex G.Don.

The leaf of *Rh. brachycarpum* is bifacial, bare in terms of ventilation system, the structure is hypostomatic, the pulp has a dorsoventral structure. The leaf covering tissue is cutinized with equal-layered cuticle; adaxial epidermis is double-layered and abaxial - single-layered. In the ventral side of the leaf, there are tightly packed and tangentially slightly stretched smaller epidermal cells. However, in the dorsal side of the leaf, there is smaller nipple-like epidermal tissue. In the abaxial covering tissue, the differential stomata apparatus is sunk toward epidermis cells.

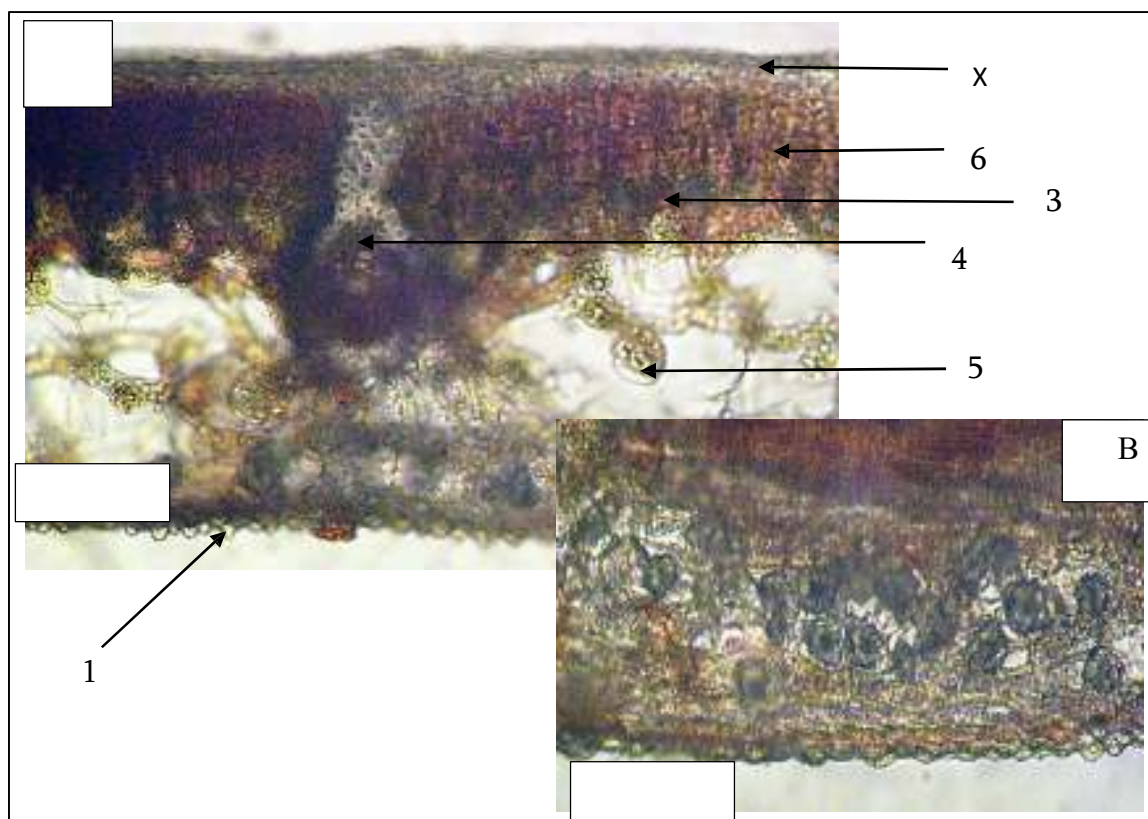
The basic texture of the leaf pulp of *Rh.* is composed of structural units of a rather thick spongy parenchyma; typical palisade parenchyma is represented by double-layered cells, separated by the cells with not typical palisade-like habitus. Significant amount of calcium oxalate druse crystals is accumulated in the leaf mesophyll of short-fruit rhododendron, especially in the spongy parenchyma, the size of druse is really impressive (pic.2).

Vascular bundles of *Rh. brachycarpum* packed in the ventral part of the leaf pulp are surrounded by starch sheath and sclerenchymal cells; the vascular bundle differentiated in the leaf is complex

and contains fibrovascular and reverse-collateral structures; rounded and weakly angular lumens of smaller caliber are arranged in the xylem (pic. 3).

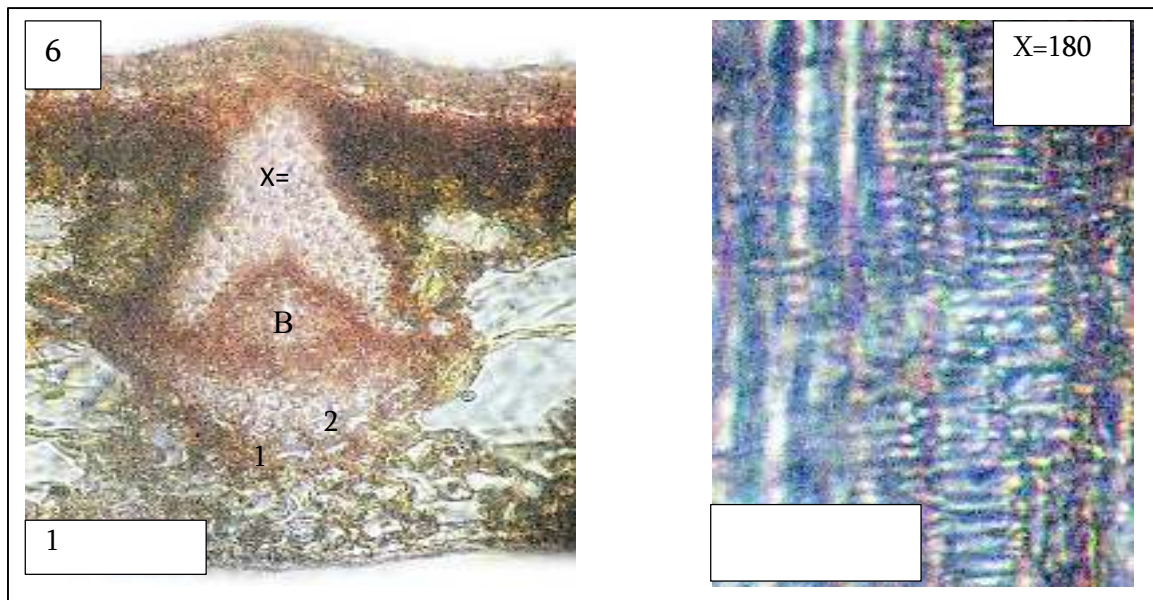
The covering tissue of the midrib of the leaf of *Rh. brachycarpum* is cutinized. The vascular structure is more or less crumbly, polygonal cells are represented. Lamellar collenchyma areas are shown in ventral and dorsal sides of the main vein. Vascular system concentrated in the costa is surrounded by mechanical tissue. The phloem is especially narrow-cellular, roundish lumens of cortex fibers and vascular vessels are arranged in the cortex, membrane of tracheary elements is spirally thickened. Radial rays differentiated in the xylem are short and single-layered (pic.4).

Cells at the base of adaxial and abaxial epidermis of the leaf of *Rh. brachycarpum* are not lined but with curved and uneven structure; abundant blade apparatus arranged in the lower epidermis of the leaf is simple and anomocytic [1] (Pic.5). The membrane of stomatal locking cells is rectilinear and thin; the clefts are mostly spindle-shaped, although sometimes oval. (Pic.5). Considering the direction of stomatal clefts toward the midrib of the leaf, the ventilation system is revealed to be chaotic. Significant amount of calcium oxalate crystals druse is accumulated in cells at the base of abaxial epidermis of the leaf of short-fruit *Rh. brachycarpum* (Pic.5).

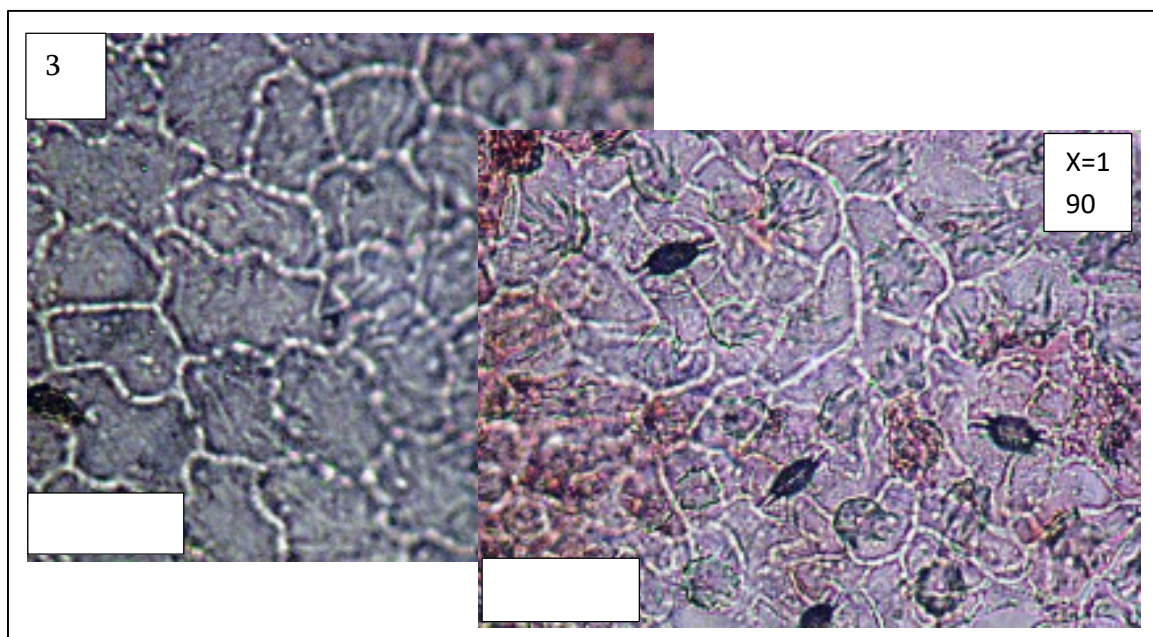


Pic. № 2. Leaf microstructure of *Rh. brachycarpum* D.Don ex G.Don.

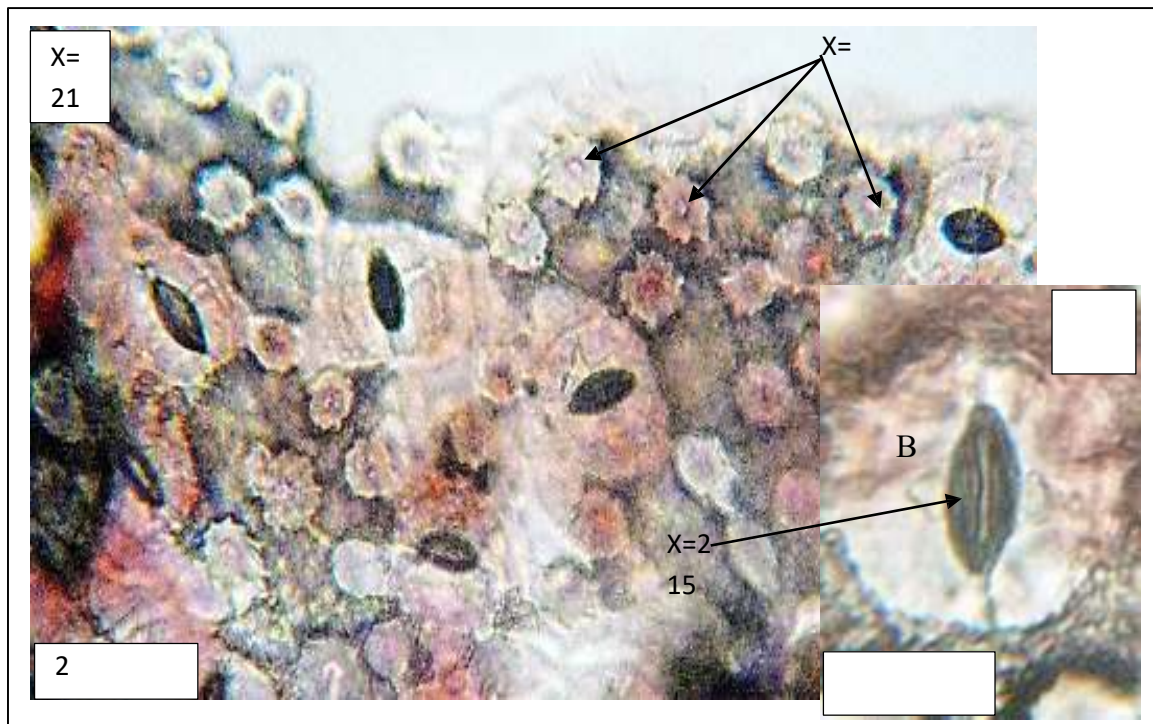
A. Dorsoventral mesophyll of a leaf; B. Druse of calcium oxalate crystals in spongy parenchyma of the leaf: 1. Upper epidermis, 2. Lower epidermis, 3. Palisade parenchyma, 4. Palisade-like parenchyma, 5. Vascular bundle, 6. Spongy parenchyma.



Pic. №3. Microstructure of the midrib of *Rh. brachycarpum* D.Don. ex G.Don leaf: A. Vascular bundle with fibrovascular and reverse-collateral structure; B. Spirally thickened vascular vessels of membrane. 1. Mechanical cells, 2. Xylem, 3. Phloem.



Pic. №4. Microstructure of the leaf epidermis of *Rh. brachycarpum* D.Don. ex G.Don : A. The texture of not lined, but curved and uneven tissue near the base of adaxial side; B. Ventilation system in not lined, but curved and uneven tissue at the base of abaxial side.



Pic. №5. Microstructure of lower leaf epidermis of *Rh. brachycarpum* D.Don ex G.Don. A. Calcium oxalate crystals druse in abaxial covering tissue; B. Simple, anomocytic stomata apparatus with a spindle-shaped stomatal cleft: 1. Druses, 2. Stomatal cleft, 3. Stomatal locking cells.

Plant anatomy is a theoretical basis for a number of biological and agronomical disciplines. Knowledge of the internal structure of a research object is extremely helpful for the study of the plant biology, as vital processes ongoing in the organism are linked with specific structural units. The diagnostic signs of the plant microstructure are the following: indumentum type, outline and interlocking features of epidermal tissue basic cells of an organ of interest; types of stomata in the covering tissue and their characteristics; leaf pulp architectonics and details of structural elements of vascular tissue differentiated in axial organs.

The study revealed the diagnostic characteristics of the internal structure of the leaf of *Rh. Brachycarpum* D.Don.:

- Leaf is naked bifacial;
- Hypostomatic arrangement of stomata;
- Leaf mesophyll has a dorsoventral structure;
- Vascular bundle is complex with fibrovascular and reverse collateral structure;
- Significant amount of calcium oxalate crystal druses are revealed in abaxial covering tissue of the leaf and spongy parenchyma;
- Abundance of mechanical cells can be found in vascular bundles and midribs;
- Lumens of vascular vessels in the xylem are mostly round-shaped, their membrane is spirally thickened;
- Radial rays of cortex are short and single-layered;
- Cells at the base of adaxial and abaxial epidermis of the leaf are not lined, but curved and uneven;
- Stomatal ventilation apparatus is anomocytic;

- Stomatal locking cells are rectilinear and clefts are mostly spindle-shaped.

It should be noted that in “The Plant List” of world international plants *Rhododendron brachycarpum* is considered as a synonym of *Rhododendron fauriei* and is also mentioned as *Rhododendron brachycarpum* subsp. *fauriei* (Franch.) D. F.Chamb.(The plant list).

The timeliness and relevance of our research is evidenced by the fact that on the worldwide portal of plants - Plants of the World Online (POWO) (povo.science.kew.org.), currently, the information about the above described species is available as follows:

Family: Ericaceae Durande.

Genus: *Rhododendron* L.

Species: *Rhododendron brachycarpum* D.Don ex G.Don

Chapter VI

Growth-Developmental Peculiarities of genus *Rhododendron* L. Species

The peculiarities of seasonal plant development, especially of introduced species, to some extent reflect the phylogenesis of species, their ecological and adaptive capabilities. It is well known that in the new environment at a particular stage of seasonal development, various physiological and morphological changes take place in plants. The alternation of phenophases is of a sequential nature, determined by a system of self-regulation. In the new environmental conditions, local soil-climatic conditions are of crucial importance (Metreveli, 2008:12).

Plant growth and development, active viability, especially if introduced in new environmental conditions, significantly depends on air temperature, lighting, humidity, precipitation, etc. rhododendrons are no exception.

Generally, rhododendrons are characterized with spreading over wide areas and high plasticity. Abundance of the representatives of this genus in the Batumi Botanical Garden is conditioned by the above-mentioned fact, although bioecological peculiarities of some of them in local soil and climatic conditions haven't studied yet. Moreover, in the collections, there are only few units of some of the species with high decorative values and the attempts of their propagation with seeds and vegetative methods have been unsuccessful for decades. We tried to fix this flaw by studying biological rhythm of growth and development of introduced hardwood plants, having theoretical and practical importance as we count.

Phenological observations were mainly conducted in 2016-2018, partially in 2019-2020

We carried out the observation on the following phenol-phases: 1) plant awakening – vegetation; 2) shoot development, leaf formation, the end of growth; 3) the beginning and the end of blooming; 4) fruit formation, ripening, the end of fruit bearing; 5) leaf color change; 6) the beginning and the end of leaf fall. Phenointervals were detected.

During the period of intensive growth, the observations and measurements were conducted for decades, whereas during the period of less intense growth – twice a month. By characterizing the periods of growth and development, shoot growth, foliage, leaf color change and leaf fall we have calculated the average annual growth rate as well as shown the dynamics of leaf and apical growth according to the decades; characterized the flower phenophase according to the development of flower

buds, flowering terms, flowering quality and duration; characterized fertility and fruiting phenophase by fruit development, seed dispersal and fruit falling periods, according to degree of fertility.

From our observations, the species under study are characterized by the development of vegetative, generative and mixed type buds. Both vegetative and generative organs are formed in the mixed type buds.

Rhododendron delavayi - turgor of vegetative buds starts in early January and lasts till the end of February. The budding takes place in the first decade of March. Apical growth of shoots begins in the first decade of March, lasts until the first decade of August, with a short recess in June. After a two-week break, the terminal buds at the tip of the shoot are reactivated and the plant enters a second growth period that lasts until the first decade of August. The average annual growth size of shoots is 10-15 cm. The appearance of new, young leaves begins in late March – early April, the plant wears massively young leaves in May. The change in the colors of last year's leaves is observed in April. Leaf fall starts in late April – early May, lasting until late August .

Mass turgor of flowers buds takes place in the first decade of March. Flowering of the flower buds starts at the end of the first decade of March, with massive flowering by the end of March and lasts until the third decade of April. The degree of flowering is mostly massive, the average duration of flowering is 40-45 days. Fruit kernels in the last decade of April, ripening starts from the second half of June and ends in the second decade of December. Seed dispersal begins in December-January and lasts through February as well. Fruit falls in January-February. However, sometimes petrified empty capsules of fruit are left on the tree all year round.

Rhododendron brachycarpum – Mass turgor of vegetative buds takes place in the third decade of March and budding starts from early April. Apical growth of shoots begins from the second decade of April, lasts until the end of July, sometimes the end of August, with a two-week recess period in June. The average annual growth size of shoots is 15-23 cm. Frondescence starts in the first half of April. Becomes massively covered with young leaves in May. Color change in old leaves is noticed in late May-early June and lasts to the third decade of August .

Mass turgor of flowers buds takes place in the first decade of April. Flowering of the flower buds takes place from the end of April, massive flowering in the first decade of May and ends in early June. Flowering duration in average 35-40 days. Flowering degree – weak. Rarely characterized by fruit formation and ripening. In 2016, a small number of fruits developed in isolation, fertility was very weak. Fruit kernels in the third decade of June, starts growth and ripening from the first decade of July and ends by the end of October. Seed dispersal begins in the beginning of November and ends at the end of November .

Rhododendron japonicum – vegetative buds are massively turgid in the first decade of March. Bud blooming (opening) begins from the second decade in March. Apical growth of shoots starts from the second-third decade of March and lasts till the end of July or the first decade of August. Frondescence begins in the third decade of February and by the end of June the plant is completely leafed with new leaves. Color change in old leaves is noticed from the first decade of September and ends by the first decade of October. Duration of vegetation comprises 8-9 months. The annual growth size of shoots is 11-13 cm .

The generative buds are massively swollen in the first decade of March and start opening/blooming at the end of the second decade of March. Flowering starts at the end of March and lasts till the end of April - beginning of May. It has massive flowering in mid-April. Flowering duration

is on average 32-34 days. It is characterized by massive and attractive flowering. Fruit is kernalled by the second decade of May. Ripening of fruit starts from the second decade of June and ends in September-October. Seed dispersal process lasts through October-November. Fruiting degree is relatively weaker compared to flowering degree. Empty capsules remain on the plant until December-January.

Rhododendron arborescens – vegetative buds are massively swollen in the first decade of March and begin opening from the end of March. Growth of shoots starts from the beginning of April and lasts till the end of August, with a two-week recess in June. The annual growth size of shoots is 9-17 cm. Leafing process starts at the end of March. Color change in old leaves begins from the beginning of September and lasts till the end of September. Fall of leaves starts from the end of September and lasts till the end of October.

The flowering buds are swollen by the first decade of May and start opening/blooming from the third decade of May. Massive flowering takes place in mid-June. Flowering ends at the beginning of July. The average duration of flowering period is 30 days. Is characterized by high degree of flowering. Fruiting does not always take place. During the years of our observation, the kernaling of fruits, ripening and seed dispersal took place in 2016: fruit ripening starts from the month of July and ends in the first decade of October. Seed dissemination starts from the end of October till mid-November. The capsule fruit remains on the plant till the end of December. In some cases, the open fruit is found on the plant all year round.

Rhododendron macrosepalum – vegetative buds are massively turgid at the beginning of March and begin opening from the third decade of March. Shoot growth starts at the end of March and ends in the first decade of July, with a two-week recess period in May. The average annual growth size of shoot is 9-14 cm. Frondescence starts from the third decade of March. The plant is massively leafed with new leaves by the last decade of June, is characterized by adhesive and puberulent leaves. The color change in leaves starts from the third decade of August and lasts till the end of September - beginning of October. Defoliation begins at the beginning of October and ends at the end of October.

The flower buds are swollen in the first decade of April and open by the third decade of April. Flowering starts in the first decade of May and lasts till the first decade of June. The flowers are characterized by adhesive glands. The average flowering duration is 31 days. The degree of flowering is average. Fruit ripening starts at the beginning of July and ends in the second decade of October. Seed dissemination starts at the end of October and ends at the end of November. Fruiting degree is average. 50% of empty fruits fall down in November-December, the rest remain on plants for a year or longer period.

Rhododendron arboretum var. *Roseum* Lindl. – vegetative buds are turgid in the second decade of April. Buds open at the end of April. The specimen is severely diminished and is characterized by slow shoot growth. The apical part of the shoot starts growth at the beginning of May and ends by the third decade of July. The average annual growth size of shoot is 5-9 cm. The plant has not been characterized by flowering or fruiting so far.

Rhododendron smirnowii Trautv. – buds are turgid in the first decade of March and start opening in the second decade of March. The apical growth of shoots begins at the end of April and proceeds in slow speed till the end of July. New fresh leaves appear in the first half of May, is massively covered with leaves in June. The change of color in old leaves is noticed in May. Defoliation starts at the end of May – early June and lasts till the end of August. The species develops very slowly due to the different geographical-climatic conditions compared to the natural habitat. Approximately

10 specimens introduced in 2015 have not flowered or fruited so far. The average annual growth size of shoot is 4-9 cm .

Rhododendron ponticum L.- buds are massively turgid at the end of February or beginning of March. Is characterized by mixed type of buds. Bud opening starts from the second decade of March. The apical part of the shoot starts growth at the end of March and ends by the end of August. Appearance of fresh leaves starts at the beginning of April and in May is massively leafed. The change of color in old leaves is noticed in May. Defoliation starts at the end of May and lasts till the end of August. At the beginning of September. The plant starts the second vegetation that is finished at the end of November. The average annual growth size of shoot is 18-23 cm.

Flowering starts at the end of March or the first decade of April and ends by the second decade of June. In October the plant starts second flowering. During the second flowering, it does not give fruit. The flowering degree is massive. Duration of the first flowering is 62-64 days whereas the second flowering – average 30-35 days. Fruit kernels in the second decade of June, ripening starts at the beginning of July and ends in October. Seed dissemination takes place from mid-October till the end of November. The majority of the fruit capsules remain on the plant all through the year while the rest fall as a result of dissemination by the end of December.

Rhododendron luteum Sweet. – buds become turgid from the first decade of March, opening/blooming takes place from the early April. The plant starts frondescence from the third decade of March. Changing of leaf colors begins from the second decade of September and the massive color change is observed at the end of September. Leaves are characterized by puberulent adhesive glands. Defoliation starts from the second decade of October and lasts till the first decade of November. The average annual growth size of shoot is 12-16 cm.

Flower buds are swollen from the third decade of March and start opening from the second decade of April. Flowering begins from the first decade of April and lasts till the first decade of June. Flowering duration is 53-66 days. The degree of flowering is massive. Like leaves, flowers are also characterized by adhesive glands. Fruit kernels in the first decade of June. Starts ripening at the end of the third decade of June and lasts till the first decade of October. Seed dissemination starts from the end of October and lasts till the second decade of November. Empty fruits remain on the plant till the end of December, often even for 7-8 months. Fruiting degree is average.

Rhododendron unguernii Trautv. – vegetative buds are massively turgid in the third decade of March and open from the second decade of April. The apical growth of shoots starts from the third decade of April and ends at the end of August. Appearance of fresh leaves starts in the first decade of May and in June is massively leafed. Change of color in the old leaves is noticed in April. Defoliation begins in the last days of May and last till the end of August. The average annual growth size of shoot is 12-24 cm.

Flower buds are turgid from the second decade of May and opening starts from the third decade of May. Flowering starts in the first decade of June and ends at the end of July. Is characterized by massive fruiting. Duration of flowering – 25-35 days. Fruits are kerned in the first decade of August. Starts ripening from the second decade of August and ends at the end of October. Seed dissemination from the capsules takes place from mid-November till December. Part of the empty fruit capsules remains on the tree till spring while the majority falls in December-January.

Rhododendron sp.1 – vegetative buds of this evergreen 2,5 m tall shrub are massively swollen at the beginning of February and start opening from the second decade of March. The apical growth of shoots starts in the first decade of April and last till the first half of June. After a three-week

recess, continues growth till the end of June. Appearance of fresh leaves begins from the second decade of April, is in full foliage in May. Change of color in the old leaves is noticed in April. Defoliation starts from the first days of May and lasts till the end of August. The average annual growth size of shoot is 9-12 cm.

Flower buds are turgid from the first decade of April and start opening at the end of the third decade of April. Flowering starts at the end of April and lasts till the end of June. Is characterized by massive flowering. Duration of flowering – 60 days. Is characterized by an inflorescence compiled from 8-14 purple-pinkish flowers. Starts the second flowering from the beginning of September, which lasts 30-35 days. Does not give fruits after the second flowering. Fruits are kernalled at the end of June. Starts ripening in August and ends at the last decade of October. Seed dissemination takes place from mid-November and ends by the first decade of December. Is characterized by abundant fruiting. Empty fruit capsules remain on the tree till the end of January. The majority of open capsules remains on the plant all the year round.

Rhododendron sp.2 – vegetative buds of this evergreen, up to 3 m tall shrub are massively turgid in late February and begin to open from the second decade of March. The growth of the apical part of the shoot starts from the second decade of April and ends at the end of June. Appearance of new fresh leaves starts from the second half of April, is massively covered with fresh leaves in late May. Change of color in the old leaves is noticed at the end of April. Defoliation starts from the first decade of May and lasts till the end of August. The average annual growth size of shoot is 8-12 cm.

Flower buds are massively turgid in early April and start opening from the third decade of April. Flowering starts from the first decade of May and ends in early July. Duration of flowering – 70-75 days. The second flowering starts at the end of September and ends in the second decade of October. Is characterized by purple-pinkish flowers. Fruit kernels in the first half of June. Starts ripening at the end of August and ends in early November. Empty fruit starts falling in November and ends at the end of December. Sometimes empty fruits remain on the plant all throughout the year.

Rhododendron sp.3 – vegetative buds of this evergreen, up to 5 m tall shrub are turgid by the second decade of March and begin to open from the end of the third decade of March. The apical growth of the shoot starts from the third decade of April till at the end of May and continues growth till the end of August after a 2-3-week recess. Is characterized by a high speed of growth. Appearance of new fresh leaves starts in early May, is massively covered with fresh leaves in late June. Change of color in the old leaves is noticed at the end of May. Defoliation starts from late June and lasts till the end of September. The average annual growth size of shoot is 9-28 cm.

Flower buds are turgid by the second decade of April and start opening in late April. Flowering starts from the first decade of May and lasts till the first decade of June. Is characterized by an average degree of flowering. Duration of flowering – 30-39 days. Is characterized by white flowers gathered in one inflorescence. Fruit kernels at the beginning of June. Starts ripening at the end of August and ends by the first decade of November. Seed dissemination from the fruit starts from the end of November and ends by the end of December. Empty fruits remain on the plant till the end of January. Is characterized by an average degree of fruiting.

Rhododendron sp. 4 - vegetative buds of this evergreen, up to 3 m tall shrub are turgid in early January and begin to open in mid-February. The growth of the apical part of the shoot starts from the first decade of March and ends at the beginning of September. Appearance of new fresh leaves starts in late April, is massively covered with fresh leaves in May. Change of color in the old leaves is

noticed in April. Defoliation starts from the end of April – early May and lasts till the end of August. The average annual growth size of shoot is 12-21 cm.

Flower buds are turgid in early March and start opening in late March. Flowering starts from the third decade of March and ends in late May. The second flowering begins in September-October that ends by late November. Is characterized by an average or sometimes massive degree of flowering. Has light purple-pinkish flowers. Fruit kernels at the beginning of June. Starts ripening at the end of August and ends by the third decade of November. Seed dissemination starts from November and ends by the end of December. Empty fruits remain on the plant all year round.

Rhododendron sp.5 – vegetative buds of this evergreen, up to 5 m tall shrub are well mature in the first decade of March, opening takes place in late April. The apical growth of shoots starts in early April and ends in late June. Is characterized by fast growth speed. Appearance of fresh leaves starts in the first half of April, is massively covered by new leaves in May-June. Change of color in the old leaves is noticed in the first half of May. Defoliation starts from early June and lasts till the end of September. The average annual growth size of shoot is 12-22 cm.

Flower buds are turgid in early April and start opening in the third decade of April. White flowers are gathered in large inflorescences. Flowering starts from late April and lasts till the first decade of June. Is characterized by an average degree of flowering. Duration of flowering – 30-35 days. Fruit kernels in the first half of June. Starts ripening in the first half of August and ends by late November. Seed dissemination starts from late November and lasts till the end of December. Is characterized by weak degree of fruiting. Empty fruits remain on the plant till late December.

Rhododendron sp.6 – vegetative buds of this evergreen, up to 4 m tall shrub are turgid by the third decade of March and begin to open in early April. The growth of the apical part of the shoot starts from the second decade of April and lasts till the beginning of June. After a two-week recess, the plant continues growth till early August. Appearance of fresh leaves starts in the last decade of April, is massively leafed in May. Change of color in the old leaves is noticed in the first half of May. Defoliation starts from early June and lasts till the end of September. The average annual growth size of shoot is 11-18 cm.

Flower buds are turgid by late April and start opening from the second decade of May. Is characterized by whitish-pinkish flowers. Flowering starts from the third decade of May and lasts till the end of June. Degree of flowering – average, duration of flowering – 35-40 days. Fruit kernels in late June. Fruit ripening starts in the first decade of August and ends in late November or mid-December. Degree of fruiting is very weak.

The winter of 2018 was not distinguished by abundant rainfall and low temperatures. Nevertheless, the development of the *Rhododendron* species under study was weak and slow. If in previous years the fruit of *Rhododendron delavayi* Franch. was fully matured in February and capsules open, the fruit did not ripen till the first decade of April, 2018. If in previous years there was an average of massive and medium flowering from the end of February to the end of April, in early April 2018 only three inflorescences were observed. Buds started to appear 5-6 weeks later than in previous years. Development of shoots was normal. In 2018 *Rhododendron brachycarpum* D.Don ex G.Don. had no flowering.

In the humid subtropical climates of Ajara, one of the main limiting factors for the development of introduced plants is always considered to be low temperatures, or an increase in precipitation and humidity. During the last five years, at the Ajara Black Sea littoral, and in particular

at the Batumi Botanical Garden, there were relatively harsh winters in 2016 and 2020, when the absolute minimum temperature in the January decade of 2016 was $-1,9^{\circ}\text{C}$, and in 2020, in the second decade of February, $-4,7^{\circ}\text{C}$, and in some places of the garden area -6°C . However, it did not have a negative impact of Rhododendrons' vegetation and flowering, on the contrary, compared to previous years, for example, *Rhododendron delavayi* had massive fruiting and seed production, which confirms the literature data on Rhododendrons that they grow and develop well at low temperatures.

The results of three years of phenological observations on all research species are presented in the following tables of the dissertation: growth and vegetative development of the species of genus *Rhododendron* L.; flowering; fruiting. The tables of growth and vegetative development show: bud development – massive turgor, opening; shoot growth – start, ending; shoot annual growth size (average); frondescence – beginning, massive; defoliation – beginning, full. Flowering tables show: massive turgor of flower buds, opening; flowering – start, massive, end; flowering duration in days; degree of flowering; for the description of fruiting the following is shown: fruit development-ripening, ripening start and end; seed dispensation – start, end; fruit falling – start, end; degree of fruiting.

In addition to the tables, based on the mathematical-statistical processing of data for each species, the results are shown in the diagrams: growth-development, flowering and fruiting dynamics at the Batumi Botanical Garden.

After studying the growth and development phases of highly decorative, rare and single specimens of the introduced and local species of genus *Rhododendron* L.: *Rhododendron arboreum* Smith f. *Roseum*, *Rhododendron dalavayi* Franch., *Rhododendron brachycarpum* D. Don ex G. Don., *Rhododendron japonicum* (A. Gray) Suringer, *Rhododendron arborescens* (Pursh.) Torr., *Rhododendron macrosepalum* Maxim., *Rhododendron ponticum* L., *Rhododendron luteum* Sweet., *Rhododendron smirnowii* Trautv., *Rhododendron unguernii* Trautv., sp.1, sp.2., sp.3, sp.4, sp.5, sp.6, in the humid subtropical climate conditions, we can conclude the following: 1) vegetative development of the species under study comprises 8-10 months; 2) they usually start growth process in March and June-July, after a 1-2-week recess period complete the second growth in August; 3) *Rhododendron macrosepalum* Maxim., *Rhododendron arboreum* Smith f. *Roseum*, *Rhododendron luteum* Sweet., are characterized by the shortest period of shoot growth; 4) according to the flowering periods and terms we can distinguish between: a) species flowering in spring: *Rhododendron dalavayi* Franch., *Rhododendron japonicum* (A. Gray) Suringer, *Rhododendron ponticum* L., and b) species flowering in spring-summer: *Rhododendron brachycarpum* D. Don ex G. Don., *Rhododendron macrosepalum* Maxim., *Rhododendron arborescens* (Pursh.) Torr., *Rhododendron unguernii* Trautv., *Rhododendron luteum* Sweet.; 5) flowering duration comprises in average 35-45 days. The longest flowering has the highly decorative rare species: *Rhododendron dalavayi* Franch., *Rhododendron ponticum* L., *Rhododendron luteum* Sweet., *Rhododendron* sp.1 and sp.2; 6) massively flowering species are: *Rhododendron japonicum* (A. Gray) Suringer, *Rhododendron* *Rhododendron ponticum* L., *Rhododendron luteum* Sweet., *Rhododendron* sp.1, sp.2 and sp.3, the average degree of flowering: *Rhododendron brachycarpum* D. Don ex G. Don., *Rhododendron arborescens* (Pursh.) Torr., *Rhododendron unguernii* Trautv., sp.5, sp.6; 7) ripening of the species under study mostly happens in September-October, November, while the seed dissemination process – in November-December; *Rhododendron dalavayi* is an exception as it finishes ripening by December and seed dissemination

lasts in January-February-March as well; 8) among the species under study, the high degree of fruiting is characterized for: *Rhododendron ponticum* L., *Rhododendron luteum* Sweet, *Rhododendron* sp.1, sp.2., sp.3, and sp.4; The average degree: *Rhododendron dalavayi* Franch., *Rhododendron macrosepalum* Maxim., *Rhododendron unguernii* Trautv., Very weak degree: *Rhododendron brachycarpum* D.Don ex G.Don., *Rhododendron japonicum*(A.Gray) Suringer, *Rhododendron arborescens* (Pursh.) Torr., sp.5, sp.6; 9) defoliation of the evergreen species under study: *Rhododendron arboreum* smith f. *Roseum*, *Rhododendron dalavayi* Franch., *Rhododendron brachycarpum* D.Don, *Rhododendron ponticum* L., *Rhododendron smirnowii* Trautv., *Rhododendron unguernii* Trautv., sp.1, sp.2., sp.3, sp.4, sp.5, sp.6, takes place in summer months and mostly ends by late August whereas the deciduous species: *Rhododendron japonicum*(A.Gray) Suringer, *Rhododendron arborescens* (Pursh.) Torr., *Rhododendron macrosepalum* Maxim., *Rhododendron luteum* Sweet., start change of leaf color in late summer and defoliation - in September-October, till November; 10) The species are characterized by very weak development, neither flower nor give fruit: *Rhododendron smirnowii* Trautv., *Rhododendron arboreum* var. *Roseum* Lindl.; 11) it is remarkable that the fall of temperature below 0° C in winter does not damage the plants or hinder the flowering process; 12) in our opinion, weak fructification of the species under study in certain years must be due to the absence of some pollinating insects in that period.

Chapter VII

The results of the propagation of the introduced species of the genus *Rhododendron* L. in the Batumi Botanical Garden

Based on literature data, rhododendrons effortlessly propagate in nature by their seeds. The seeds are too small; after appearing in wet and loose soil, they germinate rapidly and create dense sowings. Moreover, in natural conditions, they actively propagate by root sprouts too. Sometimes, due to snow weight, branches touch the ground and after getting covered with leaves, withered twigs, mosses, other organic remains, they easily get rooted. All these kinds of propagations are established successfully in practice (Володько, 2015: 40).

The propagation of some introduced species of rhododendrons has been an issue for years at the Batumi Botanical Garden since they are represented by single examples. These species are: *Rhododendron dalavayi*, *Rhododendron brachycarpum*, *Rhododendron japonicum*, *Rhododendron arborescens*, *Rhododendron macrosepalum*. Their vegetative development is well enough under the soil and climatic conditions of the Batumi Botanical Garden. However, they are characterized by not annual, poor fruit-bearing. 2020 was an exception when *Rhododendron dalavayi* showed more than average productivity.

The seed of *Rhododendron dalavayi* gets matured at the end of December, dispersal occurs in January; *Rhododendron brachycarpum* seeds are ready in October-November while dissemination starts in November; *Rhododendron japonicum* gets matured at the end of September and seeds dispersal takes place in October; *Rhododendron arborescens* gets ripen in October and dispersal occurs in October-November; *Rhododendron macrosepalum* seeds are ready at the end of October, while seeds start to spread in November.

We have studied the propagation opportunities through seeds, stem cuttings and microclonal means.

Chapter VII. 1. Propagation by seeds of the single examples of the genus *Rhododendron* L. introduced to the Batumi Botanical Garden

The seeds were collected from the fruit-bearing species existing in the collection. The biology of shoots and seedlings as well as propagation of research species by seeds was studied under orangerie conditions. We had a control version and samples treated by bio stimulators. Moreover, we conducted sowing the species (see the annex) received from different botanical gardens with the help of the Batumi Botanical Garden Seed Exchange Foundation. During sowing, we followed all the rules, which are efficient for the propagation of rhododendrons. These rules were elaborated by Belarus colleagues with a significant experience working on rhododendrons (Володько..., 2015: 40).

The sowing process was conducted in three versions:

- 1) on Petri cups
- 2) on substrates (peat, perlite) prepared by us;
- 3) in sifted soil.

Conditions of the propagation of plants by seeds:

For the preparation of the substrate, equal concentrations of peat and perlite were taken. Loose and sifted deposits were mixed and placed in wooden boxes.

Seeds storing dates have a significant influence on the germination and energy of the research species. While storing, the germination ability for the representatives of the genus *rhododendron* is decreased or lost at all. It is better to apply for newly collected seeds.

We started processing the seeds of the research objects as soon as the top of its fruit or box turned brown, despite the rest of the parts remained green, since the box immediately started to open after this condition, seeds were dispersed and we did not manage to collect them as they are characterized by too poor or no productivity.

After placing the collected fruit in paper materials, closer to the heat source, its boxes start to open and seeds are dispersed. As soon as the seeds are well-cleaned, they are sowed. We drew attention to one thing, that the seeds had humid-cell-like conditions in the laboratory or orangerie as they continuously require a high level of humidity before and after germination as well. During our annual experiment, we studied, that if *rhododendron* seeds get a bit dried, it dramatically affects their stirring and germination ability.

The boxed fruit of *Rhododendron delavayi* Franch. is 8 mm long, blackish, gets matured in Autumn. The seed is 0.5 mm. After picking the fruit, we were waiting for its box to open for ten days. The seed was sowed in March, after ten days of the box opening. Some of them were placed in the Petri dish, the other on substrates prepared by us and the rest of them in sifted soil.

The first shoots are visible after 23 days in prepared substrates. After the 5th day of its germination, two leaves are developed on sprouts, they are green with intensive trichomes, 2-3 mm long. At the end of May, the plant develops 8-9 leaves. Lower leaves are bigger than upper ones, characterized by slow-growing. After 65 days of sowing, it reaches 2-3 cm, survival coefficient is 60-70%, the leaf is 1 cm long. The shoot has a very weak main stem. After reaching 1 cm in height, it gets hard to develop and succumb to rot. They need humidity and watering 3-times a day with a little number of water drizzles.

Well-cleaned primary materials or seeds (26.02 2020) of *Rhododendron delavayi* Franch. were placed in Petri dishes on humid filter paper at 18-20°C, under intense light conditions. Every four days, it was moistened by water. Water acidity was pH - 5,6. Seed germination dynamics had been observed

for 30 days. The germination started on the 15th day. For the 30th day, the germination coefficient reached 80%. After five days, the sprouts developed embryonic stem and leaves.



Pic.6 Ripe capsula, fruit and sown seed; germination in the stereoscopic microscope

Matured seeds of *Rhododendron delavayi* Franch. need 30 days for their full germination. Then, it is necessary, to broaden a feeding area or transplant for their better growth and development. Transplantation was carried out into the orangery. Only Peat was applied as a substrate, watered three times a day by spraying (potassium permanganate and water). The transplantation of the research objects was done in the cotyledons stage.

After 15 days of transplantation (03.05.2020), the shoots developed the third and fourth leaves at the same time, while after a month (11.06.), they grew and reached 3-3,5 cm.

The fruit of *Rhododendron japonicum* A.Gray. gets matured in December-January. The fruit box is 8-10 mm long. Its seed is bigger, 1-2 mm long, unlike our other research objects. After 7-10 days of collecting the seeds, we were waiting for its boxes to open and seeds to disperse. After seven days of scattering the seeds, we sowed them in the Petri dishes in three options in soil and substrates prepared by us. Unlike *Rhododendron delavayi* Franch., stirring and germination of its seeds start earlier, in 16 days and have a faster growth rate. The materials sowed in the soil did not germinate. *Rhododendron japonicum* A.Gray. has revealed a low level of germination in Petri dishes. After developing three leaves, it immediately started to rot in the soil. Sowing them in our peat and substrate mixture came out efficient; after five days of its germination, two leaves are visible. The main stem is weak. After a month of their transplantation in separate pots, the seedlings start to die after the very first days. Only the strongest six examples are left. The plant develops 9-10 leaves after two months. It is sensitive to direct sunlight and heat. It does not like to get dried or not too much wet. The germination percentage is 80%, although the survival ability for germinated shoots is 5-10 %. 12-13 leaves appear in the main stem after three months; they are 0.5-1 cm long and 7 cm tall.



Pic. № 7 *Rhododendron dalavayi* Franch. – seedlings in the orangery of Batumi Botanical Garden

The same results with the same consequences and dates were received for *Rhododendron arborescens* (Pursh.) Torr. and *Rhododendron macrosepalum* Maxim.

Two groups based on the quality of the germination of the seeds of research objects were pointed out (see annex about other species):

- Species with a high coefficient of seed germination;
- Species not germinated.

Rhododendron delavayi Franch. *Rhododendron japonicum* A.Gray. *Rhododendron arborescens* (Pursh.) Torr. *Rhododendron macrosepalum* Maxim. are included in the first group of species as they have a high coefficient of seed germination. The second group consists of *Rhododendron brachycarpum* D.Don, the species which was not germinated and revealed no germination abilities during the experiment.

VII.2. Vegetation Propagation by Stem Grafting of Single Species of Genus *Rhododendron* L. Introduced in Batumi Botanical Garden

We tested the vegetative propagation of the introduced species of *Rhododendron* L. genus on 8 objects of our research: *Rhododendron arboreum* smith f. Roseum, *Rhododendron delavayi* Franch., *Rhododendron brachycarpum* D. Don, *Rhododendron japonicum* A. Gray, *Rhododendron arborescens* (Pursh.) Torr., *Rhododendron macrosepalum* Maxim., *Rhododendron indicum* (L.) Sweet.

We used the methods of stem grafting and layering of the vegetative propagation.

Cutting of stem grafts took place in August from the semi-ligneous apical shoots.

Grafts were treated with rooting biostimulators: "Organica" 0,05%, potassium permanganate 0,1%, sucrose 5%, GeoHumate, the so-called humic fertilizer 5% solutions.

Rooting of the grafts was recorded according to the time of grafting (spring and autumn). Graft rooting depends of the plant's age, time of graft cutting, temperature, humidity, etc.

A mixture of sand, humus and perlite was selected as the substrate. They were processed with biostimulators step by step.

GeoHumate, the so-called humic fertilizer 5% solution proved to be effective. Graft rooting was possible only on two species: *Rhododendron indicum* (L.) Sweet., development percentage comprises 30-40%; *Rhododendron brachycarpum* D. Don., development percentage comprises 10%. However, it should be noted that the latter was found to be rather weak against the infestation of pests – species of thrips, orangery thrips (*Heliothrips haemorrhoidalis*). (Pic. №8).



Pic № 8 . *Rhododendron brachycarpum* D. Don. with rooted stem grafts;
A pest - *Heliothrips haemorrhoidalis*, on the leaf of *Rhododendron brachycarpum* D. Don.

In case of *Rhododendron arboreum* smith f. *Roseum*., we applied one of the effective methods of vegetative propagation – layering. A specimen grown on the cut of 1 m tall root was used as a rootstock with its own root. In early spring we dug a 0.5 m deep pit next to the plant and covered the layered stem with loose soil. In autumn the stem was already rooted and was removed from the mother plant in two years by specimen cutting.

VII.3 . Outcomes of introducing some species of the

Genus *Rhododendron* L. to in vitro culture

The alternative method of traditional propagation methods (vegetative and generative) is clonal micro propagation having lots of advantages, in particular, getting planting materials in a short period of time, any seasons and unlimited amount.

Clonal propagation is based on the unique quality of a plant cell – giving rise to an entire organism of a plant as a result of experimental influence. Using this method is important for decorative gardening, forestry, agriculture, medicine, especially for the propagation of single and endangered plants.

Although this method is quite progressive, clonal micro propagation technology is not perfectly elaborated for many plants. *Rhododendrons* belong to this kind of plants, especially their introduced species. Even not to count single examples, their propagation will bring economic benefits as the planting material of this valuable and rare decorative plant does not exist for realization. They are almost not used in green constructions of parks, gardens, squares and boulevards of our country and Belorussia too.

The main topic in our research is that, the only method for the preservation and restoration of single and endangered species and taxon existing in the collections of the Batumi Botanical garden is elaboration of their clonal micro propagation method and creation of in vitro plant bank. While creating this type of banks, it is necessary to ensure the vitality of the samples from collections, genetic integrity and the quality of collection samples giving us the opportunity for their further scientific and economical usage.

In order to get in vitro culture and achieve efficient plant propagation, morphogenetic potential of cultivated tissues are studied and the factors are revealed, which react on their realization (physical conditions of cultivation, mineral and hormonal composition of feeding areas, etc.) and also optimal conditions for cloning and adventive root production are determined in vitro.



Pic. 9. The branches of *Rhododendron* prepared for the experiment

After numerous repetitions of the experiment, at this stage, we have achieved certain outcomes for 2 species: evergreen *Rhododendron delavayi* and deciduous *Rhododendron japonicum*.

As recognized, for microclonal propagation of the genotype of valuable wood plants, it is necessary to use primary meristems as the first materials and seeds can be applied for the propagation of species. Besides that, as less is the absolute age of the plant as intensively meristems are developed and shoots are grown. At the stage of getting in vitro culture, optimal ratio of cytokinins and auxines in the area gives an opportunity for induction of direct regeneration of shoots from the primary meristems. The said ratio has a nature of species-specificity (often breed-specificity).

Efficient technologies for micro propagation are elaborated for certain species of *Rhododendrons* and foreign selection breeds [1-3;8] .

These types of technologies are based on comprehensive studies of regeneration peculiarities and shoots morphogenesis in various types of explants. Traditionally, for the purpose of getting micro clones, a feeding area with 2-izopentiladenine and indolilaceticacid are used in vitro culture. Zeatin is used as a regulator for the growth of cytokinin activity and in recent years thidiazuron has been also applied to, which is a strong inductor of morphogenetic reactions for hardwood plants in vitro culture [4-5].

Our research goal was to optimize sterilization conditions and identify morphogenetic reactions on various growth regulators on in vitro culture, in the explants of *Rhododendron delavayi* Franch. and *Rhododendron japonicum* and create the method for in vitro culture in order to get aseptic cultures of these taxa considering the age factor of the primary plants.

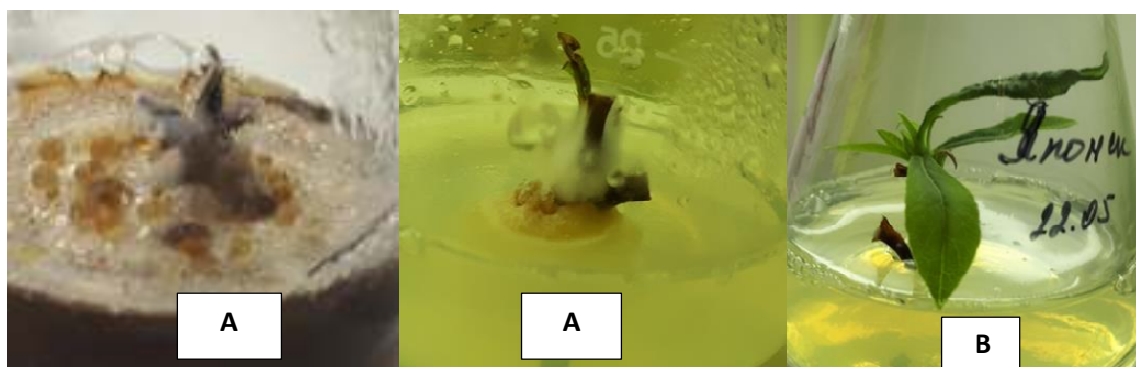
Explants were vegetative buds taken from 50-60 years old single examples. Moreover, cuttings with two bosoms from ontogenetically young and actively growing shoots were taken from these plants and delayed under room conditions. While cultivating the shoots, we used the areas in accordance with WPM (Woody plant medium) [7-8] by adding plant growth regulators – auxines and cytokinins. Before starting autoclaving, pH of all the feeding areas were reached to 4,8 - 5,0 importance. Passing of aseptic cultures on new feeding areas was the following: 0 passage – for the 12th week, from the first passage 1

– to every 8th week. Cultivation of plant materials was carried out in the climatic chamber at $25 \pm 2^\circ\text{C}$, during 16 hours photoperiod and 50% relative humidity of air.

For getting aseptic cultures of research plants, the following scheme for the sterilization of plant materials was applied to: the shoots were washed by brush with detergent through running water. Sterilization was carried out by putting the plant materials consecutively in Ditan M-45 and fungicide 0,4% solution for 60 minutes and 9% solution of $\text{Ca}(\text{ClO})_2$ (Calcium Hypochlorite) for 30 minutes. In order to saturate the surface of the explant, sterilization compound was added a droplet of tween 80. Then washed with sterile distilled water three times for 5-5- minutes. Prepared explants were placed in the feeding areas by tested growth regulators.

The following growth regulators were added to the feeding areas on relative: zeatin with the concentration 5 mg/l; 5 mg/l zeatin and 0,1 mg/l thidiazuron; 5 mg/l 2 - isopentiladenine and 0,1 mg/l thidiazuron; 5 mg/l zeatin and 0,5 mg/l thidiazuron; 5 mg/l 2 - isopentiladenine and 0,5 mg/l thidiazuron; 15 mg/l 2 isopentiladenine and 4 mg/l indolylaceticacid.

Big infection of primary explants in case of both *Rhododendrons* are indicated by us. 100% fungus infection was revealed. Applying to Ditan M-45 gave us an opportunity to decrease fungi pathogens and receive sterile primary shoots, which means that while sterilizing, without primary treatment by fungicide solution, the material was infected in 100%. After using Ditan-45, infection with fungi pathogens was decreased and sterile primary shoots were received (pic.10).



Pic №10. A) infected explants of research *Rhododendrons* (sterilization without Ditan M-45); B) sterile primary explants.

Tab. 2

Received sterile materials in accordance with the sterilization scheme, %

	9,0 % $\text{Ca}(\text{ClO})_2$	0,4 % Ditan M-45 + 9% $\text{Ca}(\text{ClO})_2$
<i>Rhododendron japonicum</i>	0	50,0
<i>Rhododendron delavayi</i>	0	35,3

Morphogenic response in the explants of *Rhododendron delavayi* was received on the area containing traditional phytohormones for the propagation of rhododendrons: 2 isopentiladenine and zeatin with the concentration of 5 mg/l added to 0,5 mg/l thidiazuron. The growth of buds in size and initiation of meristems were visible in the sterile shoots on the 7th day. Although on the 8th week, further development of shoots was detected only in 50% of explants. Areas containing 15 mg/l 2 isopentiladenine and 4 mg/l indolylaceticacid, also thidiazuron with limited concentration to 0,1 mg/l in combination with the other growth regulators were found inefficient. For *Rhododendron japonicum* was detected as follows: the growth of buds for this species was faster than *Rhododendron delavayi*. The development of shoots from the primary meristems was detected on the 4th day of cultivation on the feeding area containing zeatin. On the 14th day of the beginning of the experiment, the growths of young shoots on these areas reached 0,8-1,0 cm on average (Picture11). Activation and development of primary meristems on the area containing 15 mg/l 2 isopentiladenine and 4 mg/l indolylaceticacid, are almost the same as the feeding area with Zeatin.



Pic. 11. Growings of young shoots of explants of *Rhododendron japonicum*

Thus, it is identified, that in order to get sterile plant materials of research *Rhododendrons*, they must be preliminarily processed by fundazol, for example, 0,4 % Ditan M-45. Regarding deciduous species - *Rhododendron japonicum*, in order to get aseptic culture, there is WPM feeding area containing 5 zeatin or 5 mg/l2 izopentiladenine and 1 mg/l indolilaceticacid and the evergreen species - *Rhododendron delavayi* needs feeding area WPM 5 mg/l zeatin and added 0,5 mg/l of thidiazuron or 5 mg/l 2 izopentiladenine and 0,5 mg/l thidiasuron.

On the basis of conducted works, there are received aseptic cultures of the shoots of *Rhododendron japonicum* and *Rhododendron delavayi* (pic.12).



Pic. 12. aseptic cultures of the shoots of *Rhododendron japonicum* (A) and *Rhododendron delavayi* (B).

Chapter IX

Study of Antimicrobial Properties of the Genus *Rhododendron* L. Species

Organic substances released into the environment as a result of plant cell activity, or in most cases, their complex, are characterized by antiviral and antimicrobial properties. The investigation of *Rhododendron* species is interesting in this direction as well. Based on the collaboration with the Institute of Phytopathology and Biodiversity of Batumi Shota Rustaveli State University, we have studied the antimicrobial action of the extracts obtained from the leaves of the species under study: *Rhododendron japonicum*, *Rhododendron arborescens*, *Rhododendron brachycarpum*, *Rhododendron macrosepalum*, *Rhododendron dalavayi*, on the example of fungicidal and fungistatic actions.

The experiments were mainly conducted during the periods of active vegetation.

For the determination of fungicidal activities the following pathogenic fungi: *Phytophthora infestans*, *Alternaria alternata*, *Alternaria solani*, *Trichothecium roseum*, *Pestalotia coryli*, *Fusarium moniliforme*, *Pestalotia theae*, *Fusarium moniliforme*, causing important cultural plant diseases: Potato – *Phytophthora*, *Alternariosis*; Hazelnut – Pink Rot, *Trichotillocytosis*; Tomato – *Alternaria* Leaf Spot, *Fusarium*; Blueberry – Brown Leaf Spot, Tea – Leaf Spot, were applied for the experiment. The effect of the extract activity was determined according to the interruption of the fungus development.

The determination method of the fungicidal activity [5] in agarised feeding areas was used in order to determine antimicrobial sensitivity of extracts of cedar needles in vitro conditions. Water extracts (tincture) made of cedar needles were prepared for the research and Ethanol (40 %) extracts with various dilutions including the identification of fungistatical and minimal fungicidal concentration. Instead of herbal extracts, sterile water as a control option was used during the experiment. Sowing the fungi and their consistent cultivation were conducted within agarised feeding areas containing the extracts of research plants. Outcome analysis was carried out by the development quality of the fungus. 2% Potato Glucose Agar was used as a feeding area. Water extracts of cedar needles were prepared as follows: liquid extract of the plant was gained from newly-picked cedar

needles cleaned with distilled and sterile water, 20 cm³ boiled water was poured on 5 gr minced raw materials and left during 40 minutes in a water bath till boiling point. Then the received extract was cooled down and filtered with a sterile filter paper. 20 cm³ melted potato Agar was added to the plants extract prepared in 2 cm³ and immediately poured in sterile petri dishes. Spore suspension of the following fungi: *Phytophthora infestans*, *Alternaria alternata*, *Alternaria solani*, *Trichothecium roseum*, *Pestalotia coryli*, *Fusarium moniliforme*, *Pestalotia theae*, *Fusarium moniliforme*, was placed on the surface of cooled Agar by an injection. They were cultivated during 3 days at 25°C. The Ethanol extracts are gained by leaving (1:5) the raw materials in Ethyl alcohol (40%) during 7 days. The extracts were prepared with different concentrations: 1:1, 1:2, 1:4, 1:8. Average rate was counted according to the results.

The works are done at the Institute of Phytopathology and Biodiversity of the Batumi Shota Rustaveli State University. Fungal pathogens were locally separated from diseased plants; strains from the institute collection were also used.

Based on three times repeated studies about fungicidal activity of water and Ethanol extracts gained from *Rhododendron delavayi* and *Rh. Brachycarpum* lives extracts, on the development of phytopathogen fungi, it was detected, that the extracts prepared from the plant material collected in August are characterized with higher fungicidal and fungistatistical activity.

Concerning the water extracts (tincture), the strongest fungicidal activity was shown toward the following pathogen fungi: *Phytophthora infestans*, *Alternaria alternata*, *Alternaria solani*, *Pestalotia theae*, *Fusarium moniliforme*. In this case, the growth of the fungus mycelium was completely stopped, while the mycelium of the following fungi: *Trichothecium roseum*, *Pestalotia coryli*, *Fusarium moniliforme*, appeared difficult to grow or their development was interrupted, fungistatistical activity was revealed.

Regarding the Ethanol extracts, the highest fungicidal activity according to the conditions of both experiments, was revealed in 1:1, 1:2 diluted extracts, good result was reached in case of 1:4 and 1:8 dilutions, lysis zones were clearly shown during the experiment completed by the diffusion method. In other dilution cases, fungistatic activity was revealed except the fungus *Trichothecium roseum*, weak fungicidal activity was shown when the pathogens were placed in agarised feeding area, although there was no fungistatistical activity while conducting the diffusion testing (Tabl. 3;4).

As for the control option, the fungi pathogens were characterized with good development.

Table 3

Fungicidal activity of water and Ethanol extracts of
Rhododendron brachycarpum testing the fungal spore suspension in agarised feeding area

Nº		The growth of fungus strains in case of various diluted Ethanol extracts, water extracts and the control option
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	Phytopathogen fungus	Various diluted Ethanol extracts					Water extracts (tincture)	Control option
		1:1	1:2	1:4	1:8	1:12		
1	Phytophthora infestans	–	–	±	±	±	–	+
2	Alternaria alternata	–	–	–	±	±	–	+
3	Alternaria solani	–	–	–	±	±	–	+
4	Trichothecium roseum	–	±	±	±	±	±	+
5	Pestalotia coryli	–	–	–	±	±	±	+
6	Pestalotia theae	–	–	±	±	±	–	+
7	Fusarium moniliforme	–	–	–	±	±	±	+

Note: „+“ - Growth of the fungus mycelium; „–“ - Termination of the growth of the fungus mycelium; „±“ - Interruption of the growth of the fungus mycelium.

Table 4

Fungicidal activity of water and Ethanol extracts of
Rhododendron delavayi testing the fungal spore suspension in agarised feeding area

№	Phytopathogen fungus	The growth of fungus strains in case of various diluted Ethanol extracts, water extracts and the control option						
		Various diluted Ethanol extracts					Water extracts (tincture)	Control option
		1:1	1:2	1:4	1:8	1:12		
1	Phytophthora infestans	–	–	–	±	±	–	+
2	Alternaria alternata	–	–	–	–	±	–	+
3	Alternaria solani	–	–	–	±	±	–	+
4	Trichothecium roseum	–	±	±	±	±	±	+
5	Pestalotia coryli	–	–	–	±	±	±	+
6	Pestalotia theae	–	–	–	±	±	–	+

7	Fusarium moniliforme	–	–	–	±	±	±	+
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Note: „+“ - Growth of the fungus mycelium; „-“ - Termination of the growth of the fungus mycelium; „±“ - Interruption of the growth of the fungus mycelium.

Conclusions

Bioecological features of the specimens of genus *Rhododendron* L. introduced and local species: *Rhododendron dalavayi* Franch., *Rhododendron brachycarpum* D.Don ex G.Don., *Rhododendron japonicum* (A.Gray) Suringer, *Rhododendron arborescens* (Pursh.) Torr., *Rhododendron macrosepalum* Maxim., *Rhododendron arboreum* smith f. Roseum, *Rhododendron ponticum* L., *Rhododendron luteum* Sweet., *Rhododendron smirnowii* Trautv.ex Regel, *Rhododendron unguernii* Trautv.ex Regel, *Rhododendron* sp. *Rhododendron* sp. growing along the Ajara littoral, in particular at the Batumi Botanical Garden have been studied for the first time, resulting in as follows:

1. Biomorphological peculiarities of the species under study have been determined.
2. As a result of the study of microstructural peculiarities of *Rhododendron brachycarpum* D.Don. ex G.Don.- leaf, the following diagnostic indices of the internal structure of the leaf was determined: Hypostomatic arrangement of stomata; Leaf mesophyll has a dorsoventral structure; Vascular bundle is complex with fibrovascular and reverse collateral structure; Significant amount of calcium oxalate crystal druses are revealed in abaxial covering tissue of the leaf and spongy parenchyma; Abundance of mechanical cells can be found in vascular bundles and midribs; Lumens of vascular vessels in the xylem are mostly round-shaped, their membrane is spirally thickened; Radial rays of cortex are short and single-layered; Cells at the base of adaxial and abaxial epidermis of the leaf are not lined, but curved and uneven; Stomatal ventilation apparatus is anomocytic; Stomatal locking cells are rectilinear and clefts are mostly spindle-shaped. The study confirms the validity of the existence of *Rhododendron brachycarpum* as an independent species:
3. As a result of studying the phases of growth and development, a conclusion is drawn:
 - 1) vegetative development of the species under study comprises 8-0 months;
 - 2) they usually start growth process in March and June-July, after a 1-2-week recess period complete the second growth in August;
 - 3) *Rhododendron macrosepalum* Maxim., *Rhododendron arboreum* smith f. Roseum, *Rhododendron luteum* Sweet., are characterized by the shortest period of shoot growth;
 - 4) according to the flowering periods and terms we can distinguish between: a) species flowering in spring: *Rhododendron dalavayi* Franch., *Rhododendron japonicum*(A.Gray) Suringer, *Rhododendron ponticum* L.,and b) species flowering in spring-summer:

Rhododendron brachycarpum D.Don, *Rhododendron macrosepalum* Maxim., *Rhododendron arborescens* (Pursh.) Torr., *Rhododendron unguernii* Trautv., *Rhododendron luteum* Sweet.;

5) flowering duration comprises in average 35-45 days. The longest flowering has the highly decorative rare species: *Rhododendron dalavayi* Franch., *Rhododendron ponticum* L., *Rhododendron luteum* Sweet., *Rhododendron* sp.1 and sp.2;

6) massively flowering species are: *Rhododendron japonicum* (A.Gray) Suringer, *Rhododendron* *Rhododendron ponticum* L., *Rhododendron luteum* Sweet., *Rhododendron* sp.1, sp.2 and sp.3, the average degree of flowering: *Rhododendron brachycarpum* D.Don., *Rhododendron arborescens* (Pursh.) Torr., *Rhododendron unguernii* Trautv., sp.5, sp.6;

7) ripening of the species under study mostly happens in September-October, November, while the seed dissemination process – in November-December; *Rhododendron dalavayi* is an exception as it finishes ripening by December and seed dissemination lasts in January-February-March as well;

8) among the species under study, the high degree of fruiting is characterized for: *Rhododendron ponticum* L., *Rhododendron luteum* Sweet., *Rhododendron* sp.1, sp.2., sp.3, and sp.4; The average degree: *Rhododendron dalavayi* Franch., *Rhododendron macrosepalum* Maxim., *Rhododendron unguernii* Trautv., Very weak degree: *Rhododendron brachycarpum* D.Don, *Rhododendron japonicum*(A.Gray) Suringer, *Rhododendron arborescens* (Pursh.) Torr., sp.5, sp.6;

9) defoliation of the evergreen species under study: *Rhododendron arboreum* smith var. *Roseum*, *Rhododendron dalavayi* Franch., *Rhododendron brachycarpum* D.Don, *Rhododendron ponticum* L., *Rhododendron smirnowii* Trautv., *Rhododendron unguernii* Trautv., sp.1, sp.2., sp.3, sp.4, sp.5, sp.6, takes place in summer months and mostly ends by late August whereas the deciduous species: *Rhododendron japonicum*(A.Gray) Suringer, *Rhododendron arborescens* (Pursh.) Torr., *Rhododendron macrosepalum* Maxim., *Rhododendron luteum* Sweet., start change of leaf color in late summer and defoliation - in September-October, till November;

10) The species are characterized by very weak development, neither flower nor give fruit: *Rhododendron smirnowii* Trautv., *Rhododendron arboretum* var. *Roseum* Lindl.;

11) it is remarkable that the fall of temperature below 0°C in winter does not damage the plants or hinder the flowering process;

12) in our opinion, weak fructification of the species under study in certain years must be due to the absence of some pollinating insects in that period.

4. Among the research objects, *Rhododendron smirnowii* Trautv.ex Regel, *Rhododendron unguernii* Trautv.ex Regel., which are characterized by a perfect rhythm of growth and development in natural conditions, are distinguished by a weak degree of adaptation in the conditions of Batumi Botanical Garden.

5. Positive results were obtained in the propagation experiment of the species represented by single specimens - *Rhododendron dalavayi*, *Rhododendron brachycarpum*, *Rhododendron japonicum*, *Rhododendron arborescens*, *Rhododendron macrosepalum*: 1) by seed propagation - *Rhododendron dalavayi*, *Rhododendron japonicum*; 2) by stem graft propagation - *Rhododendron brachycarpum*; 3) by tissue culture propagation the results are obtained: in the case of *Rhododendron dalavayi*, *Rhododendron japonicum* species.

6. As a result of studying the antimicrobial action of extracts obtained from the leaves of *Rhododendron japonicum*, *Rhododendron arborescens*, *Rhododendron brachycarpum*, *Rhododendron macrosepalum*, *Rhododendron dalavayi*, on the example of fungicidal and fungistatic actions, it has been determined: extracts obtained from *Rhododendron dalavayi* and *Rhododendron brachycarpum* are characterized by very high antimicrobial action on the example of fungicidal and fungistatic activity.

6.1. The highest fungicidal activity was observed in the case of dilution of ethanol extract 1:1, 1:2, in the case of fairly high dilutions of 1:4 and 1:8, in the rest of the cases there was a clearly expressed fungistatic activity; in the case of aqueous extracts (tinctures), high fungicidal action was detected in relation with fungi *Phytophthora infestans*, *Alternaria alternata*, *Alternaria solani*, *Pestalotia theae*, *Fusarium moniliforme*, whereas fungistatic – in relation with the fungi *Trichothecium roseum*, *Pestalotia coryli*, *Fusarium moniliforme*. The rest of the species of *Rhododendron* under study are characterized by weakly expressed fungistatic activity.

6.2. The high antimicrobial activity of the extracts of the leaves of *Rhododendron dalavayi* and *Rhododendron brachycarpum* is one of the factors confirming the health-promoting importance of their environment. It is also noteworthy for the study of the content of the biologically active substances.

7. Up to 50 new species and forms of the genus *Rhododendron* L. have been obtained and cultivated on the basis of new production works in order to refill the collection of the Batumi Botanical Garden on the basis of new production works.

8. Based on the research and the introduction works, in the program of replenishment-renewal of the 2021 collection of exotic plants of the Batumi Botanical Garden, the cultivation activities of the *Rhododendron* collection are included.

9. The performed research allows us to conduct in-depth research on the practical, sustainable use and further complex approach to the species of genus *Rhododendron* L.

Annex to the dissertation – the Annex provides the following issues performed by the PhD student: new introduction species of genus *Rhododendron* L. At the Batumi Botanical Garden; results of propagation of *Rhododendron* L. Species by seeds at the Batumi Botanical Garden through the Seed Exchange Fund; determination of the age of *Rhododendron* L. sp. species; results of the soil analysis of the locations of the genus *Rhododendron* L. Species under study at the Batumi Botanical Garden; results of the study of *Rhododendron decorum* Franch at the Batumi Botanical Garden in 2016-2018.

Annex 1 – while working on the dissertation topic, the PhD student conducted introduction of new species of genus *Rhododendron* L. In 2018, 92 seedlings of 29 species, up to 15 cm tall, were imported from the Minsk Central Botanical Garden in order to increase the collection of the representatives of *Rhododendron* genus at the Batumi Botanical Garden. Of these, 21 species are new to the collection, 4 reintroduced and 4 – replenished, 2 species are on the IUCN World Red List of rare and endangered plants... bio-morphological characterization and the results of phenological observations are provided; they are quite well adapted to the soil-climatic conditions of the Batumi Botanical Garden and are in active growth and development process.

Annex 2 – provides the dynamics of the germination of up to 30 species and forms of *Rhododendron* seeds obtained through the Seed Exchange Fund between the Botanical Gardens (seed

origin, number of seeds, seed size, sowing time, germinations – first, massive, final; time required for germination; degree of germination) and the results of observations on seedling growth and development.

Annex 3 – for the purpose of further identification and study of the sp. species expressed in the dissertation, preliminary research has been carried out – age determination works, counting annual rings using the Pressler Drill, without damaging the plant. The results are given in the Annex.

Annex 4 - provides the results of the soil analysis of the locations of the genus *Rhododendron* L. species under study, at the Batumi Botanical Garden.

Annex 5 – the results of the study of the *Rhododendron decorum* Franch. species at the Batumi Botanical Garden in 2016-2018, which was the only rather old and weak specimen and perished due to mechanical damage. Attempts at various methods of reproduction, including in vitro, have failed.

List of publications:

1. M.Kandelaki, V. Filipenia, M.Metreveli, I. Valodzka, L. Goncharova, J.Jayeli, A.Meskhidze. (2020) Outcomes of Introducing Some Species of the Genus *Rhododendron* L. to In Vitro Culture, IJSRM- International Journal of Science and research methodology; New Delhi, India, ISSN 2454 2008 Vol.:16, Issue 4 pp. 93-104 DOI:10.251666Impact.Factor 6,418, <http://ijsrm.humanjournals.com/>
2. M.Kandelaki, M. Metreveli, V.Papunidze. Growth and Development Peculiarities of Rare, Single and Highly Decorative Introduced Species of *Rhododendron* L. Genus in Climatic Conditions of the Batumi Botanical Garden, (2020) Bulletin of the Georgian Academy of Sciences Tbilisi, ISSN - 0132 - 1447 vol.14, no. 4, pp.75-81 Scopus database, bulletin@science.org.ge
3. M.Kandelaki, K.Mchedlidze, K.Shalashvili, M.Metreveli (2020-2021) Microstructural Characteristics of the leaf of *Rhododendron brachycarpum* D. Don, Georgian Medical News, Tbilisi - New York, 202-2021, , [privacy \(geomednews.com\)](http://privacy.geomednews.com) (Accepted for publication)
4. M.Kandelaki, M.Metreveli, V.Papunidze, A.Meskhidze, G.Shakarishvili, L.Kodanovi (2019) Recreational dendroflora in the urban environment of Adjara; Georgian National Academy of Sciences Adjara Autonomous Republic Regional Scientific Centre , ISSN - 0132 – 1447, Transactions V, pg.64-68
5. M.kandelaki, M.metreveli “Medicinal species of *Rhododendron* L. growing in Batumi Botanical Garden, Conference Hall of Hotel “Radisson Blu”, Batumi, Georgia, (2017), International scientific Conference “ Future technologies and quality of life”, pg.119-120
6. M.kandelaki “Diversity of Genus *Rhododendron* (*Rhododendron* L.) Growing in Batumi Botanical Garden Batumi Botanical Garden”, Biodiversity and Georgia proceedings of the II scientific conference Tbilisi 2016, National Botanical garden of Georgia, pg 20-21;

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