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Green technologies of introduction and processing of some aromatic plants

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Specialty: **Plant Biodiversity**

A N N O T A T I O N

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Introduction

Theme topicality:

Aromatic plants take a special place in the world of plants. They vary with their wide range of diversity and the origins of their natural coverage. More than 2000 species of aromatic plants are known for the world, among them 43.6% grow in tropics, 9,3% in the subtropical zone, 19,5% in the temperate zone and the rest of the species can be found in various zones (*Marshall,* 2011).

Production of aromatic substances was intensively developed in the middle centuries. Essential oils were widely used in the pharmaceutical, food and especially, perfumery industry. Despite the development of synthetic substances, natural essential oils appear irreplaceable for making food, medicinal, perfumery, cosmetic production, etc. They have the biggest medicinal qualities. Medicinal-aromatic plants play a significant role all over the world in terms of economic, social, cultural and ecological aspects. These are secondary products of herbal metabolism used for producing medicinal preparations to avoid different diseases and preserve health. Besides officinal medicine, aromatic-healing plants are used in traditional medicine by 80% of the world population. Most aromatic plants used in officinal and folk medicines grow wild. Therefore, the conservation of aromatic plants in nature and their intensive cultivation has recently become a very topical issue due to increased consumption of aromatic plants, commercial activities and their influence on habitats and species (*Marshall Elaine*, 2011; *Aftab,* 2020; *Patel,* 2015).

The stock of wild-growing aromatic plants in Georgia is not big. However, the natural climatic conditions of our country allow their cultivation. This is proved by industrial plantations of geranium, jasmine, rose, basil, etc. created and cultivated by the introduction in Georgia last century. They were used to produce essential oils. Today, essential oils are imported rather than exported.

Anthropological influence on natural phytocenosis of wild-growing plant species causes catastrophic reduction of the stock or extinction of some species. The said problem is topical in terms of an economical point of view as the contemporary industry (food, pharmacy, etc.) requires a new base of guaranteed raw materials for the development, which can be achieved through the introduction or cultivation (Batumi Botanical Garden 100, 2012; *Бойкова, 2013; Баханова, 2009; Кочетов, 2008).*

Deliberate research about the introduction of some plant species containing essential oils started in the 1960s. Many scientific works done by Georgian and foreign scientists were dedicated to the mentioned issues.

Georgia has culture, traditions and experience of cultivating aromatic plants with the help of introduction and producing essential oils from them. It must be also noted, that nowadays, special attention is given to creating a healthy environment and products without chemistry but using green technologies. *Organic* quality production is possible to get from ecologically clean raw materials introduced and cultivated without poisonous chemicals, chemical pesticides, etc. Therefore, the introduction and recycling of plants containing essential oils with the help of green technologies are one of the topical issues.

Botanical gardens are the best venues for studying the process of introduction and cultivation of aromatic plants. In some countries, they were established as collection and research centers for live collections of medicinal, aromatic, edible, decorative and other plants of local and foreign flora (*Bidzinishvili,* 2012).

Gardens of medicinal plants are available in many botanical gardens of the world comprising the collections of medicinal-aromatic plants with various usage and significance. The collection of medicinal-aromatic plants is not separated in the Batumi Botanical Garden. However, lots of introduced aromatic hardwood species are available in the collections of hardwood plants of the garden. It is noteworthy, that the stock of wild-growing aromatic plants is not big in Georgia. Therefore, researches on the introduction and cultivation of aromatic plants are important to be carried out.

Research aims and objectives

The research aim is the introduction of some aromatic plants in the Batumi Botanical Garden by green technologies and elaboration of green technologies for their processing .

The following tasks were accomplished to achieve the goals:

- Selecting aromatic plants for introduction;
- Analyzing general biological and ecological characteristics of donor floristic regions;
- Analyzing host soil and climatic conditions, host plants and limiting factors of introducent aromatic plants; selecting the adaptation conditions for introduced aromatic plants
- Testing of the primary introduction of some aromatic plants;
- Elaborating agrotechnical activities for growth and development of introduced aromatic species without chemistry but natural materials;
- Studying growth and development peculiarities of introduced aromatic plants;
- Identifying the adaptation opportunities and quality of introducent aromatic plants;
- Studying microstructural characteristics of vegetative and generative organs of introduced aromatic plants;
- Studying the content of bioactive compounds of aromatic plant materials introduced by green agrotechnical activities on the locations of the Batumi Botanical Garden by Gas chromatography – mass spectrometry *GC/MS* method;
- Screening the extracts of introduced aromatic plants on antibacterial activities.

Research object:

At the first phase of the research, the research objects were 14 aromatic plant species introduced by us in the Batumi Botanical Garden.

At the second phase of the research, the aromatic species - *Polianthes tuberosa* L., *Iris pallida* Lam., *Cuminum cyminum* L. introduced by us in the soil and climatic conditions of the Batumi Botanical Garden by „green technologies" were selected as the final research objects.

Scientific novelty - The growth and development characteristics of some aromatic plants at seven different locations of the Batumi Botanical Garden with various expositions and soils and agrotechnical activities necessary for the perfect development of their life cycles without any chemical means were studied for the first time, at the base of the Batumi Botanical Garden. Most of them are introduced to the soil and climatic conditions of the Batumi Botanical Garden for the first time, the rest of them is reintroduced.

Based on the studies of the aromatic plant raw materials on the content of bioactive substances introduced without chemical means under the conditions of open soil of Batumi Botanical Garden, significant compounds were detected. They have antioxidant, antipneumonic, antimicrobial and other qualities; Part of them is known and used in medicine, perfumery, cosmetics, food industry, culinary and other various directions. Their antibacterial activities are studied. The content of essential oils received from aromatic plants is detected.

Practical values – Scientific basics of green technologies of growth-development and processing of some species of aromatic plants Polianthes tuberosa L., Iris pallida Lam., Cuminum cyminum L., has great importance for the creation of the base of ecologically clean raw materials.

Green technologies for obtaining essential oils from introduced aromatic plants are elaborated.

Introduction and processing of essential oil-containing plants by agrotechnical activities provide the creation of an ecologically healthy environment and production, which will support the preservation and enrichment of the diversity of flora. The said issue represents a challenge for the contemporary world.

Based on this paper, the first experimental plot of aromatic plants in the history of the Batumi Botanical Garden was established and the collection of aromatic-spicy plants was decided to arrange.

Thesis approbation:

Research outcomes as the basis for the thesis were presented at international scientific conferences: II Scientific-Practical Conference -Biodiversity and Georgia (Tbilisi, 2016); III Scientific-Practical Conference - Biodiversity and Georgia (Tbilisi, 2017); International Scientific Conference – Future Technologies and Life Quality (Batumi, 2017); Scientific-Practical Conference - Green Medications – By Green Technologies For Healthy Life (Tbilisi, 2019); Young scientist and Student Conference – Actual Issues of Contemporary Biomedicine (Batumi, 2019); International Symposium in Veterinary Medicine (Akhaltsikhe, 2021).

Publications - Seven 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 185 printed pages, comprising of an introduction, 11 chapters, 19 sub-chapters, conclusions, bibliography and annex. The paper also covers 9 tables, 72 photos and a bibliography, including 143 titles among them are 116 foreign ones. The thesis annex covers 6 appendices including 1 table and 56 pictures (chromatograms and mass spectra of chemical compounds)

Bibliography overview

The first chapters of the thesis analyze the results of literature overview: diversity of aromatic plants, cultivation history and practical values; Concept and importance of introduction and production of aromatic plants by green technologies; General description of aromatic plants newly introduced to the Batumi Botanical Garden; Comparative description of soil and climatic conditions of the Batumi Botanical Garden and natural spreading areas of research aromatic plants; General meteorological data of Batumi coastline in 2015-2020.

Research outcomes are included in **the experimental part**, fourth and further chapters:

Chapter IV. Research venue, object and methods

IV.1. Research venue:

At the first phase of the research, the research objects were 14 aromatic plant species introduced by us in the Batumi Botanical Garden:

1. *Cuminum cyminum* L.
2. *Polianthes tuberosa* L.
3. *Crocus sativus* L.
4. *Iris pallida* Lam.
5. *Curcuma longa* L.
6. *Pogostemon patchouly Pellet. = Pogostemon cablin.* (Blanco).
7. *Cananga odorata* (Lam.) Hook.f. & Thomson
8. *Zingiber officinale* Roscoe.
9. *Elettaria cardamomum* (L.) Maton.
10. *Cistus ladaniferus* Stokes.
11. *Coffea arabica* L.
12. *Coffea canephora* Pierre ex A.Froehner.
13. *Vanilla planifolia* Jacks. ex Andrews.
14. *Cassia angustifolia* M.Vahl.

At the second phase of the research, the aromatic species - *Polianthes tuberosa L.*, *Iris pallida* Lam., *Cuminum cyminum* L. introduced by us in the soil and climatic conditions of the Batumi Botanical Garden by green technologies were selected as the final research objects.

IV.2. Research methods and research venue

studying growth and development peculiarities and phenological phases of introduced aromatic plant species under the local soil and climatic conditions were carried out by Beideman and Serebriakov, Elagin and Lobanov's methods (*Beideman,* 1974; *Elagin,* 1979; *Serebriakov,* 1974). The research was conducted on the base of the Batumi Botanical Garden and the Institute of Phytopathology and Biodiversity of the Batumi Shota Rustaveli University.

determined the acidity of the soil, the rate of humus and the content of the main feeding elements, namely, pH, humus %, total nitrogen %, K_2O%, P_2O_5 %. The state method 26107-91 (*Methods for determination of total nitrogen*, *Moskow,* 2019) was applied for the identification of total nitrogen; Phosphorus and potassium moving particles were determined by Oniani's method, modification by the state method 26206-91 (*Phosphorus and potassium by Oniani method modified by CINAO,* 2013, *Moskow).* Phosphorus was determined by phytoelectrocolorimeter at a wavelength of 710nm; Potassium was detected by Atomic Absorption Spectrometry; humus and pH were determined by the express method. The research was carried out based on LEPL Laboratory Research Center of the Ministry of Agriculture of Ajara.

For studying anatomical structure, microstructural characteristics of vegetative and genetative organs of the research species, transversal, Transversal, longitudal 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. the research was carried out at the base of Iovel Kutateladze Parmacochemistry Institute of the Tbilisi State Medical University.

Plants screening on the content of bioactive compounds was carried out by quantitative reactions and thin-layered chromatography (*H. Wagner*, *S. Bladt*, 2nd edition, 2003; *Pharmacopoeial,* 2013; *Vachnadze,* 2012). The research was done at the base of the department of Pharmaceutical Technology of the Tbilisi State Medical University.

For identification of the content of biologically active substances, the study was conducted with gas chromatography-mass spectrometry GC/MS method at the Toxicology and Chemical Expertise Laboratory of Levan Samkharauli Court Expertise National Bureau (Georgia). Raw materials for analysis were dried, received samples were parted in following the requirements of the 10th edition of the State Pharmacopeia. Raw materials taken for analysis were parted under the requirements of the State Pharmacopeia. Each of them was weighed by electric scale *(AMERICAN WEIGH SCALES, Model No.: PNX-1001 SN; Capacity. 1000g x 0.1g Operating Humidity: 10-85 % RH; Pover: 12V DC 500Ma Max Operating temp: 10°c to 40°c).* After weighing, raw materials were placed in Erlenmayer flasks. 5,0-5,0 gr of parted plant materials were added 25,0-25,0 ml Methanol. Materials were left for 24 hours at room temperature and then filtered by ash-free filter paper. Filtrates were placed in files and left there in suction boxes for ethanol evaporation.

After evaporation of the organic solvent, mixture of derivatization solvents were separately added to dry remains: *BSTFA/ EtAc* (55:50 mkl), heated at 70^0 C for 20 minutes. After cooling 1-1 mkl Was studied by tandem chromate mass spectrometry – device: Agilent Technologies 7000 GC/MS/MS Tripe Quad; column - Elite 5-MS; 30MX250 μm X 0,25 μm; furnace temperature - 60C-310C (program regime); injector temperature - 250C; transfer line temperature – 310C; airborne – helium 1ml/m, ionization source - El-70 ev; scanning regime - TIC. For identification of the target substance in the object under study, mass spectrums of the peaks existing on chromatographs were compared with the mass spectrums of the substances existing in the database (NIST 2016).

Essential oils were received from iris roots, tuberose flowers and cumin seeds by so-called Green Extraction methods or green technologies: hydrodistillation; steam distillation; liquefied gases, microwave-assisted hydrodistillation, microwave-assisted distillation without solvents and ultrasound-assisted hydrodistillation extraction (*Войткевич,* 1999; *Хохлов и др.*, *МЕЖГОС. СТАНД. ГОСТ* 34213 — 2017; *Сафин и др.*, *Jibrin et all.,* 2014). We used the Clevenger apparatus for hydrodistillation and *EURO FOOD and BREW* machine for steam extraction. With the help of the said machine, essential oils are obtained by steam; after the steam gets cold, condensate appears in the oil collector. Technical characteristics for the machine are as follows: steam temperature - 90-95^{0}C, steam pressure - 0,2 bar, essential oil obtainability - 0,1-100 g/h., essential oil extraction time - 2 hours; microwave-assisted extraction was conducted with an extragent (water) and without it. Obtaining essential oils without extragents takes place by microwave heating energy and dry distillation methods (Pic.N1); The extraction of essential oils was carried out by a combined method: ultrasound and hydrodistillation. At first, raw materials are loaded in an evaporating flask, water is added to and treated by ultrasounds. Ultrasonicators are used as the source of ultrasounds. The amplitude of ultrasounds consists of 60% (ultrasound frequency is equal to 20 kHz). Ultrasound treatment duration is 10 min. After ultrasound treatment, essential oils are obtained by hydrodistillation with the Clevenger apparatus (Pic.N2). The obtainability of essential oils in percentages (%) was determined by calculating the absolute dry mass of raw materials. The research was done at the base of the department of Pharmaceutical Technology of the Tbilisi State Medical University.

Studying the antibacterial activities of the plant materials was conducted under *in vitro* conditions by the *Spot test (scriining)* method. Plant excerpts were prepared by the maceration method. Bacterial pathogens causing various human diseases were used while testing. For the identification of antibacterial activities, the research was carried out at the base of the department of Pharmaceutical Technology of the Tbilisi State Medical University and the George Eliava Institute of Bacteriophage, Microbiology and Virology.

Statistical processing of outcomes. The research outcomes are processed by the statistical program - Sigma STAT. Each experiment was conducted 3 times minimum and average meanings (Mean = M) and average standard deviations (Standard Deviation =SD) of received results were calculated.

Chapter V

Growth and development peculiarities of *Polianthes tuberosa* L., *Iris pallida* Lam., *Cuminum cyminum* L. introduced to the Batumi Botanical Garden at different locations.

Note: *At the beginning of the research, exotic aromatic plants unavailable for the collection of the Batumi Botanical Garden. For their propagation, growth and studying, we ordered primary materials or seeds and seedlings, up to 30 species from different websites and Seed Exchange Foundation between botanical gardens. For the first time, they were sowed and planted under closed soil conditions or orangery. Based on the observations of sowings and shoots of most species of these plants along with the importance of certain species of aromatic plants, species were selected for the research to be planted in an open soil: Cuminum cyminum* L., *Polianthes tuberosa* L., *Iris pallida* Lam., *Curcuma longa* L., *Zingiber officinale* Roscoe., *Elettaria cardamomum* (L.) Maton., *Coffea arabica* L., *Coffea canephora* Pierre ex A.Froehner., *Vanilla planifolia* Jacks. ex Andrews., *Cassia angustifolia* M.Vahl. *The first experimental plot was separated, where agrotechnical activities for growth and development of these species were carried out without chemicals but Green Technologies. Their growth and development characteristics were studied; screening of obtained raw materials - grass, tubers, flowers, seeds - on the content of bioactive substances was done by thin-layered Chromatography and Gas chromatography - Mass Spectrometry GC-MS method.*

The results of this stage of the research are available in the annex.

Based on the analysis of primary outcomes of the introduction of new aromatic plants to the collection of the Batumi Botanical Garden, we can conclude, that full vegetative and generative development under the conditions of an open soil occurs with the following species: *Polianthes tuberosa* L., *Cuminum cyminum* L., and *Iris pallida* Lam. They flower, partially develop fruit and seeds, which is an indicator of their adaptation to new environmental conditions. The said three species were selected as final objects for our further research.

Tuberose is endemic to Mexico; Pale iris is native to the Balkan Peninsula. Mediterranean cliffy locations are advantageous for them; The genus cumin is considered to be native to Minor Asia. However, the coverage area for true cumin is closer to Eastern and Mediterranean regions, where it has been actively produced and consumed since ancient times. Cumin was known 5000 years BC, its seeds were discovered in fossils in Egypt, that's why the Mediterranean region is recognized as a The research objects belong to dry and warm climates. They require sunlight for a longer time, less humidity in the air, and mild winter for their normal growth and development.

A general description of soil and climatic conditions of the Batumi Botanical Garden is available in the chapter... of the literature overview. Based on the overview, the soil and climatic conditions of Ajra littoral and, particularly, Batumi Botanical Garden is characterized by humid subtropical climate. The main limiting factor in the process of introducing subtropical and tropical plants to Ajara littoral is low winter temperature and heavy precipitation for certain years. Meteorological data of Batumi coastline in 2015-2020 is described in the chapter IV, of the dissertation literature overview. The charts (*https://www.meteoblue.com*) show, that by this period During last 5 years, a bit stricter winter was recorded in 2016 and ongoing 2020, not typical for Ajara littoral, in particular, for the Batumi Botanical Garden. The absolute minimum temperature was -1.9^{0}C in January, in 2016 and for the current year, it fell down to -4,7-6^{0}C in the 2nd decade of February. Moreover, in certain places of the littoral even -10-14^{0}C was confirmed. Average monthly precipitation was extremely high in March, July, August, September, October and December, in 2018-2019, fluctuating between 100,9 mm- 388,5mm. Average relative humidity reaching 86-96 %, which is quite high amount, was revealed in March, May, June-July, August and October, in 2017-2019, while in the other months it achieved 79-82 %.

V.1. Characteristics of different locations in the Batumi Botanical Garden, allocated for the study of the growth and development of the studied aromatic species

Since 2018, for thorough studying growth and development peculiarities and adaptation quality of tuberose, pale iris and true cumin under the conditions of the Batumi Botanical Garden, plant and seed materials obtained from research objects grown on the experimental plot of aromatic plants in 2016-2017 have been planted and sowed in seven different locations with various expositions and soil conditions of different phytogeographical sections of the garden: East Asian, Himalayan, Australian, Mediterranean (European), North American sections, central park and experimental plot. Since 2017-2018, the second experimental plot was selected for aromatic plants, which is located closer to the East, with good sunlight, but more humid than the previous one.

In all locations, the plant was placed in 15-20 cm deep and 20 cm diameter pits added to substrate peat-perlite-ground with the ratio-1:1:1. Considering literature data, hardwood plant surroundings and the degree of their phytoncidic activity was carefully observed, as phytoncids are extremely important for improving the air quality and preventing the development of pathogen microorganisms.

Location №1 lies on the Central Park territory. *Hamamelis mollis, Loropetalum chinense, Myrtus communis, Eucalyptus viminalis, Eucalyptus cinerea* create the habitat around. All of them are the plants with high microbial activity *(Metreveli, 2008).*

Location №2 is situated in Australian floristic department. *Laurocerasus officinalis, Eucalyptus cinerea, Hakea saligna, Abelia grandiflora and Aucuba japonica* create the habitat around. Cherry laurel, eucalyptus and abelia are distinguished by enough high antimicrobial activity.

Location №3 can be found in Himalayan floristic department, in the habitat of pine trees: *Pinus massoniana, Pinus pinaster, Pinus pallasiana, Vaccinium arctostaphylos, Styrax yaponica.* Pine trees are well-known environment improving coniferous plants.

Location №4 is positioned in East Asian floristic department. The following plants creat the habitat: *Parrotiopsis jacquemontii, Corylopsis veitchiana, Mahonia lomariifolia.* All the three species share high antimicrobial activities *(Metreveli, 2008).*

Location №5 can be found in North American floristic department. The habitat is created by the group of *Hamamelis virginianas*, also *Pinus taeda, Pinus sylvestris, Crataegus macrosperma var. Pastorum, Crataegus lucorum, Crataegus pringlei, Catalpa bignonioides.* Hamamelis and pine trees possess strong antimicrobial activities *(Metreveli, 2008).*

Location №6 lies in European floristic department. *Parrotia persica, Cerasus avium* create the habitat around. Iranian ironwood is distinguished by high phytoncidicity (*Metreveli, 2008,2017).*

Location №7 - Unlike other locations, the experimental plot is located in an open, vast territory with good sunlight, no shades by other plants.

Bio preparations are considerably beneficial for the growth and development process of tuberose. Therefore, we applied for the bio preparation called GeoHumate. It is 100% natural preparation, special liquid humic fertilizer for active growth and development of the plant, distinguished by high penetrability in the soil without the risk of phytotoxicity. Physical and chemical composition of the bio fertilizer is the following: 12% liquid; fraction of total mass of organic compound, not less than 12.5% including humic acid not less than 34%, fulvic and other organic acids reach not less than 25%; fraction of total mass of mineral compound is not less than 1.0% including fraction of total mass of macro elements: $N \geq 1,2\%$, $P_2O_5 \geq 0,55\%$, $K_2O \leq 16,5$, $CaO \geq 0,56\%$, $S < 2,1\%$, $MgO \leq 0,32\%$, $Fe_2O_5 \leq 0,5\%$. Fraction of total mass of microelements: $ZnSO_4 \leq 0,41\%$, $CuSO_4 \leq 0,08\%$, $MnSO_4 \leq 0,08\%$, $CoSO_4 \leq 0,03\%$, $(NH_4)_2MoO_4 \leq 0,7\%$, $H_3BO_4 \leq 0,3\%$, $KIO_3 \leq 0,03\%$.

20 ml bio preparation was diluted in 5 l water; tuberose was watered in the morning hours, the plants were watered every three days, some plants were isolated for control.

For the analysis of the outcomes of phonological observations of the plants, it is important to study the soil content along with exposition peculiarities, as the presence of elements in the soil is essential for plant metabolism and full life cycle; the soil is one of the main factors for plant introduction. Therefore, we studied soil samples taken from all seven locations.

The outcomes of the analysis of soil samples are available in Table №1, it's clear that some results according to locations differ from each other.

The Himalayan, East Asian and European phytogeograpical sections are distinguished by fertile solil among selected locatins.

Table №1

The outcomes of the analysis of soil samples taken from different locations of the Batumi Botanical Garden

№	Location	Arrangement of the locations in the Batumi Botanical Garden	Rate of the content of acidity, humus and basic nutrients of soil				
			pH	Humus %	Common nitrate %	K_2O%	P_2O_5%
1	№1	Central park	4	2	0.1	0.06mg/l	35
2	№2	Australian phyto-geographical department	4.5	1	0.05	0.08 mg/l	12
3	№3	Himalayan phyto-geographical department	5	5.0	0.25	0.08 mg/l	18
4	№4	East Asian phyto-geographical department	4.5	3	0.15	0.08 mg/l	10
5	№5	North American phyto-geographical department	5.0	1	0.05	0.08 mg/l	20
6	№6	Mediterranean (Europe) phyto-geographical department	5.0	3	0.15	0.08 mg/l	35
7	№7	Experimental plot of aromatic plants	4.5	1	0.05	0.06 mg/l	18

V.2. Growth and development peculiarities of *Polianthes tuberosa* L. in different locations of the Batumi Botanical Garden

The tubers of *Polianthes tuberosa L.* were planted in seven different locations with various expositions and soil conditions of different phytogeographical sections of the Batumi Botanical Garden: East Asian, Himalayan, Australian, Mediterranean (European), North American sections, central park and experimental plot. The vegetative development took place very well at all locations.

On the basis of phonological observations conducted in 2019, it was identified, that flowering stem was developed only in the locations of the experimental plot and East Asian section, in the second decade of July. Massive flowering period started in the third decade of August. The first decade of September was a period of decreased flowering; flowering period was finished in the third decade. Flowering stems appeared again in the 1st decade of November, on the territory of the experimental plot. Massive flowering started in the 3rd decade of November and finished in the 3rd decade of December. The flowering stem was 85-93 cm tall. The first blooming period was longer than the second one. Its blooming period was finished earlier in the East Asian section than in the experimental plot; in the end of August, the flowering period was fully finished. The flowering stem was 45-49 cm tall.

In accordance with 2020 observations, tuberose developed multiple child tubers underground in the 2nd decade of January, in the experimental plot and all locations. Its aboveground part appeared in the 2nd decade of May reaching 5-15 cm in the 3rd decade of May. The shoots were best visible in the experimental plot, central park and East Asian section. The flowering stems were well-developed in the experimental plot, at the end of July –beginning of August. Massive flowering started in the end of August. The main distinguishing points of the flowering period of tuberose compared to the other years, were multiple catkins on the flowering stem as well as its typical subtle, sweet, strong aroma, that could be smelled for the first time (Pic. №3). East Asian phyto-geographical section was noted as the location with less flowering quality, while in the other locations, flowering stems appeared difficult to develop.

While blooming, a spike-shaped catkin is developed on the top of a flowering stem of tuberose containing about 10-40 flower buds. Tubular flowers are 50 mm wide and 60 mm long. Pinkish smooth and dense crown petals have sharp tops. Buds are gradually opened, ones located in the bottom of the catkin are first to open. They stay open during 3-4 days, then finish flowering and the other buds start to open; flowering period continues for a long period, during 2-3 months and more. At the place of shriveled flower, there are developed box-type oblong, oval-shaped fruit-like formations. However, at this stage, it is impossible to develop the seeds in the conditions of the Batumi Botanical Garden. Therefore, only vegetative propagation is available with the help of fresh child tubers.

We planted the tubers with conical shapes; their surfaces are covered with dense, brown scales, 50-60 mm in diameter. Based on our observations, lifespan of tubers includes up to 2 years – after the processes of their germination, stem development, leafing, bud development, flowering, end of flowering and withering of above-ground organs, child tubers start to develop; the same cycle is repeated for the next year and for the third year, the lifespan of the main tuber is finished and child tubers are used for planting materials.

The best development (flowering) of tuberose was revealed at the experimental plot, while the least efficient appeared the dry, inclined slope of the East Asian section. Following the table (№3), the experimental plot isn't distinguished by the content of microelements. We think, that, unlike other locations, the experimental plot is located in more open, a sunlit vast territory with no shades from the other plants, which has one of the crucial significance and also, treatment with Geohumate rich in organic and mineral substances helping plants to grow and develop properly. It is also noted in the instruction, that the said bio preparation protects plants from harmful diseases.

Harmful diseases typical to tuberose are frequently discussed in the literature. Some pests affect badly with crops. They are *Thysanoptera* and *Red spider – Tetrahychus,* possible to control by insecticides. In recent years, manufacturers from Mexico have noticed, that some damages are caused by *Scyphophorus acupunctatus* (*Camino, 2002*). *Fusarium oxysporium* is known as a fungus disease typical to tuberose, causing quite a big loss of crops (*Muthukumar, 2006*). No harmful diseases were detected on our research objects, which may be conditioned by phytocindic plants in the surroundings and the positive influence of bio preparations.

V.3. Growth and development peculiarities of *Iris pallida* Lam. at different locations of the Batumi Botanical Garden

The bulbs of *Iris pallida* Lam. were planted in seven different phytogeographical sections with various expositions and soil conditions of the Batumi Botanical Garden East Asian, Himalayan, Australian, Mediterranean (European), North American sections, central park and experimental plot. (Pic. №50). The vegetative development phases were conducted normally at all locations, flowering differs according to the locations, vegetative and generative development is more efficient among the examples treated with bio preparations. Full vegetative and generative development was revealed at the experimental plot of aromatic plants, while Australian and Himalayan phytogeographical sections appeared less efficient. Under the conditions of the Batumi Botanical Garden, from the second decade of January, *Iris pallida* starts to develop its above-ground organs, this process is revealed at every location; its flowering stem is developed in the second decade of April, massive flowering starts in the end of April and the first decade of May. In 2020, the first fruit-bearing was pointed at the experimental plot and Himalayan phytogeographical section. Flowering ends at the end of May in parallel with the fruit development. The fruit starts ripening in the middle of July, brown spots are visible on some areas of the fruit. The first fully ripen fruit was detected at the end of July. As for the Himalayan phytogeographical section, the first fully ripen fruit appeared at the end of August. The height of the flowering stem reaches 75-95 cm at the experimental plot and 40-45 cm at the Himalayan phytogeographical section. The seed is in the box, which turns brown while gets ripen. Iris developed perfect seeds at the experimental plot among seven locations of the Batumi Botanical Garden. The seed was matured at the end of July at the said location, although for the Himalayan phytogeographical section, seed ripening occurred at the end of August and the first half of September.

V.4. Growth and development peculiarities of *Cuminum cyminum* L. at different locations of the Batumi Botanical Garden.

Cuminum cyminum L. were sowed at seven different phytogeographical sections with various expositions and soil conditions of the Batumi Botanical Garden East Asian, Himalayan, Australian, Mediterranean (European), North American sections, central park and experimental plot. *Cuminum cyminum* L. was sowed in March-April, 2019. The first shoots at these locations were visible at the beginning of April and massively appeared in the third decade of April. *Cuminum cyminum* started to develop its first leaves in the first decade of May, while flowering started at the end of July and the beginning of August. Massive flowering starts from the end of August continues for a long period and some examples at the experimental plot are still in blossom in the second decade of January, while some of them develop fruits. Based on literature sources (*Rezvani,* 2014), for increasing cumin seed production, it is better to sow them in Autumn. Therefore, we conducted one experiment by sowing the seeds in Autumn (19.11. 2019). The first plantings appeared in the third decade of December. The leaves started to develop in the second decade of February, flowering started in June and August was pointed out as its massive flowering period. The seed got matured in Autumn (IX-X). If compared the plants sowed in open soil in Spring and Autumn, it is obvious, that plants sowed in Spring accomplish the full cycle of their development sooner. Plants treated with bio preparations are much better in growing and fruit-bearing too.

The plants seeded in Spring, 2019, especially at the locations of the experimental plot and the Central Park, are distinguished by normal vegetative and generative development, based on observations done in Autumn, 2020. The most efficient appears the development of plants treated with bio preparations, they bloom and form seeds.

If compared the development peculiarities of the plantings of *Cuminum cyminum* L. from various locations of the garden, sowed in 2019-2020 and the plantings at the experimental plot taken from the orangery in 2016-2017, we can conclude, that the best development, massive flowering and seed-bearing was revealed at the first experimental plot (Pic. №53), where plants were placed 15 cm distance from each other and the seeds were sowed densely. The seeds were distinguished by the high quality of development, the plantings were not replaced, which caused the weakening of their development. Therefore, after appearing the very first shoots of *Cuminum cyminum* L., it is necessary to move them to another place.

Growing introduced aromatic plants *Polianthes tuberosa* L., *Iris pallida* Lam., and *Cuminum cyminum* L. by green technologies at seven different phytogeographical sections with various expositions and soil conditions of the Batumi Botanical Garden is possible to carry out successfully, they get on quite well with soil and climatic conditions of the Batumi Botanical Garden (see the certain paragraphs of the conclusions about growth and development in the conclusions) (Table №2).

Table №2

Basic phenophases and phenointervals of vegetative and generative development of *Polianthes tuberosa L.*, *Iris pallida* Lam., *Cuminum cyminum* L. in the Batumi Botanical Garden

№	Species	Phenophase	Month											
			1	2	3	4	5	6	7	8	9	10	11	12
1	*Polianthes tuberosa* L.	Vegetation duration												
		Flowering												
		Fruit and seed ripening period												
2	*Iris pallida* Lam.	Vegetation duration												
		Flowering												
		Fruit and seed ripening period												
3	*Cuminum cyminum* L.	Vegetation duration												
		Flowering												
		Fruit and seed ripening period												

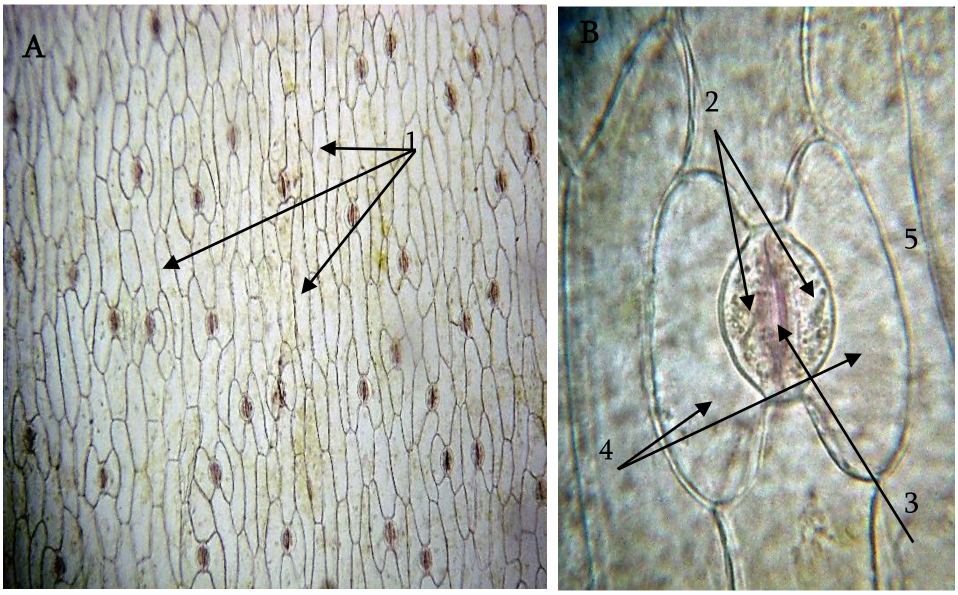
Chapter VI

Microstructural characteristics of vegetative and generative organs of *Polianthes tuberosa* L., *Iris pallida* Lam., *Cuminum cyminum* L.

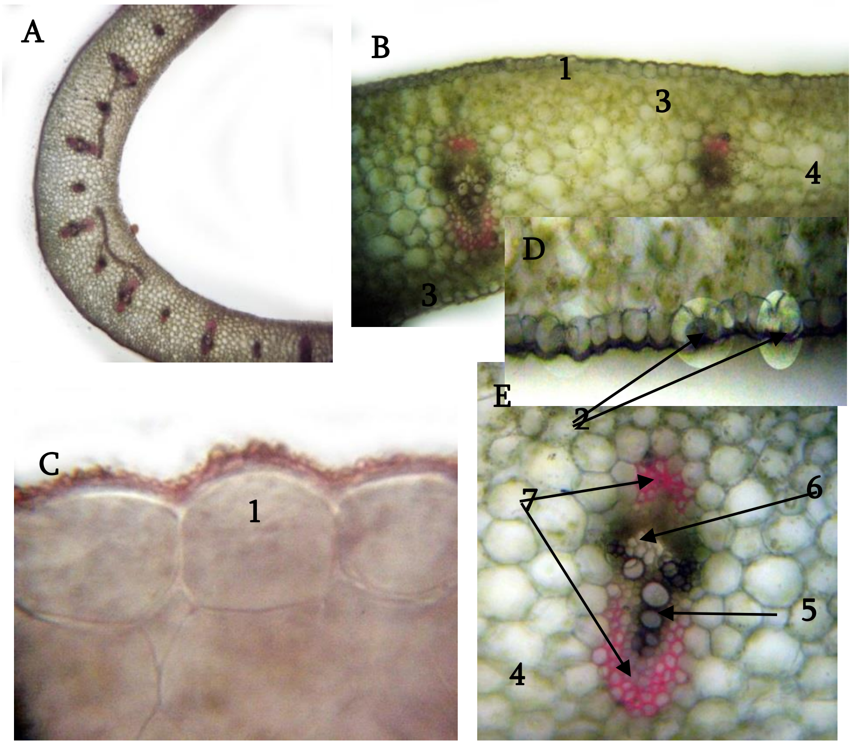
We studied microstructural characteristics of underground and aboveground vegetative organs - root, tuber and the leaf of *Polianthes tuberosa* L.; Macro and microstructural characteristics of vegetative and generative organs - leaf, stem and fruit of *Iris pallida* Lam.; Macro and microstructural characteristics of underground and above-ground vegetative organs - leaf, stem and the leaf of *Cuminum cyminum* L. (methodology, see the chapter IV)

Research raw materials are obtained from *Polianthes tuberosa* L., *Iris pallida* Lam., *Cuminum cyminum* L. introduced to the Batumi Botanical Garden by green technologies in 2020 during the active blooming phase. The method is described in chapter №5.

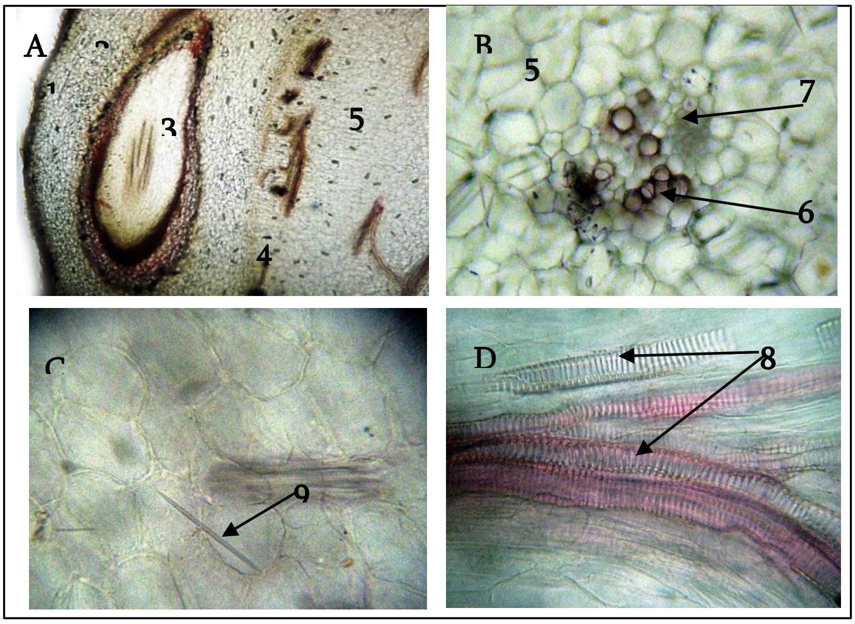
Microstructural characteristics are described in three chapters of the thesis including 12 subpoints, which is a huge amount of materials collected by decoding and describing of below-mentioned materials based on microscopic research. (Pic. №1-14).



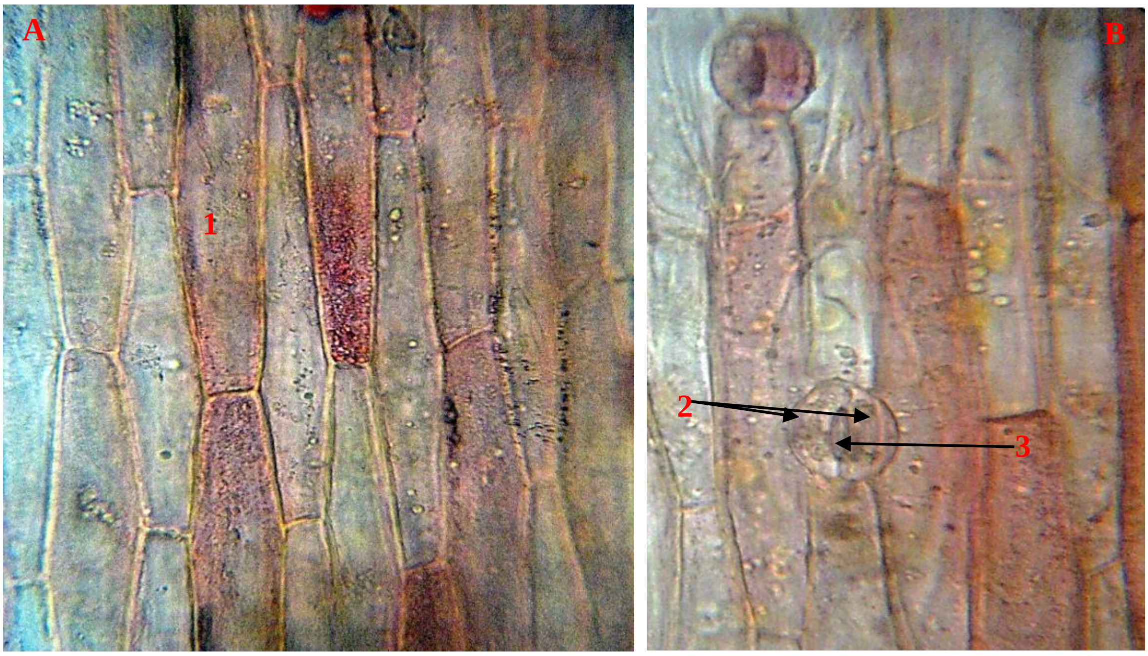
Pic. №1. Epidermis microstructure of the leaf of Polianthes tuberosa L.: *A.panorama of root-based tissue of a leaf epidermis; B. lamina apparatus; 1. not quilted, rectilinear, thimble-rhombus type cells; 2. lamina locking cells; 3. crossluminal hole; 4. satellite cells; 5. chloroplasts*



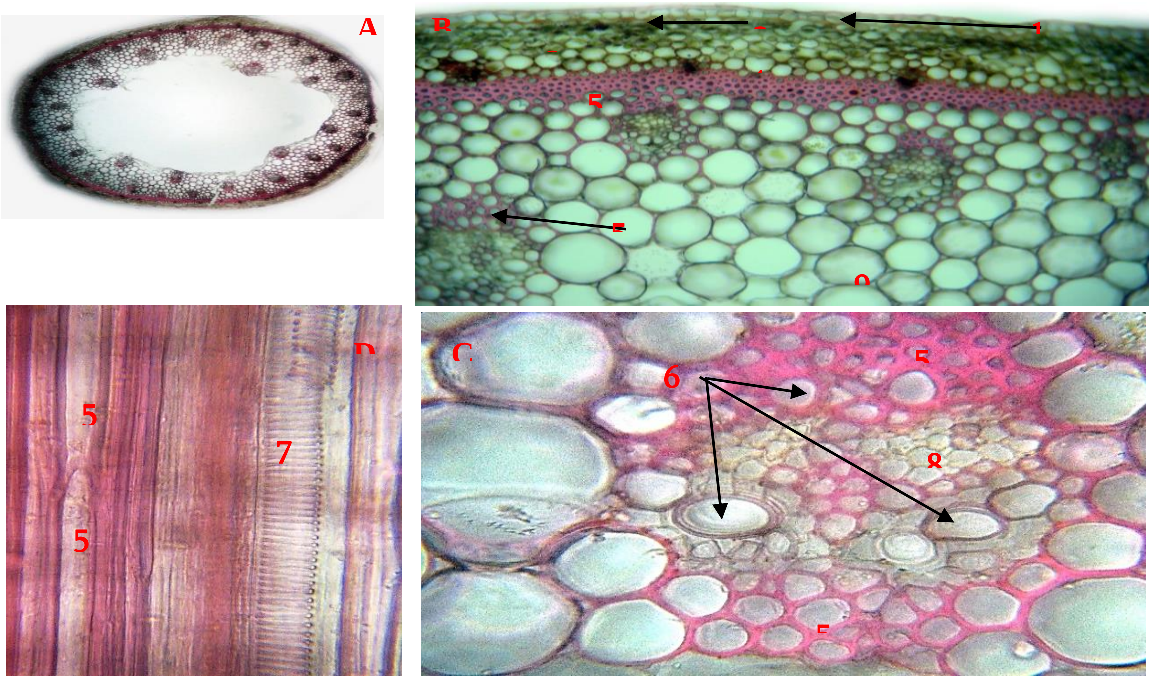
Pic. №2. Microstructure of the leaf of Polianthes tuberosa L.*: A. fragment of transverse cut of a leaf; B. view of a leaf pulp; C. fragment of the cutinized epidermis; D. luminal histology in covering tissue; E. vessel-fiber collateral vascular bundle of a leaf. 1.cuticle, epidermis; 2. lamina apparatus; 3. cells of the palisade parenchyma; 4. spongy parenchyma; 5. wood; 6. phloem; 7. sclerenchemical tissue.*



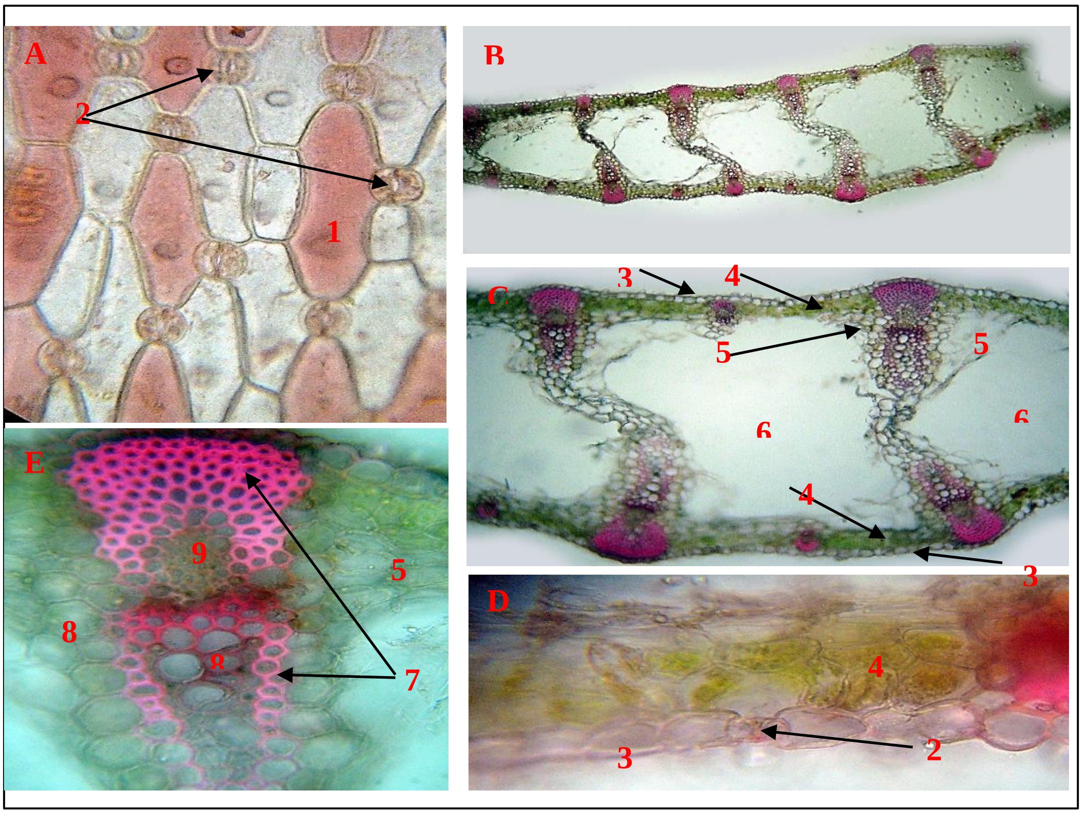
Pic. №3. Microstructure of the tuber of *Polianthes tuberosa* L.: *A. structural panorama of the tuber; B. vascular bundle; C. raphid; D. fragment of vascular bundle in a longitudal view:1. periderm; 2. differentiated embryo 3. bark parenchyma; 4. cambium; 5. central cylinder 6. wood; 7. phloem; 8. vascular vessels with spirally thickened membrane; 9. Raphids.*



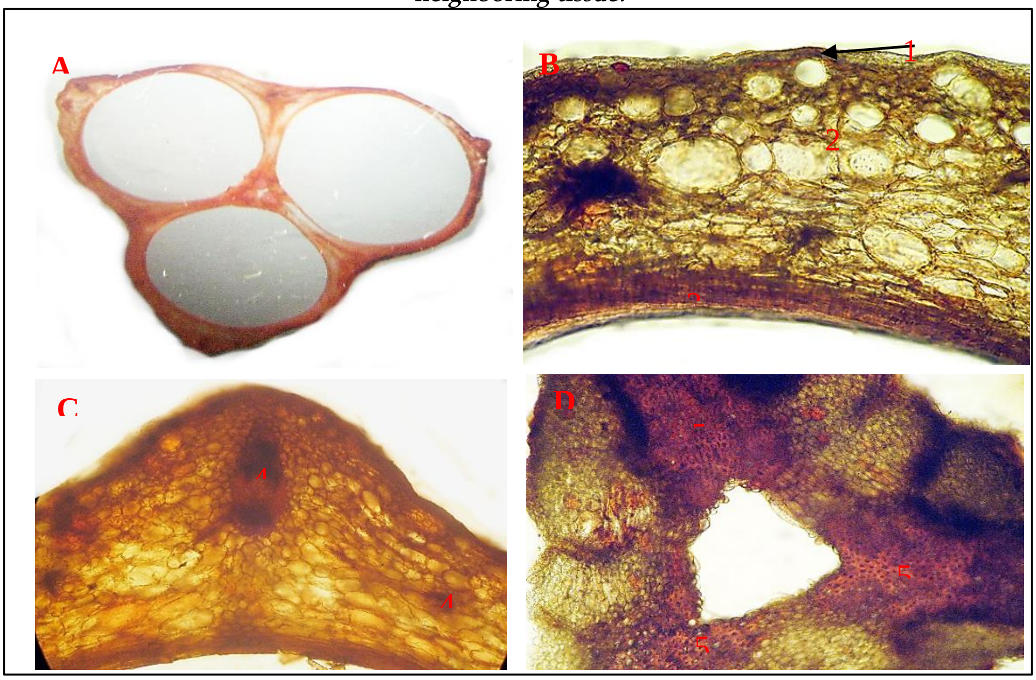
Pic. №4. Microstructure of stem covering tissue of Iris pallida Lam.: *A. Structural panorama of the steam epidermis; B. Fragment of luminal histology; 1. quilted, rectilinear, root-based cells; 2. anomicytic lamina closing cells 3. and interlaminar hole.*



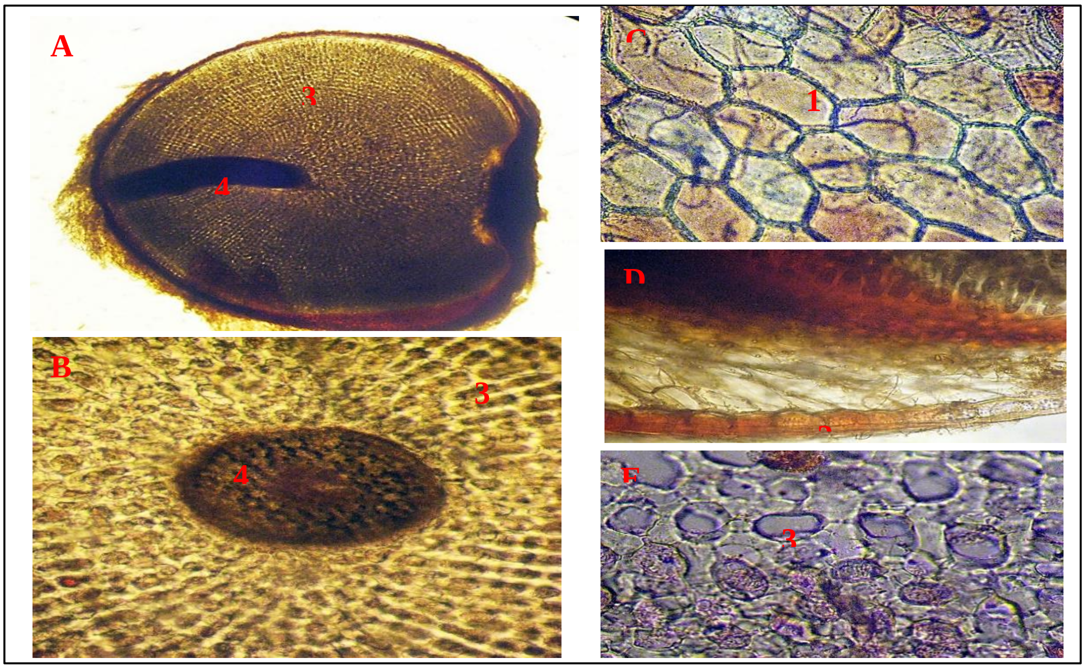
Pic. №5. Microstructure of the stem of Iris pallida Lam.: *A.stem structure panorama; B. fragment of main structural elements of a stem; C. fragment of vascular bundle in longitudal and D. transverse expositions; 1. epidermis; 2. colenchima; 3. chlorenchyma 4. bark parenchyma; 5. sclerenchemical tissue; 6. bark lumina of vascular bundle 7. vessel with spirally thickened membrane; 8. phloem. 9. polygonal cells.*



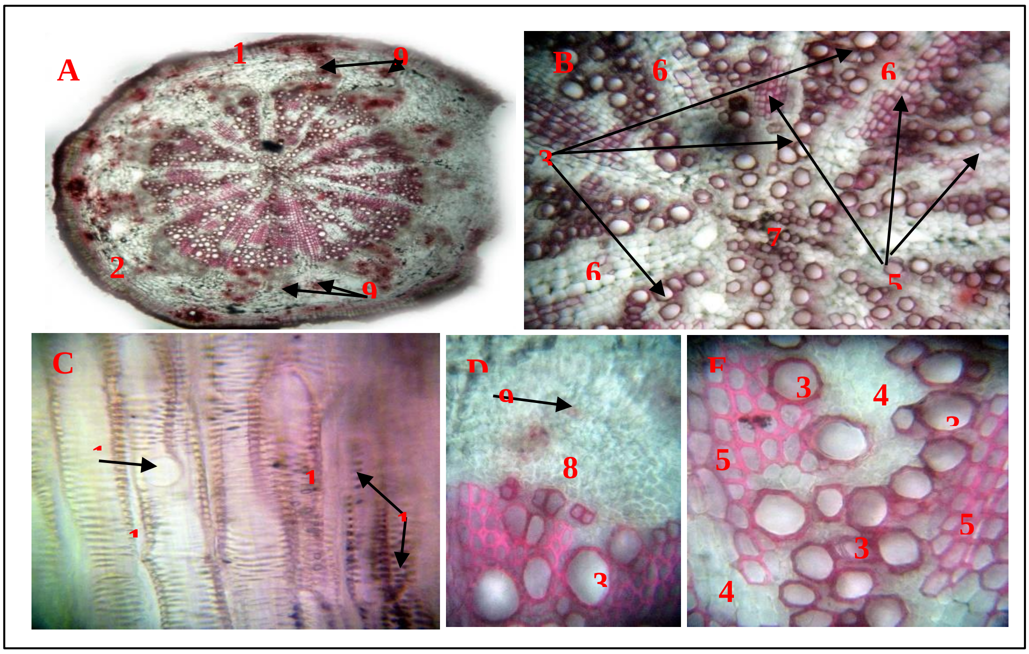
Pic. №6. Microstructure of the leaf of Iris pallida Lam.*: A. Leaf structure panorama; B. fragment of leaf pulp f; C. view of vascular bundles; D. fragment of leaf epidermic tissue and palisade parenchyma; E. vessel-fiber vascular bundle; 1. rhombus-type root-based cell; 2. anomotytic lamina; 3. epidermis; 4. palisade and 5. spongy parenchyma cells; 6. area of obliterated cells; 7. sclerenchemical cells; 8. bark lumens 9. phloem; 10. neighboring tissue.*



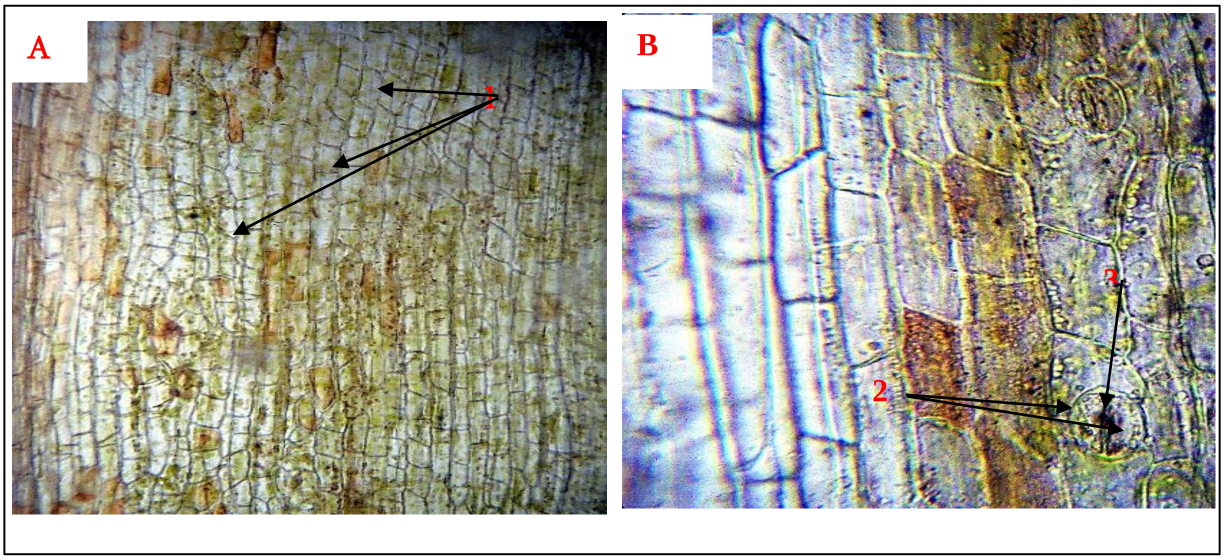
Pic. №7. Microstructure of the fruit neighboring of Iris pallida Lam.*: A. common view of fruit neighboring B. fragment of fruit neighboring; C. fragment of the wall of fruit neighboring; D. meeting spot for the nests of fruit neighboring; 1. exocarpium; 2. mesocarpium; 3. endocarpium 4. vascular bundle; 5. sclerenchyma.*



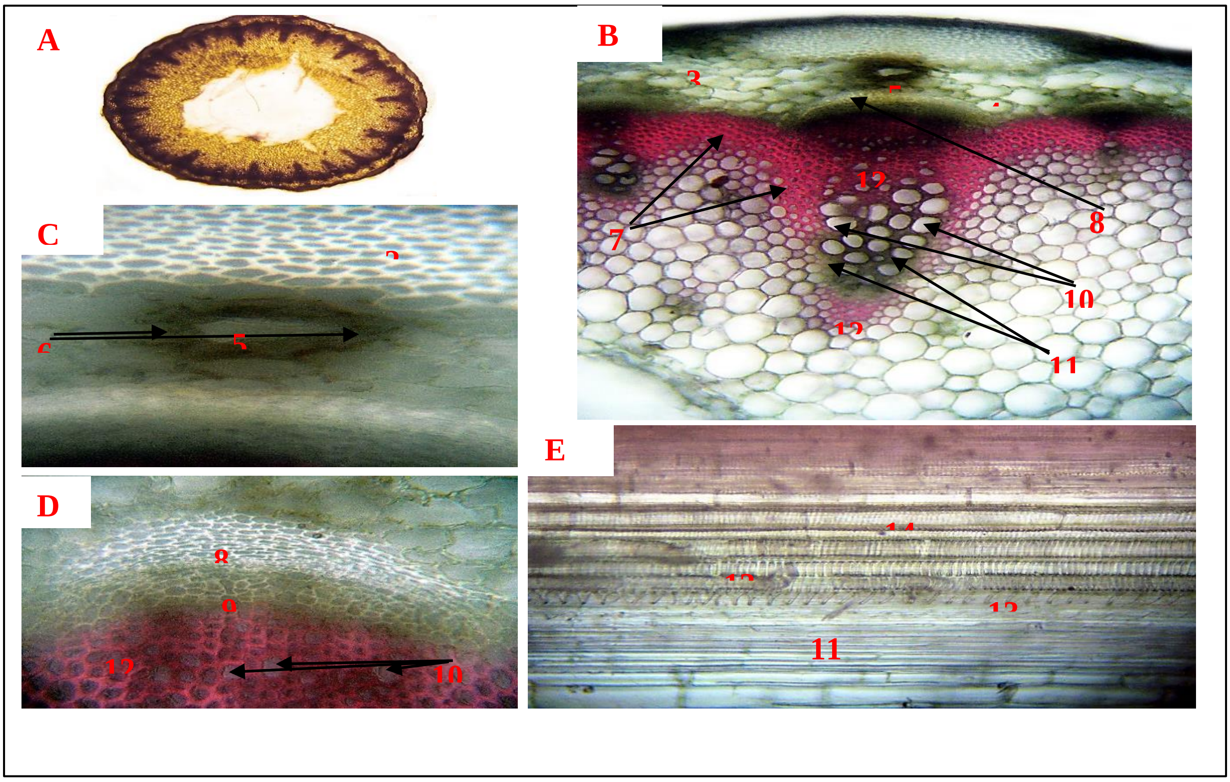
Pic. №8 Microstructure of the seed of Iris pallida Lam.*: A. view of a seed structure in longitudal and B. transverse cuts C. structure of seed epidermic tissue in an exogenous tree and D. transverse cuts; E. basic parenchyma of seed; 1. linear, rhombus-type root-based cells; 2. epidermis; 3. parenchymal cells with equally thickened membranae 4. Embryo.*



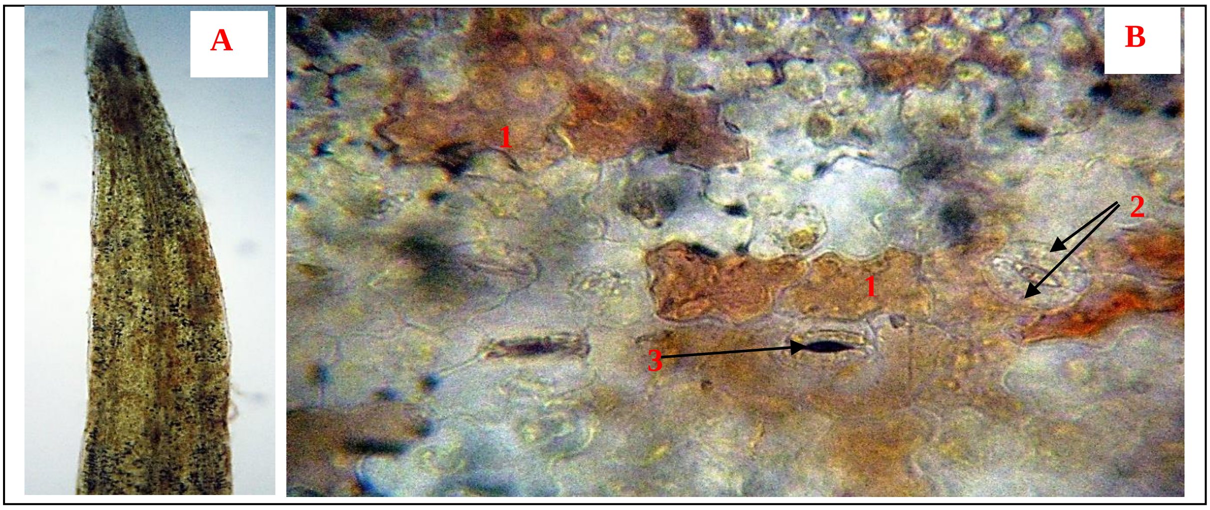
Pic. №9. Microstructure of the root of Cuminum cyminum L.*: A. panorama of root structural architectonics; B. fragment of the central cylinder with polyarchic structure; C. fragment of the vascular vessel in longitudal exposition; D., E. structural elements of the vascular vessel; 1. periderm; 2. bark parenchyma; 3. wood vascular bundles; 4. wood parenchyma; 5. libriform; 6. radial rays; 7. tracheal elements of primary wood; 8. phloem; 9. idioblastic cell of secretory storage; 10. reticulate and 11. porous vessels; 12. pores with petrified membrane; 13. simple perforated plate.*



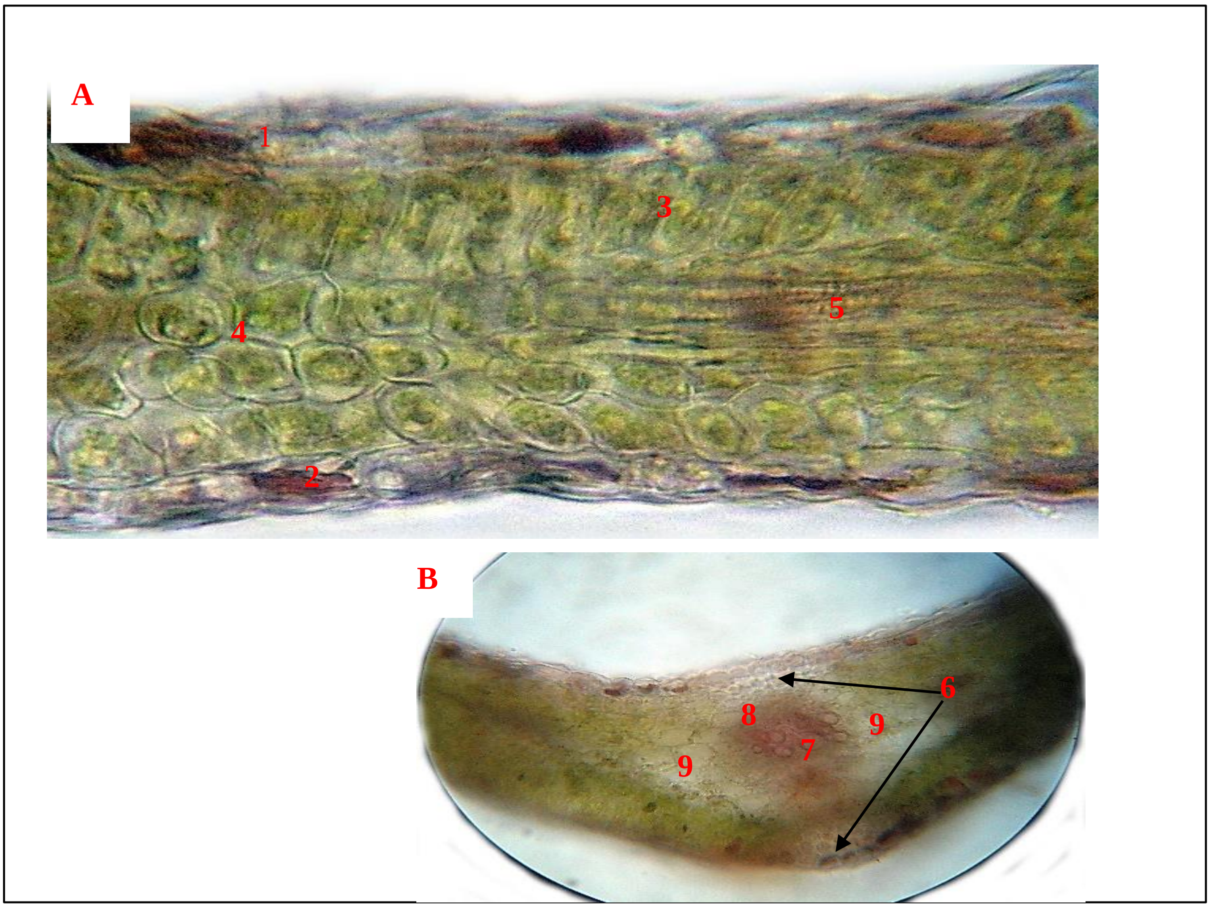
Pic. №10. Microstructure of the stem covering tissues of Cuminum cyminum L.*: A. panorama of Cumunium cyminum; B. fragment of ventilation stem in stem covering tissue; 1. linear, square-shaped, inclined epidermal cell; 2.anomocytic lamina closing cells and 3. interlaminar hole.*



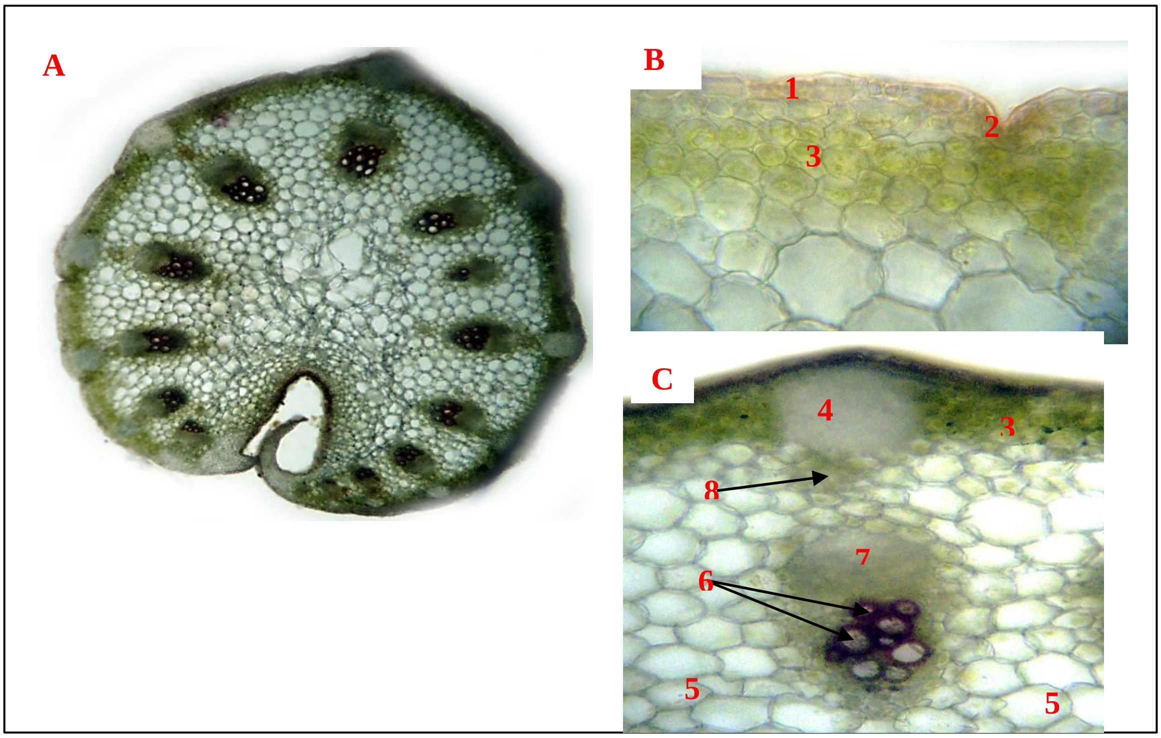
Pic. № 11. Microstructure of a stem structure of Cuminum cyminum L.*: A.; B. fragment of basic structural elements of the stem; C. view of idioblastic cell secretory storage; D. fragment of aggregates of vascular tissues in transverse and E. longitudal expositions; 1. cuticula with epidermis; 2. collenchyma; 3. chlorenchyma 4. parenchymal cells of bark; 5. idioblastic cell and its 6. covering tissue; 7. sclerenchyma cells; 8. phloem; 9. cambium; 10. vascular bundles lumina of wood; 11. wood parenchymal cells; 12. wood sclerenchyma; 13. spiral and14. reticulate vascular vessels.*



Pic. №12. Microstructure of the covering tissue of the leaf of Cuminum cyminum L.: *A. fragment of leaf segment; B. Fragment of epidermic tissue; 1. non-linear and inclined-walled root-based cells; 2. anomocytic lamina closing cells and 3. interlaminar hole.*



Pic. №13. Microstructure of the leaf of cuminum cyminum L.: *A. dorsoventral structure of a leaf pulp; B. median vascular bundle; 1. epidermis with cuticle 2. lamina; 3. palisade and 4. spongy parenchymas 5. spiral anastomosis; 6. collenchyma; 7. wood; 8. phloem; 9. neighboring tissue.*



Pic. №14. Microstructure of the leaf stem of cuminum cyminum L.:*A. panorama of a leaf stem cuts; B., C. fragments of stem structural elements; 1. epidermis with cuticle; 2. lamina 3. chlorenchyma 4. collenchyma 5. polygonial cells of main tissue; 6. wood vascular vessels lumina; 7. phloem 8. idioblastic cell of secretory storage.*

Chapter VII

Studying the content of bioactive compounds of aromatic plant materials introduced on the locations of the Batumi Botanical Garden by Gas chromatography – mass spectrometry (*GC/MS)* method

Based on the studies of growth and development peculiarities of research aromatic plants: *Polianthes tuberosa L.*, *Iris pallida* Lam., and *Cuminum cyminum* L. at seven different locations of the Batumi Botanical Garden, we concluded, that growth and development of plants along with the quantity and quality of obtained raw materials depend on the rate of soil acidity, humus and main feeding elements; well-provided with warmth, sunlight and humidity; good results were received after applying a liquid humic bio preparation. We set goals to study the content of bioactive substances of raw materials of research objects, namely, tuberose flowers and tubers, iris tubers and roots with good quality and quantity obtained from the locations.

For the determination of the content of bioactive substances of tuberose flowers and tubers, iris tubers and roots, the research was carried out by Gas Chromatography - Mass Spectrometry *GC-MS* Method under the methodology described in Chapter 4.

For the identification of the content of bioactive substances in tuberose flowers, iris roots and cumin seeds collected from various locations of the Batumi Botanical Garden, the best quality and quantity raw materials were selected for the experiment.

Subchapter VI. 1 - VI. 4 and annotation includes the experiment results in general, and chromatograms, while the dissertation thoroughly analyzes the outcomes of the experiment including mass spectrum materials of dominant substances.

VII.1. Study of flowers of *Polianthes tuberosa* L. the content of bioactive compounds

High-quality flowers materials of *Polianthes tuberosa* L *Cuminum cyminum* L. were obtained from the experimental plot of aromatic plants and from North American phyto-geographical department locations.

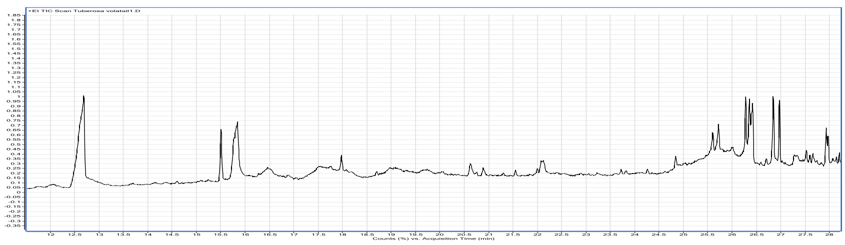
Important compounds identified in tuberose flowers by *GC-MS* researches are valuable for cosmetics, perfumery, medicine, and plenty of different fields.

Non-derivatized:

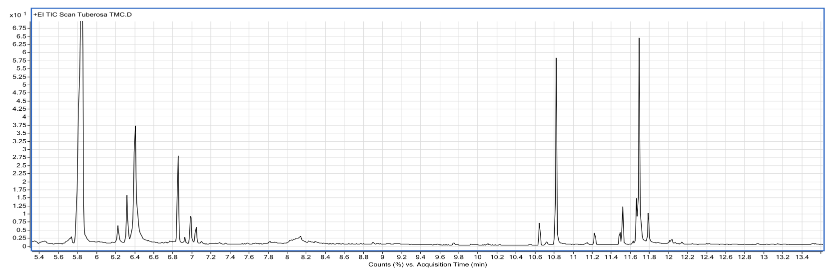
Pyranone: 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-Pyran-4-one; Coumarin: 2.3 – DihydroBenzofuran, 5-Hydroxymethylfurfural, Cuminol: p-Cymen-7-oli, d-Glycero-l-gluco-heptose; 1,3-di-iso-propyl naphthalene, 1,7-di-iso-propylcnaphthalene, Myristic-acid, tetradecanoic acid, Benzyl Benzoate, β-Hydroxylauric acid, D-Melezitose: α-D-Glucopyranoside, O-α-D – Glucopyranosyle-β – D-fructofuranosyle; Methyl palmitate (Pic. №15).

Derivatized:

Phloroglucinol: 1,3,5-trihydroxybenzol (Trimethylsilyl ether); Thymol: 2-isopropyl-5-methylphenol; Carvacrol: 2 – methyl – 5 (1-methylethyl) phenol; 2-Methoxy-4 vinylphenol; Methyl linoleate: Linoleic acid Methyl ether; Methyl isostearate: 16- methyl ester; linoleic Acid; Oleic Acid; Stearic acid (Pic. №16).



Pic. №15. *GC-MS* chromatography of the extracts of *Polianthes tuberosa* L.- flowers (Non-derivatized)



Pic. №16. *GC-MS* chromatography of the extracts of *Polianthes tuberosa* L.- flowers (Derivatized)

VII.2. Study of tubers of *Polianthes tuberosa* L. the content of bioactive compounds

High-quality raw materials from the tubers of *Polianthes tuberosa* L. were obtained from the locations of the Experimental Plot of Aromatic Plants, Central Park, Himalayan, Australian and Mediterranean (European) phytogeographical sections.

Based on Gas Chromatography - Mass Spectrometry (*GC-MS)* research of the methanol extracts of the tubers of *Polianthes tuberosa* L. received from five different locations of the Batumi Botanical Garden, primary and secondary compounds of biosynthesis were detected, in particular:

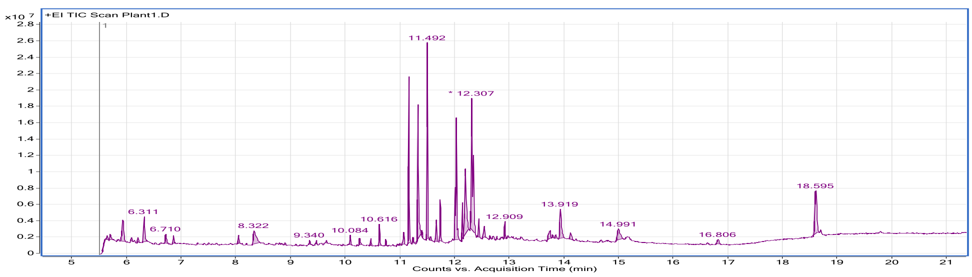
Following *GC-MS* research of the tubers of *Polianthes tuberosa* L. received from the experimental Plot of Aromatic Plants, the content of various compounds was identified, namely: fatty acids, Elaidic acid ethylether (not detected in raw materials from any other locations), methylizostearate, lactose, paranol. Unlike other locations, no furfural was identified here (Pic. №17).

Based on *GC-MS* research of the content of the tubers of *Polianthes tuberosa* L. obtained from the Himalayan department of the Batumi Botanical Garden, the following compounds were detected: piranone, furaneol, valeraldehyde, 5 hydroxymethylfurfural, 6 ethyl 2 methyl pyrazole, suprosa, melezitose, glucose, palmitinic acid, palmito oleic acid, ethylpalmitate, palmito oleic acid, ethylpalmitate, oleic acid, linolenic acid, ethyloleate (Pic. №18).

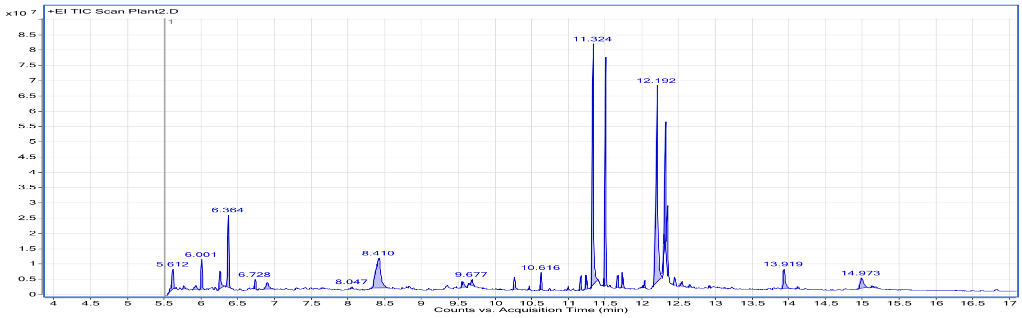
Based on *GC-MS* research of the tubers of *Polianthes tuberosa* L. obtained from the Australian Department of the Batumi Botanical Garden, the various compounds were identified, such as furfural with less quantity compared to the other locations and too little amount of pyrazine (Pic. №19).

In accordance with *GC-MS* research of the tubers of *Polianthes tuberosa* L. received from the Central Park, the content of various compounds was identified, namely: methyl 2 ethyl 5 ethyl pyrazine (dominant), little amount of 5 hydrox methyl furfural (unlike the European section RT 7.2) (Pic. №95-96). Quantitatively not different from the other locations (Pic. №20)

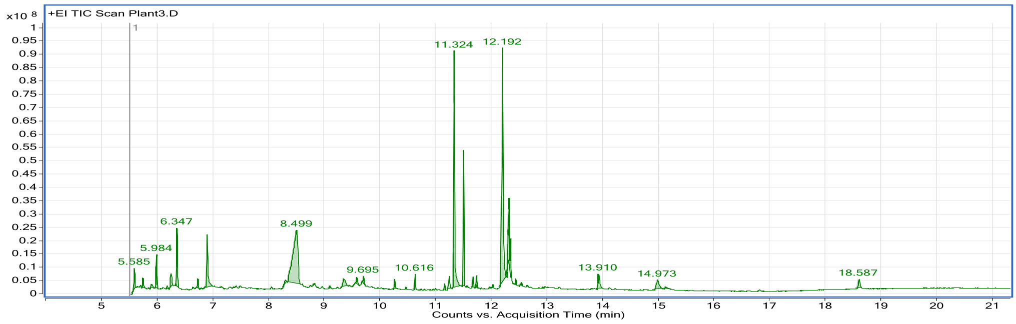
Based on *GC-MS* research of the content of the tubers of *Polianthes tuberosa* L. obtained from the Mediterranean or European phytogeographical department of the Batumi Botanical Garden, the following compounds were detected: piranone (dominant) (Pic. №22), furaneol, valeraldehyde, 5 hydroxymethylfurfural, RT-6.9 (dominant), 6 ethyl 2 methyl pyrazole, suprosa, melezitose, glucose, massoilactone, palmitinic acid, palmito oleic acid, ethylpalmitate, oleic acid, linolenic acid, ethyloleate (Pic. №21).



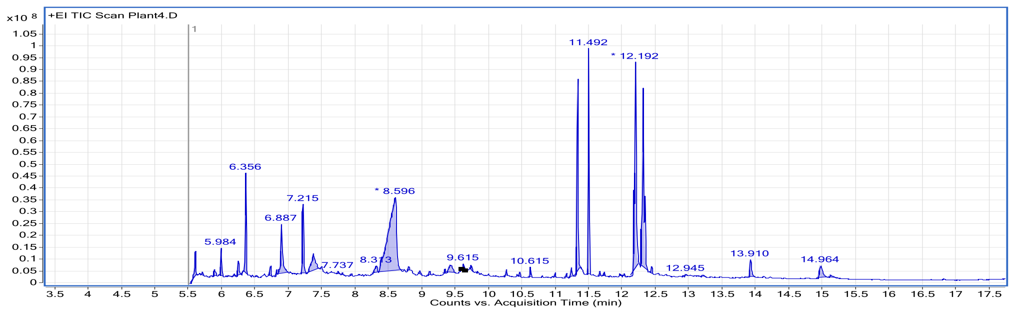
Pic. №17. *GC-MS* chromatography of the extracts of *Polianthes tuberosa* L.- tubers from Experimental plot of aromatic plants at Batumi Botanical Garden(BBG)



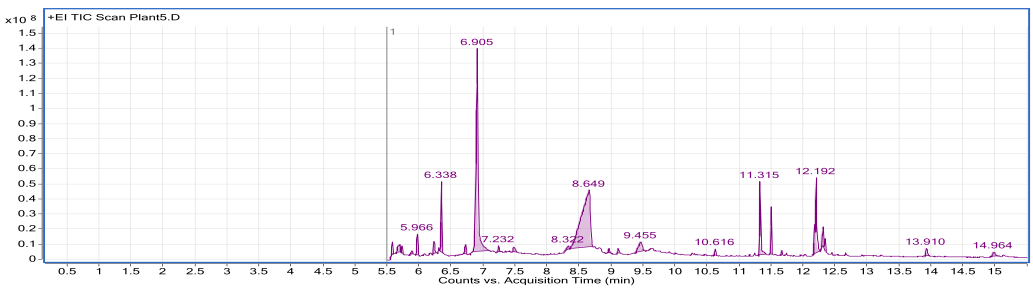
Pic. №18. *GC-MS* chromatography of the extracts of *Polianthes tuberosa* L.- tubers from Himalayan phyto-geographical department at BBG



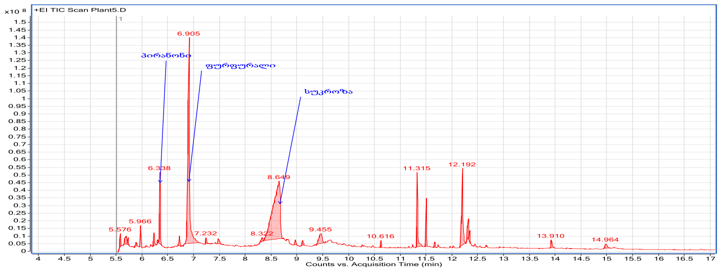
Pic. №19. *GC-MS* chromatography of the extracts of *Polianthes tuberosa* L.- tubers from Australian phyto-geographical department at BBG



Pic. №20. *GC-MS* chromatography of the extracts of *Polianthes tuberosa* L.- tubers from Central park at BBG



Pic. №21. *GC-MS* chromatography of the extracts of *Polianthes tuberosa* L.- tubers from Mediterranean (Europe) phyto-geographical department at BBG

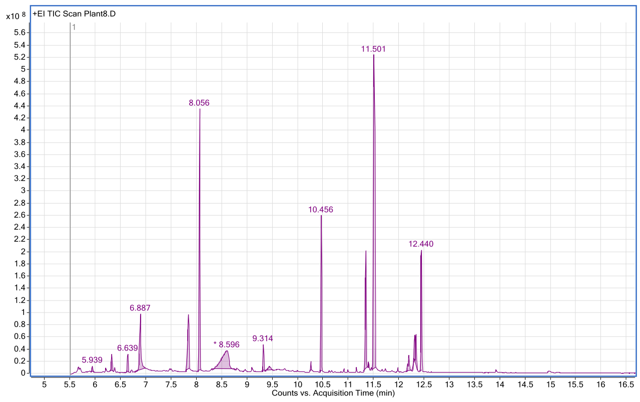


Pic. №22. *GC-MS* chromatography of the extracts of *Polianthes tuberosa* L.- tubers from Mediterranean (Europe) phyto-geographical department at BBG, dominant substancesare marked

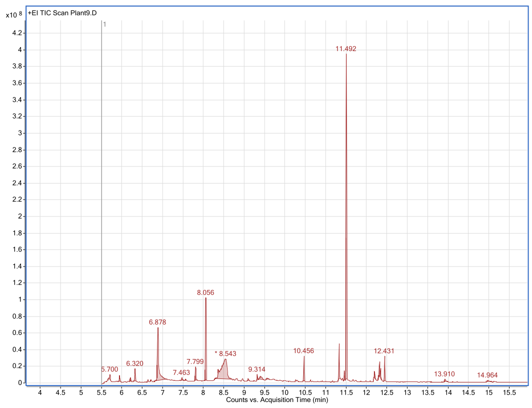
High-quality raw materials from the tubers of *Iris pallida* Lam. were obtained from the locations of the Experimental Plot of Aromatic Plants, Central Park, Himalayan, Australian and Mediterranean (European) phytogeographical sections.

Based on Gas Chromatography - Mass Spectrometry (*GC-MS)* research of methanol extracts of the tubers of *Iris pallida* Lam. obtained from six different locations of the Batumi Botanical Garden, primary and secondary compounds of biosynthesis were detected, namely:

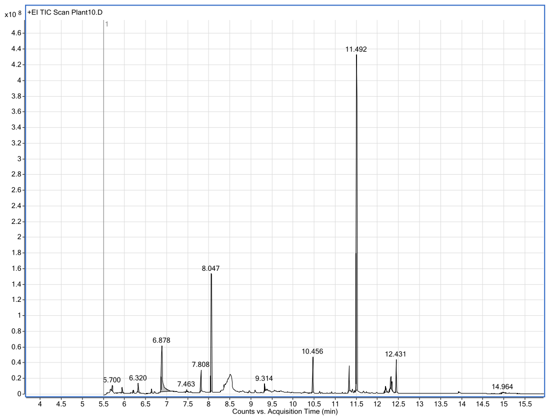
As a result of *GC-MS* researches of tubers and roots of *Iris pallida* Lam. obtained from the examples grown in the Batumi Botanical Garden, the content of different compounds was identified, such as caprylic acid, caprylic acid ethylether, 5 furan carboxaldehyde (little amount), lauric acid, ethylstearate, sucrose, maltose, lactose, arachidic acid ethylether, myristic acid, stearic acid, oleic acid, maltol, dihydrobensofuran, citral, xylopyranozide, gamacitosterol (Pic. №23-28).



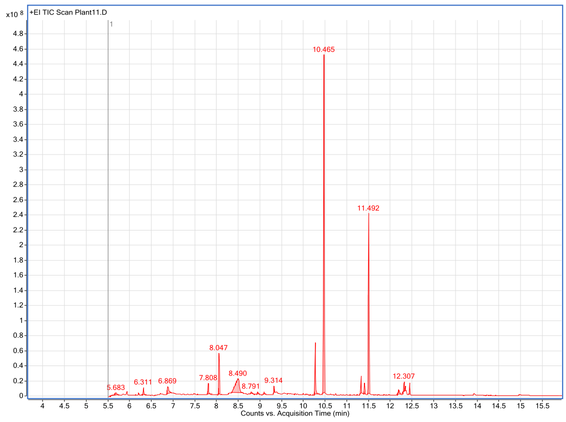
Pic. №23. *GC-MS* chromatography of the extracts of *Irris pallida* roots from Himalayan phyto-geographical department at BBG



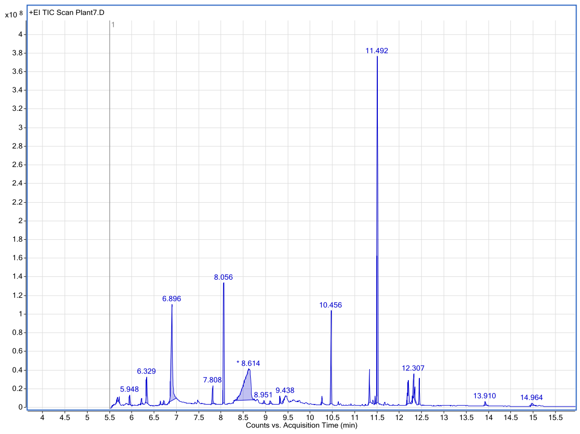
Pic. №24. *GC-MS* chromatography of the extracts of *Irris pallida* roots from North American phyto-geographical department at BBG



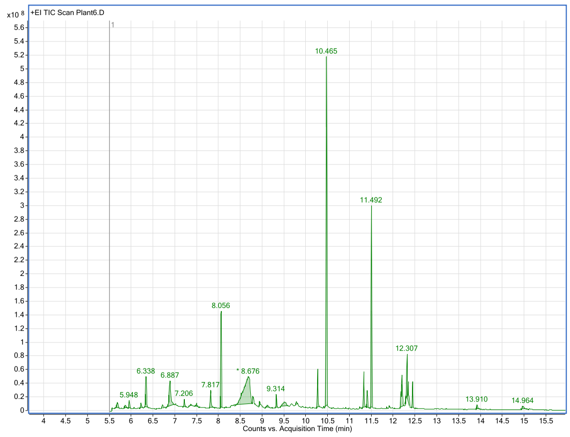
Pic. №25. *GC-MS* chromatography of the extracts of *Irris pallida* roots from Australian phyto-geographical department at BBG



Pic. №26 *GC-MS* chromatography of the extracts of *Irris pallida* roots from Experimental plot of aromatic plants at Batumi Botanical Garden(BBG)



Pic. №27. GC-MS *GC-MS* chromatography of the extracts of *Irris pallida* roots from Mediterranean (Europe) phyto-geographical department at BBG

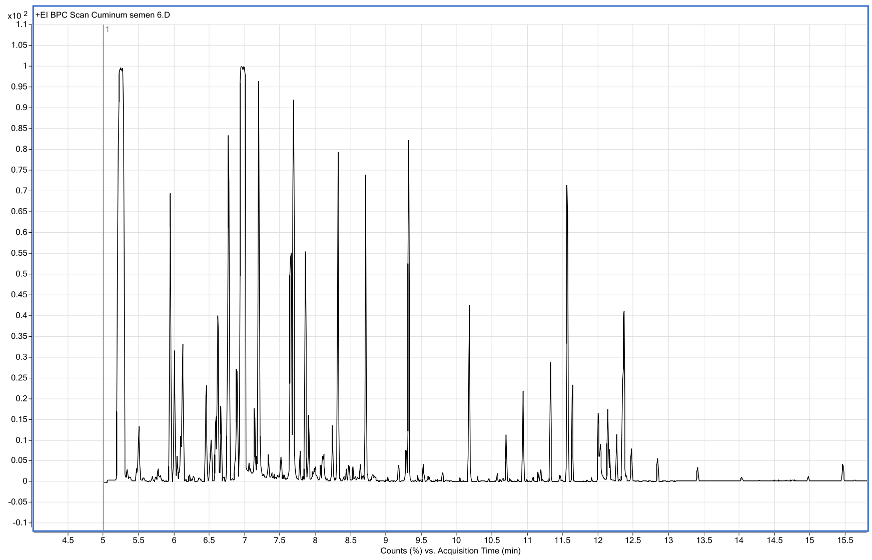


Pic.. №28. *GC-MS* chromatography of the extracts of *Irris pallida* roots from Central park at BBG

VII.3. Study of seeds of *Cuminum cyminum* L. the content of bioactive compounds

High-quality seed materials of *Cuminum cyminum* L. were obtained from the experimental plot of aromatic plants.

The following compounds were identified by GC-MS studies of the seeds of ***Cuminum cyminum***: D-Limonene, Trans-p-Mentha-2,8-Dienol, 1-Vinylcyclohexanole, Cis-p-Mentha-2,8-Den-1-Ol, Cis - Carveol, Trans-p-Mentha-1(7),8-Dien-2-Ol, Trans-p-Mentha-2,8-Dienol, Carveol, Trans-Dihydrocarvone, Trans-carveol, (-)-Carvone **(Dominant)**, p-Mentha-1,8-Dien-3-On, (+)-, Phenilole, Limonene-6-Ol, Terpinyl Butyrate, Eugenole, Cumaldehyde, Lavamenthe, Caryophylene Oxide, Isocaryophyllene, Humulene, Geranyl Isovalerate, aceteugenol, Isoaromadendrene, Myristic acid, Palmiitic acid, Linolein acid, Olein acid, Stearin acid, Squalen (Pic. №29).



სურ. №29. *GC-MS* chromatography of the extracts of *Cuminum cyminum* L. seeds

After doing GC-MS researches of tuberose, *Polianthes tuberosa* L., flowers; of Cuminum, *Cuminum cyminum* L. Seeds, of Iris*, Iris pallida* Lam. Roots, introduced in different locations of the Batumi Botanical Garden with various exposition and soil content, there were identified important compounds valuable for cosmetics, perfumery, medicine, and of different fields.

Chapter VIII

Study essential oils received by green technologies from iris roots (*Iris pallida* Lam.), cumin seeds (*Cuminum cyminum* L.) and tuberoze flowers (*Polianthes tuberosa* L.) by Chromato-Mass spectrometry *GC/MS* method

We had an opportunity to study essential oils received by green technologies from iris roots (*Iris pallida* Lam.), cumin seeds (*Cuminum cyminum* L.) and Polianthes tuberosa flowers by Gas Chromatography-Mass spectrometry *GC/MS* method (See chapter IV). The best results are revealed.

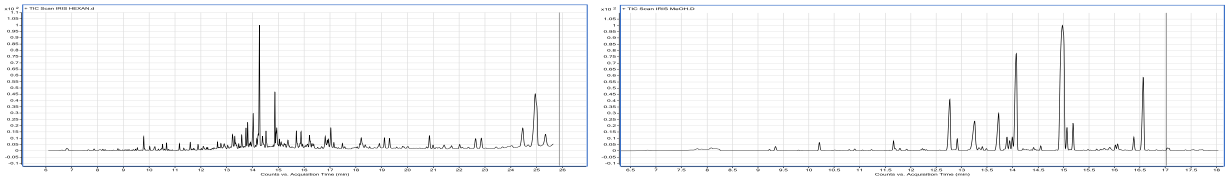
The following important compounds were revealed in cumin; (-)-β-Pinene; (.(+)-(R)-Limonen, 3-Carene; 5,4 - β-Linalool; trans-p-Mentha-2,8-dienol; 5,73 -Limonene epoxide; 6-Camphenone; cis-Carveol; 6,23 - p-Menth-8en-2-ol; trans Dihydro-Carvone; cis-Carveol; Dihydrocarveol; (-)-Carvone **dominant**; Perillaldehyde; (-)-cis-Carvyl Acetate; Limonene-1,2-diol; β-Elemene; Caryophyllene.

Citronellol, Citral, (±)-cis-Verbenol; β-GERANIOL; 2,3-epoxygeranial, α-Citral; (R)-lavandulyl acetate; Dihydropseudoionone); Geranyl vinyl ether were detected in hexane samples of iris.

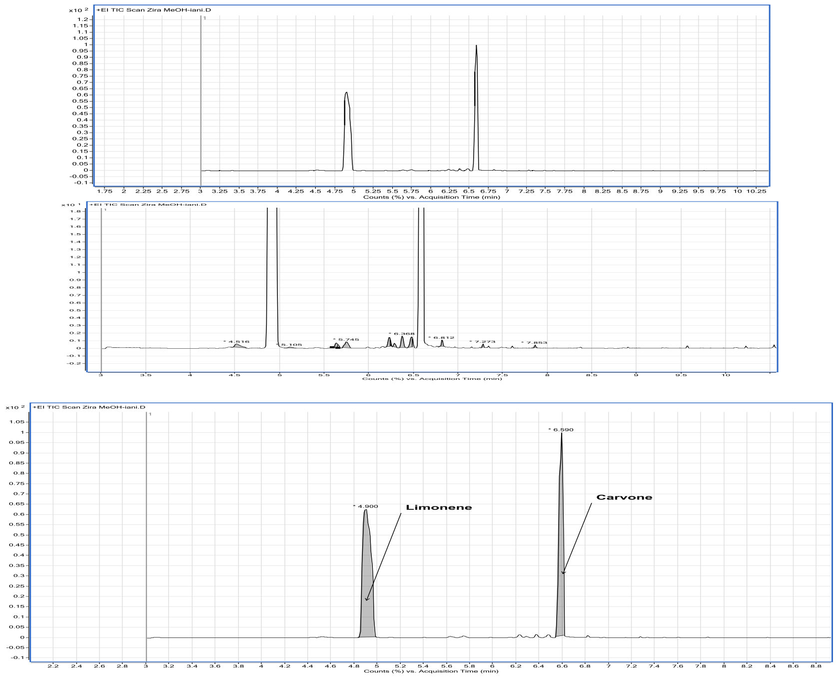
The following compounds are identified in ethanol samples of iris: benzofuran; Thymol; Methyl cis-cinnamate; Vanillin; Vanillyl methyl ketone; p-Coumaric acid methyl ester, dominant; Methyl p-coumarate dominant; Ferulic acid methyl ester dominant; Methyl 3,4-dimethoxycinnamate.

The following volatile compounds were identified in essential oils of tuberose flowers: methyl benzoate; pyranone; phloroglucinol; dihydrocoumarone (coumaron), p-cymene - 7 - ol.

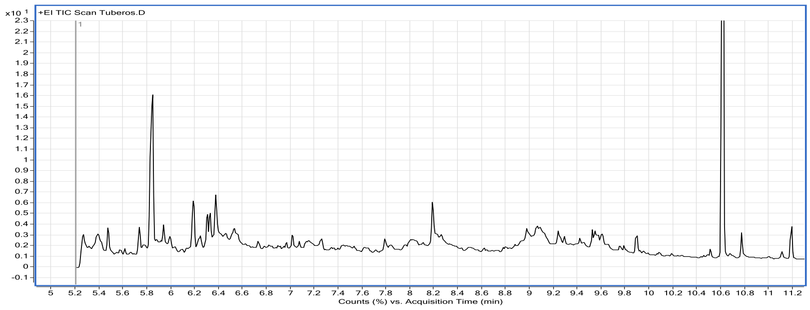
In the dissertation work we give all the chromatograms and masspectrs, some of them are given in annotation (Pic. №30-32).



Pic. №30. *GC-MS* chromatographys of the essential oils of *Iris pallida* Lam. roots



Pic. №31. *GC-MS* chromatographys of the essential oils of *Cuminum cyminum* L.seeds



Pic. №32. *GC-MS* chromatographys of the essential oils of *Polianthes tuberosa* L. flowers

Chapter IX

Obtaining essential oils from the roots of iris (*Iris pallida* Lam.), flowers of tuberose (*Polianthes tuberosa* L.) and seeds of cumin (*Cuminum cyminum* L.) by green technologies

This stage of the research aimed to obtain essential oils from the research objects by green technologies and their comparative description.

Research objects were tuberose flowers, iris roots and cumin seeds (see the description of methods in chapter IV).

Technological evaluations of the methods for obtaining essential oils from tuberose flowers, iris roots and cumin seeds are shown in tables №3-5. The obtainability of essential oils in % was determined by calculating the absolute dry mass of the raw materials.

Table №3

Technological evaluation of the methods for obtaining essential oils from iris roots (*Iris pallida* Lam.).

Extraction method	Characteristics			
	humidity content in the raw materials	the size of particles of parted materials, mm	pouring time. min	obtainability of essential oils, %.
Hydrodistillation of fermented materials (2 years)	5,24	2	120	0,11
Hydrodistillation of fermented materials at 60^{0}C during 24 h	3,12	2	120	0,10
a water-steam distillation of fermented materials at 60^{0}C during 24 h	3,12	2	105	0,09
a steam distillation of materials treated with a 20% solution of sodium chloride in water	89,12	2	105	0,11
microwave-assisted hydrodistillation of materials treated with 20% solution of sodium chloride in water	89,12	2	120	0,10
microwave-assisted extraction of materials treated with 20% solution of sodium chloride in water without extragent	89,12	2	120	0,10
ultrasound extraction + hydrodistillation of materials treated with 20% solution of sodium chloride in water	89,12	2	130	0,12
Extraction of fermented materials (2 years) with liquefied gas (freon-12)	5,24	0,5	360	0,13

The duration of the hydrodistillation process of obtaining essential oils from the roots of iris was experimentally detected based on studying the changes in dynamics of obtainability of essential oils. The outflowing intensity of essential oils is high enough during the first 30 minutes of hydrodifussion and ends after 2 hours.

The maximum obtainability of essential oils from iris roots can be achieved by two methods: 1. ultrasonic treatment and further hydrodistillation of the materials treated with 20% solution of sodium chloride in water; 2. Extraction of fermented materials (2 years) with liquefied gas (freon-12); (Table N3); The obtainability is almost equal for either case. Moreover, we gave a priority to a combined method (ultrasound+hydrodistillation) among green technologies. The essential oil "Concretia" obtained by the said method contains 20% liquefied essential oil. Further researches were carried on using essential oils received by the given method.

Table №4

Technological evaluation of the methods for obtaining essential oils from flowers of tuberose (*Polianthes tuberosa* L.)

Extraction method	Characteristics				
	extragent	Humidity content in the raw materials	The size of particles of parted materials, mm	Pouring time. min	Obtainability of essential oils, %.
Hydrodistillation	Water	88%	8-10	90	0,06
a water-steam distillation	„...."	88%	8-10	90	0,07
microwave-assisted hydrodistillation	„...."	88%	8-10	90	0,07
microwave-assisted extraction	-	88%	8-10	90	0,07
ultrasound extraction + hydrodistillation	Water	88%	8-10	100	0,09
Extraction with liquefied gas (freon-12)	Freon-12	5,4%	1-2	360	0,05

Following the data in Table N4, a combined method: ultrasound extraction + hydrodistillation is distinguished among the methods applied for the extraction of essential oils from tuberose flowers. The obtained essential oil is Concrete.

Table №5

Technological evaluation of the methods for obtaining essential oils from seeds of cumin (*Cuminum cyminum* L.)

Extraction method	Characteristics				
	extragent	Humidity content in the raw materials	The size of particles of parted materials, mm	pouring time. min	obtainability of essential oils, %.
Hydrodistillation	Water	3,46	0,7	90	2,5
a water-steam distillation	„....“	3,46	0,7	90	2,8
microwave-assisted hydrodistillation	„....“	3,46	0,7	90	2,6
microwave-assisted extraction	-	3,46	0,7	90	2,7
ultrasound extraction + hydrodistillation	Water	3,46	0,7	100	2,6
Extraction with liquefied gas (freon-12)	Freon-12	2,31	0,7	360	2.5

Confirmed by the experimental research data (Table №5), the water-steam distillation method is reasonable for obtaining essential oils from cumin seeds.

Organoleptic and physical-chemical rates of essentials oils received by methods with high obtainability were studied at the next stage of the research. "Absoliu" was prepared with iris and tuberose Concretes by ethyl extraction. Results are shown in Table №6.

Table №6

Organoleptic and physical-chemical rates of essentials oils received from iris, tuberose and cumin by different technologies

Characteristics	Product name				
	Essential oil	Concret		Absoliu	
	Cumin	Iris	Tuberose	Iris	Tuberose
Appearance	easily movable fluid	thick, ountment-kind mass	thick, pasta-kind mass	movable, transparent fluid	movable non-Newtonian fluid
Color	light yellow, greenish-brown	dark yellow	brown	yellow	red-pink

Ador	strong, soft, with the appearance of spicy, fatty new greens	deep purple	heavy, sweet, floral aroma	deep purple	heavy, sweet, floral aroma
The density of essential oil, 20 ^{0}C	0,924	0,912	0,952	0,934	0,983
Refraction rate of essential oil, 20 ^{0}C	1,505	1,439	1,447	1,496	1,495
Acidity rate, mg KOH/g	2,9	178	157	6,2	78
Ether rate, mg KOH/g	38	32	108	52	137

Based on the studies of organoleptic and physical-chemical characteristics of essential oils of iris, tuberose and cumin, the research objects meet the relevant quality requirements (Table №6).

In the end we can say: Essential oils from iris roots, tuberose flowers, and cumin seeds were obtained by hydro distillation, steam distillation, microwave distillation with liquefied gases, solvent-free microwave distillation, and Ultrasound-assisted hydro distillation extraction.

Based on the comparative studies of extraction methods, it is identified that combined "Green Technique" – ultrasound-assisted hydro distillation extraction appears optimal for getting essential oils from iris roots or tuberose flowers.

The maximum amount of essential oils from cumin seeds is obtained by steam distillation of raw plant materials.

Organoleptic and physical-chemical properties of the essential oils from iris, tuberose, and cumin are studied. It is detected, that the research objects meet the required standards in terms of good quality rate.

Chapter X

Screening of essential oils on antibacterial activity obtained from the roots of *Iris pallida* Lam., flowers of *Polianthes tuberosa* L. and seeds of *Cuminum cyminum* L. by green technologies

We aimed at studying antibacterial activities of essential oils received from the seeds of iris (*Iris pallida* Lam.), flowers of tuberose (*Polianthes tuberosa* L.) and seeds of cumin (*Cuminum cyminum* L.) introduced by us in the Batumi Botanical Garden and grown by green technologies.

For studying the antibacterial activities of essential oils received from iris roots and tuberose flowers by an optimal combined Green Method, ultrasound-assisted hydrodistillation extraction and cumin seeds by steam distillation, the research was carried out in the George Eliava Institute of Bacteriophage, Microbiology and Virology

In vitro evaluation of antimicrobial activities of research objects was conducted by Spot Test method toward certain clusters of bacterial strains on the following cultures: *Streptococcus spp., Streptococcus pyogenes, Escherichia coli , Salmonella typhimurium, Proteus vulgaris, Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella Klebliella Spp., Proteus spp., Streptococcus spp., Enterococcus spp., Shigella spp., Staphylococcus spp., Enterobacter spp., Pseudomona spp.*

0.7%) agar and placed on Petri dishes with 1 cm diameter circles drown preliminarily. After hardening the agar (20 min), 0.01 ml dilutions of research filtrates 10^{-2} 10^{-4} 10^{-6} 10^{-8} are dropped in circles. The dishes are left half-open until droplets get dry. After that, the dishes are incubated in a thermostat at 37^0C for 18-24 hours. The influence of the preparation on bacterial growing is shown by lysis areas. The Spot Test allows the determination of the activity quality of research filtrates.

Determination of lysis activity and spectrum of the preparation – For the determination of lysis activity and spectrum of the preparation, 24-hour leached material of bacterial culture from indirect agar is diluted 10X (108 CFU/ml), a lawn is produced on Petri dishes based on the Spot Test method and 10 µl preparation is poured on it. After 18-24 hours of incubation at 37°C, lysis activity is determined according to the quality of lysis zones created by the preparation.

Outcomes of the screening of the antibacterial activity of essential oils obtained from the roots of iris (*Iris pallida* Lam.), flowers of tuberose (*Polianthes tuberosa* L.) and roots of cumin (*Cuminum cyminum* L.) by green technologies are shown in the table №7.

Based on the table, the research objects have expressed antibacterial effects. Essential oils obtained from the seeds of cumin and roots of iris are distinguished by a wide range of the spectrum.

Thus, based on the studies of antibacterial activities of essential oils received from the seeds of iris (Iris pallida Lam.), flowers of tuberose (Polia¬nthes tuberosa L.) and seeds of cumin (Cuminum cyminum L.) introduced by us in the Batumi Botanical Garden and grown by green technologies, a certain cluster of bacterial strains toward the following cultures: *Streptococcus spp., Streptococcus pyogenes, Escherichia coli, Salmonella typhimurium, Proteus vulgaris, Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella Spp., Proteus spp., Streptococcus spp., Enterococcus spp., Shigella spp., Staphylococcus spp., Enterobacter spp., Pseudomona spp.*

Based on the studies (by green technologies) of antibacterial activities of essential oils obtained from iris roots (*Iris pallida* Lam.), tuberose flowers (*Polianthes tuberosa* L.) and cumin seeds (*Cuminum cyminum* L.) by green agrotechnical activities and tested on a certain cluster of bacteria stems toward the following cultures: *Streptococcus spp., Streptococcus pyogenes, Escherichia coli, Salmonella typhimurium, Proteus vulgaris, Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella Spp., Proteus spp., Streptococcus spp., Enterococcus spp., Shigella spp., Staphylococcus spp., Enterobacter spp., Pseudomona spp., it was identified as follows*: 1) research objects have antibacterial effect; 2) essential oil obtained from cumin seeds and iris roots is distinguished by a wide spectrum of antibacterial activities; 3) the following essential oils obtained from a) cumin - *Escherichia coli*; *Staphylococcus aureus; Pseudomonas aeruginosa; Enterococcus spp.*; *Enterobacter spp.*; b) iris roots - *Escherichia coli; Salmonella typhimurium* ; *Proteus vulgaris; Streptococcus spp.; Enterobacter spp.*; and c) tuberose flowers - *Streptococcus pyogenes; Enterococcus faecalis* are characterized with the highest antibacterial activities toward bacteria stams.

Table №7

Results of Screening of essential oils on antibacterial activity obtained from the roots of Iris pallida Lam., flowers of Polia¬nthes tuberosa L. and seeds of Cuminum cyminum L. by green technologies

Strain	Title of essential research essential oils		
	Iris	Tuberoza	Cumin
Streptococcus. spp	-	-	R
Streptococcus pyogenes	-	4+	-
Escherichia coli	4+	-	4+
Salmonella typhimurium	4+	-	3+
Proteus vulgaris	4+	-	-
Enterococcus faecalis	-	4+	-
Staphylococcus aureus	3+	3+	4+
Pseudomonas aeruginosa	3+	-	4+
Klebliella Spp.	3+	-	3+
Proteus spp.	3+	-	3+
Streptococcus. spp	4+	-	-
Enterococcus spp	4+	-	4+

Shigella spp	3+	-	3+
Staphylococcus spp	R	-	R
Enterobacter spp	4+	-	4+
Pseudomona spp	2+	-	2+

Conclusions

Green technologies of introduction and processing of some aromatic plants are studied for the first time based on the Batumi Botanical Garden.

The following conclusions are drawn based on the researches:

1. To assess the first results of the introduction of aromatic plants, new species for the collection of the Batumi Botanical Garden - *Polianthes tuberosa* L., *Elettaria cardamomum* (L.) Maton., *Coffea arabica* L., *Coffea canephora* Pierre ex A.Froehner., *Cuminum cyminum* L., *Cassia* angustifolia Delile., *Iris pallida* Lam., *Vanilla planifolia* Jacks., *Zingiber officinale* Roscoe., *Curcuma longa* L., having studied their features of growth and development in the open field, it was established:

a) All species developed shoots from seeds and tubers;

b) Full vegetative and generative development under the conditions of open soil is passed by the following species: *Polianthes tuberosa L.*, *Cuminum cyminum* L., *Iris pallida* Lam. They can flower, develop the fruit and partially seeds, which proves their full adaptation to new environmental conditions;

c) *Cassia* angustifolia Delile. flowers under the conditions of open soil, but without developing seedboxes. In December, the plant gets frozen and withered. However, under the orangery conditions, it can flower, bears fruit and develops seeds;

d) Orangery conditions are advantageous for the following species: *Elettaria cardamomum* (L.) Maton., *Coffea arabica* L., *Coffea canephora* Pierre ex A.Froehner., *Vanilla planifolia* Jacks., Vegetative development occurs normally, while generative development is noticeable with Arabic coffee - *Coffea arabica* L. and - *Coffea canephora* Pierre ex A. Froehner.

e) Vegetative organs of *Curcuma longa* L..; *Zingiber officinale* Roscoe.; and *Elettaria cardamomum* (L.) reach their full development, but without flowering.

2. Based on the analysis of important aromatic-spicy species of seeds, flowers and leaves of *Cassia acutifolia* Delile., seeds of *Cuminum cyminum* L., leaves and fruits and *Coffea canephora* Pierre ex A. Froehner., and *Coffea Arabica* L., leaves of *Vanilla planifolia* Jacks., and leaves of *Elettaria cardamomum* (L.) Maton., introduced by green technologies under the soil and climatic conditions of the Batumi Botanical Garden and selected especially for studying the content of bioactive substances, the following things were detected:

a) The content of essential oils, glycosides, flavonoids, and aglycones are detected as a result of screening of bioactive substances by thin-layered chromatography;

b) Bioactive substances of various classes are identified based on studying the content of bioactive substances by Gas Chromatography – mass spectrometry (*GC-MS)* method. The content of essential oils is detected in every research species.

3. Based on the studies of growth and development peculiarities of aromatic plants - *Polianthes tuberosa L.*, *Iris pallida* Lam., and *Cuminum cyminum* L., introduced into 7 different locations of the Batumi Botanical Garden in the conditions of various expositions and soil, such as: East Asian, the Hymalayan, Australian, Mediterreanean (European), North American phytogeographical sections, Central Park and the experimental plot, without the use of chemicals, we can conclude, that:

a) *Polianthes tuberosa* L., *Iris pallida* Lam., and *Cuminum cyminum* L., get on quite well with soil and climatic conditions of the Batumi Botanical Garden;

b) Research objects pass the full cycle of their growth and flowering on well-lit and fertile locations. Moreover, their vegetative-generative development is significantly improved while using liquid humic biopreparations containing mineral and organic substances;

c) Locations surrounded by hardwood plants with high antimicrobial activities condition healthy growings of research species free from harmful diseases;

d) Full cycle of growth and development for *Polianthes tuberosa* L. includes the period from May till the end of December; For *Iris pallida* Lam. – from January till the end of September, and for *Cuminum cyminum* L., the period lasts almost all year long.

e) Among research objects, full generative development occurs with *Iris pallida* Lam. and *Cuminum cyminum* L., while *Polianthes tuberosa* L. bears no fruit, but flowers.

f) Propagation of *Polianthes tuberosa* L., *Iris pallida* Lam., and *Cuminum cyminum* L. under the conditions of the Batumi Botanical Garden is possible with the help of seeds and planting materials of local reproduction received by green technologies, while *Cuminum cyminum* L., and *Iris pallida* Lam., are reproduced by seeds, and *Polianthes tuberosa* L. – by multiple child tubers produced during the vegetation process.

g) Successful cultivation of the aromatic plants, such as *Polianthes tuberosa* L., *Iris pallida* Lam., and *Cuminum cyminum* L., introduced into the humid subtropical climatic conditions of the Batumi Botanical Garden is possible by green technologies.

4. Microstructural characteristics of underground and aboveground vegetative organs namely, roots, tubers, leaves of tuberose -*Polianthes tuberosa* L., are studied; Macro and microstructural characteristics of generative and vegetative organs namely, leaves, stems and fruits of *Cuminum cyminum* L.; Macro and microstructural characteristics of underground and aboveground vegetative organs, namely, roots, stems, leaves of cumin. Diagnostic characteristics are identified, which will help to identify suitable raw materials and establish authenticity.

5. Thus, based on the Gas Chromatography - Mass Spectrometry *GC-MS* method research of the content of bioactive substances of the aromatic plants *Polianthes tuberosa L.* (tuberose), cumin seeds *(Cuminum cyminum* L.) *and Iris pallida* Lam. (pale iris), in particular, tuberose flowers and tubers, iris roots, grown only by biological methods to different locations of the Batumi Botanical Garden, significant compounds were identified. Most of them are known and used in medicine, perfumery, cosmetics, the food industry, culinary and many other fields; they have antioxidant activities, anti-infectious, antimicrobial and other valuable qualities.

6. In the end we can say: Essential oils from iris roots, tuberose flowers, and cumin seeds were obtained by hydro distillation, steam distillation, microwave distillation with liquefied gases, solvent-free microwave distillation, and Ultrasound-assisted hydro distillation extraction. Based on the comparative studies of extraction methods, it is identified that combined "Green Technique" – ultrasound-assisted hydro distillation extraction appears optimal for getting essential oils from iris roots or tuberose flowers. The maximum amount of essential oils from cumin seeds is obtained by steam distillation of raw plant materials.

7. Organoleptic and physical-chemical properties of the essential oils from iris, tuberose, and cumin are studied. It is detected, that the research objects meet the required standards in terms of good quality rate.

8. As a result of the study of essential oils obtained by green technologies from the roots of iris (*Iris pallida* Lam.), Cumin seeds (*Cuminium cyminum* L.) and tuberose flowers (*Polianthes tuberosa* L.) by chromatography-mass spectrometry, GC / MS, it was established that essential oils contain: terpenes, terpenoids, phenolic derivatives, aliphatic and aromatic components.

9. Based on the studies (by green technologies) of antibacterial activities of essential oils obtained from iris roots (*Iris pallida* Lam.), tuberose flowers (*Polianthes tuberosa* L.) and cumin seeds (*Cuminum cyminum* L.) by green agrotechnical activities and tested on a certain cluster of bacteria stems toward the following cultures: *Streptococcus spp., Streptococcus pyogenes, Escherichia coli, Salmonella typhimurium, Proteus vulgaris, Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella Spp., Proteus spp., Streptococcus spp., Enterococcus spp., Shigella spp., Staphylococcus spp., Enterobacter spp., Pseudomona spp., it was identified as follows*: 1) research objects have antibacterial effect; 2) essential oil obtained from cumin seeds and iris roots is distinguished by a wide spectrum of antibacterial activities; 3) the following essential oils obtained from a) cumin - *Escherichia coli*; *Staphylococcus aureus; Pseudomonas aeruginosa; Enterococcus spp.*; *Enterobacter spp.*; b) iris roots - *Escherichia coli; Salmonella typhimurium* ; *Proteus vulgaris; Streptococcus spp.; Enterobacter spp.*; and c) tuberose flowers - *Streptococcus pyogenes; Enterococcus faecalis* are characterized with the highest antibacterial activities toward bacteria stams.

10. Based on this paper, the first experimental plot of aromatic plants in the history of the Batumi Botanical Garden was established and the collection of aromatic-spicy plants was decided to arrange.

11. Elaboration of the scientific basis for green technologies of growth-development and processing the aromatic plants *Polianthes tuberosa* L., *Iris pallida* Lam., *Cuminum cyminum* L., introduced to the Batumi Botanical Garden, has great importance for creating the base of ecologically clean raw materials and obtaining essential oils from them.

Published articles on the topic of the dissertation:

1. L. Kodanovi, M. Jokhadze, M. Metreveli, D. Berashvili, A. Bakuridze ,, ,,Introduction of aromatic plants in the Batumi Botanical Garden and their research for the content of biologically active compounds“, Georgian Medical News - 2020, Tbilisi - New York, ISSN 1512-0112; No7-8 (304-305), pp.153-157; privacy (geomednews.com)
2. L. Kodanovi, A. Bakuridze, M. Metreveli, M. Jokhadze, D. Berashvili, A. Meskhidze ,,Biological characteristics of growth and development of *Polianthes tuberosa L.* in soil and climatic conditions of the Batumi Botanical Garden", IJSRM - International Journal of Science and research methodology India; ISSN 2454-2008, Vol.:17, Issue 1, pp. 93-104, privacy (geomednews.com)
3. L. Kodanovi, M. Metreveli, ,,The Study Results of Some Introduced Medical-Atomatic Plants in Conditions of Batumi Botanical Garden“, Tbilisi State Medical University, Tbilisi, Georgia, 2019, International Scientific Conference ,,Green Medications – By Green Technologies –For Healthy Life", https://tsmu.edu/conference2019/index.php?lang=en
4. L. kodanovi, ,,Introduction and Cultivation of some Medicinal-Aromatic Plants in the Batumi Botanical Garden“, Conference of young scientist and students, Actual issue of modern Biomedicine, Batumi Shota Rustaveli State University, 29 September, 2019
5. Kodanovi L. Introduction of therapeutic, aromatic, exotic species in Batumi botanical garden and its results. Comference Hall of Hotel “Radissin Blu", Batumi, Georgia, 2017, International scientific Conference ,,Future technologies and quality of life", pp. 124-125, https://tsmu.edu/lifeconference2017/ABSTRACTS.pdf
6. L. kodanovi, A. Bakuridze, ,,Introduction of medical and aromatic plants at Batumi Botanical Garden“, Biodiversity and Georgia, Proceedings of the II scientific conference, National Botanical Garden of Georgia, Tbilisi, 2016, Pp. 93-95
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