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NOVEL COROTINOID CONCENTRATES FROM SUBTROPICAL PLANTS

CHIKOVANI D., KOBAKHIDZE M.

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(Received September 7, 2009)

Abstract

Chemical composition of pressed mandarin (*Citrus Laus unshiu mau)* fruits and fallen leaves of Caucasian persimmon *(Diospyrus kaki* L.) were determined. Carotenoid concentrates were obtained from these raw materials. These concentrates may be used in food industry as yellow food colorants as well as food additives for vitaminization of products.

Key words: Carotenoid concentrate, pressed mandarin, Caucasian persimmon.

Introduction

Carotenoids are organic pigments which are widely distributed in plant kingdom. In humans, carotenoids such as β-carotene are a precursor to vitamin A, they can also act as antioxidants.

Carotenoid concentrates are used as food additives for vitaminization and for intensification of natural colour of yellow colored food products [Kunitsina, 2001]. There are several methods of production of carotenoid concentrates from fruits and leaves of plants [Savinov, 1948; Kudritskaya, 1990]. It has been shown that old leaves of plants are characterized by higher content of carotenoids than the fresh ones [Fishman et al., 1996]. Carotenoid concentrates can be prepared both in the form of liquid (oil-based carotenoid concentrates) and in the form of powder. Liquid carotenoid concentrates are more effective for vitaminization of food products [Zagorodsky, 1997].

The aim of the presented work was to obtain carotenoid concentrates from the pressed mandarin (*Citrus Laus unshiu mau*) fruits and fallen leaves of Caucasian persimmon (*Diospyrus kaki* L).

Materials and Methods

Pressed mandarin (*Citrus Laus unshiu mau)* fruits (byproduct) and fallen leaves of Caucasian persimmon *(Diospyrus kaki* L.) were served as materials.

Carotenoids were determined according to the methods of [Savinov, 1948; Kudritsky, 1990].

Pectin substances were determined by the methods of calcium pectate precipitation.

P-active substances were determined by the methods of cyanidine as described in ref [Ermakov, 1987].

Polyphenols were determined by using the method of Folin-Denis as described in ref [Ermakov, 1987].

Ascorbic acid (Vitamin C) was estimated by the method described in ref [Ermakov, 1987].

Mineralization of samples was done in a combustion oven [Ermakov, 1987].

Cellulose was determined by the modified methods of Ermakov [Ermakov, 1987]

Carotenoid concentrate from pressed mandarin fruits was obtained by the following procedure: pressed mandarin fruits were crushed and extracted with petroleum ether several times. The obtained extract was evaporated under the vacuum up to 1/10 of its initial volume and saponified with 8-15% of KOH in ethanol at room temperature for 4 h. The saponified fraction was removed and the remained fraction (carotenoid fraction) was washed with water and concentrated using vacuum evaporator.

In order to **prepare the powder of Caucasian persimmon leaves** the leaves of this plant were dried at 60-80° and powdered.

Results and Discussion

Chemical composition of pressed mandarin fruits is shown in Table 1.

Table 1. Chemical Composition of Pressed Mandarin Fruit

Chemical constituent	% of fresh material
Pectin substances	4.0
P-active substances	0.123
Carotenoids	0.0037
Minerals	0.92

Carotenoid concentrates obtained from pressed mandarin fruits was dark orange liquid with density equal to 0.91 g/cm^3. It solved easily in oil. This concentrate was found to contain quite large amount of carotenoids (26.42 mg/100g).

As it can be seen from Table 2 the powder of Caucasian persimmon leaves is rich in carotenoids as well as other biologically active substances including polyphenols and pectic substances.

Table 2. Chemical Composition of the Powder of Caucasian Persimmon Leaves

Chemical constituent	% of dry material
Total sugar	23.700
Pectic substances	13.700
Cellulose	9.500
Polyphenols	6.400
Minerals	5.300
Total carotenoids	0.019

The powder of Caucasian persimmon leaves should be kept in the hermetically sealed containers, protected from direct action of the sun rays and at temperature between 5 and 15°C. It was shown that during the storage of the powder under such conditions for a year the content of carotenoids and polyphenols did not change (Table 3).

Table 3. Changes in Content of Carotenoids and Polyphenols in the Powder of Caucasian Persimmon Leaves During Storage

Duration of storage, month	Carotenoids, mg % of dry matter	Polyphenols, % of dry matter
Initial	19.0	6.4
3	19.0	6.4
6	18.9	6.38
9	18,9	6.35
12	18.9	6.29

Thus, the liquid carotenoid concentrate from the pressed mandarin fruits and the powder of Caucasian persimmon leaves which are rich in carotenoids may be used in food industry as yellow food colorants as well as for vitaminization of food products. The Caucasian persimmon leaf powder may also be used for enrichment food products with biologically active compounds.

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ახალი კაროტინოიდული კონცენტრატები სუბტროპიკული მცენარეებიდან

ჩიქოვანი დ., კობახიძე მ.

აგრარული ტექნოლოგიების სამეცნიერო-კვლევითი ინსტიტუტი შოთა რუსთაველის სახელმწიფო უნივერსიტეტი

(მიღებულია 07.09.2009)

რეზიუმე

დადგენილია მანდარინის (*Citrus Laus unshiu mau.*) ნაყოფის გამონაწნეხისა და კავკასიური ხურმის (*Diospyrus kaki* L.) ჩამოცვენილი ფოთლების ქიმიური შედგენილობა. აღნიშული ნედლეულიდან მიღებულია კაროტინოიდული კონცენტრატები, რომლებიც შეიძლება გამოყენებულ იქნეს კვების მრეწველობაში, როგორც ყვითელი საღებავები და აგრეთვე კვების დანამატები პროდუქტების ვიტამინიზაციისათვის.

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COMPARISON OF SCREENING METHODS FOR RELIABLE DETECTION OF GENETICALLY MODIFIED ORGANISMS

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(Received July 20, 2009)

Abstract

Three PCR primer pairs suitable for common regulatory elements of genetically modified organisms (GMOs) were compared for selection of the most effective screening method for GMO. Two of the primer pairs 35S1/35S2 and 35S-cf3/35S-cr4 are specific to 35S promoter from Cauliflower Mosaic virus and NOS1/NOS2 is specific to the NOS terminator from *Agrobacterium tumefaciens*. The Roundup Ready soybean was used as GM material. The comparison of the primer pairs was carried out in two different PCR conditions PCR1 and PCR2. The results obtained exhibited 35S promoter specific primer pair 35S-cf3/35S-cr4 as the most specific, sensitive and efficient tool for GMO screening in the both PCR conditions applied.

Keywords: Screening of GMO, genetically modified organisms (GMOs), PCR analysis, regulatory elements.

Introduction

The intentional modification of plant genetic material by recombinant DNA technology has resulted in the development of genetically modified organisms (GMO). They are also called transgenic plants and products of biotechnology. GMOs occur as seeds, plants, grains, food and feed. The introduction of candidate genes into the plants and their regulated expression generated GMOs with useful traits, such as: herbicide tolerance (Roundup Ready), insect resistance (Bt), resistance to illness, high yield, resistance to extreme conditions, etc. [Popping, 2002], therefore they have significant agronomical and commercial interests. The vast proportion of GM crops grown commercially in the world has been accounted for by four species: soya, maize, cotton and rapeseed. To date, 107 GM-events of 21 crops have been approved in different countries and new GM-varieties are added annually [ISAAA Brief 37-2007]. However, unregulated distribution and use of GMOs may pose serious threat to environment, human and animal health. Correspondingly, development of reliable GMO detection methods for monitoring of biotechnological products is in urgent need.

There is particular interest in the GMO screening methods due to the increasing number of transgenic events. GMO Screening determines if a sample contains a GMO material or not, correspondingly screening tests are targeted to the GMO common elements. The standard polymerase chain reaction (PCR) based screening method detects 35S promoter from Cauliflower

Mosaic virus and the NOS terminator from *Agrobacterium tumefaciens* [Pietsch et al., 1997; Studer et al., 1997], because these sequences do not naturally occur in the plants genome and they often are used in construction of vectors for cloning in plants. Nowadays several PCR-based screening systems have been already developed and validated, however they differ in terms of sensitivity and specificity [Lipp et al., 1997; Lipp et al., 2001; Windels et al., 2001; Marmiroli et al., 2008]. There is a lack of information regarding comparative evaluation of the different screening methods. In this study three commonly used PCR-based GMO screening methods were compared for detection of roundup ready soybean.

Materials and Methods

Transgenic material. Roundup Ready soya was used as GM plant. The genetically modified soya bean powder set containing 0-5% Roundup Ready as Certified reference material (ERM-BF-410) was obtained from Fluka.

DNA extraction. The certified reference materials were in dried powdered form. DNeasy plant mini kit (Qiagen) was used for genomic DNA extraction from 100 mg of samples. The DNA quality and quantity was assessed by agarose gel electrophoresis.

PCR primers. The sequences of the soybean-specific primers lect1/lect2 and three pairs of GMO-specific primers 35S1/35S2, 35S-cf3/35S-cr4 and NOS1/NOS2 were selected based on the published data [Pietsch et al., 1997; Lipp et al., 1999]. The primers were synthesized and provided by MWG Biotech.

Amplification conditions. The amplification reactions were performed with a thermal cycler Techne TC-412. The PCR was carried out in the final volume of 25 μl using 0.8 U GoTaq™ DNA polymerase with 1x green GoTaq™ reaction Buffer (pH 8.5) (Promega), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP (Promega), 0.4 μM of each primer, and 1μl (50-70 ng) of genomic DNA.

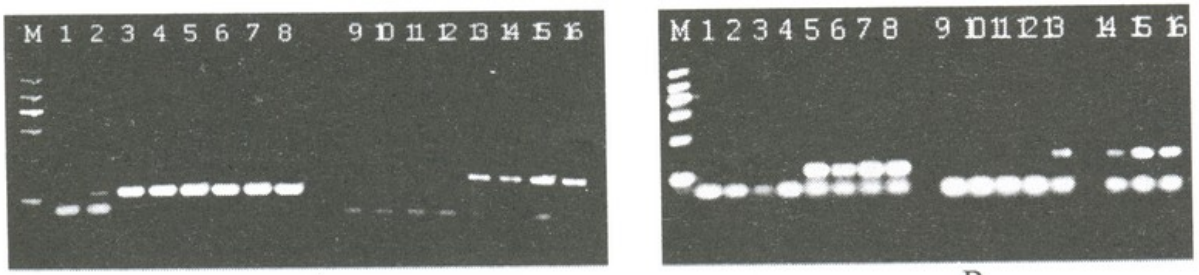
Two different PCR conditions were applied for all the three primer pairs analyzed, that were as follows: PCR1: denaturation for 3 min at 95°C, 50 cycles of amplification for 25s at 95°C, for 30s at 63°C, for 30s at 72°C; final extension step for 7 min at 72°C, and PCR2: denaturation for 3 min at 95°C, 50 cycles of amplification for 25s at 95°C, for 35s at 60°C, for 45s at 72°C; final extension step for 7 min at 72^0C.

Gel electrophoresis. The PCR products were analyzed using agarose gel electrophoresis. The gel was prepared with 2.0% of agarose (Promega), in Tris Borate EDTA (TBE) 1x buffer with 1μg/ml of Ethidium Bromide (EtBr). After electrophoresis the DNA bends were visualized and photographed by Digital still camera (DSC-S600, Sony).

Results and Discussion

Three PCR primer pairs suitable for GMO common regulatory elements were compared for selection of the most effective screening test for genetically modified organisms. Two of the primer pairs such as: 35S1/35S2 and 35S-cf3/35S-cr4 are specific to 35S promoter from Cauliflower Mosaic virus and one primer pair NOS1/NOS2 is specific to the NOS terminator from *Agrobacterium tumefaciens*. The roundup ready soybean was chosen as a model GMO because it is the most popular transgenic crop and its genome contains both 35S promoter and NOS terminator. In our previous studies the pairs 35S1/35S2 and NOS1/NOS2 were found as suitable for reliable detection of genetically modified organisms [Datukishvili et al., 2007; Kutateladze et al., 2009]. The comparison of the primer pairs was carried out in two different PCR conditions PCR1 and PCR-2 which are mainly different in the amplification temperature and duration as mentioned above in the materials and methods.

Fig.1 shows the results obtained in the PCR1 condition. The certified reference materials containing 1% and 5% of RRS were used as GMO material in this set of experiments while water and 0% RRS were examined as negative controls. Each sample was tested in duplicate. The primer pair lect1/lect2 gave expected one amplicon in size of 118 bp for all soybean samples (0% RRS, 1% RRS, 5% RRS) while water control did not give DNA bend. One PCR product in size of 195 bp, 123 bp and 180 bp was obtained for the GMO specific primer pairs 35S1/35S2, 35S-cf3/35S-cr4 and NOS1/NOS2 respectively, as was expected. However PCR with primers 35S-cf3 and 35S-cr4 produced more intensive DNA bends suggested that these primers are more efficient for GMO screening. Our result corresponds with the early published data [Lipp et al., 2001]. The both water and 0% RRS negative controls did not give any PCR fragment indicating high specificity of these primers for GMO screening in the PCR1 conditions.



A B

Fig. 1. Comparison of the primers for GMO screening in PCR1 condition. *Lanes 1-2, 9-10.* water; *lanes 3-4*, 11-12. 0% GMO*; lanes 5-6, 13-14.* 1% GMO; *lanes 7-8, 15-16.* 5% GMO. A. *lanes 1-8.* primers lect1/lect2; *lanes 9-16.* primers 35S1/35S2; B *lanes 1-8.* primers 35S-cf3/35S-cr4; *lanes 9-16.* primers NOS1/NOS2; *M.* PCR markers 1.2 kb, 900 bp, 700 bp, 500 bp, 300 bp, 100 bp (Qiagen).

The results of the comparison of three GMO specific primers analyzed in the PCR2 conditions are presented in Fig.2. In these experiments the certified reference materials containing 0.1% and 5% of RRS were used as GMO material. The testing of water and 0% RRS as negative controls, exhibited PCR fragments for primers 35S1/35S2, while no PCR products was seen for primers 35S-cf3/35S-cr4 and NOS1/NOS2 that suggested unspecificity of the primers 35S1 and 35S2 in the condition PCR2. This might be explained by the lower annealing temperature (60°C) in the conditions PCR2 than in the PCR1 (63°C). The expected one PCR fragment was produced for all GMO samples by each screening method except of 0.1% RRS, its PCR with NOS1/NOS2 primer pair did not give any visible PCR product.

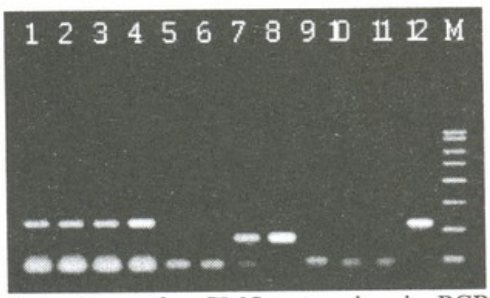


Fig. 2. Comparison of the primers for GMO screening in PCR2 conditions. *Lanes 1-4.* primers 35S1/35S2; *lanes 5-8.* primers 35S-cf3/35S-cr4; *lanes 9-12.* primers NOS1/NOS2; *lanes 1, 5, 9.* water; *lanes 2, 6, 10.* 0% GMO; *lanes 3, 7, 11.* 0.1% GMO; *lanes 4, 8, 12.* 5% GMO; ***M.*** PCR markers: 2 kb, 1.5 kb 1 kb, 750 bp, 500 bp, 300 bp, 150 bp, 50 bp (Sigma-Aldrich).

In conclusion, the results obtained exhibited 35S promoter specific primer pair 35S-cf3/35S-cr4 as the most specific, sensitive and efficient tool for GMO screening in the both PCR conditions applied.

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რეზიუმე

გენეტიკურად მოდიფიცირებული ორგანიზმების (გმო) საერთო რეგულატორული ელემენტების შესაბამისი პოლიმერაზული ჯაჭვური რეაქციის (პჯრ)-ის პრაიმერების სამი წყვილი შედარებულ იქნა გმო-ს სკრინინგისათვის ყველაზე ეფექტური მეთოდის შესარჩევად. პრაიმერების ორი წყვილი, კერძოდ 35S1/35S2 და 35S-cf3/35S-cr4 სპეციფიკურია ყვავილოვანი კომბოსტოს მოზაიკური ვირუსის 35S პრომოტორის, ხოლო პრაიმერების ერთი წყვილი სპეციფიკურია აგრობაქტერიის - *Agrobacterium tumefaciens* - NOS ტერმინატორის. Roundup Ready სოია გამოყენებულ იქნა გმ მასალად. პრაიმერული წყვილების შედარება მოხდა პჯრ-ის ორ სხვადასხვა პირობებში პჯრ1 და პჯრ2. მიღებული შედეგების შედარებით გამომჟღავნდა, რომ 35S პრომოტორის სპეციფიკური პრაიმერების წყვილი 35S-cf3/35S-cr4 ყველაზე სპეციფიკური, მგრძნობიარე და ეფექტურია გმო-ს სკრინინგისათვის პჯრ-ის ორივე პირობებში.

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NATURAL FOOD RED COLORANT WITH RADIOPROTECTIVE PROPERTIES

MCHEDLISHVILI N. [1], NIKOLAISHVILI M. [2], OMIADZE N. [1], ABUTIDZE M. [1], GULUA L. [1]

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Abstract

The chemical composition, antioxidant activity and radioprotective properties of natural red colorant from green tea and pokeberry were investigated. The colorant was found to consist of phenolic compounds (15.8%), pectin substances (18.0%), free aminoacids (16.3%), reducing sugars (27.0%), organic acids (7.8%), minerals (8.0%). It contains small amounts of radionuclides and toxic elements. The natural colorant revealed high antioxidant activity and showed only about 4 times less antioxidant potential than ascorbic acid. The factor of doze changing was found to be equal to 1.25 indicating the high radioprotective activity of the natural food red colorant. It was shown that the natural red colorant from pokeberry and green tea may be successfully used in food industry.

Keywords: food colorant, antioxidant activity, radioprotective properties, trace elements

Introduction

Protection against deleterious effects of ionizing radiation is the subject of numerous publications, which offer a scientifically sound approach for prevention of radiation-induced injuries with the aid of plant extracts [Jagetia et al., 2002; Shimoi et al., 1996]. Beneficial effects of these extracts is attributed to plants secondary metabolites flavonoids as well as to other polyphenolic compounds [Kevin, Kroft,1999] such as catechins, including epicatechin, epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate, which are the most active plant flavonoids [Bombardelli et al., 2000]. The mechanism of the observed radioprotective effects of these compounds is mainly attributed to their antioxidant properties [Blokhina et al, 2003]. It has been established, that vitamins C, E and catechins of green tea, in particular (-) epigallocatechingallate (EGCG) have high antioxidant activity. The latest compound is characterized by 200 times higher antioxidant activity than vitamin E [Okuda, 1993]. It has also been shown that some phenolics (anthocyanins, catechins) and pectin substances bind heavy metals and facilitate their excretion from the organism. In some cases the mixture of various natural substances has more sharply expressed medical effect, than separate compounds [Guthrie et al., 2001; Vercauteren et al., 1998].

The aim of the presented work was to determine chemical composition of red colorant from pokeberry and green tea and to investigate its antioxidant and radioprotective activities as well as to test its ability to color different kinds of food products.

Materials and Methods

Pokeberry fruits were field collected in west Georgia.

Green tea extract was commercially produced at the JS "Kolkheti-93" (Tsalenjikha, Georgia). Green tea leaves were subjected to the high temperature (100 ^{0}C) treatment for 5 min, as a result, enzymes present in the leaves were inactivated and the chemical composition was fixed. Next, the leaves were undergone extraction process with 70 ^{0}C water, the extract was filtered, concentrated and spray dried.

Male Wistar-line rats weighing 150-200g were housed in the vivarium and were given seeds of sunflower and bread swollen in milk.

Preparation of red colorant samples. Fresh juice of pokeberry fruits was boiled twice for the purpose of detoxification. Green tea dry extract was gradually added to the hot juice with permanent stirring until complete dissolution. Ratio of green tea extract and pokeberry juice dry matters was 70:30. The obtained mixture was filtered through cheesecloth, concentrated under vacuum and spray-dried. Final product was red dry powder.

Pectin substances were quantitatively analyzed by the method which is based on the reaction of galacturonic acid with carbasole in sulphuric acid medium [Demchenko et al. 1981].

Red coloring substances were determined spectrophotometrically at λ= 538 nm using cobalt sulphate as a standard [Bokuchava et al., 1976].

Total polyphenol content in plant materials was investigated as described in ref [Jinjolia et al., 1983].

The Ferric Reducing Ability of Plasma (FRAP) assay was used to measure the concentration of **total anioxidants** [Benzie and Strain, 1996].

Amino acids were determined by method based on colour reaction of free amino acids with nynhydrin reagent [Burkina et al., 2000] with some modifications.

Organic acids were determined by titration with 0.1 N NaOH and calculated on the base of tartaric acid.

Reducing-sugars were estimated by the methods of [Somogyi 1952, Nelson 1944].

Trace elements and radionuclides were determined by flame atomic absorption spectrometry.

Mineralization of samples was done at 525°C in a combustion oven.

Average lifetime of Wistar-line rats was determined as described in ref [Davidov et al., 1991]. Wistar-line rats were injected intraperitoneally for three days during the first half of the day. 1ml of 10% water solution of food red colorant was used for injection. The same amount of the physiological solution was injected into control animals. The rats were irradiated with 4 and 7 Gy in the special organic glass containers under the irradiation conditions: 200 KB, 15 Ma, filters of copper and aluminum 0.5 mm and 1.5 mm respectively using an equipment RUM-17. The source-to-skin distance was - 40 cm and dose rate - 1.23 Gy/min at room temperature (23±2°C). 20 rats were used for each treatment group. After death the irradiated animals were dissected and investigated. Some of the tested rats were sacrificed on the day of control rats death and investigated.

Results and Discussion

The natural food red colorant from pokeberry and green tea was found to consist of quite a large amount of biologically active compounds including polyphenolic compounds, pectin substances and amino acids (Table 1)

Table 1. Chemical composition of food red colorant from pokeberry and green tea

Dry substances, %	Coloring substances, % of dry substances	Polyphenols, % of dry substances	Soluble pectic substances, % of dry substances	Reducing sugars, % of dry substances	Free amino, % of dry substances	Minerals, % of dry substances	Organic acids % of dry substances
95.0	4.1	15.8	18.0	27.0	16.3	8.0	7.8

This plant composite was shown to contain small amounts of radionuclides (32 Bq/kg cesium-137 and 24 Bq/kg strontium-90) and toxic elements and rich in beneficial elements such as Fe and Mg (Table 2).

Table 2. Content of trace elements in natural red food colorant from pokeberry and green tea (mg/kg)

Hg	As	Cd	Zn	Cu	Fe	Mg
0.02	0.04	0.18	9.50	27.30	400.00	500.00

The red colorant revealed high antioxidant potential and showed only about 4 times less antioxidant potential than ascorbic acid. Antioxidant potential of the colorant was equal to 2.5 [Fe^{2+}] mM per 1 g/l concentration of the sample while antioxidant potential of vitamin C was about 9.4 [Fe^{2+}] mM per 1 g/l concentration. It should be mentioned that antioxidant activity of natural red colorant did not change for 12 months storage.

The colorant was found to possess high radioprotective properties expressed by increase of lifetime of irradiated Wistar-line rats. The lifetime of the Wistar-line rats injected with the colorant and exposed to 4 and 7 Gy was increased by 8-9 and 4-5 days respectively as compared with control animals (Fig.1 and Fig.2). It had positive effect on the acute radiation disease and facilitated this process as well as reduced the decrease of the weight of animals in case of acute radiation disease. The factor of doze changing or the factor of doze decreasing was found to be equal to 1.25 indicating the high radioprotective activity of the natural food red colorant.

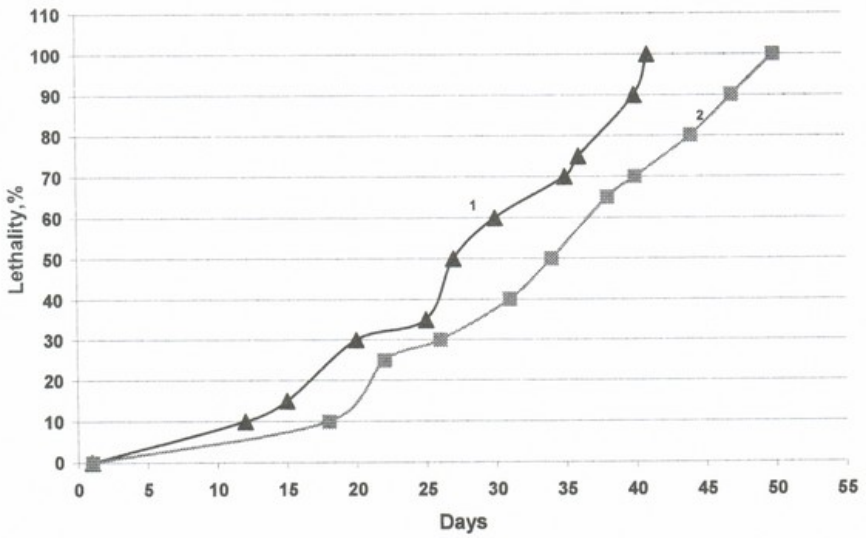

Fig.1. Lifetime of Wistar-line rats irradiated with 4 Gy
1-conrtol animals, 2- injected with natural colorant animals

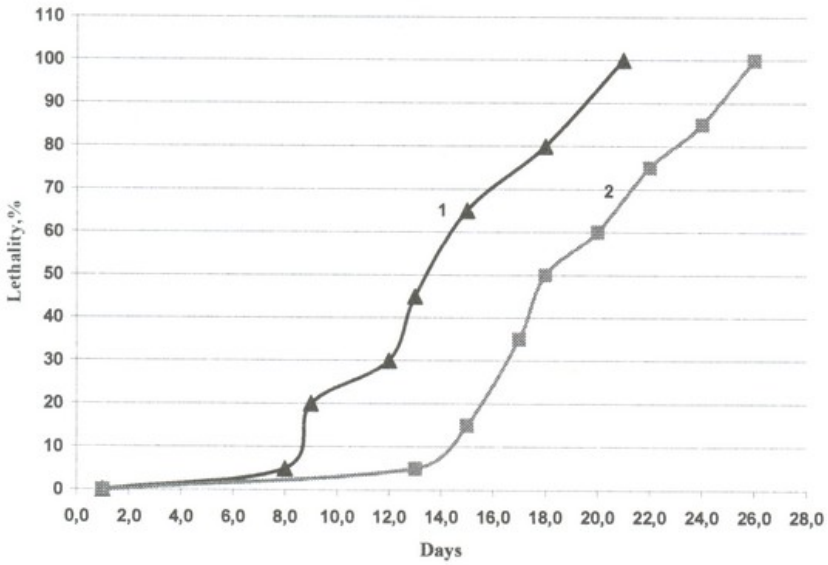

Fig. 2. Lifetime of Wistar-line rats irradiated with 7 Gy
1-conrtol animals, 2- injected with natural colorant animals

The colorant was used to color different kinds of food products including lemonade, Georgian mineral water, juices, liquor, cream, ice cream, jelly, yoghurt and mousse (egg whites). The colorant was added in a dry form, without preliminary dissolution in water at the room temperature at concentration of 0.1-0.25%.

Natural red colorant from pokeberry and green tea which contains a large amount of red coloring pigments (Table 1) revealed high ability to color different kinds of food products such as diary products, soft and alcoholic drinks with pH ranging between 3-7. The colour of the products dyed with the natural food red colorant from pokeberry and green tea was stable and according to the concentration of the colorant it varied from light pink to dark reddish (claret). At used concentrations the colorant did not change organoleptic characteristics of the colored food product.

Thus, natural red colorant from pokeberry and green tea which is rich in polyphenolic compounds and pectic substances possess high antioxidant activity and radioprotective properties. The biologically active natural red colorant from pokeberry and green tea may be successfully used in food industry to color different kinds of food products.

Acknowledgements. This work was fulfilled by financial support of the Science and Technology Centre in Ukraine and Georgia National Science Foundation, project # 4329.

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რადიოდაცვითი თვისებების მქონე კვების ბუნებრივი წითელი საღებავი

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[2] რადიობიოლოგიისა და რადიაციული ეკოლოგიის სამეცნიერო ცენტრი

(მიღებულია 27.07.2009)

რეზიუმე

გამოკვლეულია ჭიაფერადან და მწვანე ჩაიდან მიღებული ბუნებრივი წითელი საღებავის ქიმიური შემადგენლობა, ანტიოქსიდანტური აქტივობა და რადიოდაცვითი თვისებები. აღნიშნული საღებავი შეიცავს ფენოლურ ნაერთებს (15.8%), პექტინის ნივთიერებებს (18.0%), თავისუფალ ამინომჟავებს (16.3%), აღმდგენელ შაქრებს (27.0%), ორგანულ მჟავებს (7.8%), მინერალურ ნივთიერებებს (8.0%). რადიონუკლიდები და ტოქსიკური ელემენტები მასში წარმოდგენილია მცირე რაოდენობით. ბუნებრივმა საღებავმა გამოავლინა მაღალი ანტიოქიდანტური აქტივობა და აჩვენა ასკორბინის მჟავასთან შედარებით მხოლოდ 4-ჯერ ნაკლები ანტიოქსიდანტური პოტენციალი. დოზის შემცირების ფაქტორი აღმოჩნდა 1.25-ის ტოლი, რაც ბუნებრივი წითელი საღებავის მაღალ რადიოდაცვით თვისებებზე მიუთითებს. ნაჩვენებია, რომ ჭიაფერადან და მწვანე ჩაიდან მიღებული ბუნებრივი წითელი საღებავი შეიძლება წარმატებით იქნეს გამოყენებული კვების მრეწველობაში სხვადასხვა პროდუქტის შესაღებად.

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A NEW RECORDS OF GEASTRUM PERSOON (GASTEROMYCETES) FOR GEORGIA

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Abstract

The paper presents diversity of the genus Geastrum in Georgia. Currently only 14 species are identified, 4 of which (*Geastrum berkeleyi* Massee, *Geastrum kotlabae* V.J. Staněk, *Geastrum lageniforme* Cooke, *Geastrum quadrifidum* Pers.) are new for Georgia.

Key words: Georgian Gasteromycetes, *Geastrum*, taxonomy.

Introduction

The genus Geastrum Persoon is a member of Gasteromycetes. Currently, 50 species of Geastrum are known in the world [Hawkswort et al., 1995]. A few studies of Geastrum species have been carried out in Georgia so far encompassing only *G. corollinus* (Batsch.) Holl., *G. coronatum* Pers., *G. fimbriatum* Fr., *G. fornicatum* (Huds.) Hook., *G. minimum* Schwein., *G. pectinatum* Pers., *G. pseudolimbatum* Hollós, *G. rufescens* Pers., *G. striatum* Quél. and *G. triplex* Jungh. [Nakhursrishvili, 1986; Jorjadze, 2002]. While field studies undertaken during 2007 - 2008 on different territories of Georgia, 7 Geastrum species were found. 4 of them are new records for the Georgian mycobiota. These species are given below.

Materiales and Methods

The morphological and ecological characteristics of the specimens were recorded and photographed in natural habitats. The small sections of the hymenium were processed with Melzer's reagent (5% KOH) for microscopical examination. The specimens were identified using fungi keys [Sosin, 1979; Ellis and Ellis, 1990]. The material is stored at the Herbarium of Tbilisi Botanical Garden and Institute of Botany.

Results and Discussion

Characterization of the following species (class - Gasteromycetes, subclass - Lycoperdales, family – Geastraceae, genus Geastrum) is given below: *Geastrum berkeleyi* Massee, *Geastrum kotlabae* V.J. Staněk, *Geastrum lageniforme* Cooke, *Geastrum quadrifidum* Pers.

1. **Geastrum berkeleyi** Massee - Fig. 1

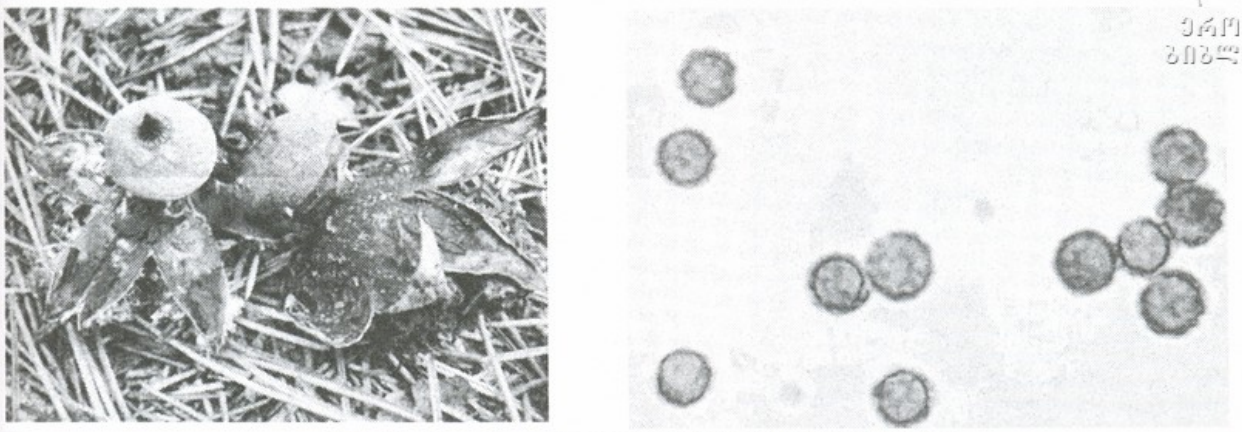

Fig. 1. *Geastrum berkeleyi* (Fruitbody, Spores)

Fruitbody first spherical, opened 5-8 cm diameter. Exoperidium firm, thick, torn on 6, 7, 8 unequal sharp lobes, outer surface covered with soil remnant, inner surface brown, smooth or scaly. Endoperidium 1-2.5 cm diameter, nearly roundish, slightly flattened, bright brown or grey, clearly warted, apophysis indistinct, with short stalk. Peristome long, conical, depressed silky zone, striate, disk whitish. Gleba dark brown. **Spores** brownish, spherical, warty, 4-6 µm diameter.

Habitat – on soil, under pines.

Distribution in Georgia – Vashlovani National Park (12.03.2008), Algeti National Park (02.05.2008).

2. ***Geastrum kotlabae*** V.J. Staněk - Fig. 2

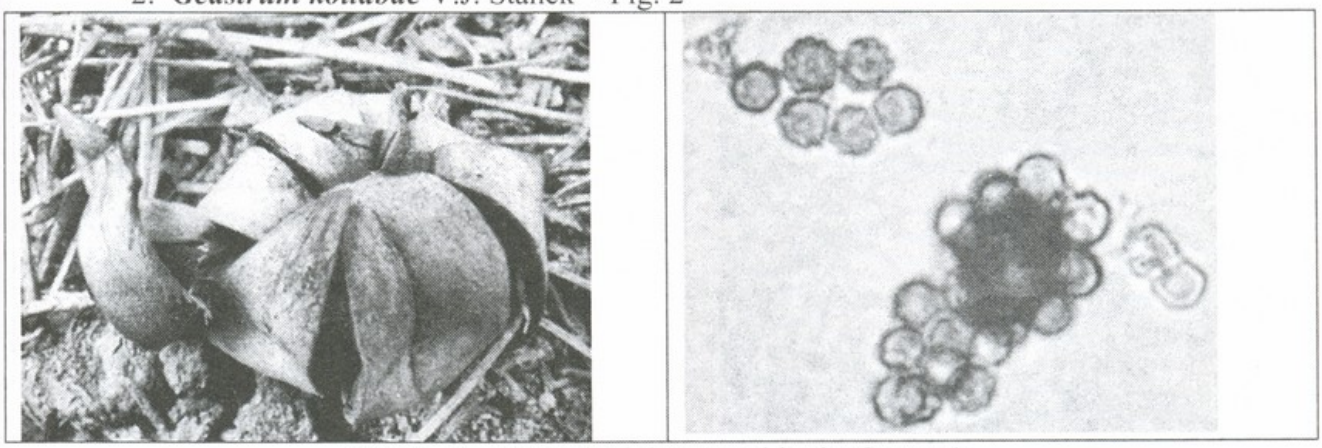

Fig. 2. *Geastrum kotlabae* (Fruitbody, Spores)

Fruitbody first flattened spherical, 1-2 cm diameter, opened 2-3 cm diameter, 5-10 lobes. Exoperidium firm, thick, outer surface whitish-grayish, inner surface dark chestnut-coloured, smooth or rarely scaly, hygroscopic, opened out when moist and closed when dry. Endoperidium spherical, slightly warty, old smooth, sessile, nut-coloured. Peristome conical, depressed silky zone, slightly expressed disk. **Spores** brown, spherical, warty 3.5-6.5 µm. diameter.

Habitat – on soil, under broad-leaf and coniferous trees.

Distribution in Georgia – Tbilisi Botanical Garden (29.04.2008)

2. ***Geastrum lageniforme*** Cooke - Fig. 3

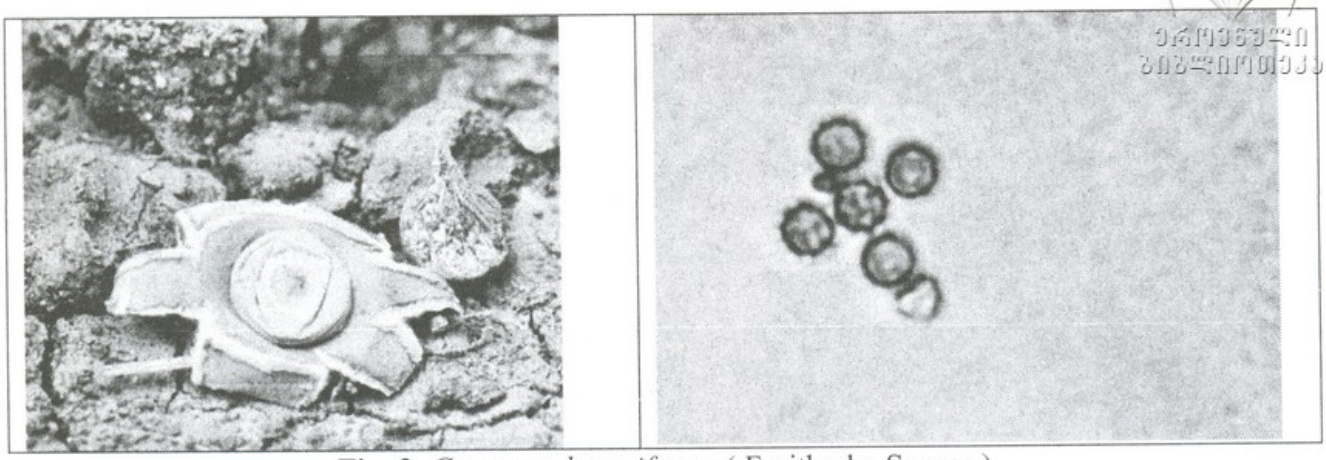

Fig. 3. *Geastrum lageniforme* (Fruitbody, Spores)

Fruitbody first bulb-shaped, with long sharp tip, opened 4-6 cm diameter, 6-9 lobes. Exoperidium outer surface whitish, inner surface ochraceous, slightly scaly. Endoperidium spherical, 1-2 c. diameter, whitish, sessile, parchment-coloured. Peristome grayish, fibrous, disk present. Gleba greenish. **Spores** spherical, yellowish, warty, 3-6.5 µm diameter.

Habitat – on soil, under juniper.

Distribution in Georgia – Vashlovani National Park, Herbarium materials (04.11.1989, I. Nakhursrishvili)

3. ***Geastrum quadrifidum*** Pers. - Fig. 4

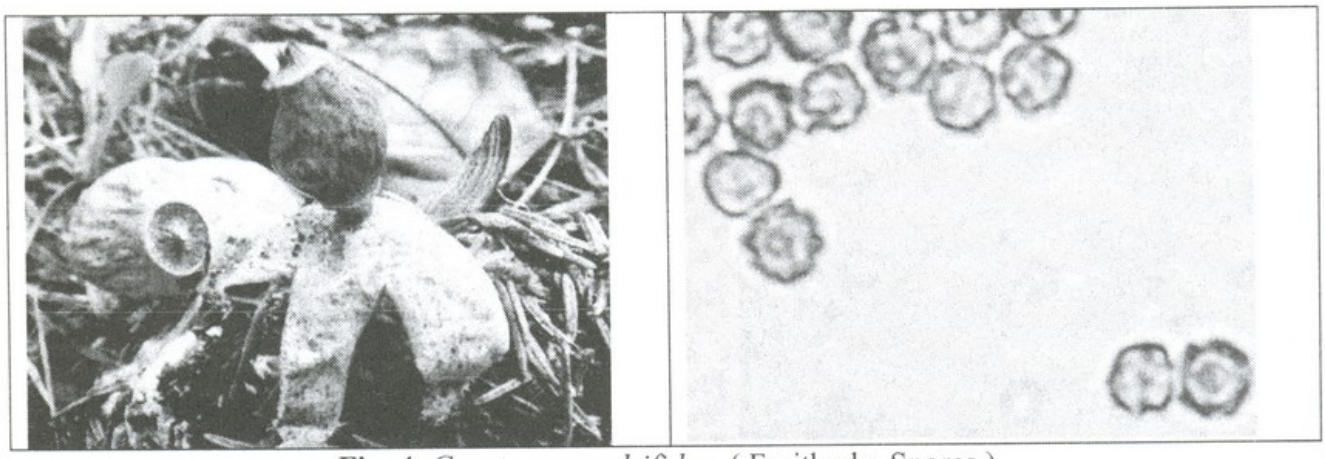

Fig. 4. *Geastrum quadrifidum* (Fruitbody, Spores)

Fruitbody first spherical, opened 2.5-4 cm diameter, 4 lobes. Exoperidium with 2 layers, cream, yellowish brown, both layers 4-lobed, outer layer saucerlike, often disappearing, inner layer lobes leaned on the outer layer. Endoperidium egglike, 1-2.5 cm diameter, bluish-grey, covered with white crystals, easily washed off in the rain. Stalk short, apophysis slightly expressed, peristome fimbriate, disk clearly expressed. **Spores** spherical, brown, warty, 3-6 µm diameter.

Habitat – on soil, under coniferous trees.

Distribution in Georgia – Kartli-Tsemi (02.08.2008)

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GEASTRUM PERSOON (GASTEROMYCETES)-ის ᲐᲮᲐᲚᲘ ᲡᲐᲮᲔᲝᲑᲔᲑᲘ ᲡᲐᲥᲐᲠᲗᲕᲔᲚᲝᲡᲗᲕᲘᲡ

ჯორჯაძე ა.

თბილისის ბოტანიკური ბაღი და ბოტანიკის ინსტიტუტი

რეზიუმე

(მიღებულია 15.06.2009)

სტატიაში განხილულია საქართველოში გვარი Geastrum-ის მრავალფეროვნება და აღწერილია საქართველოს მიკობიოტისთვის Geastrum-ის 4 ახალი სახეობა (*Geastrum berkeleyi* Massee, *G. kotlabae* V.J. Staněk, *G. lageniforme* Cooke, *G. quadrifidum* Pers.).

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EFFECT OF SEED STRATIFICATION ON EMBRYO GROWTH DYNAMICS AND DORMANCY BREAK IN *TAXUS BACCATA* L.

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(Received May 11, 2009)

Abstract

Seeds of English yew *Taxus baccata* L. germinate in the second or even the third year after ripening due to shift to dormant stage soon after maturation. In order to break dormancy seeds were warm (25° C, 90 d) and cold (5° C, 90 d.) stratified in moist conditions. At the end of cold prechilling mean embryo size increased 3 times, embryo reached the full length of the seed, seed coat spitted along the ridges, soon after sowing embryo became green and early germination phase occurred. Over 60% of the stratified seeds had a pregermination potential. Data obtained make it possible to suggest that a practical and easy method to overcome dormancy of yew seeds for mass propagation is a wet warm followed cold stratification. The adoption of this technique might be useful for yew conservation strategies.

Key words: dormancy breaking, stratification, *Taxus baccata* L.

Introduction

English yew – *Taxus baccata* is a dioecious forest conifer with limited distribution in Georgia. Except for pure stand of protected Batsara area, most of the yew populations are small and degraded. Yew regenerates poorly and is extremely slow growing. Usually yew seeds have a prolonged dormant period that could last up to 5 years. An efficient regeneration of the species is problematic. As an endangered species yew is included in Red Data Book of Georgia [Georgia Red Data Book, 1982]. In addition to the poor sexual reproduction, overexploitation of the yew resources (barks and needles of the species contain an anticancer alkaloid taxol) is an another cause in decreasing size and number of yew populations in many parts of Europe [Fetto-Neto et al., 1992; Lores et al., 1993; Zhiri et al., 1994, Majeda, 2000]. Vegetative propagation [Maden, 2002] solves the conservation issues partly, because only the seed germination and seedling establishment provides genetic diversity of the species.

Special pretreatment is necessary for seed dormancy breaking in *Taxus* species. Methods applied for overcome dormancy are divided into the two categories: those that use exogenous hormonal stimulation and those that use mechanical and thermal pretreatment – scarification and stratification. Numerous studies have been carried out on the dormancy breaking in *Taxus* species. Most of them concerns with *in vitro* embryo growth with the emphasis on hormone balance during chemical treatment [Le Page-Degivry, 1968; Le Page-Degivry, Garello, 1973; Dirr et al., 1987; Hu et al., 1992; Fett-Netto et al.; 1992, Zarek, 2007]. These methods require chemicals and equipment for embryo cultures. From mass propagation perspective rather simple, inexpensive and effective is to resort to scarification and stratification technique under the different thermal conditions. A

review of papers reporting germination of seeds in the genus Taxus revealed that the data on duration of stratification are not defined. Thus, in case of *T. baccata* timing varies from 8.5 month for both warm and cold stratification [Chien at al., 1994] to one year for cold stratification only [Chang, Yang, 1996].

The objective of this research was to study the effects of stratification conditions on seed dormancy breaking in *T. baccata*. We aimed to identify the optimum length of warm and cold pretreatment and statistically assay the embryo growth dynamics at pregermination stage.

Materials and Methods

Mature seeds of *T. baccata* were collected from a female trees growing in the Tbilisi Botanical Garden in September 2008. Seeds were divided into two categories: 1 - with fleshly red arils; 2 – with unripe green, or slightly pinkish arils at the time of collection. Ripe arils were manually removed from seeds, after which seeds were rinsed under running water with following storage in plastic bags filled with moist sawdust both for warm and cold stratification. Firstly seeds were stratified at 22 ° C for 3 month and then moved into the refrigerator at 5 ° C for 3 month. The control for the stratification treatments was non-stratified seeds stored in cold (8° C) basement. Seeds were examined for dormancy breaking every month from January to March 2009. Seed coat permeability was tested using 10% water solution of bromophenol blue. A free hand longitudinal sections were made with a razor blade. These sections where checked for staining of endosperm and embryo under a stereomicroscope Karl Zeiss DV 4 equipped with Canon A 470 digital photocamera. The embryo growth dynamics was analyzed by one-sample t-test with SPSS version 13.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Seeds of *T. baccata* selected in mid September were both mature (with red juicy arils) and immature (with underdeveloped green arils) (Fig. 1, a). Permeability test revealed that bromophenol blue accumulates in the seed coat in stratified and nonstratified seeds during 2 weeks of exposure. In case of seeds to be fully developed at the last stages of cold stratification, bromophenol blue penetrated both chalazal and micropilar part. The last was wide open due to seed coat cracking (Fig.1, b, c). However, the dye did not overcome the thin endotesta layer and did not reach the endosperm and embryo. As to nonstratified seeds, dye permeability was extremely low and it was localized only in chalazal end of the seed coat without penetration into the endosperm tissue (Fig. 1, d).

The dynamics of the embryo growth was monitored monthly from September to March (Table 1). Embryos in freshly matured seeds were 1.3±0.1 (mean± SE) mm long, ~ 21.5% of the length of the seed. Embryos grew to 2.1±0.4 mm during 90 d. of warm stratification and to 4.4±0.3 mm during 90 d. of cold stratification (Fig.1, e). Thus, embryo length increased by ~ 340% between seed maturity and pregermination. Cracking of the seeds occurred after 60 d. of cold stratification, when embryos had grown enough to start splitting the seed coat (Fig. 1, f). At the end of the third month of cold prechilling embryos reached maximal length and were ready to germinate (Fig. 1, g).

The level of maturation at the time of seed collection has significant effect ($p<0.001$) on embryo growth: embryos from the seeds with red arils increased over than tree times of the initial length, while embryos from seeds with green or slightly pinked arils remained underdeveloped after warm plus cold stratification.

Table 1. Embryo length dynamics analyzed by one-sample t-test

Days of stratification	Number of measurements	Mean embryo length (mm)	Std. Deviation	Std. Error Mean
30	30	1.2963	.06398	.01168
60	30	1.5730	.14135	.02581
90	30	2.1477	.19544	.03568
120	30	3.1533	.24316	.04439
150	30	4.3850	.16461	.03005
180	30	4.4203	.18342	.03349

During the warm stratification embryo increased partly. It seems that both warm and cold pretreatment are needed for postdevelopment of the embryo after the seeds mature. However, cold stratification is necessary also for dormancy break of the seed coat. After the embryo becomes fully nondormant, it has sufficient growth potential to push through all the layers of the seed coat that surround it. Thus, during dormancy breaking white and minute embryo underwent significant changes: considerably increased, extended the almost full length of the seed (maximum length attained was approximately 4.8 mm (Fig. 1, h)), turn to green and became ready to be photosynthetically active after sowing.

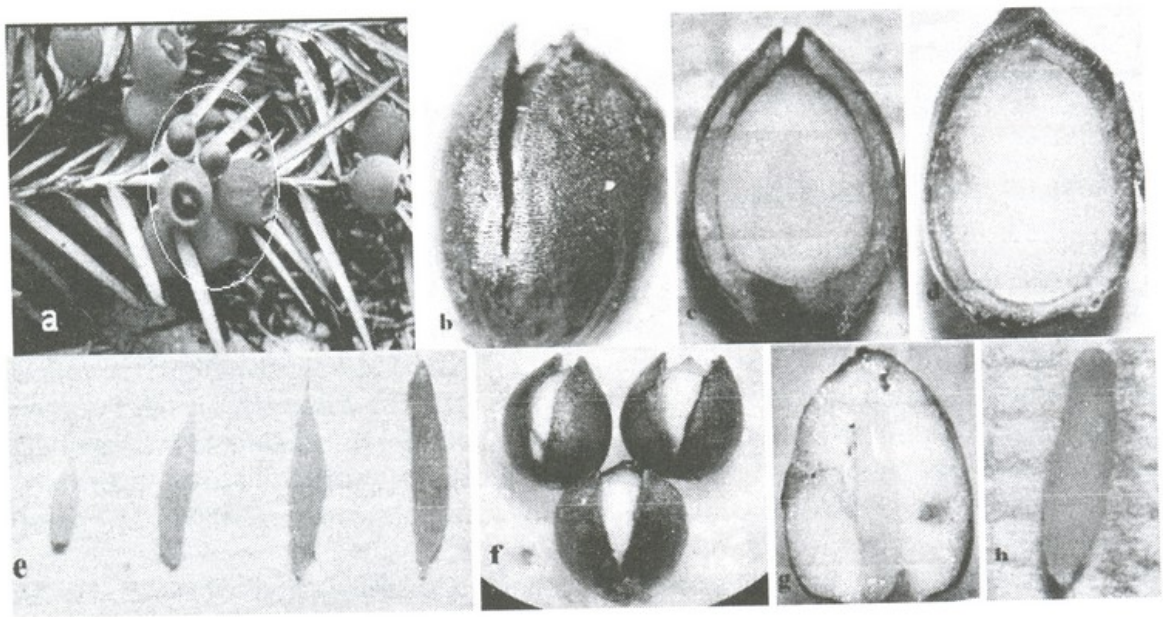

Fig 1. a - seeds at time of ripening, natural size; **b, c** -accumulation of bromphenol blue in sclerotesta and endotesta, x 7; **d**- longitudinal section of non-stratified seed after 14 days of staining, x 6.5; **e** – embryos at different stages of the development, x 5; **f** - splitting of the seed coat after warm (3 month) plus cold (3 month) stratification, x 3; **g** - fully developed embryo just before germination, x 5; **h** – 4.7 mm long embryo, x 5.7.

There are five classes of seed dormancy: physiological, morphological, morphophysiological, physical and combinational [Baskin, Baskin, 2003]. Each type of dormancy is influenced by genetic and environmental factors and interaction between the two. At the time of seed ripening embryo of *T. baccata* is differentiated but underdeveloped and seeds require pretreatment both for pregermination growth of the embryo and coat-imposed dormancy. This kind of dormancy is known as morphopysiological [Nikolaeva, 1967; 1977]. Seed coat of *T. baccata* is

known to be water-impermeable (physical dormancy) when mature [Thomas, Polwart, 2003]. However, according to our observations it seems that seed coat of *T. baccata* is not fully impermeable to water. Thus bromphenol solution penetrated cuticular and sclereid layers but did not surmount barrier of the thin endotesta layer. Moreover, in case of split seeds dye was accumulated in the wide open micropilar end but did not enter the endosperm and embryo (Fig. 1, b, c). When the embryo becomes fully undormant, it can exert enough force to break through the mechanical barrier. Our results of the permeability test revealed that seed coat has a mechanical dormancy, which is known to be a component of physiological dormancy and is caused by the resistance of the seed coat to embryo expansion and thus to germination [Baskin, 1998]. It seems that mechanical dormancy here plays an important role not only in the protection of embryo from fungal infection; it also keeps out moisture thereby delaying germination. Under natural conditions, seed coat dormancy may be overcome by prolonged exposure to soil moisture or saprophytic soil organisms. It also may be overcome by exposure to digestive acids in the gut of a bird or other animal. We suggest that warm and cold thermal pretreatment has a similar effect and breaks the morphophysiological dormancy via artificial simulation of the prolonged natural conditions.

The dormancy of *T. baccata* seeds was hypothesized to be caused by abscisic acid (ABA) or an ABA-like compound in the embryos [Lepage-Degivry, 1968; 1973]. It has been reported that gibberellins (GAs) play an essential role in many aspects of plant growth and development, and particularly in seed germination [Haba et al., 1985; Khafagi et al., 1986; Kumar, Neelakandan, 1992; Maske et al., 1997]. Apparently, the warm stratification caused the underdeveloped embryo and ABA concentration to decline, whereas the cold stratification induced the accumulation of GAs and/or increased the sensitivity of seeds to GAs, thus resulting in the release of physiological dormancy and enhancing seed germination. In vitro embryo growth experiments show that embryos from cold-treated seeds germinated earlier and at a higher frequency than those from control seeds [Flores et al, 1993].

In *T. maieri* seeds require not only 6 month of warm stratification at alternating temperatures of 25/15 ° C but also 3 months of cold stratification at 5° C to overcome the combined morphological and physiological dormancy. Alternative temperatures and cold stratification for 8.5 months produced a 50% germination rate in *T. mairei* [Chien et al., 1994]. Our observations revealed that pregermination growth requirements differ in *T. baccata:* the optimal requrement for the dormancy break was 90 d warm plus 90 d cold stratification under a wet comditions.

We can explain the phenology of seed germination in *T. baccata* by temperature requirements of seeds from our results. High temperatures are needed to elongate partly underdeveloped embryos and to enhance the permeability of the seed coat. Low temperature is necessary for completion of dormancy breaking because germination occurs after the full elongation of embryo at the end of cold stratification period.

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სტრატიფიცირების გავლენა ჩანასახის ზრდის დინამიკაზე და თესლის მოსვენების მდგომარეობიდან გამოსვლაზე უთხოვარში - *Taxus baccata* L.

ნ. შაქარიშვილი

თბილისის ბოტანიკური ბაღი და ბოტანიკის ინსტიტუტი

რეზიუმე

(მიღებულია 11.05.2009)

უთხოვრის - *Taxus baccata*-ს თესლი მომწიფების შემდეგ მალევე გადადის მოსვენების ფაზაში, ამიტომ მისი გაღივება ხშირად მხოლოდ მეორე ან მესამე წელს არის შესაძლებელი. მოსვენების პერიოდის ხელოვნური შეწყვეტის მიზნით გამოყენებული იყო თბილი (25°C, 90 დღე) და ცივი (5°C, 90 დღე) სტრატიფიცირება ნოტიო პირობებში. ცივი სტრატიფიცირების დასასრულს, რომელიც სამ თვეს გრძელდება, ჩანასახის სიგრძე საშუალოდ სამჯერ იმატებს, თესლის გარსი იხსნება წიბოს გასწვრივ, დათესვიდან ძალიან მალე ჩანასახი იძენს მწვანე ფერს და იწყება გაღივების ადრეული ფაზა. მიღებული შედეგები საშუალებას იძლევა გავაკეთოდ დასკვნა, რომ დიდი რაოდენობით უთხოვრის თესლის გაღივებისთვის ძალზე პრაქტიკული და მარტივი მეთოდია ნოტიო პირობებში თბილი და ცივი სტრატიფიცირების შედეგად მოსვენების მდგომარეობის შეწყვეტა. სტრატიფიცირებული თესლების 60% გაღივების უნარს იძენს. ამ მეთოდიკის გამოყენება სასარგებლო იქნება უთხოვრის თესლით გამრავლებისა და კონსერვაციისთვის.

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DYNAMICS AND SPECIES COMPOSITION OF PHYTO- AND ZOOPLANKTON OF THE TBILISI SEA, KUMISI AND LISI LAKES IN VICINITY OF TBILISI, GEORGIA

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Abstract

Three multifunctional water reservoirs in vicinity of Tbilisi, Georgia - Tbilisi Sea, Kumisi and Lisi Lakes- have been studied during 30 months in 2006-08 for phytoplankton and zooplankton abundance and dynamics, species composition and their role in assessing the ecological status of the reservoirs. Kumisi Lake, a brackish water body, appeared to be most rich in phytoplankton species - 124 species of 8 taxonomic groups were detected, leaded by Bacillariophyta. Cyanophyta dominated by abundance comprising 95% of the total phytoplankton numbers in the Kumisi Lake and causing periodical massive blooms involving toxic forms *Oscilatoria planctonica, O. limnetica, O. brevis, Microcystis aeruginosa* etc. 90 species of 7 taxonomic groups of microalgae were identified in the Tbilisi Sea, presented by Bacillariophyta (42% of species), Chlorophyta (17%), Cyanophyta (17%) and other groups. The spring and autumn algal blooms in 2006-07 were dominated by diatoms *Fragilaria crotonensis* and *F. capicin,* while in summer 2008 the massive bloom of Chrysophyta was detected. Similar phytoplankton composition but slightly lower diversity was observed in the Lisi Lake with 81 species of 8 taxonomic groups leaded by Diatom algae. Several abundance peaks during 2006-08 were due to significant increase in Diatoms, specifically, *Nitzschia holsatica* numbers and also by pyrrophyte algae (*Peridinium bipes, Ceratium carolinenanum*, etc*)*. The zooplankton analysis showed presence of three main groups (Rotatoria, Copepoda and Cladocera) in all three reservoirs with certain difference in species composition and abundance. Rotatoria comprised up to 62%, 55% and 63% of total number of zooplankton species in Kumisi and Lisi Lakes and Tbilisi Sea, respectively. The rest was presented by Copepoda (15-19%) and Cladocera (7-15%) groups, also by larvae of Nematodes, Chironomidae and Oligochaeta. In all three reservoirs the indicator phyto- and zooplankton species have been detected, and the saprobic class of water reservoirs was specified. Tbilisi Sea has been attributed to oligosaprobic-β-oligosaprobic reservoirs, while Lisi Lake was classified as β-oligosaprobic-β-mesosaprobic, and Kumisi Lake as α, β-mesosaprobic, eutrophic water reservoirs.

** - Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

Key words: Georgia, water reservoirs, phytoplankton, zooplankton, species composition, saprobic level, pollution.

Introduction

Aquatic life in inland freshwater reservoirs is affected by various factors such as physical habitat conditions, water chemistry, and biological interactions, therefore, biological assessment, along with other physical and chemical assessments, is crucial.

It is known that planktonic community plays important role in functioning of fresh and brackish water eceosystems in temperate zones [Jadin, 1940; 1949; Kasimov, 1972]. The phytoplankton represents the key biological element in water reservoirs in terms of production of organic substances, formation of oxygen regime and as a food source for other hydrobionts. The life cycle of algae is relatively short, they react quickly on changes in water quality and respectively can serve as effective indicators of water pollution [APHA, 1998; Golubovskaya, 1972; Water pollution microbiology, 1972]. Zooplankton is another important link of the food chain of a water reservoir and its abundance depends on food quantities and numbers of predators. Saprobic and trophic level of a water reservoir can be defined by biomass and diversity (species composition) of phyto- and zooplankton along with the other indicators of water pollution [APHA, 1998; Bick, 1963; Liebman, 1962; Ruoppa, Heinonen, 2006]. Noteworthy that the certain species of phyto and zooplankton can form associations with the bacteria, among them the human pathogens such as *Legionella, Campylobacter, Salmonella, Vibrio, E.coli*, etc [Colwell et al., 2003; Fedorov, 1979; Water pollution microbiology, 1972]. In associations with planktonic cells (on the surface or inside their cells) bacteria are resistant to the negative impact of the environment and can also reproduce hence the planktonic organisms can be the reservoirs of human pathogens.

During the 2006-2008 three water reservoirs in vicinity of Tbilisi: Tbilisi Sea, Kumisi and Lisi Lakes were studied for abundance and seasonal dynamics, and speciation of phytoplankton and zooplankton, and their role in assessing the ecological status of these water reservoirs. Tbilisi Sea, Kumisi and Lisi lakes differ from each other by the geochemical and hydrological parameters as well as by the anthropological load and consequently, the pollution rate.

Kumisi Lake, a natural brackish reservoir with total space 96.6 km^2 and maximal depth 4.5 m, is situated in Gardabani District, in the basin of river Mtkvari at the altitude of 470 m, in distance of 35 km from Tbilisi. The lake is endorheic with salt beds around it, hydrographic net consists of small rivers and functions mainly during the rainy seasons [Apkhazava, 1975; Barach, 1964].

Lisi Lake is situated North-West of Tbilisi, at the altitude of 624 m in the river Mtkvari basin. The hydrographic net of the lake is weak - there is only one constant input source, the lake is filled with rain and ground waters [Apkhazava, 1975; Barach, 1964]; the north side of the lake is swamp. Starting from 2002 two additional input sources (ground water) have been added [Vasser et al., 1989].

Tbilisi Sea is artificial freshwater reservoir created in the 50's of last century from river Yori water. It is situated East from Tbilisi in parallel of river Mtkvari ravine, in the place of three small lakes. Deep lowland is filled with the waters coming from Makhata Mountain and from Kakheti Plateau. The length of Tbilisi water reservoir is 11 km, maximum width – 2.5 km, depth - 45 m [Apkhazava, 1975; Barach, 1964].

All three water reservoirs are intensively used for recreation and fishing, also for irrigation. In addition, Tbilisi Sea represents a drinking water supply reservoir for a number of Tbilisi districts. The Kumisi Lake is used as fish farming reservoir and is also known for the therapeutic qualities of its grey mud. There are few data about the abundance and seasonal

dynamics of phyto- and zooplankton species of the above mentioned water reservoirs in relation with changing climatic conditions and the increased anthropological load.

Materials and Methods

The sample collection started in July, 2006 and continued including October, 2008. Measurement of physical-chemical parameters (water temperature, salinity, pH, conductivity, total dissolved solids - TDS, Dissolved oxygen) was done *at site* using portable multi-Log system YSI 556 (Yellow stone Instruments, USA).

The samples were taken from the surface of water bodies and the 100-150L of water was filtered through the 64 μ and 200 μ mesh size plankton nets. The concentrated plankton samples were fixed by 3.7- 4% formalin solution and examined using high-resolution light microscopes: Leica DMLS, Leica stereomicrioscope M55 (Germany), and Kruss Biological inverse microscope MBL 3100 (Germany) at the magnification X400.

Algae and the zooplankton species were studied using standard methodologies, including registration and enumeration of planktonic organisms in Nageotte or Bogorov counting chambers. Identification of plankton species was performed based on using the widely accepted morphometric characteristics [Identification guide of Azov and Black Sea Fauna, 1968; 1969; 1972; Jadin, 1940; 1949; Gorunova et al., 1968; Dedusenko-Shegoleva, Gollerbakh, 1962; Kiselev, 1954. Kiseleva, 2004; Kutikova, 1970; Moshkova, Gollerbakh, 1986; Kasimov, 1972; Ruoppa, Heinonen, 2006]. Focus was made on identification of indicator and dominant species. Saprobic type of a water reservoir was determined by direct assessment, according to the list of indicator organisms for a given saprobic level [Bick, 1963; Liebman, 1962; Sirenko, Gavrilenko, 1978; Willen, 2000].

Results and Discussion

The measurements of the physico-chemical parameters in three studied reservoirs (data not presented in this paper) revealed expected seasonal fluctuation in water temperature in all three reservoirs. A slight variation of total salinity and conductivity, and pH values was observed everywhere while variability of dissolved oxygen was more remarkable in Kumisi Lake. The difference between studied water bodies was revealed by total salinity: in the Tbilisi Sea this parameter was varying in the range of 0.09-0.18 ‰ and corresponded to the accepted value for the freshwater reservoirs. In the Lisi lake total salinity comprised 1.5 – 1.8‰ and 3.3 – 4.8‰ in Kumisi Lake. Significant difference was observed for the chlorophyll-a (Chla) data, with maximal value in Kumisi lake in summer time – up to 48 mg/L, and the minimum – in winter months in Tbilisi sea (as low as 0.39 mg/L).

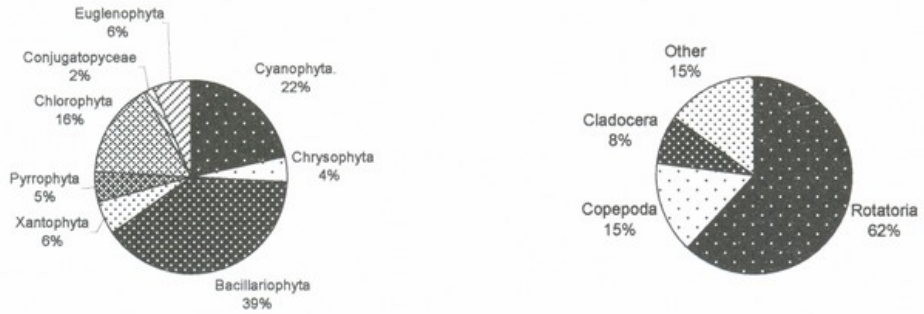
The results of monthly monitoring for phyto- and Zooplankton abundance and speciation are given separately for each reservoir.

Dynamics and speciation of Phyto- and Zooplankton in the Kumisi Lake

The monthly monitoring in 2006-2008 showed that the Kumisi Lake is quite rich with phytoplankton species. During 30 months representatives of almost all freshwater taxonomic groups have been registered, namely, 8 groups with total of 124 species (Fig. 1). The most diverse was *Bacillariophyta* group with 49 species detected (39% of total species revealed) followed by *Cyanophyta* with 26 species (22%).

The overall high phytoplankton abundance in the Kumisi Lake should be noted specifically. Although the quantitative characteristics of phytoplankton were changing by the season, expressed in couple of abundance peaks, this wasn't linked with the temperature

fluctuations (Fig. 6). Interestingly, during the whole observation period 95% of all algae in the Kumisi Lake were represented by *Cyanophyta,* both 64 μ and 200 μ fractions, with practically the same number of algae except August 2008 when in 64μ fraction 5.7 times more cells were detected compared to 200 μ fraction. The periods of massive phytoplankton blooms in the Kumisi Lake mainly coincided with summer and autumn months and were caused by *Cyanophyta.* High numbers of α and β-mesosaprobic species: *Oscilatoria planctonica, O. limnetica, O. brevis, Microcystis aeruginosa, M. pulverea, Spirulina minima, Anabaenopsis Elenkini, Anabaena flos–aqua* and others were registered. The polysaprobic forms such as *Euglena viridis* was also identified in the Kumisi Lake indicating that the water reservoir is eutrophic. *Cyanophyta* algae cause the major problem for the freshwater reservoirs, especially during the periods of massive blooming. Many species of this group are characterized by toxicity, especially in the process of aging and decomposing. The mucus of *Cyanophyta* can support propagation of many pathogenic bacteria. The bloom of toxic forms of *Cyanophyta (M. aeruginosa, A. flos–aqua, O. planctonica, O. limnetica)* could be a probable reason of massive fish death periodically observed in Kumisi Lake. And finally, fish reproduced in such environment also can be dangerous for human health [Golubovskaya, 1972; Gorunova et al., 1968; Sirenko, Gavrilenko, 1978].



a b

Fig.1. Percent distribution of phytoplankton and zooplankton taxonomical groups in the Kumisi Lake (2006-2008). a) phytoplankton groups; b) Zooplankton

Zooplankton in the Kumisi Lake during the study period was represented by 24 species of *Rotatoria, Copepoda and Cladocera* (Fig.1). The maximum number (16) of species belonged to Rotatoria, comprising 62% of total number. In the *Copepoda* group 3 species (15%) were identified and only 2 species (8%) in *Cladocera* group. The rest of 15% of zooplankton were presented by the larvae of different species - *Nematodes*, *Chironomidae* and *Oligochaeta.* Similarly to phytoplankton, seasonal changes in zooplankton abundance and diversity were also registered in Kumisi Lake (Fig.6). Notable increase in average number of zooplankton (from 138 ind/m^3 to 1413 ind/m^3) in 2007 can be explained partly by changes in study design, particularly by including 64μ plankton fraction in zooplankton enumeration. In this lake ecosystem change of the species correlated with the change of the temperature, although it was not the defining factor of population dominance. Especially clearly seasonal factor was showing in *Rotatoria* dynamics - their numbers were decreasing from summer to winter. The maximum Zooplankton abundance in Kumisi lake was registered in August 2008 when the number of *Rotatoria* (mainly *Brachionus angularis angularis,B. plicatus B.caluciflorus*) reached 6380 ind/m^3. During the study nauplii period of *Rotatoria* and *Copepode* were permanent components of zooplankton community with periodic increase in numbers of *Crustacea* larvae indicative of their high reproductive ability. In summer months several species of *Rotatoria* group, characteristic for oligo and β-mesosaprobic zones, prevailed such as *Asplanchna brightwelli, Brachionus angularis angularis, B. plicatus*

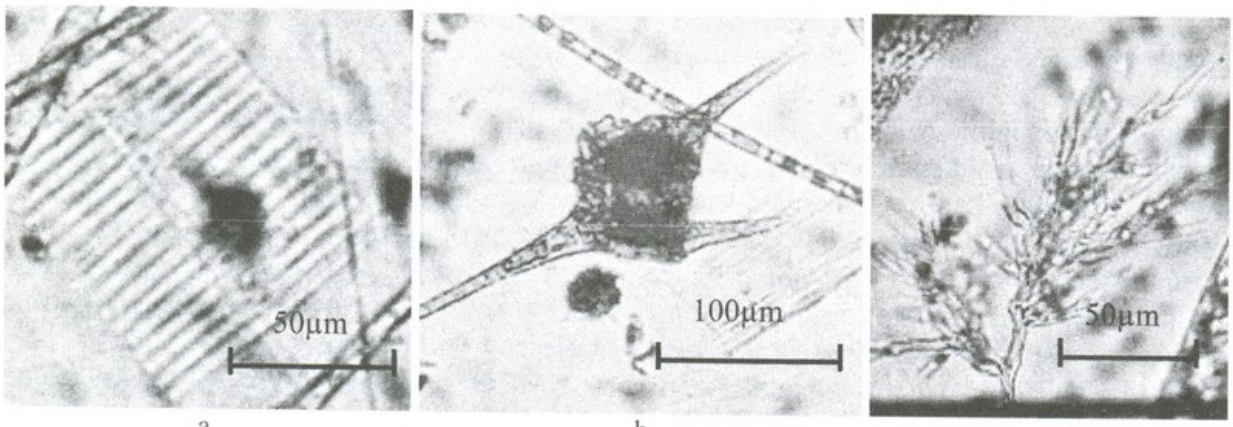
B.caluciflorus, Keratella cochlearis, K.tropica reducta, K.tropica tropica. β-mesosaprobic aerobic forms of *Rotatoria* are bioindicators of water pollution by the biogenic factors [Bick, 1963; Kutikova, 1970]. High content of detritus and chitin *of Canthocyclops bisetosus* in the Kumisi Lake should be mentioned as well.

Phytoplankton and Zooplankton abundance and diversity in the Tbilisi Sea

During 30 months monitoring in the Tbilisi Sea 90 species of microalgae were identified. These species belong to 7 taxonomic groups (Fig. 4), dominated by Diatoms (*Bacillariophita*: to this taxon 42 % of total number of species was attributed). *Chlorophyta* (17%), *Cyanophyta* (17%), *Pyrrophyta* (10%), *Xanthophyta* (7%) along with others were also represented.

The massive phytoplankton blooms in the Tbilisi Sea occurred (Fig.6) in spring and autumn caused mainly by diatoms. Namely, the maximum numbers were detected in 64μ size plankton fraction in October 2006 (48014 ind/m^3) and in April 2007 (45402 ind/m^3), with the numbers of *Fragilaria crotonensis* and *F. capicin* equaling 25000 cells/ml. In the same periods numbers of Daitoms in 200 μ size fraction were considerably low - only 1156 cells/ml and 207 cells/ml respectively. Existence of *Fragilaria crotonensis* and *F. capicina* in Tbilisi Sea has been considered as an important indicator for classification of this water reservoir. These microscopic algae belong to halophilic oligosaprobic forms and are widely spread in considerably clean oligotrophic freshwater reservoirs with low primary production.

The ecological situation in the Tbilisi Sea has been changed in 2008 when in early summer period the massive bloom of *Chrystophyta* was detected. Among identified 10 species *Hyalobrion ramosum* (200-230 cell/ml), *H.voigitii* (96-125 cell/ml), *Dinobrion berhningii swir.* (145-165 cell/ml) and *D.cylindricum* (100-110 cell/ml) were dominant (Fig.2). Occurrence of unicellular algae *Mallomonas misolepsis, M.robusta* and others should be mentioned as well. *Chrysophyta* are mainly autotrophic organisms and are spread in all climate zones - in swamps, lakes and rivers, coloring water in grey during the blooming [Matvienko, 1954]. Among *Chrysophyta there* are the toxic forms although during our study in Tbilisi Sea toxic forms haven't been detected.

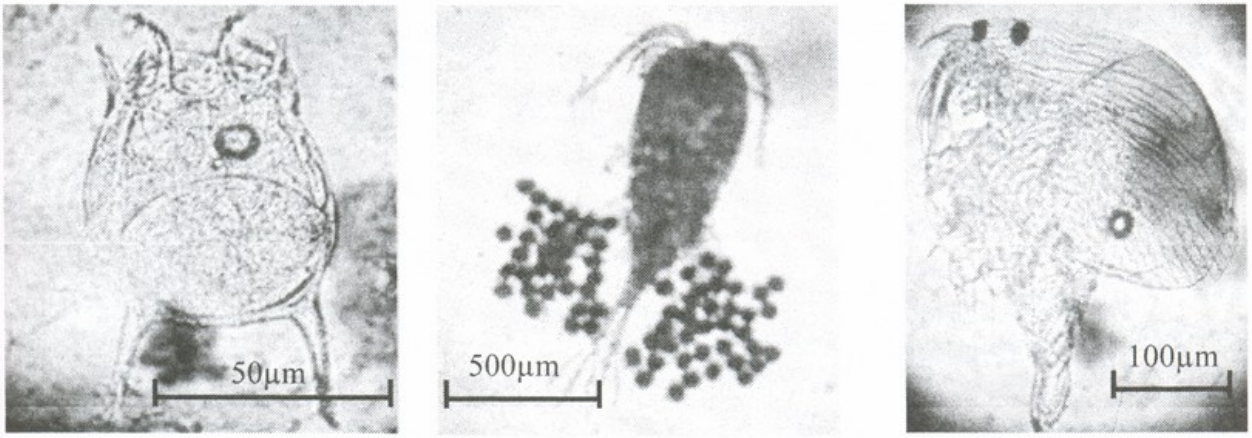


a b c

Fig. 2. Phytoplankton species form freshwater reservoirs around Tbilisi: a) *Fragilaria capucina Desm*; b) *Ceratium robustum (Amberg) Bachm*; c) *Hyalobrion ramosum Laut.*

Zooplankton of Tbilisi Sea was represented by 3 main groups: *Rotatoria, Copepoda* and *Cladocera* (Fig.4). Total of 27 zooplankton species were identified during 2006-2008, majority belonged to *Rotatoria* -17 species, while 6 and 3 species have been attributed to *Cladocera* and *Copepoda,* respectively. In 2006-2008 the numbers of zooplankton have been changing significantly in Tbilisi Sea (Fig. 6). In particular, the maximum numbers of zooplankton in 2007 was 1208ind/m^3 and reached 2529ind/m^3 in 2008. The total number of *Rotatoria* species and

dominant species were changing as well. For example, in 2006 the dominating species was *Lecane (s.str.) luna,* in 2007 - *Polyarthra trigla* and in 2008 - *Keratella cochlearis, Polyarthra trigla, Cyclops strenuous* (Fig. 3).



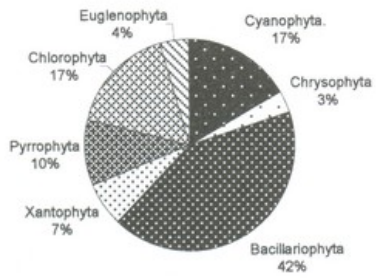
a b c

Fig. 3. Zooplankton species found in freshwater reservoirs around Tbilisi: a) *Keratella quadrata (Rotatoria*); b) *Cyclops strenuous F.,*1851 (*Copepoda)*; c) *Alona rectangular Sars (Cladocera).*

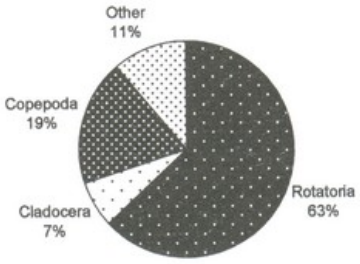
Phytoplankton and Zooplankton in the Lisi Lake

8 taxonomic groups of phytoplankton, total of 81 species, were registered in the Lisi Lake during 30 months monitoring period in 2006-2008 (Fig. 5). Diatom algae were characterized by highest diversity (42% of all species revealed) while *Chlorophyta* and *Pyrrophyta* algae prevailed by numbers. Cyanophyta species comprised 12% of total phytoplankton species in the Lisi Lake, the others were less or poorly represented. *Chlorophyta* are mainly phototrophs with e few saprophyte and endophyte species and live in freshwater reservoirs in plankton as well as in benthos [Moshkova, Gollerbakh, 1986; Sirenko, Gavrilenko, 1978]. Among these groups *Ankistrodesmus longissima, A.angustus, Scenedesmus quadricaudata, S.acuminatus* and others were often registered in Lisi Lake samples, some of them are toxic forms. Several peaks of phytoplankton abundance were observed in Lisi Lake during the study period (Fig. 6), among them two in 2007, in July and October, due to significant increase in Diatoms. More specifically, *Nitzschia holsatica* numbers reached 5700cells/ml. The blooming of pyrrophyte algae *Peridinium bipes, Ceratium carolinenanum* (Fig. 2) also took place, while relatively low numbers of *Cyanophyta* occurred. Such combination is understandable since the freshwater forms of *Pyrrophytae* require high content of oxygen and they can't live in water reservoirs with the excess numbers of Cynophyta [Kiselev, 1954]. In June, 2008 massive diatom bloom was registered in the Lisi Lake. Total phytoplankton numbers reached 39080 cell/ml, with practically equally distributed numbers in 64 ή and 200 ή fractions.

Zooplankton in the Lisi Lake was quite similar to the Tbilisi Sea zooplankton in both, the total abundance, and the variety of species and their numbers. Main zooplankton groups in this lake also were *Rotatoria, Copepoda* and *Cladocera*, among them *Rotatoria* accounted for 55% of total number of species (Fig. 5). Average numbers of zooplankton per annum during 2006, 2007 and 2008 were 105 ind/m^3, 959 ind/m^3 and 2129 ind/m^3, respectively. High numbers of *Keratella quadrata, Brachionus angularis angularis* were detected, along with *Cyclops streenus* from the *Copepoda* group (Fig. 3). Especially high numbers of zooplankton (3414 ind/m^3) were detected in summer, 2008 (Fig. 8). This was mainly caused by the increase in abundance of *Rotatoria* group species - *Polyarthra minor, Hexarthra fennica, Asplanchna brightwelli* and *Brachionus calyciflorus*. The number of *Copepoda Nauplius* also increased and reached 32% (484 ind/m^3).

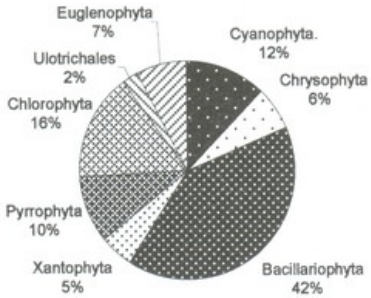


a



b

Fig. 4. Percent distribution of phytoplankton and zooplankton taxonomical groups in the Tbilisi Sea (2006-2008). a) phytoplankton groups; b) zooplankton.



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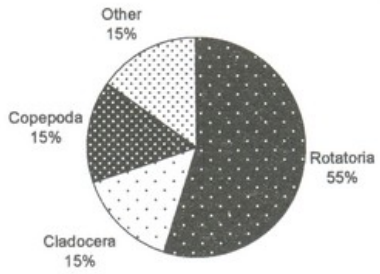

b

Fig 5. Percent distribution of phytoplankton and zooplankton taxonomical groups in the Lisi Lake (2006-2008). a) phytoplankton groups; b) zooplankton groups.

Conclusion

Summarizing the collected data we can conclude that the periodical variation of phytoplankton and zooplankton abundance was less evident in eutrophic water reservoir (Kumisi Lake) then in oligotrophic ones, e.g. Tbilisi Sea. The algal planktonic flora in these reservoirs was characterized by a large number of ubiquitous, cosmopolitan elements, together with several algae and zooplankton species having well defined ecological preferences, and serving as indicators of the trophic status and saprobic level of the water body. Among the three monitored reservoirs near Tbilisi Kumisi Lake appeared to be very rich in phytoplankton species. During 30 months of study 124 species of 8 taxonomic groups were detected, among them Bacillariophyta was identified as the most diverse group with 49 species registered. Dominant group in Kumisi Lake by abundance was Cyanophyta (95% of the total phytoplankton numbers) with periodical massive blooms involving toxic forms - *Oscilatoria planctonica, O. limnetica, O. brevis. Microcystis aeruginosa, M. pulverea, Spirulina minima, Anabaenopsis Elenkini, Anabaena flos–aqua.* Among 24 zooplankton species found in Kumisi Lake high numbers of Rotatoria, namely β-α-mesosaprobic species *Brachionus caluciflorus* were found.

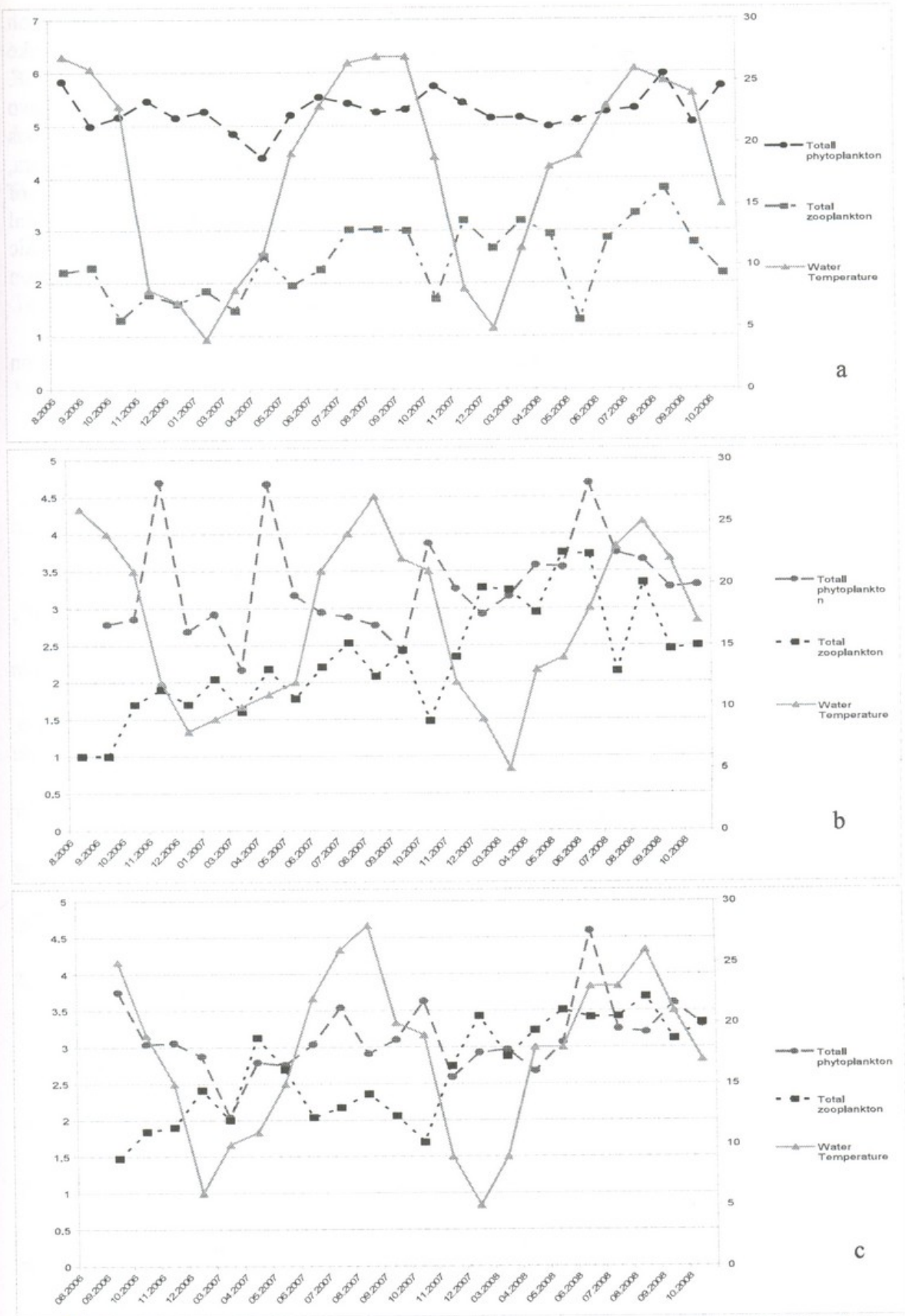

Fig. 6. Phyto- and zooplankton dynamics in fresh water lakes during 2006-2008: a) Kumisi lake, b) Tbilisi Sea, c) Lisi lake

According to the qualitative and quantitative characteristics of phyto- and zooplankton Kumisi Lake was attributed to eutrophic/polysaprobic water reservoirs. In Tbilisi Sea and Lisi Lake oligo- and β-saprobic algae have prevailed, among them Diatom algae *Fragilaria crotonensis, F. capicina, Nitzschia holsatica* and others dominated. The phytoplankton blooms in these two reservoirs occurred in spring and autumn. Zooplankton in the Tbilisi Sea and Lisi Lake was represented by 3 main groups of continues population: Rotatoria, Copepoda and Cladocera, characterized by high reproduction and prevalence of larvae which indicates the vital capacity of the water reservoirs. By phyto- and zooplankton abundance and speciation, also by water microbial parameters (Jaiani et al., 2009, paper under preparation) Lisi Lake was attributed to β-mesosaprobic water reservoirs, while Tbilisi Sea during the period of our observation varied between oligosaprobic and β-oligosaprobic levels.

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თბილისის მიდამოებში არსებული წყალსატევების (თბილისის ზღვა, კუმისი და ლისის ტბები) ფიტო- და ზოოპლანქტონის დინამიკა და სახეობრივი შემადგენლობა

კალანდაძე რ.[1], მიქაშავიძე ე.[1], მგელაძე მ.[1], ჯაიანი ე.[2], ჯანელიძე ნ.[2], ვაითჰაუსი ქ.[4], ჰაქი ა.[3], თედიაშვილი მ.[2]

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[3] მერილენდის უნივერსიტეტის პათოგენების კვლევის ინსტიტუტი, კოლეჯ პარკი, მერილენდი, აშშ

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(მიღებულია 27.07.2009)

რეზიუმე

2006-08 წლებში, 30 თვიანი მონიტორინგის შედეგად შესწავლილ იქნა თბილისის მიდამოებში არსებული სამი მრავალფუნქციური წყალსატევის - თბილისის ზღვის, კუმისისა და ლისის ტბების ფიტო და ზოოპლანქტონის

რიცხოვნობა, სეზონური დინამიკა, სახეობრივი შემადგენლობა და მათი როლი ტბების ეკოლოგიური სტატუსის განსაზღვრაში. მომლაშო კუმისის ტბა ყველაზე მდიდარი აღმოჩნდა ფიტოპლანქტონის სახეობრივი შემადგენლობის მხრივ. გამოვლენილია 8 ტაქსონომიური ჯგუფის 121 სახეობა, მათგან წამყვანია Bacillariohyta. რაოდენობრივი სიჭარბით და პერიოდული მასიური ყვავილობით გამოირჩეოდა Cyanophyta-ს ჯგუფის წყალმცენარეები (საერთო რიცხოვნობის 95%), მათ შორის იყო ტოქსიკური ფორმები *Oscilatoria planctonica, O. limnetica, O. brevis, Microcystis aeruginosa* და სხვა. მიკროწყალმცენარეების 7 ტაქსონომიური ჯგუფის 90 სახეობა გამოვლინდა თბილისის ზღვაში, მათგან 42% მიეკუთვნებოდა Bacillariophyta-ს, მას მოსდევდა Chlorophyta, Cyanophyta და სხვა მცირერიცხოვანი ჯგუფები. გაზაფხულისა და შემოდგომის ყვავილობა 2006-07 წლებში ძირითადად გამოწვეული იყო დიატომოვანი წყალმცენარეებით *Fragilaria crotonensis* და *F. capicin*, ხოლო 2008 წლის ზაფხულში - ოქროსფერი წყალმცენრეების (Chrysophyta) მასიური გამრავლებით. ლისის ტბაში გამოვლენილ იქნა ფიტოპლანქტონის მსგავსი შემადგენლობა, კერძოდ, 8 ტაქსონომიური ჯგუფი 81 სახეობით და დიატომეების უპირატესობით. 2006-08 წწ. წყალმცენარეთა რაოდენობრივი შემცველობის რამდენიმე პიკი იყო აღრიცხული, რაც ძირითადად დიატომეების, კერძოდ, *Nitzschia holsatica*-ს რაოდენობრივი მატებით იყო გამოწვეული, თუმცა აღინიშნა პიროფიტული წყალმცენარეების *(Peridinium bipes, Ceratium carolinenanum)* ყვავილობაც. ზოოპლანქტონის ანალიზმა ცხადყო, რომ სამივე წყალსატევში ძირითად ჯგუფებს წარმოდგენდნენ Rotatoria, Copepoda და Cladocera, მცირედი განსხვავებებით სახეობრივი შემადგენლობისა და რიცხოვნობის მხრივ. კუმისისა და ლისის ტბებში, და თბილისის ზღვაში Rotatoria შეადგენდა ზოოპლანქტონის სახეობათა ჯამური რიცხვის 62%, 55% და 63%-ს, შესაბამისად. დარჩენილი ნაწილი წარმოდგენილი იყო Copepoda-ს (15-19%) და Cladocera-ს (7-15%) სახეობებით, ასევე ნემატოდების, ქირომონიდების და ოლოგოქეტების ლარვებით. სამივე წყლის რეზევუარის კვლევისას დადგენილ იქნა ფიტო- და ზოოპლანქტონის ინდიკატორი სახეობები და განსაზღვრულ იქნა მათი საპრობულობის კლასი. თბილისის ზღვამ დაიკავა შუალედური ადგილი ოლიგოსაპრობულ და β-ოლიგოსაპრობულ წყალსატევებს შორის, ლისის ტბა კლასიფიცირდა როგორც β-ოლიგოსაპრობული-β-მეზოსაპრობული, ხოლო კუმისის ტბა როგორც α,β-მეზოსაპრობული, ევტროფული წყალსატევი.

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ANTAGONISTIC ACTIVITY IN THE AJARA (WESTERN GEORGIA) AND KAKHETI (EASTERN GEORGIA) NATURAL POPULATIONS OF WINE YEAST

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Abstract

Antagonistic activity of wine yeast strains isolated from natural populations of ecologically distinct regions of Georgia was studied. Wine yeast populations were isolated from small farm wine-cellars where the fermentation of grape juice proceeds spontaneously. K, N and S phenotype strains are revealed in the natural populations of wine yeast. Composite structures of populations appeared distinct.

Key words: wine yeast, killer system, yeast population

Introduction

A lot of wine yeast strains have the killer system synthesizing and isolating in the environment toxic proteins which kill the cells of sensitive strains [Marquin et al., 2002]. Killer strains compete with sensitive strains in getting aliment in the natural environment [Lenski, Riley, 2002]. First the killer strains were revealed in *Saccharomices cerevisiae* [Bevan, Makower, 1963; Naumov, Naumova, 1973], further in other yeast genera, namely: Candida, Cryptococcus, Debaryomyces, Hanseniaspora, Hansenula, Kluyveromyces, Ustilago, Pichia [Schmitt, Breining, 2002]. Detection of the effect of killer strains is possible via sensitive strains and is dependant on the definite conditions (acidity, salinity, temperature of environment). An exotoxin reveals its action in acid medium (pH 3-5.5) [Yasuyuki, 1995]. The killer system in saccharomycetes is very complicated; it is controlled by systems located in cytoplasm, as well as by the nuclear genes [Magliani, 1997; Marquin et al., 2002].

In saccharomycetes the killer activity is caused by symbiotic virus like particles consisting of dsRNA. Their molecular structure and action mechanism is well studied [Magliani et al., 1997; Schmitt, Breining, 2002]. In *Saccharomices cerevisiae* three types of killer system K1, K2, K28 are observed [Magliani et al., 1997; Gulbiniene et al., 2004]. According to the data obtained from the museum collections and wineries, also from the strains isolated from the natural populations it is established that some killer systems occupy the definite ecological niche. Antagonistic relationships determine the composition of strains participating in wine fermentation [Naumov, Naumova, 1973; Menabde et al., 2004; Rib'ereu-Gayon, 2006; Shatirishvili et al., 2007].

Materials and Methods

Isolation of strains from the natural population. Material (wine crust) was taken from 10 various small farm wine-cellars of the village according to the method described previously [Menabde et al., 2004; Shatirishvili et al., 2007]. Yeast strains isolated from the wine crust of separate wine-cellars (isolates) represent a micropopulation. Wine-cellars and vineyards from where the farmer has been harvesting were far from each other (at more than 500 m distance). Between those points gene streams (transmission of wine yeast cells via drosophila) are limited. Wine crust was taken after grape juice fermentation is over. In studied farms fermentation proceeds spontaneously and inlet of industrial strains does not occur. Then wine crust was put in sterile vessels.

From the vessels wine crust was transferred by microbiological loop into the sterile flask of 4 ml filled with grape juice. Dilute material was sown on the agar overlaid grape juice medium according to the streak method. Incubation of culture lasts for 4 days at 25^0C. Isolation of typical colonies and their cloning was conducted on the YEPD medium. Determination of species was carried out by existed criteria [Krasilnikov, Shcholokova, 1991]. Data obtained during 2008 were analyzed.

Yeast strains. Antagonistic activity was determined by the following test-strains: K7 (MATαarg9(KIL-1)); sensitive to K1 killer S14 (MATα) strain; M437 (wild type HM/HM (KIL-2)); sensitive to K2 killer 7A-p192 (MATα ade2) strain; MS 300 (MATα leu2 ura3 (KIL-28)); sensitive to K28 killer S6 (wild type HM/HN) strain.

Nutrient media. Cultivation was carried out on YEPD medium (glucose - 2%, bactopeptone - 2%, yeast autolysate - 1%, agar - 2%). Induction of sporulation was conducted on acetated medium (Na acetate – 1%, KCl – 0.5%, agar – 2%). Killer activity was observed on YEPD-MB medium (YEPD with methylene blue – 0.003%, acetate-phosphate buffer). Pure culture was isolated on the agar overlaid grape juice medium (grape juice - 50%, agar - 2%).

Assays of killing and resistance. Identification of K1 and K2 killer systems was carried out at pH 4.6 and of K28 system at pH 5.2 [Bevan, Makower, 1963]. Test-strains were sawn on Petri dishes as lawns; strains isolated from the natural population were brought in on them as streaks. Killer strains lysed both sensitive and unrelated killer test-strains. Around the streaks sterile zones of various sizes are formed. Neutral strains reveal resistance against the killer strains and do not lyse sensitive test-strains. Sensitive strains are killed on the killer test-strain lawn, and as a result the streak is dyed blue.

Results and Discussion

Antagonistic activity of wine yeast populations of Ajara (Kapreshumi, Batumi) and historically wine producing region Kakheti (Kvareli) was studied (Tables 1 and 2). Strains composing the both populations were divided into the three phenotypic classes: killer (K) producing exotoxins, sensitive (S) which are affected lethally by toxins and neutral (N) resistant to toxins.

Strains studied according to the killer test-strains were divided into morphs. Kapreshumi population consists of 21 and that of Kvareli 18 morphs. The morphs are distinguished by the number of strains united within them. Strains with neutral phenotype form big groupings. Determination of strain phenotype is carried out according to the relation to the test-strain. Strain consisting of K2 plasmid lyses strain with K2 plasmid, as well as sensitive strain. Strain consisting of K1 plasmid lyses both sensitive strain and strain with K1 plasmid. As is seen from Table 1, four strains grouped in II and V morphs of Kvareli population have K1 plasmid and 22 killer strains grouped in the rest I, III, IV, VI morphs have K2 plasmid. 3 strains grouped in I and III morphs of

Kapreshumi population have K1 plasmid, and 26 strains united within II, IV-X morphs have K2 plasmid (Table 2).

We have researched 10 composite micropopulations of Kvareli and Kapreshumi populations (Tables 3 and 4). Micropopulations composing the same populations differ from each other by killer-sensitivity.

In Kvareli micropopulations K, N and S phenotype strains occur with various frequencies. Neutral strains (N phenotype) are presented with high frequency in the micropopulations. Strains of K phenotype are not presented in three micropopulations (I, III and IV) at all. Their number as compared with strains of neutral phenotype is many fewer (2-18%) in the rest micropopulations. Sensitive strains occur in all 10 micropopulations, their frequencies vary from 6% to 40%. K an N phenotype strains are presented with high frequencies in all micropopulations and they determine the rate of fermentation.

In Kakheti region low vine is grown. Historically Kakheti (Kvareli) is viticulture region and the place where gene pool of wine yeast was formed and preserved. This process was supported by the existence of diversity of vines, soil-climatic conditions, technological peculiarities of wine-making (fermentation in a pitcher with grape mass). Eastern Georgia geographically is isolated as it is bordered with the countries without viticulture traditions, so the gene flow is weakened very much. Wine yeast of Kakheti appeared to be significant and interesting isolate.

Kapreshumi micropopulations differ from Kvareli micropopulations by their composition. Kapreshumi micropopulations differ from each other by S phenotype strain frequencies. I micropopulation consists of only sensitive strains. In VII, VIII and X micropolulations the killer strains do not occur. They consist of only N and S phenotype strains. It should be mentioned that in VII and X micropopulations S phenotype strains are observed with high frequency. K phenotype strains are found only among six micropopulations. Kapreshumi, and Batumi district generally is not viticulture region. High vine is cultivated here. Vines go along the trees and are rather high from the ground (3-5 m). Due to high precipitations characterizing this region yeast fungi occur not much on the grapes here. Spontaneous fermentation is often conducted by strains occasionally got into the grape juice. In six micropopulations of Kapreshumi sensitive strains take part basically in fermentation process.

As distinct from Batumi region the main feature of populations and composing micropopulations of viniculture regions of Georgia is that frequencies of K and N phenotype strains is high and they dominate in fermentation [Menabde et al., 2004; Shatirishvili et al., 2007].

Ability of toxin synthesis in killer strain always correlates positively with resistance against it. Resistance of the producer cell to its toxin does not assume the resistance to the different type of toxins isolated by other strains. Every strain can be characterized by resistance-sensitivity against toxin, and killer strains by the action spectrum on the other killers and strains [Bevan, Makower, 1963; Magliani et al., 1997; Gulbiniene et al., 2004]. Exotoxins affect the young yeast cells being in the phase of division [Baeza et al., 2008].

The killer systems are well studied in *Saccharomices cerevisiae*. In saccharomycetes the killer activity is caused by symbiotic virus like particles consisting of dsRNA. They are surrounded with protein capsids. Two types of such particles occur. One of them has major L genome, and the other one – minor M genome. L genome encodes polymerase and capsid consisting protein. M genome encodes exotoxin and determines resistance against toxin. L genome defines expression of M genome. The killer strains have particles of both types. Neutral strains have M genome; they can not synthesize toxin, or the toxin synthesized due to mutation induction is inactivated. About 20 nuclear genes participated in this process are revealed. Killer activity is controlled by nuclear-cytoplasm systems [Magliani, 1997; Sommer, Wickener, 1984]. It depends on the definite ecological conditions (acidity, salinity, temperature of environment). [Magliani, 1997; Marquin et al., 2002]. In the local vine populations the high frequency of neutral strains is caused by

inactivation of cytoplasm determinant, as well as by action of nuclear genes. Intrapopulational polymorphism of wine and brewer's yeasts is detected and confirmed by many scientists [Naumov, Naumova, 1973; Magliani et al., 1997; Gulbiniene et al., 2004; Shatirishvili et al., 2007]. Some killer systems occupy definite ecological niche [Naumov, Naumova, 1973; Gulbiniene et al., 2004; Shatirishvili et al., 2007].

Table 1. Determination of the frequencies of antagonistic activity of Kvareli (Kakheti) natural population

Number of morphs	Number of strains	Test-strains (lawn)			
		M437	7A-P192	K7	S14
I	5	N	K	N	N
II	3	K	N	N	N
III	10	N	N	K	N
IV	5	N	N	N	K
V	1	K	N	S	N
VI	1	S	K	N	N
VII	354	N	N	N	N
VIII	34	N	N	S	N
IX	30	N	S	N	N
X	16	S	N	N	N
XI	20	N	N	N	S
XII	6	S	N	N	S
XIII	1	N	S	N	S
XIV	4	N	S	S	N
XV	6	S	N	S	N
XVI	2	S	S	S	N
XVII	2	S	S	S	S

Table 2. Determination of the frequencies of antagonistic activity of Kapreshumi (Batumi) natural population

Number of morphs	Number of strains	Test-strains (lawn)			
		M437	7A-P192	K7	S14
I	1	N	N	N	K
II	10	N	K	N	N
III	2	K	K	S	S
IV	1	N	K	S	N
V	2	N	K	N	S
VI	3	S	K	N	N
VII	4	S	K	S	S
VIII	1	S	K	N	S
IX	3	N	K	S	S
X	1	S	K	S	N
XI	74	N	N	N	N
XII	27	N	N	S	N
XIII	18	S	N	N	N
XIV	3	N	S	N	N
XV	11	N	N	N	S
XVI	16	N	N	S	S
XVII	16	S	N	N	S
XVIII	22	S	N	S	N
XIX	2	N	S	S	N
XX	31	S	N	S	S
XXI	2	N	S	S	S

Table 3. Determination of the frequencies of K, N and S phenotypes in Kvareli micropopulation

Micro-population	Number of analyzed strains	Killer (K)		Neutral (N)		Sensitive (S)	
		Number	%	Number	%	Number	%
I	50	-	-	42	84	8	16
II	50	2	4	31	62	17	34
III	50	-	-	27	54	23	46
IV	50	-	-	45	90	5	10
V	50	1	2	33	66	16	32
VI	50	5	10	25	50	20	40
VII	50	9	18	23	46	18	36
VIII	50	3	6	41	82	6	12
IX	50	4	8	43	86	3	6
X	50	1	2	44	88	5	10
Total	500	25	5.0	354	70.8	121	24.2

Table 4. Determination of the frequencies of K, N and S phenotypes in Kapreshumi (Batumi) micropopulation

Micro-population	Number of analyzed strains	Killer (K)		Neutral (N)		Sensitive (S)	
		Number	%	Number	%	Number	%
I	25	-	-	-	-	25	100
II	25	2	8	-	-	23	92
III	25	2	8	-	-	23	92
IV	25	18	72	2	8	5	20
V	25	4	16	7	28	14	56
VI	25	1	4	20	80	4	16
VII	25	-	-	13	52	12	48
VIII	25	-	-	19	76	6	24
IX	25	2	8	3	12	20	80
X	25	-	-	9	36	16	64
Total	250	29	11.6	73	29.2	148	59.2

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ანტაგონისტური აქტივობა აჭარისა და კახეთის ღვინის საფუარის ბუნებრივ პოპულაციებში

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რეზიუმე

ეკოლოგიურად განსხვავებული ორი რეგიონის კახეთისა (ყვარელი) და აჭარის (კაპრეშუმი) ღვინის საფუარის ბუნებრივი პოპულაციიდან გამოყოფილ შტამებში შესწავლილია ანტაგონისტური აქტივობა. პოპულაციები გამოყოფილია მცირე ფერმერულ მეურნეთა მარნიდან, სადაც ყურძნის წვენის ფერმენტაციის პროცესი სპონტანურად მიმდინარეობდა. ღვინის საფუარის ბუნებრივ პოპულაციებში გამოვლენილია K, N და S ფენოტიპის შტამები. ორივე პოპულაციაში გვხვდებოდა K1 და K2 სისტემის მქონე შტამები. პოპულაციები შემადგენელი სტრუქტურით აღმოჩნდა განსხვავებული.

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ASSOCIATION BETWEEN SEROPOSITIVITY FOR CYTOMEGALOVIRUS (CMV) AND CD4$^+$ CYTOTOXIC T CELLS EXPANSIONS IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTE LEUKAEMIA (B-CLL) AND HEALTHY CONTROLS

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Abstract

B cell chronic lymphocytic leukaemia (B-CLL) is characterized by the clonal expansion of CD5$^+$CD19$^+$CD23$^+$ B cells. During the course of B-CLL, the expansion of neoplastic clone is accompanied by a disbalance between CD4$^+$/CD8$^+$ T cells and by deficiency of T cell function. We have previously shown an expansion of CD4$^+$ perforin (PF)$^+$ cytotoxic T cells (cytT) with undefined specificity in patients with B–CLL. It has been demonstrated by others that the expansion of CD4$^+$PF$^+$ T cells in control individuals is often associated with chronic viral infections. Taking into consideration that B-CLL patients are immunocompromised, with frequent viral infections, we investigated the role of CD4$^+$PF$^+$ cytotoxic T cells in immune responses to one of the most common chronic viral infections – human cytomegalovirus (hCMV). We studied an association of cytT cell frequencies with the chronic CMV infection in 32 B-CLL patients and 18 age-matched healthy controls. Peripheral blood mononuclear cells (PBMCs) were immunostained with anti-CD4~PerCP monoclonal antibodies (mAb), fixed, permeabilised and immunostained with anti-PF~FITC mAb. Cells were fixed and analyzed by flow cytometry. Serum samples were routinely tested for anti-IgG antibodies to CMV. Here we show that CD4$^+$PF$^+$ T cell expansions appeared to be strongly associated with CMV seropositivity in healthy individuals, and, particularly, in B-CLL patients. The immunocompromised status of the majority of B-CLL patients may facilitate expansion of this unusual population of cytotoxic cells to combat reactivation of a chronic CMV infection.

Keywords: cytotoxic CD4$^+$ T cells, B-CLL, perforin, CMV

Introduction

B cell chronic lymphocytic leukaemia (B-CLL) is characterized by the clonal expansion of CD5$^+$CD19$^+$CD23$^+$ B cells in peripheral blood and bone marrow [Dighiero, Binet, 1996; Caligaris-Cappio, Hamblin, 1999]. During the course of B-CLL, the expansion of neoplastic clone is

accompanied by a disbalance between $CD4^+/CD8^+$ T cells and by the deficiency of T cell function [Zaknoen, Kay, 1990] such as mitogenic stimulation [Kay, Perri, 1988], helper T cell function [Prieto et al., 1993], mixed lymphocyte reaction [Rossi et al., 1996]. Previously, we have shown an expansion of $CD4^+$ perforin $(PF)^+$ T cells in patients with B–CLL [Porakishvili et al., 2001]. Subsequently, we have demonstrated, that $CD4^+PF^+$ T cells are able to kill B-CLL cells via PF-mediated pathway when co-cultured with anti-CD3/CD19 bispecific antibodies, although the specificity of this cell subset remained unidentified [Porakishvili et al., 2004].

Later it has been shown that the expansion of $CD4^+PF^+$ T cells is often associated with chronic viral infections [Appay et al., 2002; Appay et al., 2004; Gamadia et al., 2003; Amyes et al., 2003; Landais et al., 2004] leading to the suggestion that $CD4^+PF^+$ cytotoxic T cells are involved in the immune control of chronic viral infections in both healthy and immunocompromised individuals [Kalina et al., 2005]. One of the most common chronic viral infections is human cytomegalovirus (hCMV) - a β-herpesvirus that infects 60-90% of population [Ho, 1990].

Taking into consideration, that B-CLL patients are immunocompromised with frequent chronic viral infections [Zaknoen, Kay, 1990], we studied here the role of $CD4^+PF^+$ cytotoxic T cells in anti-CMV responses both in B-CLL patients and in healthy individuals of the relevant age. Our preliminary unpublished data indicated that the percentages of $CD4^+PF^+$ T cells in $CD4^+$ T cell population significantly increases in B-CLL cells treated with Campath (anti-CD52 monoclonal antibody, mAb) known to re-activate CMV infection.

From the results discussed below it becomes evident, that CMV infection drives expansions of the cytotoxic $CD4^+PF^+$ T cells in healthy individuals, and, particularly, in B-CLL.

Materials and Methods

Patients. We studied peripheral blood from 32 B-CLL patients, at various stages of the disease (Rai I-IV), aged 54-80 (median age 67), care of Professor Darejan Ghirdaladze, Institute of Haematology and Blood Transfusiology, Tbilisi, Georgia. WBC count in patients varied from 4.9×10^9/L to 120×10^9/L. Twenty four patients were untreated during six months prior to the study, and 8 were treated with COPP (n=5), ACOP (n=3). Ten ml of blood were collected in heparinised tubes (Sigma), stored at room temperature (RT) and processed the same day. The control group consisted of 18 healthy age-matched volunteers (median age 63).

Serology. Serum samples from B-CLL patients and healthy controls were routinely tested for anti-IgG antibodies to CMV using standard ELISA kits: BioElisa CMV-IgG (Biokit SA). In our cohort of patients, 24 were seropositive (SP) and 8 seronegative (SN); within the control group, 11 were SP and 7 SN.

Isolation of peripheral blood mononuclear cells (PBMCs). Whole blood was mixed at a 1:1 ratio with Hank's balanced salt solution (Sigma, UK) and overlayed onto Histopaque (1.077 g/ml) solution (Sigma) at a ratio 3:1. After centrifugation at 400g for 30 min at RT the interface was aspirated and the resultant cell suspension diluted with Hank's balanced salt solution at 1:4 ratio. The cell suspension was washed twice by centrifuging at 300g for 10 min at 4°C (Sigma 4K15) and re-suspended into RPMI 1640 medium (Sigma), supplemented with 10% fetal calf serum (FCS, Sigma).

Percentages of PF^+ T cells in ex vivo $CD4^+$ T cell subset. PBMC were re-suspended in RPMI-1640 (supplemented with 10% FCS) at a concentration $1x10^6$ cells/ml and incubated with 10 μl anti-CD4~PerCP monoclonal antibody (mAb) (BD Biosciences) for 30 min, on ice in the dark. Cells were washed twice in 2ml PBSA (PBS, pH 7.2, supplemented with 1% Bovine serum albumin and 0.01% Sodium azide (all Sigma)), centrifuged at 300g for 5 minutes at 4°C and re-suspended as above. For intracellular staining cells were fixed with 80 μl of medium A (Caltag Fix

and Perm Kit, Invitrogen, UK) for 15 min at room temperature (RT) in the dark and washed twice as above. This was followed by permeabilisation with 80 μl of medium B (Caltag Fix and Perm Kit) for 15 min at RT, and incubation with 10 μl of anti-PF~FITC or IgG2a-FITC isotype control mAbs (BD Biosciences) for 30 min on ice in the dark. Cells were washed as above, fixed with 400 μl 2% paraformaldehyde solution (PFA, Sigma) and analysed by Flow Cytometry (FACScan, BD). Background staining with IgG2b-FITC mAb was subtracted from the results.

Statistical Analysis. Data was analysed using the 1-tailed Mann-Whitney U test. P-values were considered significant at 0.05 or below. The data are expressed as mean ± standard deviation.

Results

$CD4^+PF^+$ T cell expansion is strongly associated with CMV seropositivity in B-CLL patients and healthy controls

Consistent with our previous data [Porakishvili et al., 2004], the proportion of $CD4^+$ cells expressing PF *ex vivo* was higher in B-CLL patients (13.53±15.5, n=32) compared to healthy age-matched controls (4.6±6.3, n=18), p=0.0018. However, PF expression by $CD4^+$ T cells was found to be strongly associated with CMV seropositivity. $CD4^+PF^+$ cell frequencies were significantly increased in seropositive (SP) as compared to seronegative (SN) B-CLL patients ($p<0.001$) as well as in SP compared to SN healthy age-matched controls ($p<0.05$) (Figure 1). $CD4^+PF^+$ cell populations in almost half (11 out of 24) SP patients accounted for 20-50% of the total percentages of $CD4^+$ cells. Meanwhile no significant $CD4^+PF^+$ expansions were observed in either SN B-CLL patients or SN control individuals. The low frequency of $CD4^+PF^+$ T cells in CMV SN patients was not treatment related, as PF expression was significantly higher in $CD4^+$ cells from untreated SP patients (n=18) compared to treated SN patients (n=6, $p<0.05$).

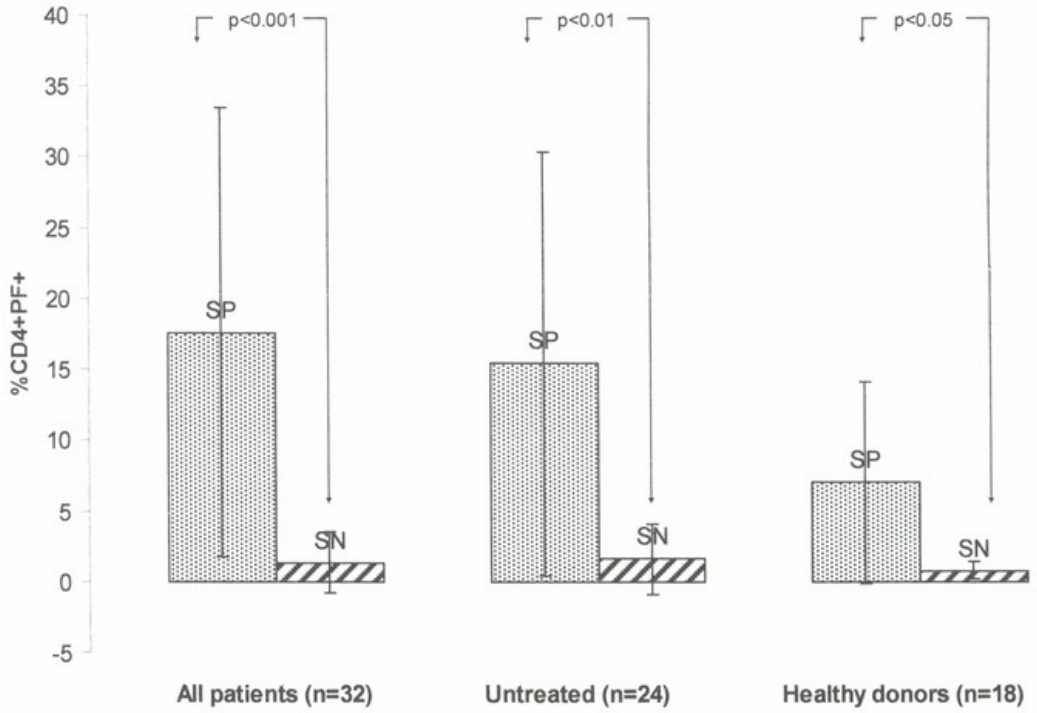

Fig. 1. CMV seropositivity is associated with $CD4^+PF^+$T cell expansion in B-CLL patients and healthy controls. PBMC from B-CLL patients and controls were immunostained with anti-CD4~PerCP mAb, fixed, permeabilised and immunostained with anti-PF~FITC or IgG2a-FITC isotype control mAbs. Samples

were fixed and data were acquired by flow cytometry. Values shown indicate percentages of $CD4^+PF^+$ cells in the total $CD4^+$ T cell population. Data were analysed using the 1-tailed Mann-Whitney U test.

Discussion

hCMV is widely accepted as a potent immune stimulator that can have dramatic effects on the T cell numbers and function. However, little is known about the role of $CD4^+$ T cells in B-CLL with regard to CMV specificity. We have previously described expansion of $CD4^+PF^+$ T cells in peripheral blood of B-CLL patients [Porakishvili et al., 2001]. Here we demonstrate that there is a strong association between the relative number of $CD4^+PF^+$ cells and CMV seropositivity. The lack of an appreciable $CD4^+PF^+$ population in CMV SN individuals strongly indicates that CMV is a major inducer of $CD4^+PF^+$ T cell expansions. However, since large $CD4^+PF^+$ T cell populations are not present in all SP B-CLL patients it appears that CMV-induced $CD4^+PF^+$ T cell expansions may be dependant on other factors such as re-activation of a chronic CMV infection, or co-infection with other viruses such as Epstein-Barr Virus (EBV) [Appay et al., 2002; 2004].

Interestingly, CMV-associated, $CD4^+PF^+$ T cell expansion was not limited to B-CLL patients, but was also observed in aged-matched, SP healthy controls (Figure 1).

Our data implies that $CD4^+PF^+$ T cells expand and accumulate due to a continuous stimulation with CMV in SP individuals. The extent to which this takes place reflects the efficiency of immune control over the virus. Insufficient immunosurveillance of CMV infection in immunocompromised B-CLL individuals may lead to the extensive $CD4^+PF^+$ cell expansion, observed in B-CLL patients.

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ციტომეგალოვირუსზე სეროდადებითობის კავშირი $CD4^+$ ციტოტოქსიკური T უჯრედების ექსპანსიასთან B-ქრონიკული ლიმფოციტური ლეიკემიით დაავადებულებში და ჯანმრთელ დონორებში

კულიკოვა ნ.[1], ცერცვაძე თ.[1], ცაგარეიშვილი პ.[1], სერედა ლ.[1], თევზაძე მ.[1], ქარდავა ლ.[1], გაჩეჩილაძე ნ.[1], ლუხუმაიძე მ.[1], ღირდალაძე მ.[2], ამაღლობელი ნ.[1], ფორაქიშვილი ნ.[1]

[1]ივანე ჯავახიშვილის თბილისის სახელმწიფო უნივერსიტეტი
[2]თბილისის ჰემატოლოგიისა და ტრანსფუზიოლოგიის ინსტიტუტი

(მიღებულია 10.07.2009)

რეზიუმე

B უჯრედული ქრონიკული ლიმფოციტური ლეიკემია (B-ქლლ) ხასიათდება $CD5^+CD19^+CD23^+$ B უჯრედების კლონური ექსპანსიით. B-ქლლ-ის დროს ნეოპლასტური კლონის ექსპანსიას თან ახლავს $CD4^+/CD8^+$ T უჯრედული პოპულაციების შეფარდების დისბალანსი და T უჯრედული ფუნქციის დეფიციტი.

წინა კვლევებში ჩვენ ვაჩვენეთ, რომ B-ქლლ-ით დაავადებულებში შეინიშნება დაუდგენელი სპეციფიკურობის ციტოტოქსიკური $CD4^+$ პერფორინ$(PF)^+$ T უჯრედების (ციტT) პოპულაციის ექსპანსია. სხვა ავტორების მიერ ნაჩვენები იყო, რომ $CD4^+PF^+$ ციტT უჯრედების ექსპანსია ჯანმრთელ დონორებში ხშირად

ასოცირებულია ქრონიკულ ვირუსულ ინფექციებთან. იმ ფაქტის გათვალისწინებით, რომ B-ქლლ-ით დაავადებულ პირებში დაქვეითებულია იმუნური სტატუსი და ხშირია განმეორებადი ვირუსული ინფექციები, გადავწყვიტეთ შეგვესწავლა $CD4^+PF^+$ T უჯრედების როლი ერთ-ერთი ყველაზე გავცელებული ვირუსული ინფექციის - ადამიანის ციტომეგალოვირუსის (ცმვ) საწინააღმდეგო იმუნურ პასუხში. ამისთვის შევისწავლეთ ციტოტოქსიური $CD4^+$ T უჯრედების პოპულაციის პროცენტული რაოდენობის დამოკიდებულება ციტომეგალოვირუსის (CMV) არსებობაზე 32 B-ქლლ-ით დაავადებულის და 18 ჯანმრთელი დონორის შემთხვევაში. ანტი-CD4~PerCP მონოკლონური სხეულებით (მკა) ვახდენდით პერფერიული სისხლის მონონუკლეარული უჯრედების იმუნოფენოტიპირებას და მომდევნო ფიქსაციის და პერმეაბილიზაციის შემდგომ მეორად შეღებვას ანტი-PF~FITC მკა-თ. ფიქსირებული უჯრედების ანალიზი ხდებოდა გამდინარე ციტომეტრიის მეთოდით. შრატი იყო რუტინულად ტესტირებული ცმვ-ს საწინააღმდეგო ანტი-IgG ანტისხეულებზე. ნაჩვენებია, რომ ციტომეგალოვირუსული ინფექცია განაპირობებს ციტოტოქსიკური $CD4^+PF^+$ T უჯრედების პოპულაციის ექსპანსიას როგორც B-ქლლ-ით დაავადებულებში, ასევე ჯანმრთელ დონორებში. დაქვეითებული იმუნური სტატუსი B-ქლლ-ით დაავადებულების უმრავლესობაში, შესაძლებელია, განაპირობებს ამ უჩვეულო ციტოტოქსიკური უჯრედების პოპულაციის ექსპანსიას, იმისთვის, რომ მოახდინონ ქრონიკული CMV ინფექციის რეაქტივაციის დათრგუნვა.

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ANTIMICROBIAL ACTIVITY OF ACTINOMYCETES ISOLATED FROM DIFFERENT AZERBAIJAN SOILS

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Abstract

The antimicrobial activity of actinomycetes isolated from some soils of Azerbaijan was studied. Strains tested have shown different activities against the different test strains. They were more active against the Gram positive bacteria. Activity against the Gram negative bacterium *E. coli* was relatively low.

Key words: actinomycetes, Actinomadura, Microbispora

Introduction

At present studies on searching the new biologically active connections are carried out intensively. Majority of these connections are the metabolites of microorganisms. More considerable sources of the new antibiotic substances are actinomycetes [Zakalyukina et al., 2004] representing the original group of prokaryotic microorganisms with molecular, chemical and physiological properties of prokaryotes, and morphological characteristics of eukaryotic fungi [Zenova et al., 2001; Goodfellow, Kim, 1999].

Cultures of the scarce genera are of a great interest as an unstudied source of new natural connections [Galatenko et al., 1990; Hayakawa et al., 1991]. Recently intensive investigation of biological activity of some actinomycete strains of scarce genera showed that they are perspective in search of new connections and they should be researched more widely [Zenova et al., 2002; 2004].

The aim of the work is to investigate antimicrobial activity of actinomycetes isolated from different soils of Azerbaijan.

Materials and Methods

The actinomycetes isolated from soil samples taken from Ismayilli, Shamakhi and Gebele regions of Azerbaijan were researched. For studying the antimicrobial activity of actinomycetes the disk method was used. Strains were isolated in Gauze-1 mineral agar.

Test cultures were estimated as a stable in zones with diameter up to 10 mm, as a less sensitive in zones of 11-15 mm and sensitive in zones of 15-25 mm diameter [Dobrovolskaya et al., 1989].

As the test cultures the following Gram positive bacteria - *Bacillus mesentericus*, *Staphylococcus albus* and *Sarcina ventriculi* and Gram negative bacterium - *Escherichia coli* were

used. All the test cultures were taken from the department of Microbiology of Biology faculty of Baku State University. The diameters of all lysed zones were measured in each Petri dish and results were calculated statistically [Dobrovolskaya et al., 1989; Lakin, 1980].

Results and Discussion

During the investigation 335 actinomycete strains were isolated. Among them 138 strains were recovered up to pure culture, 14 identified as a genus Actinomadura and 3 as a Microbispora and their antimicrobial activities were tested against the abovementioned test cultures. Strains tested for their antimicrobial activity showed different activities against the different test strains. They were more effective against the Gram positive bacteria. Activity against the Gram negative bacterium *E. coli* was relatively low.

Actinomadura strains were most active against *Staphylococcus albus* and *Sarcina ventriculi* – 12-20 mm (Table 1). *Actinomadura* sp.1 was more active against *Bacillus mesentericus* – 16 mm. It is also the most active against the Gram positive bacteria among the first five strains (*Actinomadura* sp.1-5). Its effect on *E. coli* is less – 5 mm. Activity of the next four strains (*Actinomadura* sp.2-5) against the all test cultures are relatively low and similar, it varies between from 5 to 8 mm.

Among the other Actinomadura strains *Actinomadura* sp.7, *Actinomadura* sp.8, *Actinomadura* sp.12 and *Actinomadura* sp.14 were inactive against *Bacillus mesentericus. Actinomadura* sp.8 and *Actinomadura* sp.12 were also inactive against *Sarcina ventriculi,* and *Actinomadura* sp.11 against *Escherichia coli.* Activities of *Actinomadura* strains from sp.6 to sp.14 against *Staphylococcus albus* vary between 12-20 mm, against the *Sarcina ventriculi* within 10-18 mm, and against *Escherichia coli* within 8-12mm.

So, among the Actinomadura strains the most active strain against *Bacillus mesentericus* was *Actinomadura* sp.1 – 16 mm, against *Staphylococcus albus Actinomadura* sp.6 – 16 mm, against *Sarcina ventriculi Actinomadura* sp.9 – 20 mm and against *Escherichia coli Actinomadura* sp.8 – 12 mm.

Among the Microbispora strains *Microbispora* sp.1 is less active against the test cultures (Table 2). Its activity close to *Actinomadura* sp.1-5 and changes between 5-8 mm. Like the Actinomadurae *Microbispora* sp.2 and *Microbispora* sp.3 most strongly affect *Staphylococcus albus* and *Sarcina ventriculi.* The effect of *Microbispora* sp.1 and *Microbispora* sp.3 on *Escherichia coli* are nearly similar to Actinomadura's – 7-8 mm. Among them *Microbispora* sp.2 was most effective – 12 mm. In comparasion with other strains *Microbispora* sp.3 is the most effective against Gram positive bacteria, its activity is 10, 20 and 18 mm against *Bacillus mesentericus*, *Staphylococcus albus* and *Sarcina ventriculi* accordingly.

So, received results showed that among the all tested actinomycete strains *Actinomadura* sp.1 is more active against *Bacillus mesentericus*, *Actinomadura* sp.10 and *Microbispora* sp.3 are more active against *Staphylococcus albus*, *Actinomadura* sp.9 and *Microbispora* sp.3 are more active against *Sarcina ventriculi* and *Microbispora* sp.2, *Actinomadura* sp.8 and *Actinomadura* sp.12 are more active against *Escherichia coli.*

Table 1. Antimicrobial activity of actinomycetes of genus Actinomadura (diameter of lytic zone, mm)

Actinomycetes	Test strains			
	Bacillus mesentericus	*Staphylococcus albus*	*Sarcina ventriculi*	*Escherichia coli*
A.sp.1	16±0.36	12±0.42	10±0.23	5±0.14
A.sp.2	7±0.22	5±0.13	5±0.08	8±0.21
A.sp.3	7±0.38	8±0.38	8±0.22	7±0.30
A.sp.4	6±0.21	7±0.19	6±0.25	6±0.22
A.sp.5	5±0.55	6±0.24	7±0.26	8±0.36
A.sp.6	10±0.38	16±0.41	12±0.43	8±0.26
A.sp.7	-	14±0.36	10±0.34	8±0.20
A.sp.8	-	12±0.30	-	12±0.34
A.sp.9	11±0.26	10±0.21	20±0.86	7±0.18
A.sp.10	11±0.31	20±0.41	14±0.35	10±0.45
A.sp.11	9±0.24	15±0.57	10±0.25	-
A.sp.12	-	13±0.45	-	11±0.33
A.sp.13	9±0.45	14±0.67	14±0.51	-
A.sp.14	-	15±0.49	13±0.71	-

Table 2. Antimicrobial activity of actinomycetes of genus Microbispora (diameter of lytic zone, mm)

Actinomycetes	Test strains			
	Bacillus mesentericus	*Staphylococcus albus*	*Sarcina ventriculi*	*Escherichia coli*
M.sp.1	5±0.23	8±0.42	8±0.19	7±0.28
M.sp.2	7±0.16	14±0.42	15±0.57	12±0.37
M.sp.3	10±0.44	20±0.68	18±0.72	8±0.29

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აზერბაიჯანის სხვადასხვა ნიადაგიდან გამოყოფილი აქტინომიცეტების ანტიმიკრობული აქტივობა

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რეზიუმე

გამოკვლეულია აზერბაიჯანის სხვადასხვა ნიადაგებიდან გამოყოფილი აქტინომიცეტების ანტიმიკრობული აქტივობა. შესწავლილი შტამები ავლენენ სხვადასხვა აქტივობას ტესტ-კულტურებისადმი. მათ გააჩნდათ მაღალი აქტივობა გრამდადებითი კულტურების მიმართ და შედარებით სუსტი - გრამუარყოფითი *Esherichia coli*-სადმი.

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CROWN GALL DISEASE OF GRAPEVINE IN GEORGIA

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Abstract

Elimination of the thinness of vineyards caused by disease, and particularly by the crown gall, is one of the considerable potentials for increasing the vine capacity. During the long term investigations it was established that crown gall is widespread in the main vine-growing regions of Georgia. Spreading of the disease has a focal character; it varies by regions from 16-18% to 55-65% and causes premature withering of grapevines and thinness of vineyards. The crown gall damages basically the overground organs of vine. The tumors are found near the root neck, on the trunk and on the 1-year old canes. They are found less frequently on the roots and annual shoots. The causative agents of the disease were isolated from different organs of diseased and externally sound vines which points to the presence of the pathogen in the plants and its long-term remaining in a latent state. The pathogens were isolated from xylem exudates, tumors and rhizosphere. The taxonomy of the causative agents has been established – they have been identified as *Agrobacterium tumefaciens*.

Keywords: crown gall, Agrobacterium tumefaciens

Introduction

Viticulture is one of the main branches of the agriculture of Georgia and it plays an important role in the economy of the country. At present, in some regions of the country it becomes a main financial source for Georgian farmers.

One of the most important things to increase the crop capacity in vineyards is to destruct the thinness and to protect the plants from the different pathogens and diseases, among which we can indicate the crown gall disease.

Crown gall disease of grapevine was first detected in Germany in 1922, later in France in 1953 [Regala, 1970]. Today this disease is widespread all over the world in the main grape- and wine-producing areas: in Romania [Zinca, 1969], in Hungary [Lehoczky, 1968], in Bulgaria [Malenin, 1970], in Armenia [Nagapetyan, 1973], in Moldavia [Lemanova, 1980] and other counties.

In Georgia, crown gall disease was first detected by L. Kanchaveli (1945) and later by N. Kobiashvili (1956). From 1965, group of scientist-microbiologists started their researches [Tsilosani et al., 1971; Dzhighauri, 1968; Palavandishvili, 1976; Giorgobiani and Eliashvili, 1985; 2008].

Materials and Methods

Objective of our research was the crown gall disease of grapevine and its causative agents. Expeditions were carried out in the main vine-growing regions (Kakheti, Kvemo and Shida Kartli, Imereti) of Georgia to determine the spreading area of the disease. Affected plants were counted to determine the spreading area of the crown gall disease. The following formula was used: P=H100/N, where P - number of diseased plants (%), N - total number of plants (healthy and diseased), H - number of diseased plants. Frequency of damaging was counted according to the number of plant samples (50-100).

To determine the harmfulness of the disease plants on thinned areas were recorded. In the same areas yield of diseased and healthy grapevines were recorded. Also the sugar content was determined.

Bacteria were isolated from the diseased grape samples using methods described by Beltiukova et al. (1968) and Izrailskii (1968). Bacteria were isolated from the grapevine bleeding sap, during the circulation of sap, in spring. Bleeding sap was collected in test-tubes from outwardly healthy plants. One drop of the grapevine bleeding sap was disseminated on the agar medium plate in sterile conditions.

Bacteria were isolated from rhizosphere of diseased and healthy grape plants [Ezhov, 1974].

Pathogenicity of the isolated bacteria was determined by the artificial infecting of the host-plants and test-plants (kalanchoe, carrots and tomatoes).

Morphological, cultural, physiological and biochemical characteristics of the pathogenic strains were studied for their identification [Beltiukova et al., 1968; Izrailskii, 1968].

Results and Discussion

The goal of this work was to investigate the distribution area of the disease and to determine its harmfulness, to isolate the bacteria and determine the pathogenicity of these strains, to identify the pathogenic strains and determine the reserve of crown gall causative microorganisms infection.

Disease was occurred almost in all main viticulture regions of the country (Gurjaani, Kvareli, Sagarejo, Telavi, Sighnaghi, Bolnisi, Zestaponi, Mtskheta, and Kharagauli). Distribution of the disease had a focal character and it varied from 16-18% to 55-65%.

Crown gall damages basically the overground parts of grapevines. Crown galls commonly form on the trunks of vines; however, they even develop on 1-year-old canes and near the root neck. At the beginning of the disease on the damaged part of the plant, under the bark a small, white tumor, which is soft and smooth surfaced is developed. With aging the tumor grows, becoming darker and solid. Sometimes, small tumors are developed all over the trunks and canes, then accrete and create a full picture. (Fig. 1 and 2). At the end of the disease, tumors become darker, dry and they fall to pieces. Grape gives the yield before the withering and complete death (Fig. 3).

To determine the harmfulness of the disease we made the observations on the thinned areas. They were occurred mostly in the vineyards diseased with crown gall and in some cases reached to 20.5-44.5%. Thinness of vineyards was caused by the outrooting of the diseased wither plants. We also studied the effect of the crown gall disease of grapevine on the crop capacity and sugar content (Table 1).

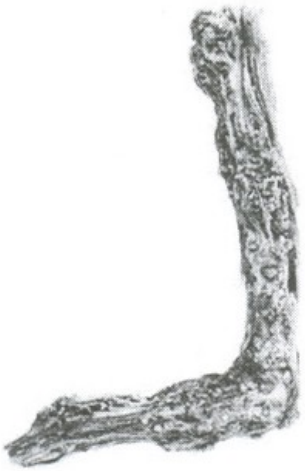

Fig. 1. Diseased trunk of grapevine

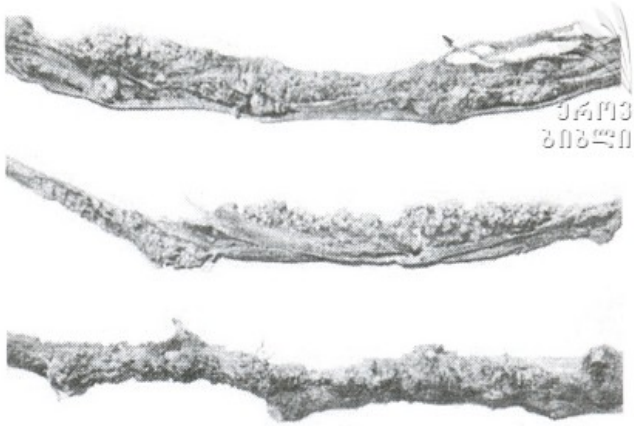

Fig. 2. Diseased canes of grapevines

Table 1. Effect of crown gall of grape on the crop capacity and sugar content

Investigated area	Healthy and diseased plants	Total number of plants	Average yield per plant, kg	Sugar content, %
Kvareli	healthy	100	3.8	18.8
	diseased	100	2.2	18.2
Zestaponi	healthy	100	3.5	18.6
	diseased	100	2.4	18.4
Sagarejo	healthy	100	4.2	18.7
	diseased	100	2.6	18.5

Figure 3. Crown gall disease of grapevine.

As is seen from the table the crown gall disease decreases grape productivity. Bunch of the diseased plant becomes more thin and fine grained, as to sugar content, there was no significance difference.

According to Malenin (1980), Lemanova (1986) and our data it was established that the causative agent of the disease can stay for years within the plant without any outward expression of disease.

To detect the latent form of the infection the bacteria were isolated directly from tumor, also from bleeding sap of diseased and healthy grapes, from propagation material and grape rhizosphere.

Pathogenicity of the isolated strains was tested *in-vitro* and *in-planta* (host and test plants) conditions (Table 2).

It occurred that the plant pathogenic bacteria can be isolated as directly from tumors, also from rhizosphere and from the bleeding sap of diseased (Fig. 4) and healthy (Fig. 5) grapevines. So, causative agent of the crown gall disease can be found in bleeding sap in the outwardly healthy plants and it can remain in the plant in an inactive state during a long period. The disease is detected only when the environmental factors cause the deterioration of physiological conditions of plant.

Table 2. Pathogenicity of the isolated strains

Part of plant, from where bacteria was isolated	Artificially infected plants	Number of tested strains	Number of pathogenic strains	Incubation period, days
tumor	grapevine	50	39	20-25
	kalanchoe	40	25	15-16
	carrot	30	21	15-20
	tomato	20	17	20-25
bleeding sap	grapevine	20	14	20-25
	kalanchoe	12	9	15-20
	carrot	15	11	15-20
	tomato	20	13	15-20
rhizosphere of grape	grapevine	10	7	25
	kalanchoe	25	18	15-20
	carrot	25	16	15-20
	tomato	20	15	15-20

According to our data, *Agrobacterium tumefaciens,* the causative agent of the crown gall disease keeps viability and pathogenicity in soil during all vegetation period. Consequently, permanently agrobacteria can be found in the rhizosphere of grapevines. In spring, this bacterium gets into the soil, when the circulation of sap is starting, where the pathogen can remain until the next vegetation period i.e. until getting the new reserve of bacteria.

To identify the species we have studied their morphological, cultural, biochemical and physiological characteristics. The bacteria presented the following characteristics: rod-shaped, aerobic, gram-negative. On meat-peptone agar medium whity-yellow colonies, convex, middle consistence, non sporing. Positive results were obtained for starch hydrolysis, acidity without gas from lactose, glucose, arabinose, xylose, sucrose, starch, sediment formation on meat-peptone agar medium. Negative results were obtained for gelatine liquefacation, nitrates reduction. Some strains produce sulphuretted hydrogen and ammonia, some do not. Bacteria turn sour the milk, milky litmus turns blue, later reducing it.

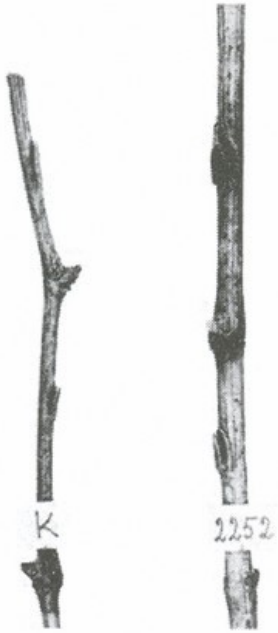

Fig. 4. Artificially infected grapevine. Pathogenic bacteria were isolated from healthy grapevine bleeding sap

Fig. 5. Artificially infected grapevine. Pathogenic bacteria were isolated from diseased grapevine bleeding sap

These characteristics allowed the identification of the bacteria according to Krasilnikov (1949) and Bergey (1980). Finally, the results indicate that the bacteria isolated from tumors were identical to the pathogens isolated from the rhizosphere and from the bleeding sap (latent state) of grapevines and they were identified as *Agrobacterium tumefaciens* (Smith and Townsend) Conn.

Acknowledgements. This research was supported by USAID-CDR Grant No. TA-MOU-03-CA23-037.

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ვაზის ბაქტერიული კიბო საქართველოში

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(მიღებულია 15.06.2009)

რეზიუმე

მეჩხერიანობის ლიკვიდაცია და მცენარის დაცვა მავნებელ-დაავადებებისაგან, რომელთა შორის აღსანიშნავია ვაზის ბაქტერიული კიბო, წარმოადგენს ვენახის მოსავლიანობის გაზრდის მნიშვნელოვან პოტენციალს. ჩვენს მიერ განხორციელებული ხანგრძლივი კვლევის შედეგად დადგენილია, რომ ვაზის ბაქტერიული კიბო ფართოდაა გავრცელებული საქართველოში. დაავადების გავრცელებას აქვს კერობრივი ხასიათი და რაიონების მიხედვით მერყეობს 16-18%-დან 55-65%-მდე, იწვევს ვაზის ნაადრევ ხმობას და ნაკვეთების მეჩხერიანობას. ბაქტერიული კიბოთი ავადდება ძირითადად ვაზის მიწისზედა ორგანოები. სიმსივნეები გვხვდება ფესვის ყელთან, შტამბზე და რქებზე. დაავადების გამომწვევი პათოგენური შტამები გამოყოფილი იქნა უშუალოდ როგორც სიმსივნეებიდან, დაავადებული ვაზის წვენიდან და რიზოსფეროდან, ასევე სადი ვაზის წვენიდან, რაც მიუთითებს პათოგენის არსებობას ვაზის მცენარეში ფარული ინფექციის სახით. განსაზღვრულია პათოგენური შტამების სახეობრივი კუთვნილება - *Agrobacterium tumefaciens* (Smith and Townsend) Conn.

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CREATION AND STUDY OF ANTIMICROBIAL-ANTIFUNGAL COMPOSITE AGAINST MIXED INFECTION USING BIODEGRADABLE POLYMER AS A MATRIX

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Abstract

Application of bacteriophages in complex with antifungal preparations for treatment of associated bacterial and fungal infections is being considered as a novel approach to deal with this problem. A biocomposite film was created by using the biodegradable polymer containing new bacteriophage combination (Pyobacteriophage+Klebsiella, Enterococcus, Acinetobacter bacteriophages), Pimafucin and Trypsin. Among the antifungal substances pimafucin was chosen, because it is less toxic in comparison with other antifungal remedies and it does not cause phage inactivation. At a certain stage trypsin contributes to the wound healing process and does not decrease phage activity significantly in laboratory conditions. Therefore this enzyme was chosen for incorporation into the complex. The biocomposite film is characterized by high activity towards bacterial and fungal flora and appeared to work according to the controllable phage release principle provided by the enzyme used in its composition.

Key words: biodegradable polymer, bacteriophage, biocomposite film

Introduction

Today widespread multiple drug resistance of microorganisms complicates treatment and prevention of bacterial infections. The etiological structure of infectious diseases, and particularly of hospital-acquired (nosocomial) infections, has changed because of the natural and acquired multiple resistances [Song, Srinivasan, 2003; Jones, 2001; Cosgrove, 2006]. At the same time infectious diseases caused by conditionally pathogenic fungi remain a serious problem of contemporary clinical therapy. Candida spp. is responsible for a significant part of those diseases. Often Candida spp. is in association with bacterial agents. The use of antibiotics for treatment of associated microbial flora is less effective, because very often they provide the process of fungus development. Candida in association with microbe is more resistant to antifungal preparations, then in monoculture.

The novel approach to this problem is application of bacteriophages in complex with antifungal preparations [Alavidze, Meiphariani, 1998]. It's important that bacteriophages may be used for therapy and also for prevention without any side-effects. [Alavidze, Danelia, 1998; Cislo,

Dabrowski, 1982]. Bacteriophages are considered to be a powerful, specific, long-lasting cure for infectious diseases [Meiphariani, 1989; Kutter, Sulakvelidze, 2005; Rigvava, Tchghkonia, 2005]. Phage therapy can be used to lyses specific pathogens without disturbing normal bacterial flora [Alavidze, Kutateladze, 2002; Alavidze, Morris, 2002]. Also it is very important that for successful treatment of many topical infections highly concentrated medications and their evenly distribution on the damaged area are required [Alavidze, Meiphariani, 2007].

One of the most advantageous approaches to solve the above-mentioned problem is a PhagoBioDerm which was developed against the wound infections in 1997 [Alavidze, Meiphariani, 1998]. PhagoBioDerm is biocomposite based on high molecular biodegradable polymer and Pyobacteriophage [Tsitlanadze, Khosroashvili, 1996]. The preparation successfully passed through the clinical trials and was registered in Georgia.

Biodegradable (or biodegradible) polymers are more suitable for use as matrices for sustained/controlled release devices [Katsarava, Beridze, 1991; Barrows, 1994; Shalaby, Jonson, 1994]. Film devices (so called "artificial skin") prepared from these composites provides a high therapeutic effect:

Action of polymer material as a protector from external actions (e.g. mechanical, etc.) and bacterial invasion, as well as prevention of heat and moisture loss from injured surfaces; and high and constant local concentration of active substances

The principle aim of our research is including of the antifungal substances and enzymes into the biocomposite, which is developed on the base of biodegradable polymer and bacteriophages [Goderdzishvili, Alavidze, 2008].

Materials and Methods

A total of P.aeruginosa-135, Proteus-105, Klebsiella-95, E.coli-107, Streptococcus spp.-115, Enterococcus spp.-123 Staphylococcus aureus-140 and Acinetobacter spp.-45; Candida albicans-76 samples (Total 941); antifungal substances - Terbizil, Mikogal, Mikoflu, Pimafucin, Nistatin, Nizorale; enzymes - Karipazim, α-cemotrypsin, trypsin and lipase were included in this study.

The phage isolation, titration (Gratia's method), concentration, multiplication and study of sensitivity of the obtained bacterial strains towards the bacteriophages was carried out by standard methods [Kutter, Sulakvelidze, 2005].

For study the interaction of bacteriophages and antifungal substances different doses of antifungal substances, and also different expositions (10m, 24h and 48h) were taken. In experiment liquid bacteriophage was added with certain concentration of the above-mentioned materials and let for a desirable period. Then each sample was tittered on a solid nutrient agar by Gratia's method. For control the same amount of bacteriophage was taken without supplement at least 3 times.

For draying the mixture of bacteriophage and antifungal substances Saccharose gelatin (SG), Lactose gelatin (LG) and Glucose gelatin (GG) solutions were used. Each solution added with proportion 5:1 (5-phage mixture and 1-SG, LG or GG solutions). As a result we established the optimal and minimal proportion of saccharose-gelatin (5g Saccharose, Lactose or Glucose+ 1g gelatin + 200 ml $D.H_2O$).

For making the biocomposite first of all we dissolve polymer with chloroform (at a concentration 100 mg polymer in 1 ml of chloroform) and than we added dry phage-antifungal substances with proportion 1:1, and enzyme trypsine with concentration 10mg/ml. This suspension was pored onto the smooth glass Petri-dishes hydrophobized by dichlorodimethylsilane, and the solvent was allowed to evaporate completely at room temperature.

For checking the activity of composites against bacterial strains we put 12mm diameter biocomposite films on of bacterial strains on nutrient agar on Petri dishes. The activity was

determined with creation of lysis zones around the film after 18 hour incubation at 37^0 C. As control we have taken liquid pyobacteriophage and polymer without any additions (Fig 1).

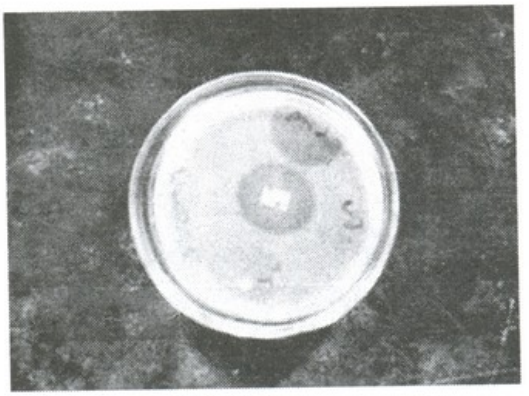


Fig.1 Checking of the activity of composites against bacterial strains

To study the kinetics 10cm diameter biocomposite films were putt in 10ml 0.2 N phosphate buffer (pH 7.4) at 37^0 C for 1-120 hour. Buffer was changed after 48 h and 72. Samples were taken after 1, 3, 5, 24, 48, 72 and 120 hours and tittered by Gratia's method on a solid agar media.

To obtain the biodegradable polymeric matrices suitable for the construction of biocomposites containing bacteriophages and other bioactive components and acting in drug sustained/controlled release fashion, the we carried out synthesis of biodegradable poly (ester amide)s (PEAs) of general formula:

$$-[CO-(CH_2)_yCO-NH-CH(R)-CO-O-(CH_2)_x-O-CO-CH(R)-NH-]_n-$$

where: $\mathbf{x} = 4, 6$; $\mathbf{y} = 4, 8$; $R = CH_2C_6H_5$ **(Phe)**, $CH_2CH(CH_3)_2$ **(Leu)**

Results and Discussion

Bacterial strains were obtained from different clinics of Georgia (Diagnostic center "Diagnosis 90", Central clinical Hospital, Diagnostic center -"Cito", Hospital for infections). Sensitivity of the obtained bacterial strains was tested towards the commercial Pyobacteriophage and bacteriophages existing in the laboratory collection. The results obtained have shown that 105 bacterial strains out of 198 were resistant and 93 were sensitive (Fig. 2).

Beside Staphylococcus aureus, Streptococcus spp, E.coli, Proteus, Pseudomonas strains we also got Acinetobacter, Klebsiella and Enterococcus spp. bacterial strains. Our commercial phage preparations don't contain phages against these bacteria but we have appropriate bacteriophages in our laboratory collection.

New clones of Staphylococcus, Streptococcus, E.coli, Proteus, Pseudomonas, Klebsiella and Acinetobacter bacteriophages have been isolated from sewage water on the resistant strains. As a result of adaptation, concentration and multiplication of freshly-isolated clones of bacteriophages new combination of highly active bacteriophage preparation was created.

We established the sensitivity of phage combination to newly isolated bacterial strains. Incorporation of Acinetobacter, Klebsiella and Enterococcus bacteriophages into the existing Pyobacterophage content significantly increased its activity. 85% of 327 bacterial strains

(Staphylococcus, Enterococcus, E.coli, Proteus, Pseudomonas, Klebsiella and Acinetobacter) are sensitive to new phage combination (Fig. 3).

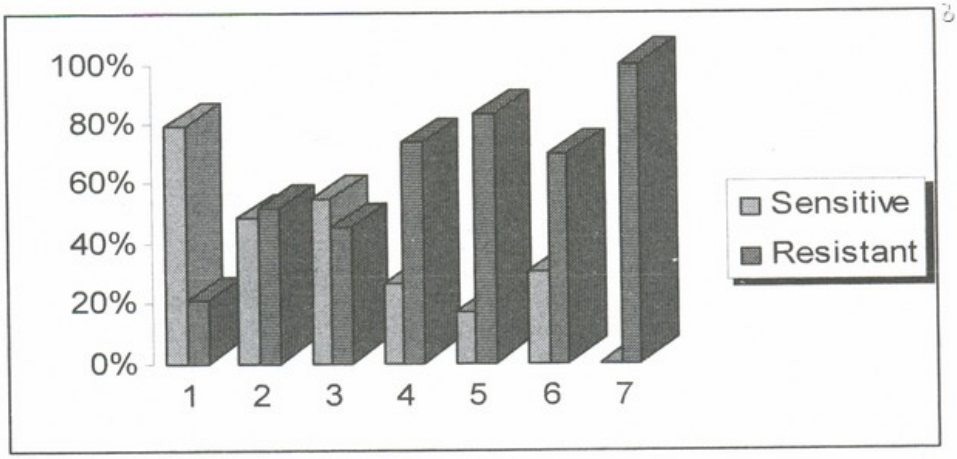


Fig. 2. Sensitivity of bacterial strains to bacteriophages from the laboratory collection. 1-Pseudomonas, 2-E.coli, 3-Staphylococcus, 4-Enterococcus, 5-Klebsiella, 6-Proteus, 7-Acinetobacter

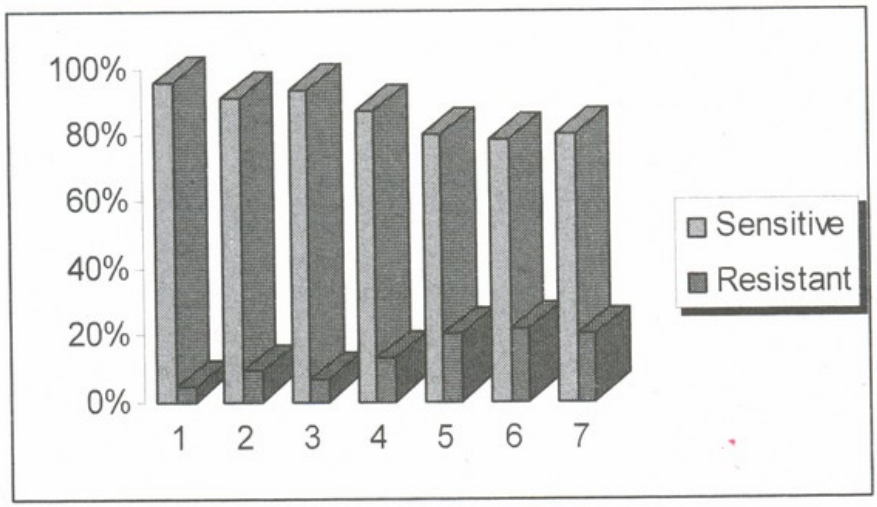
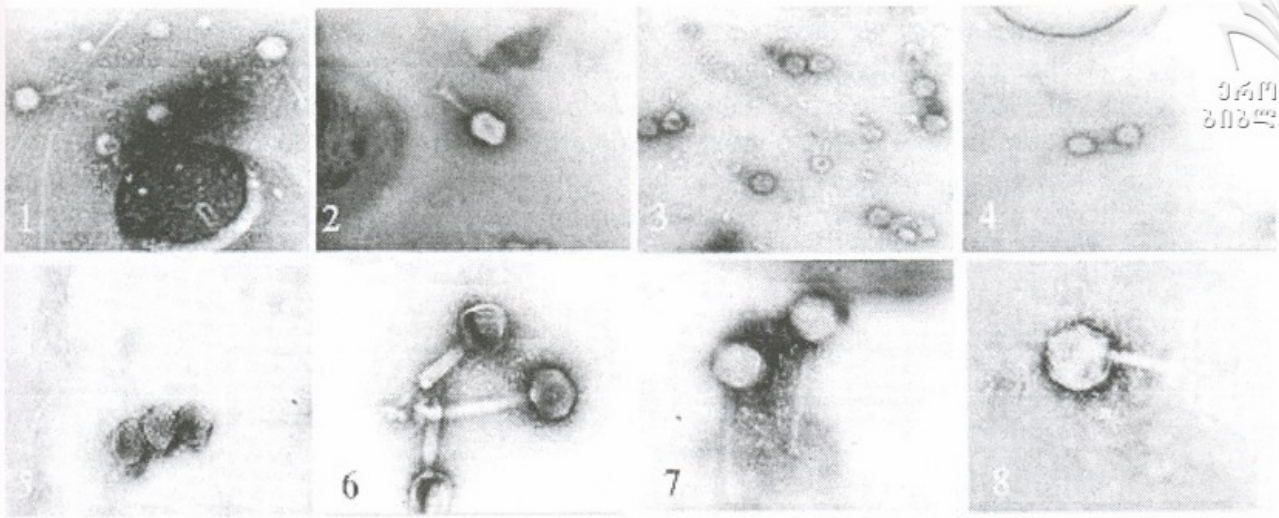


Fig. 3 Sensitivity of freshly-isolated bacterial strains to new bacteriophage combinations. 1-Pseudomonas, 2-E.coli, 3-Staphylococcus, 4-Enterococcus, 5-Klebsiella, 6-Proteus, 7-Acinetobacter.

Electron microscopy of new isolated bacteriophages showed that phages belonged to different morpho-types, namely to the families of Podoviridae, Sipoviridae and Mioviridae (Fig. 4).



Morphology of: 1. Klebsiella bacteriophage; 2.E.coli bacteriophage; 3. Streptococcus bacteriophage; 4. Proteus bacteriophage; 5.Acinetobacter bacteriophage; 6. Staphylococcus bacteriophage; 7.Enterococcus bacteriophage; 8. Pseudomonas aeruginosa bacteriophage.

Fig. 4. Morphology of bacteriophages

To investigate a possible impact of antifungal substances such as: terbizil, mikogal, mikoflu, pimafucin, nizoral and nistatine into the biocomposite we studied interaction between phages and antifungal preparations by using the different proportions of them. The results obtained have shown, that supplement of any dose of different antifungal materials didn't inactivate phage completely (Fig. 5). We determined the optimal concentration of antifungal substances; for Nistatine and Nizorale it was 50mg/ml, for Pimafucin - 10mg/ml and for Mikoflu - 50mg/ml. Before determination of the optimal concentration of antifungal substances for including into the biocomposite, we take into consideration the possible concentration of antifungal substances for children. Among the antifungal substances pimafucin was chosen, as it is less toxic in comparison with other antifungal remedies and it does not cause phage inactivation. Therefore, pimafucin was incorporated into the film containing biodegradable polymer and bacteriophages.

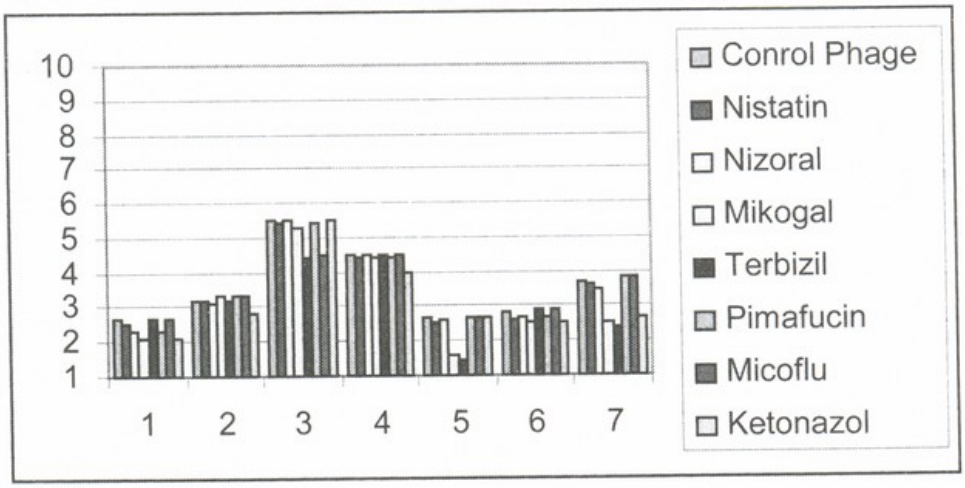


Fig.5. The effect of various antifungal materials on bacteriophage activity. Y axis: phage titer 1- 10^4; 2- 10^5; 3- 10^6; 4- 10^7; 5-10^8; 6- 10^9; X axis: 1-Proteus; 2-Streptococcus; 3-P.aeruginosa; 4-E.coli; 5-Acinetobacter; 6-Enterococcus; 7-Klebsiella

As the phages can be incorporated into biocomposite only in a powder form, we developed the technology for drying of phages. For this purpose we employed solutions of various concentrations of saccharose-gelatin, lactose-gelatin and glucose-gelatin and added liquid bacteriophages. These composites were dried by lyophilization technology. As results of drying we defined phage activity in each case. Within the drying process the titer of some phages were reduced but it was not critical and the saccharose-gelatin was selected because it showed better result (Fig 6). 50 mg dry bacteriophage equals to 1ml liquid bacteriophage with titer $2 \cdot 10^6$.

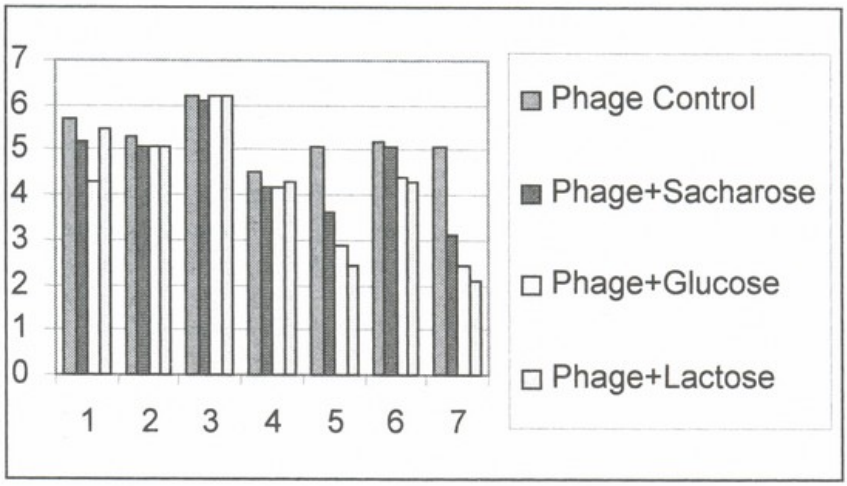


Fig. 6. Activity of dry bacteriophages. Y - phage titer $1\text{-}10^3$; 2- 10^4; $3\text{-}10^5$; 4- 10^6; $5\text{-}10^7$; $6\text{-}10^8$; $7\text{-}10^9$; X - 1-Streptococcus; 2- Staphylococcus; 3- Pseudomonas; 4- Proteus; 5- E.coli; 6- Acinetobacter; 7- Klebsiella

As a result of natural biodegradation of the polymer, phages are gradually released from the film. To study the biodegradation profile (kinetics) of the PEAs(poly(ester amide)s), in vitro biodegradation of pure **8-L-Phe-6** catalyzed by α-chymotrypsin, trypsin and lipase was studied. The results indicated that in vitro biodegradation of **8-L-Phe-6** catalyzed by this enzymes follows the zero order kinetics that is ideal for constructing drug sustained/controlled release device. Based on this study the 80/20 w/w blend was found as an optimal one to be used as biodegradable matrix for constructing biocomposites containing bacteriophages and other bioactive substances.

We determined the interaction between phages and enzymes α-chymotrypsin, trypsin, Karipazim and lipase. For this purpose we took different concentrations of enzymes: 100mg/ml, 300mg/ml and 700mg/ml of karipazim, 1mg/ml, 2mg/ml, 10mg/ml of α-chimotrypsine and Trypsine, 1mg/ml, 3mg/ml, 10mg/ml of Lipase. As our investigations showed, enzymes do not decrease phage activity significantly in laboratory conditions (pH 7.2-7.4; 37^0C) (Fig. 7). The optimal concentrations of the enzymes were determined (Lipaza – 10g/ml, Karipazim – 300mg/ml, Trypsin – 10mg/ml, α-chemotrypsine - 1mg/ml). It is well known that at a certain stage trypsin contributes to the wound healing process. Therefore this enzyme was chosen for incorporation into the complex.

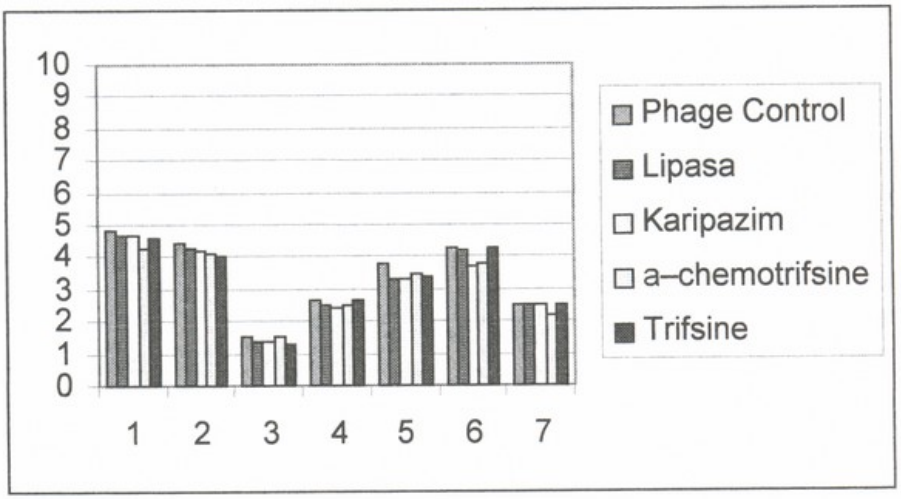


Fig.7. The effect of various enzymes on bacteriophage activity. Y - phage titer 1-10^5; 2- 10^6; 3-10^7; 4-10^8; 5- 10^9; X - 1-Ps.aeruginosa; 2-Klebsiella; 3-Acinetobacter; 4-Staphylococcus; 5-Streptococcus; 6-E.coli; 7-Proteus

A biocomposite film was created by using the biodegradable polymer **8-L-Phe-6** containing new bacteriophage combination, pimafucin and trypsin.

The activity of biocomposite film was checked against the bacterial and fungal flora. For that we used bacterial strains from laboratory collection and freshly isolated strains of P.aeruginosa, Proteus, E.coli, Klebsiella, Staphylococcus, Streptococcus, Enterococcus, Acinetobacter (total: 941 bacterial and fungal strains). Experimental results showed, that the film was characterized by high activity (89.3 %) (Fig. 8).

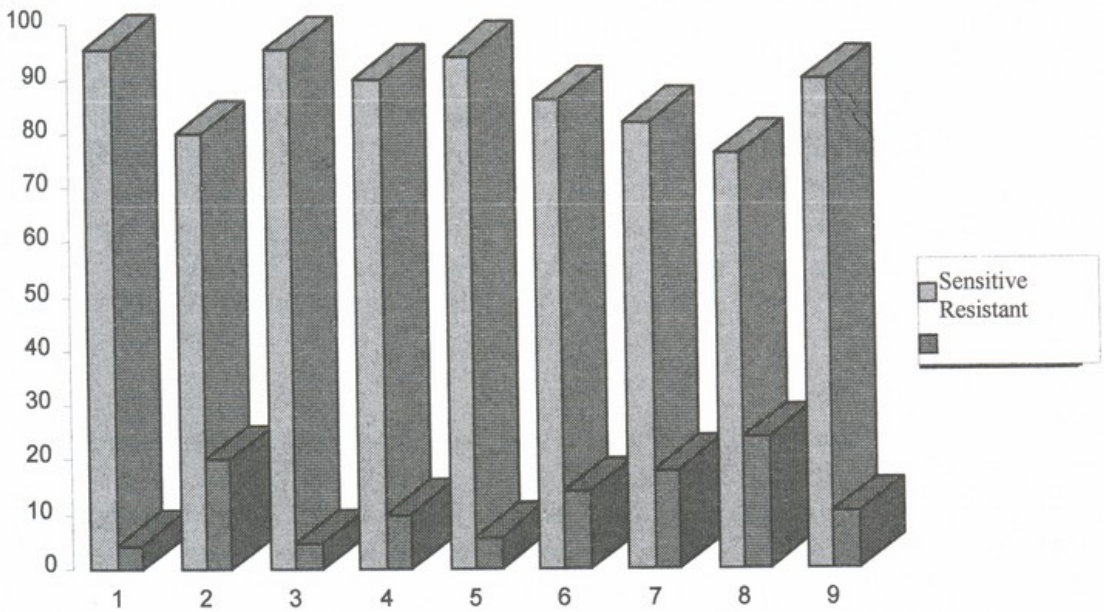


Fig. 8. Sensitivity of biocomposite film (Phage+Polymer+Trypsin+Pimafucin) against bacterial and fungal flora. X - 1-Staphylococcus; 2-Streptococcus; 3-P.aeruginosa; 4-E.coli; 5-Proteus; 6-enterococcus; 7-Klebsiella; 8-Acinetobacter; 9-Candida albicans. Y - Percentage of sensitivity.

After studying the kinetics of phage (P.aeruginosa, Proteus, E.coli, Klebsiella, Staphylococcus, Streptococcus, Enterococcus, Acinetobacter) release from film we established that effectiveness of biocomposite is based on the controllable phage release principle which is provided by the enzyme used in its composition (Fig. 9).

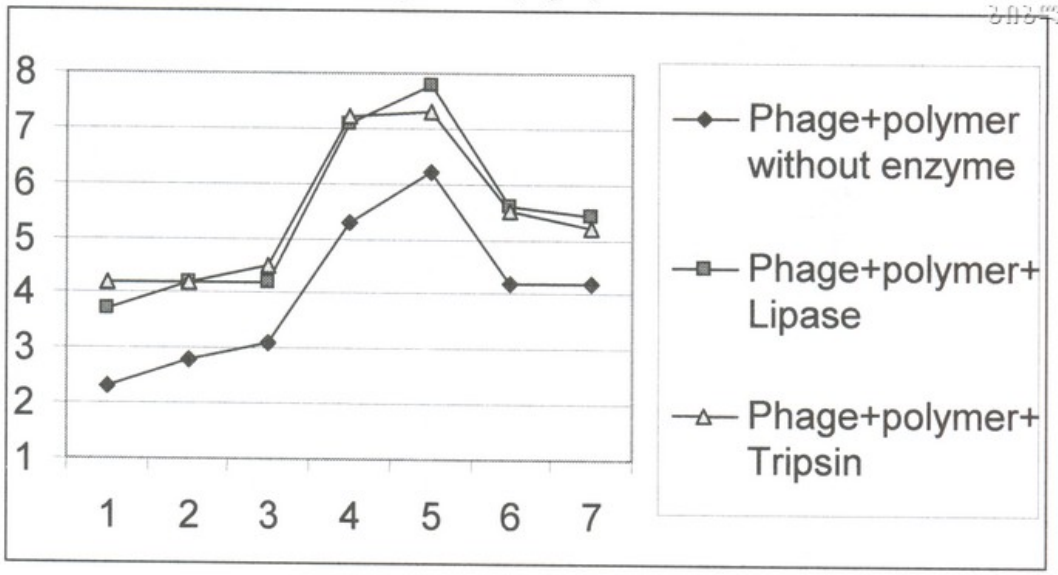


Fig. 9. Release of the Acinetobacter bacteriophage from Biocomposite. Film (Ó = 9s-m) Phosphate buffer 10 ml pH 7.4, t=37^0 C. **Y** - phage titres **1**- 10^2; **2**- 10^3; **3**- 10^4; **4**- 10^5; **5**-10^6; **6**- 10^7; **7**-10^8. **X** - time zone **1**- 1hour; **2**- 3 h; **3**- 5 h; **4**- 24 h; **5**- 48 h; **6**- 72 h (24 h in new portion of buffer); **7**- 120 h (48 h in new portion of buffer)

Based on the results obtained from pre-clinical trials of the first generation of "Phagobioderm" [Alavidze, Meiphariani, 1998], which consists of biodegradable polymer and Pyobacteriophage (P.aeruginosa, Proteus, E.coli, Staphylococcus, Streptococcus,) we predict that a biocomposite film thus created as the new generation of "Phagobioderm", (contaning biodegradable polymer **8-L-Phe-6,** new bacteriophage combination (Pyobacteriophage+ Klebsiella, Enterococcus, Acinetobacter, pimafucin and trypsin.) will be an effective tool for treatment of mixed infections (bacterial and fungal) of skin surfaces.

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ანტიმიკრობულ-ანტიფუნგალური ბიოკომპოზიტი შერეული ინფექციების წინააღმდეგ ბიოდეგრადირებადი პოლიმერის საფუძველზე

გოდერძიშვილი მ.[1], ალავიძე ზ.[1], მეიფარიანი ა.[1], ჭყონია ი.[1], ძიძიშვილი ლ.[1], ქვათაძე ნ.[1], ჟღენტი დ.[1], რიგვავა ს.[1], დვალიძე თ.[1], კუსრაძე ი.[1], ქარუმიძე ნ.[1], ნადირაშვილი ნ.[2], ქაცარავა რ.[2]

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[2]სამედიცინო პოლიმერებისა და ბიომასალების ცენტრი

(მიღებულია 20.07.09)

რეზიუმე

ბაქტერიოფაგებისა და ანტისოკოვანი ნივთიერებების კომპლექსური პრეპარატის შექმნა შერეული ბაქტერიული და სოკოვანი ინფექციების სამკურნალოდ ახალი მიდგომაა ამ პრობლემისადმი. ბიოდეგრადირებადი პოლიმერის საფუზველზე შექმნილია ბიოკომპოზიტის ფირი, რომლის შემადგენლობაშიც შედის ბაქტერიოფაგები (პიობაქტერიოფაგი + Klebsiella, Enterococcus, Acinetobacter ბაქტერიოფაგები), პიმაფუცინი და ფერმენტი ტრიფსინი. პიმაფუცინი ხასიათდება ნაკლები ტოქსიკურობით სხვა ანტიმიკრობულ ნივთიერებებთან შედარებით, ხოლო ტრიფსინი ხელს უწყობს ფირის ბიოდეგრადაციას და აჩქარებს ჭრილობის შეხორცების პროცესს. ფირი ხასიათდება მაღალი აქტივობით როგორც ბაქტერიული, ისე სოკოვანი ფლორის მიმართ და მოქმედებს ფაგის კონტროლირებადი გამოყოფის პრინციპით.

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QUANTITATIVE AND QUALITATIVE CONTENT OF MICROSCOPICAL FUNGI IN KUMISI ALKALI SOILS (EASTERN GEORGIA)

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Abstract

The qualitative and quantitative content of microscopical fungi in alkali soils of Kumisi Lake has been studied. It has been established that the intensity of spreading of microscopical fungi in alkali soils on the territory adjoining to Kumisi Lake depends on the moisture and salinity of these soils.

Key words: Georgia, alkali soils, microscopical fungi

Introduction

At present the study of qualitative and quantitative content of soil microflora has a great importance giving an opportunity to establish the features of the biochemical and microbiological processes taking place in it.

In this regard we draw attention on the alkali soils for which the existence of specific flora and fauna is characteristic, which in its turn has a considerable influence on the processes taking place in alkali soils.

The fungi actively participate in the biochemical processes taking part in the soil which appear to be a main component of the biocenosis. They have an active role in the decay of plant remains, nitrogen transformation, formation of soil structure [Gusev, Mineeva, 2003]. In the process of vital activity the fungi isolate physiologically active substrates [Lengeler, et al., 2005].

The goal of our investigation was to study the quantitative and qualitative content of microscopical fungi in alkali soils, the effect of salinity of the soil on the peculiarities of spreading of these microorganisms.

Materials and Methods

On the territory of Georgia the soils of alkali type are situated on intermontane declines, on the elements of closed lakes and mortlakes, and alluvial plains. The alkali soils on the territory adjoining to Kumisi Lake belong to the alkali soils of sulphate type where sodium sulphate is presented in the form of soluble salts.

The object of the investigation were 5 samples taken from the alkali soils on the territory adjoining to Kumisi Lake, three of them (N1, N2, N3) were taken on the west and north-west coast of the lake in the immediate zone of the flow at the distance of 5 m (N1), 30 m (N2) and 60 m

(Figure N1, a little arrow), while two samples (N4, N5) - on the south (N4) and north (N5) slopes of the hill on the north-east coast. The samples were taken on June 8, 2008 in conditions of the following indices of weather forecast: variable nebulosity, the east wind - 3m/sec, atmospheric pressure - 722-723 mercury column, temperature - 15-27°C, humidity - 64%.

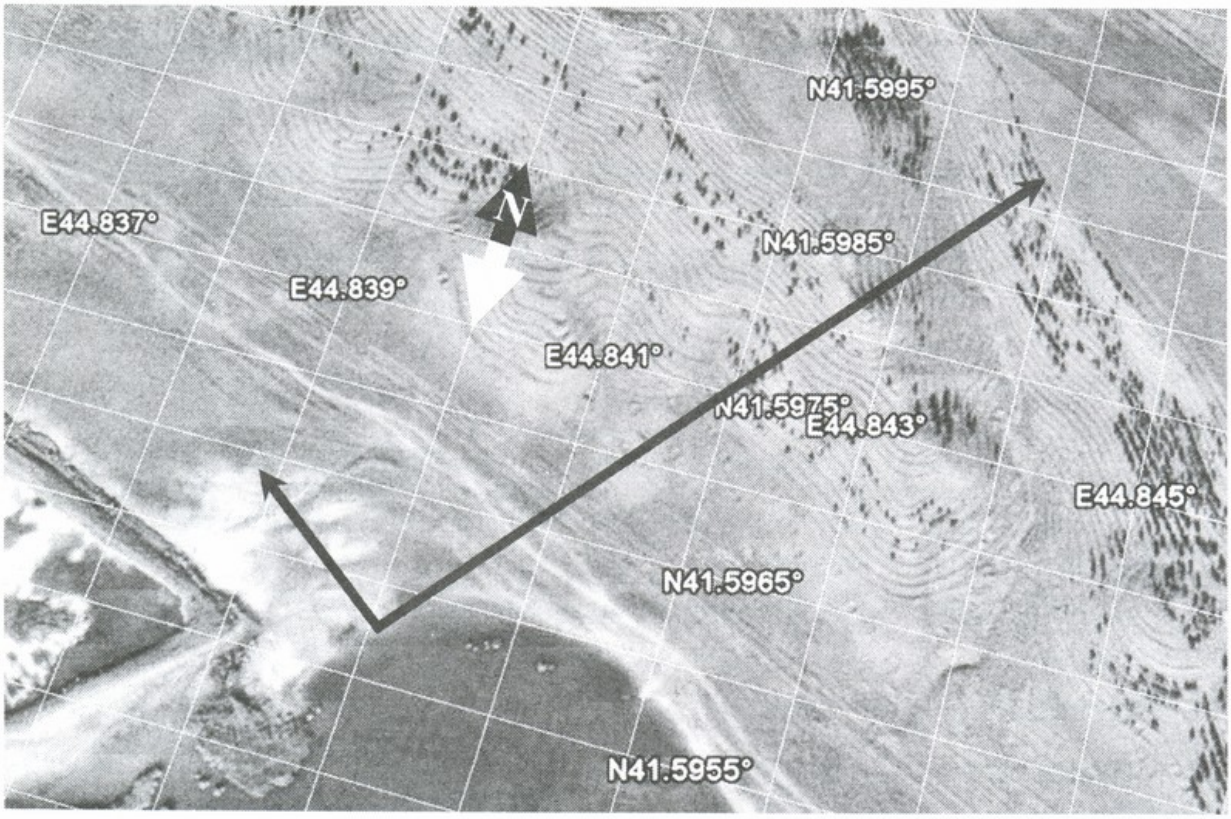

Fig. 1. Kumisi Lake and its adjoining territory. The route of the taking of soil samples are shown by the arrows.

The determination of humidity in the samples was accomplished by weighing method in % [Tepper, 2003].

The preparation of soil suspension was performed according to the well-known method adopted in microbiological practice [Tepper, 2003].

The isolation of microscopical fungi from the soils was done on Chapek modified media [Tepper, 2003].

The quantity of the microorganisms was determined using the method of statistical procession [Tepper, 2003].

The specific analysis of the microscopical fungi was fulfilled in the qualifier of the microscopical fungi according to above-mentioned methods [Litvinov, 1967].

Results and Discussion

The amount of humidity in the samples of soils on the territory adjoining Kumisi Lake is given on Figure 2.

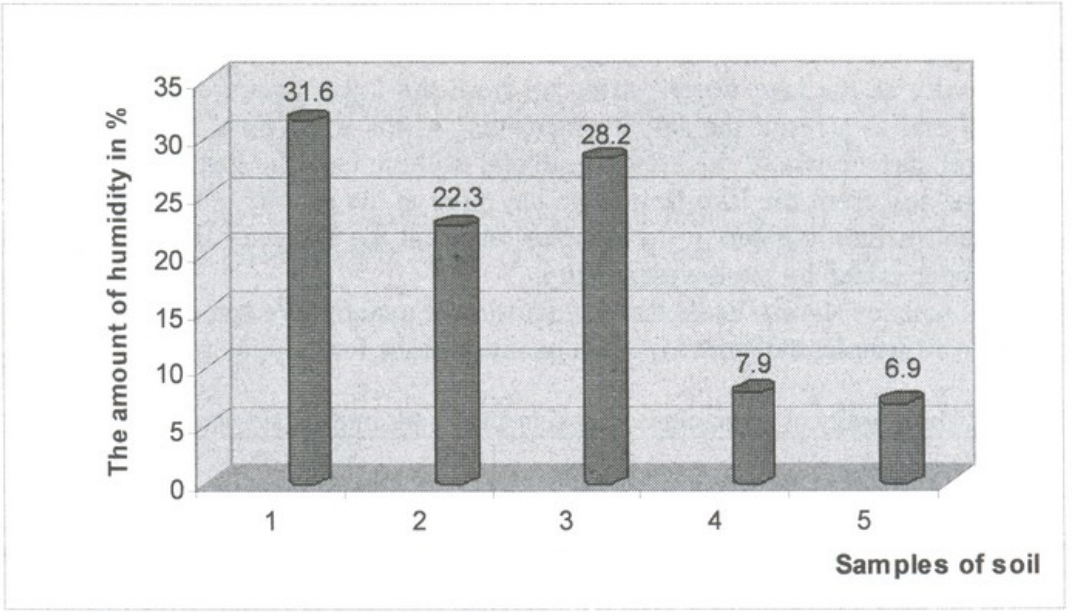

Fig 2. The amount of humidity in the samples of soils on the territory adjoining Kumisi Lake.

Proceeding from the analysis of the data obtained it is established that the amount of humidity in the samples of soils on the territory adjoining Kumisi Lake is different which is influenced by the nearness of the lake. For example, in the soil samples of the zone of the flow the amount of humidity is quite high. It is noteworthy the sample N3 where the amount of humidity is higher than in the sample N2. It is explained by the fact that the water is leaking from the channel adjoining to the place of sample taking.

In the soil samples taken on the slopes of the hill adjoining to the lake the amount of humidity is very low.

According to the samples taken on definite perimeter the salinity of the soil has been studied, as it is known that the salinity of the soil depends not only on the amount of humidity in it but also on the mobility of this moisture and its direction.

The salinity of the samples of the soil adjoining to the lake is in Figure 3.

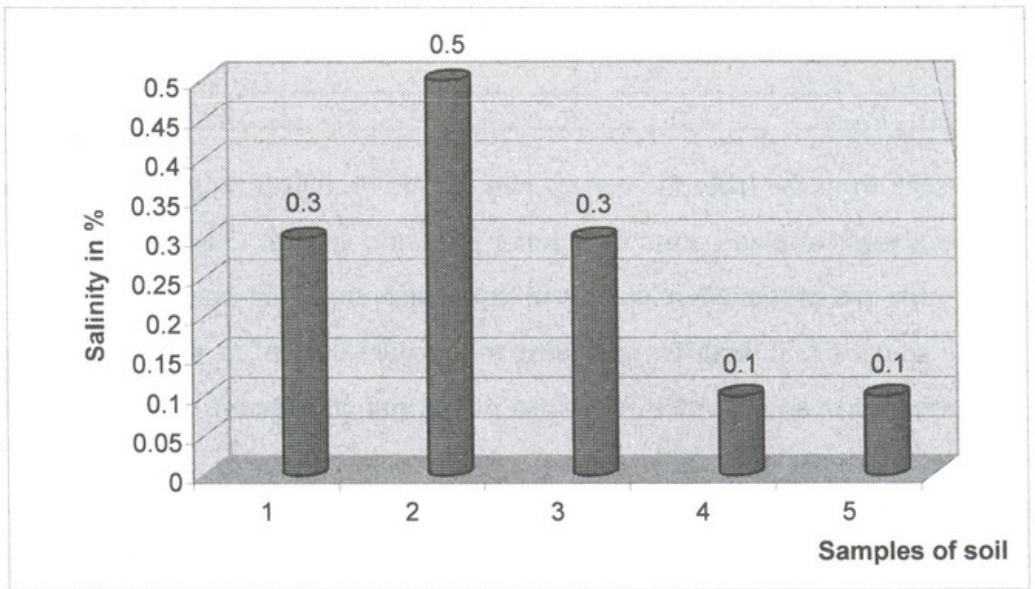

Fig. 3. Salinity of the samples of the soil adjoining to the lake.

The analysis of the data presented in Fig. 3 shows that the samples of the soil on the territory adjoining to the lake differ according to the salinity. Particularly, in the zone of the flow the soil is very salty as the lake water getting up from the lower layers evaporates in the upper layers of the soil and as a result the salinity increases. At the same time, the crust of dry salt is created on the soil surface whose thickness is several millimeters. The further the point of taking the samples of the soil from the lake flow zone the more is the salinity. The exception is sample N3. Quite different picture is noted in the samples taken on the slopes of the hill adjoining to the lake. They are characterized by the lowest salinity.

In alkali soils of Kumisi Lake the peculiarities of quantitative spreading of microscopical fungi are in Table 1 (sample, moisture in %, the quantity of the fungi in the soil).

Table 1. The quantity of microscopical fungi in alkali soils of Kumisi Lake

Sample	Humidity (%)	Number of fungi
1	31.6	986.9420
2	22.3	933.0757
3	28.2	208.9137
4	7.9	3963.0836
5	6.9	15145.0054

As is seen in the table, the samples of investigating soils differ according to the quantity of microscopical fungi. The maximal index was registered in sample N5 (the north slope from the lake), while minimal - in sample N3.

At the following stage the identification research of the microscopical fungi was carried out. The results are given in Table 2.

Table 2. The qualitative content of microscopical fungi in the soil samples on the territory adjoining to the lake and their ratio

Sample	Catenularia (Grove)	Botritis (Africana)	Gliomastix Gueguen
1	29.63%	70.37%	-
2	13.79%	96.21%	-
3	-	-	-
4	-	-	-
5	-	96.48%	1.77%

As is seen from the table the two species of microscopical fungi - *Catenularia (Grove)* and *Botritis (africana)* - in alkali soils of Kumisi Lake are spread. Other species, for example, *Gliomastix Gueguen* are presented relatively in small quantity. The analysis of the results has shown that alkali soils of Kumisi differ according to moisture content as well as to salinity. On its turn these parameters have an influence on the qualitative and quantitative content of microscopical fungi.

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მიკროსკოპული სოკოების რაოდენობრივი და თვისობრივი შედგენილობა კუმისის მლაშე ნიადაგებში

თბილისის ბოტანიკური ბაღი და ბოტანიკის ინსტიტუტი

მელია ნ., სურმავა ს., აბაშიძე ვ., გოროზია ი., ლომთათიძე ზ.

(მიღებულია 19.06.2009)

რეზიუმე

შესწავლილია კუმისის მლაშობ ნიადაგში მიკროსკოპული სოკოების თვისობრივი და რაოდენობრივი შედგენილობა. დადგენილია, რომ კუმისის მიმდებარე მლაშობ ნიადაგებში მიკროსკოპული სოკოების გავრცელების ინტენსივობა დამოკიდებულია ნიადგში არსებულ ტენსა და მარილოანობაზე.

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THE STUDY OF DNA SYNTHESIS IN FUNCTIONALLY DISTINCT FLIGHT MUSCLES OF LOCUST

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Abstract

DNA synthesis in functionally different monofunctional (MOF) and bifunctional (BIF) flight muscles (dorsal longitudinal 112, tergosternal 113, tergocoxal 119,120) of the locust in the process of imago development (one-day and mature locust) has been investigated. Using H^3-timidin as a marker it has been determined that the intensity of DNA synthesis in the locust functionally different MOF and BIF flight muscles of one-day imago (the wings are not functioning yet) is 2-4 times higher than that of mature locust. It indicates that up to this period of development the process of formation of flight apparatus is not finished yet. In MOF muscle 112 of one-day imago as well as in the case of mature locust the level of DNA synthesis was low. The differences among DNA synthesis in other muscles (113,119,120) were not observed.

Key-words: flight muscles, monofunctional muscle, bifunctional muscle, DNA synthesis, locust.

Introduction

In last years the functionally different flight muscles of insects are being intensively studied. Proceeding from their specialization the flight muscles are divided into MOF and BIF muscles [Wilson 1962]. The functional difference of these muscles is that BIF muscles participate in the movement of wings and extremities while MOF muscles – only in wings movement. For present the ultrastructure [Mandelshtam et al., 1986; Mandelshtam, Papidze 1987; Papidze, Mandelshtam, 1987; Papidze 1988], histochemistry [Shumova 1973; Shumova, Mandelshtam., 1974; Shumova et al.,1982; Mandelshtam 1983], physiology [Grigorev,1980], cation consistence [Leontiev et al., 1990] morphometry [Papidze 2003, Papidze 2004], the consistence of miofibrillar proteins [Papidze et al. 2006] of locust MOF and BIF flight muscles are investigated quite in detail.

These muscles were shown to differ with some parameters (ultrastructure, histochemistry, morphometry).

In this work we intended to study the intensity of DNA synthesis in locust MOF and BIF flight muscles during the process of imago development (one-day and mature insects).

Materials and Methods

The experiments were conducted using the laboratorial culture of locust (*Locusta migratoria migratorioides* R.F.). The insects were kept in the hothouse during the whole year at 28-30^{0}C with eternal photoperiod (12 hours light and 12 hours darkness). In such conditions the culture develops well during the whole year.

For investigations we used dorsal longitudinal 112 and tergosternal 113 muscles from MOF muscles and tergocoxal 119 and 120 muscles from BIF muscles. The designation of muscles is given according to anatomical nomenclature of Snodgrass [Snodgrass 1935].

The muscles for investigation were been taken on the first day after the imago moult (while the wings were not functioning yet) and from mature insects. The intensity of DNA synthesis was studied by means of radiometric method using H^3-timidin. Isotope was brought in among segments of bellies of insects (only one injection 5μCi per an insect) and then they were returned to the hothouse. 24 hours after the injection of marker the muscles were prepared, thoroughly washed and placed in scintillating solution. The amount of impulses was recorded on the autoanalyser of radioactivity "Beta-2" (Russia) during one minute and evaluated per gram of tissue. For precision of results the muscles from right and left sides of torax have been taken separately and were compared with each other according the number of impulses. This allowed to control the intensity of isotope inclusion more precisely. Obtained results have been elaborated statistically.

Results and Discussion

Our investigation showed that intensity of DNA synthesis in MOF muscle 112 was lower than in other MOF and BIF flight muscles of mature locust. The intensities of DNA synthesis in MOF muscle 113 and BIF muscles 119 and 120 do not differ (Fig.1, Table 1). In the case of one-day imago the picture is the same: the intensity of DNA synthesis in MOF muscle 112 was lower than in other flight muscles. Concerning the other MOF muscle - muscle 113, the intensity of DNA synthesis in it is higher. On that phase of development muscles 113, 119, 120 don't differ (Fig.1). The locust flight muscles on the investigated stages of imago development differ in the intensity of DNA synthesis. Particularly, DNA synthesis in MOF and BIF muscles of one-day imago is 2-4 times higher than in those of mature insects (Fig.1, Table 1). The obtained results are in good accordance with our previous investigations about ultrastructure and morphometry of functionally distinguished flight muscles [Mandelshtam, Papidze 1987; Papidze 2003; Papidze. 2004]. During the process of locust imago development DNA synthesis in MOF muscle 112 is characterized by low intensity. There were no differences in DNA synthesis among the other muscles (113,119,120) despite the stage of development.

Hence, DNA synthesis in MOF and BIF muscles of one-day imago, when the wings are not yet functioning, is several times higher than in those of mature insects, that indicates that up to this period of development the process of formation of flight apparatus is not finished yet. So, in DNA synthesis of functionally distinguished flight muscles of locust at studied periods of imago development significant differences were not observed.

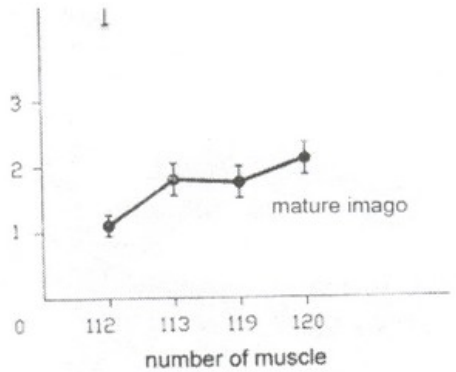

Fig.1. The intensity of DNA synthesis in the Locust functionally distinguished flight muscles.

Table 1. The intensity of DNA synthesis in the locust functionally distinguished flight muscles (imp/g/min).

N	Muscles	The stages of development of locust imago	
		One-day imago	mature imago
1	dorsal longitudinal 112 (MOF)	404573.72 ± 38488.08 n - 11	104229.19 ± 11913.61 n - 21
2	tergosternal 113 (MOF)	623570.42 ± 49596.34 n - 7	184201.9 ± 18058.42 n - 21
3	tergocoxal 119 (BIF)	700681.9 ± 48140.70 n - 10	168124.8 ± 14054.14 n - 20
4	tergocoxal 120 (BIF)	561442.5 ± 74569.93 n - 8	211909.2 ± 16356.36 n - 15

notice: n – the number of investigated muscles

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კალიის ფუნქციურად განსხვავებულ საფრენ კუნთებში დნმ-ის სინთეზის შესწავლა

პაპიძე გ.[1], სუმბაძე ც.[2], გაჩეჩილაძე ნ.[1]

[1]მოლეკულური ბიოლოგიისა და ბიოლოგიური ფიზიკის ინსტიტუტი
[2]ნათიშვილის ექსპერიმენტული მორფოლოგიის ინსტიტუტი

(მიღებულია 06.07.2009)

რეზიუმე

შესწავლილია დნმ-ის სინთეზის ინტენსიობა (გამოყენებული იყო H^3-თიმიდინი) კალიის (ერთდღიანი და სქესმწიფე) იმაგოს განვითარების პროცესში ფუნქციურად განსხვავებულ მონოფუნქციურ (მოფ) და ბიფუნქციურ (ბიფ) საფრენ კუნთებში (დორსალური სიგრძივი-112, ტერგოსტერნული-113, ტერგოკოკსული-119, 120). ნაჩვენებია ერთდღიანი იმაგოს (ფრთები ჯერ არ ფუნქციონირებს) მოფ და ბიფ კუნთებში დნმ-ის სინთეზის ინტენსიობის 2-4 – ჯერ უფრო მაღალი დონე სქესმწიფესთან შედარებით, რაც იმაზე მიუთითებს, რომ განვითარების ამ პერიოდისათვის ჯერ კიდევ არ არის დასრულებული საფრენი აპარატის ფორმირების პროცესი. მოფ კუნთ 112-ში ერთდღიან და სქესმწიფე იმაგოს შემთხვევაში გამოვლინდა დნმ-ის სინთეზის შედარებით დაბალი დონე. სხვა კუნთებს (113, 119, 120) შორის დნმ-ის სინთეზის ინტენსიობაში განსხვავება არ აღინიშნება.

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PRELIMINARY RESULTS OF EFFECT OF DIFFERENT WATER VOLUMES ON CONIDIAL MASS PRODUCTION OF *BEAUVERIA BASSIANA*

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Abstract

Mass production of entomopathogenic fungi is an important component of successful microbial insecticide program. The objective of this study was to evaluate the conidia production of strain *Beauveria bassiana* (CA-603) on millet growing media at different water volumes (substrate : water 1:0.25; 1:0.5; 1:0.75 and 1:1). The results showed that there were significant differences in conidia production at the water volume of cooking. Conidia production of *B. bassiana* was significantly higher (3.4 x107 /g millet) at 1:1 water ration and spore viability achieved 90.3%. Although, higher (94.7%) spore viability was observed too in the variant 1:0.25.

Key words: mass production, *Beauveria bassiana*, millet, water volume, spore productivity, spore viability.

Introduction

At present the biological formulations on basis of entomopathogenic fungi (EPF), are considered as one of the effective means against agriculture, greenhouse, forest and urban pest insects [Butt et al., 2001; Inglis et al., 2001]. Different factors help to produce a successful microbial insecticide, including: virulence against the key insect pests, viability of high production quantities [Goettel and Roberts 1992, McCoy, 1990; Jenkins, et al., 1998]. Other factors can be considered such as, environmental conditions and cultural techniques [Maniania 1991].

Mass-production of microbial formulations is very important part of the microbial control. But microbial industry is exclusively based on the special equipment for submerged fermentation of microorganisms using the liquid media. Unfortunately all our fungi do not produce valuable spores under conditions of submerged fermentation. For these fungi the solid substrata is needed. The big industry does not have suitable outfit or this outfit and technology demand a lot of manual labor. Mass-production of fungal formulations for plant protection can realize on small individual or collective farms. For this people can use different agricultural waste material and simple equipment [Gouli & Gouli, 2003; 2004; El Damir et al., 2003].

The several Hyphomycetes fungi such as *Beauveria bassiana* are well-known insect pathogens [Gillespie & Moor-house, 1989; Tanada & Kaya, 1993; Lisansky & Haall, 1983; Weiser et al., 2003; Wegensteiner, 2000; Zimmermann, 1993]. This group includes numerous species that

frequently cause epizootics in populations of insect pests and are considered as important biocontrol agents of insect pests.

Material and Methods

Fungal isolates

The strain of entomopathogenic fungi *Beauveria bassiana* (CA-603) was obtained from the Entomology Research Laboratory collection at the University of Vermont. The strain was isolated from soil collected in an avocado orchard in California and is active against pest insect. For the experiment as sowing material fungal culture has to grow 12 days (temperature 25^0C), on the Potato Dextrose Agar (PDA) media (Petri dishes - Ø 9cm).

Prepearing of liquid substrate/ culture media

100 ml PDA media (39g PDA + 1 l distil water) was poured into a 250ml shake flasks and autoclaved. Further one flask with media was inoculated with 1 piece of *B.bassiana* cultural media (cultural media in Petri dishes was dissected on 24 pieces). Flasks were incubated on a horizontal flask shaker at room temperature 22±2°C, 200 rpm. Cultures were harvested after 5 days'growth. Blastospore concentrations were measured microscopically using a hemacytometer.

Evaluation of Spore viability

For determination of alive conidia of *B.bassiana* (CA-603) germination test was used [Humber, 1997]. The blastospores showering into PDA media, was covered with a cover-slip and incubated at room temperature 22±2°C. After 24 hours alive conidia, at least 100 conidia per replicate, were counted under light microscopy.

Selection of moisture levels

500ml (450g) of "Organic Hulled Millet" was placed in an autoclavable plastic bags and water was added at different ratios to select the optimum moisture content for spore production. Millet and four ratios of water were tested: 1:0.25; 1:0.5: 1:0.75 and 1:1 (V:V). After adding the water 0.4 ml $liter^{-1}$ of citric acid was added to minimize bacterial contamination. Bags were cooked at 70-80 °C for two hours and then autoclaved for 1 hour.

Inoculation of plastic bags

After cooling up to room temperature, the bags were inoculated with 5ml spore suspension containing 2.6×10^7 spores ml^{-1}. To provide air exchange the opening of each bag was covered with a layer of paper towel, cloth, and aluminum foil that were held in place with a rubber band. Every 3 days, the contents were thoroughly mixed to promote growth throughput the medium.

Determination of spore productivity and spore viability

The dry millet was poured into a fine polyester monofilament mesh (~300 μm), and put in a large plastic bag. Powder spores settled to the bottom of bag and then they are transferred in sterile 100ml cryogen tubes. Haemocytometer counts of the spore suspension gave estimates of the total conidial yield.

Results and Discussion

Four different moisture levels were tested for their effects on fungal growth on millet grains.

Observations on the texture, moisture content and stickiness of the millet grains in experiments are summarized in Table 1.

Table 1. Effect of water (V:V) ratios on the texture of millet grains and their suitability as substrates for mass production of *B.bassiana* (CA-603).

Grain type	Grain:water ratio	Observation	Remark
Millet	1: 0.25	Very dry, grain not opened	Not good
	1: 0.5	Good, not sticky but slightly dry	Fair
	1: 0.75	Ample moisture, grains opened	Very good
	1: 1	Good moisture, grain opened, slightly sticky	Fair

According to our observation, after cooking in a water bath, optimum moisture content for millet was determined as 1 : 0.75. Grains were best moistened with hot tap water. In this combination, millet's physical characteristics such as grain size and hydration properties were high - ample moisture and grain opened. pH of the mixture was adjusted to approximately ≈ 5.5

Autoclaved thermostatic plastic begs with cooking millet substrate were inoculated with 5ml spore suspension containing 2.6 $x10^7$ spores ml^{-1} mixed thoroughly.

The standard growth condition was 22 ± 2°C under natural photoperiod for 3 weeks. Aluminum covers were removed from the bags after 5-7 days to minimize vapor formation. Grains were mixed every 3-5 days to maintain an even distribution of spore production. Bags were also inspected everyday for the presence of any unusual growth of fungi or bacterial contaminants until five days after inoculation. After 12 days all covers of the bag openings were removed to dehydrate further the culture, as spore viability is reduced under moist conditions. The amount of spores produced inside plastic begs was determined by taking 15 day old culture. For each bag, volume of 10 ml of millet was transferred into sterile Erlenmeyer flasks containing 100 ml of sterile 0.01% Tween 80. Spores were harvested by gently shaking the resultant suspension for 30 minutes using an orbital shaker. 10 ml of the suspension was taken out and filtered. Haemocytometer counts of the recovered spore suspension gave estimates of the total conidial yield. Serial dilutions of PDA media were used as estimates of the viable conidial yield. Viability was determined by counting the percentage of germination of conidia after 24 hours incubation at 22°C. The result of viability of spores is given in Table 2.

Table 2. Viability of spores production of *B.bassiana* (CA-603) in different water rations within 24 hours at 22°C

#	Grain:water ratio	Viability of spores (%)
1	1: 0.25	94.7
2	1: 0.5	87.5
3	1: 0.75	89.5
4	1: 1	90.3

The results showed that *B.bassiana* (CA-603) had high germination rate (94.7%) in 1:0.25 (V:V) and (90.3%) in 1:1 (V:V). Germination speed could bias the viability of a spore.

After 3 weeks plastic bags were opened in the mass production room and conidia powder was collected by sieving. The results of highest amount of spores per gram and total pure spores in the different water volumes are given in Table 3.

Table 3. Correlation of different water volumes with grain and spores mass production of *B.bassiana* (CA-603)

#	Dry millet (500 ml or 450 g)	Tap water ml	Pure spore ml/beg	Pure spore g/beg	Dry pure spore g/beg	Spore productivity /1g millet	Total Pure spore /beg $x10^7$
1	500	125	6	0.273	0.244	$2.6 x10^6$	170
2	500	250	15	0.502	0.449	$1.2 x10^7$	600
3	500	375	51	0.513	0.459	$2.2 x10^7$	1100
4	500	500	87	0.502	0.449	$3.4 x10^7$	1700

The results showed that ratio 500:500 (1:1) gave higher spore yield ($3.4x10^7$) than other water volumes of cooking. In particular, in variants 1:1 the number of yielded spores was ten times more than when grown on 500:125 ($2.6x10^6$). Correlation between pure spores production of *B.bassiana* CA-603 in millet and different water volume is given in Figure 1.

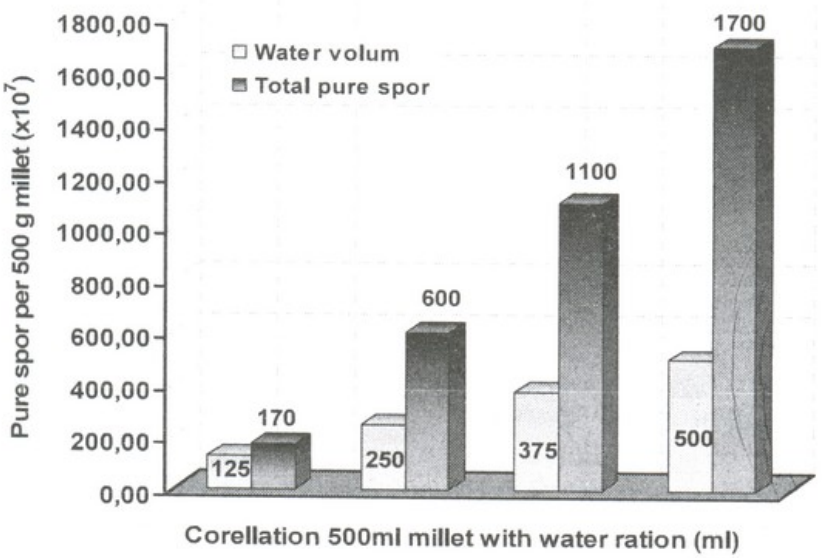

Fig.1. Total pure spores of *B.bassiana* CA-603 in millet and different water volumes

The study showed that millet is good substrate for mass production and productivity of conidia is higher. These results provide useful information to develop a good mass production technique for *B.bassiana* CA-603 and showed promising results for the mass production. Knowledge of these factors is critical in the selection of potent strain available for biological control.

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მასობრივი გამრავლების წინასწარი შედეგები და წყლის სხვადასხვა რაოდენობის ეფექტურობა *Beauveria bassiana*-ს კონიდიების წარმოქმნაზე

ბურჯანაძე მ.

ვასილ გულისაშვილის სატყეო ინსტიტუტი, მიხდელის 9, თბილისი, 0186

(მიღებულია 15.06.2009)

რეზიუმე

ენტომოპათოგენური სოკოების მასობრივი გამრავლება არის მნიშვნელოვანი კომპონენტი მიკრობიოლოგიური ინსექტიციდების წარმოების პროგრამაში. წარმოდგენილი სამუშაოს მიზანია მარცვლეულ საკვებ არეზე *Beauveria bassiana* (CA-603)-ს კონიდიების პროდუქტიულობის განსაზღვრა წყლის ოთხი სხვადასხვა მოცულობის გამოყენებით (სუბსტრატი : წყალი 1:0.25; 1:0.5; 1:0.75 და 1:1). ცდების შედეგებმა გვაჩვენა, რომ წყლის სხვადასხავ მოცულობის შემთხვევაში წარმოქმნილი კონიდიების რაოდენობა სხვადასხვაა. *B. bassiana*-ს კონიდიების მაღალპროდუქტიულობა $3.4x10^7$/გრ აღინიშნებოდა მარცვლეულისა და წყლის 1:1 თანაფარდობის შემთხვევაში და სპორების სიცოცხლისუნარიანობა 90.3%-ს შეადგენდა. ასევე სპორების გაღივების მაღალი პროცენტი დაფიქსირდა (94.7%) მარცვლეულისა და წყლის 1:025 თანაფარდობის შემთხვევაში.

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VILLAFRANCHIAN VERTEBRATE FAUNA OF GEORGIA AND ITS BIOSTRATIGRAPHIC SIGNIFICANCE

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Abstract

The paper deals with Villafranchian vertebrate fauna of Georgia, biostratigraphic significance of its environment and attempt of reestablishment of the Pliocene. Obtained data indicate that the Dmanisi fauna is older than the end of the Late Villafranchian. It is shown that during the Plio-Pleistocene extensive faunal interchanges occurred via the territory of Transcaucasus.

Key words: Georgia, Villafranchian fauna, biostratigraphic, Villanyan

Introduction

Georgia lies between the Major Caucasus ridge in the north, the Minor Caucasus ridge in the south, the Black Sea in the west, and the Kura-Araks plain in the east. Physiographically, the country is divided into Western Georgia, with a warm and humid Mediterranean climate, and Eastern Georgia, where the climate is more continental. Georgia's complex topography of river valleys, foothills, and mountains is mirrored in the strict altitudinal zonality of its biotic resources, displaying all climatic zones from coastal to mountainous ones, up to altitude of 5.201 m a. s. l.

The Biostratigraphy of the Vertebrate Faunas

The Dmanisi faunal assemblage consists mainly of Villafranchian mammals the majority of which can be attributed to the top of Middle Villafranchian or Villanyan and to the Lower Biharian. The inventory includes: *Megantereon megantereon, Homotherium crenatidens, Pantera gombaszoegensis, Ursus etruscus, Equus stenonis, Dicerorhinus etruscus* and several others which are found throughout the entire Villafranchian and therefore can not provide an accurate geological age for the Dmanisi complex. Instead, the following species we consider as better chronological markers: the very large ostrich - *Struthio dmanisensis,* which is similar in size to *Struthio transcaucasicus* from Kvabebi [Vekua, 1972], and also is reminiscent of the gigantic ostrich - *Struthio olduwaji* from Olduvai [Leakey 1967], *Mimomys ostramosensis, Mimomys reidi, Kowalskia* sp., *Pachycrocuta perrieri, Cervus perrieri and Cervidae cf. Arvernoceros, Gazella cf. borbonica* and finally, *Giraffidae cf. Paleotraginae.*

These species are widely represented in the layers 4-5. In Layer 2, to date, we found only *Pachycrocuta perrieri, Cervus perrieri,* and *Giraffidae cf. Palaeotraginae.* These data, as discussed below, indicate that the Dmanisi fauna is older than the end of the Late Villafranchian.

It should be noted that in the East Paratethys region ostriches are not known from deposits younger than the Pliocene [Dubrovo, Kapelist, 1979]. In the Caucasus their late representatives, namely, the large type of Struthio, are found in Lower Apsheronian of Taribana (correlated with the Dmanisi horizon) and of Palan-Tukan in west Azerbaijan [Burchak-Abramovich, 1953].

Mimomys reidi has a rather broad stratigraphic spread [Kordos, 1994], from the beginning of the Middle Villafranchian to the middle of the Upper Villafranchian, but it is more characteristic of the second half of the Middle Villafranchian or Upper Villanvan, where the biozone named after *Mimomys cf. reidi* [Agusti, Moya-Sola, 1992] was identified. The resent discovery of *Mimomys ostramosensis* at Dmanisi [Muskhelishvili, 2000] indicates that the Dmanisi fauna cannot be younger than basal Biharian.

The genus Kowalskia is known mainly from the Late Miocene and the Lower Pliocene [Mein, 1989]. It occurs less commonly in the Late Pliocene and has not been found to date in the Pleistocene. The discovery of this species at Dmanisi clearly supports the antiquity of the Dmanisi complex.

The remains of giraffes at Dmanisi were also unexpected because, in Georgia, late representatives of this taxon are known from the lower part of the Upper Pliocene [Gabunia, Buachidze, 1970].

Pachycrocuta perrieri and *Cervus perrieri,* characteristic of the early and middle Villafranchian faunas of Western Europe, and middle-sized deer, reminiscent of *Arvernoceros ardei,* all also indicate affinities with middle Villafranchian and basal late Villafranchian faunas [Turner, 1994].

Our recovery of *Gazella cf. borbonica* is significant because in Western Europe this taxon is not found in deposits younger than the Middle Villafranchian [Agusti, Moya-Sola, 1992].

The archaic faunal component at Dmanisi also includes *Archidoskodon meridionalis taribanensis,* a southern elephant who possibly requires a new species designation [Alekseeva, 1977].

Comparatively younger forms are also present at Dmanisi, such as Soergelia, which first appears in the Upper Villafranchian and lasts through the Middle Pleistocene. It is important to note, however, that the palaeontological history of this species has been poorly studied to date, and it may be present earlier than has previously been thought. One form was recently identified in Venta-Micena (Spain) in the context of Early Pleistocene fauna [Agusti, Moya-Sola, 1992]. Considering that *Soergelia* is probably of Asian origin and that its migration required some time, the appearance of early representatives of this genus in Transcaucasia at the beginning of the Pleistocene or even at the end of the Pliocene is not surprising.

In Europe the first appearance of *Pantera gombaszoegensis* coincides with the disappearance of *Pachycrocuta perrieri* [Tumer, 1994]. In Dmanisi both species coexisted. It is possible that *P.Gombaszoegensis* as well as *Cam's etruscus* dispersed here from the East. Traces of *P.gombaszoegensis* in Transcaucasus are older than those from Western Europe, where the earliest appearance of this species are reported from Olivola and Tegelen [Turner, 1994] and where they are found together with *Pachycrocuta brevirostris.*

In Western Europe *Dama nesti* is limited mainly to the Late Villafranchian [Azzaroli et al., 1988] but its presence in Transcaucasia at the beginning by this time or even at the end of the Middle Villafranchian cannot be excluded. It seems that at Dmanisi it is present at the beginning of Apsheronian (The basal part of the Lower Pleistocene). The same can be said for some other members of the Dmanisi fauna with comparably broad stratigraphical spread (*Hypolagus*

brachygnathus, Apodemus dominans, Megantereon megantereon, Canis etruscus, Ursus etruscus and others).

The Dmanisi bovid is a new genus and species that was named *Dmanisibos georgicus* [Burchak-Abramovich, Vekua, 1994]. It is difficult to assess its bio-stratigraphic implications. However, such features as the flatness of its forehead, an occiput notably stretched in length, and a distinctive position of the horn pivot, place it in an intermediate position between the *Tshaudian Eobison* [Flerov, 1979] and the more archaic *Adjiderebos* [Dubrovo, Burchak-Abramovich, 1984] from the Late Akchagylian. This nicely correlates the chronological horizon of the Taribana-Dmanisi fauna.

A small complex of vertebrates from the Late Akchagyl and Lowest Apsheronian of Taribana (Kotsakhuri region) consists of Testudo sp., Emys orbicularis, Struthio sp., Archidiskodon meridionalis taribanensis, Equus stenonis stenonis, Dicerorhinus cf. etruscus, Camelus sp., Protoryx sp., Leptobos sp., and others [Gabunia, Vekua, 1981; Vekua, Kvavadze, 1981]. The Dmanisi fauna is very close to that of Taribana and may date to the basal part of the Apsheronian. These two faunal localities are geographically quite close to each other and, as discussed below, represent the same paleoenvironment.

The small faunal collection from Tsalka is also assigned to the Dmanisi-Taribana complex. It contains *Homotherium cf. crenatidens, Dama cf. nestii, Eucladoceros* sp., *Leptobos* sp. [Vekua et al., 1985]. Long-range comparisons extend through Europe and the Near East. When the Dmanisi complex is compared to Eastern Europe, it corresponds to the late phase of the Khaprov faunal complex [Alekseeva, 1977], where Hipparion and Annancus are already absent. However, the presence of *Elasmotherium*, *Paracamelus* and some other species separates them from the Dmanisi complex and reflects the more severe climate that prevailed in Khaprov. The Dacic basin (Romania) is of special interest because its Tatou complex is close to the Dmanisi fauna [Radulescu, Samson, 1990] and also contains artifacts. The fauna includes *Archidiskodon meridionalis, Ursus etruscus, Pachycrocuta perrferi, Homotherium crenatidens, Megantereon megantereon, Equus stenonis, girafid Mitilanotherium.* Another site, Shandalia (former Yugoslavia), is also similar to Dmanisi in terms of the mammalian fauna and the remains of a lithic inventory [Malez, 1975]. These examples offer evidence that at the beginning of the Pleistocene Transcaucasia was more closely linked with the Dacian-Balkan region than with Eastern Europe.

In spite of the absence of good faunal correlations with Western Europe, the assemblages of Le-Coupet, Olivola, Seneze, and Tasso are generally comparable as they all contain typical forms of *Archidiskodon meridionalis, Equus stenonis, Ursus etruscus* and other taxa [Guerin, 1982; Bonadonna and Alberdi, 1987; Azzaroli et al., 1988]. These complexes are also characterized by other species which reflect regional specificities in western Mediterranean and, in some cases, differences in the geological ages. They are dated to the Middle Upper Villafranchian, from the upper part of Zone 17 and Zone 18 as defined by Guerin (1982). Among the Late Villafranichian mammalian faunas of Western Europe, the Venta-Micena complex is more similar to Dmanisi [Martinez, 1992]. It differs from Dmanisi in its regional characteristics but may represent the comparatively advanced evolution of representatives of some common forms.

The Dmanisi fauna bears a distant resemblance to Ubeidya (Israel) which contains different forms of fossil vertebrates, rare remains of Homo sp., and rich lithic assemblages [Tchernov et al., 1986]. Ubeidya, however, is not only younger than Dmanisi, but also differs by the presence of *Archidskodon meridionalis tamanensis, Hippopotamus* and other taxa which indicate different ecological conditions. At the same time both sites contain several common species: *Hypolagus brachygnatus,Canis etruscus,Ursus etruscus, Dicerorhinus etruscus.* The discovery of *Parameriones cf. obeidiensesis* at Dmanisi [Muskhelishvili, 2000] is of special interest. Tchernov and colleagues (I986) consider this taxon as endemic to the Ubeidiya region.

Overall, the Dmanisi fauna contains species with diverse points of origin where Palearctic elements prevail over Paleotropical ones. Although it is difficult to assign specific origins to the Palearctic species, most of them likely dispersed here from Asia. The origin of *Megantereon* remains moot. Some researchers argue that this taxon belongs to the African *Megantereon whitei* [Martinez-Navarro, Palmkvist, 1996]. We remain to be convinced. The sum of these data, however, do indicate that during the Plio-Pleistocene extensive faunal interchanges occurred via the territory of Transcaucasus.

The Paleogeographic Context

Transcaucasia at the end of the Pliocene was a geographic outpost of Asia Minor. To west and east it was flanked by the Kujalnihk (Black) and Akchagyl (Caspian) seas. The Caucasian range lay to the south where the waters of the Manich Strait washed the northern mountains. The Kura Strait of Akchagyl, transformed by the Apsheron basin at the beginning of Pleistocene, occupied large parts of East Georgia and created a landscape of mountain masses dissected by deep valleys and ravines. Dmanisi at this time was situated 60 km. southwest of the Caspian Sea. Paleoenvironmental data indicate a semi-dry and warm climate during this time, similar to the present Mediterranean climate, with many rivers and lakes and rich animal and vegetable resources.

In Transcaucasia, as in all Mediterranean regions at the end of the Middle Pliocene (the second part of the Middle Akchagylian), an abrupt climatic change took place, marked by cooling. This event is reflected in the disappearance of several mammalian species and the introduction of some new forms. Species that became extinct included *Hipparion crusafonti* (the last Hipparion), *Dicerorhinus megarhinus, Nyctereutes megamastoides, Ursus arvernensis,Propotamochoerus provincialis* and several others [Gabunia, Vekua, 1981]. The new arrivals included the first horses *(Equus stenonis vireti).* It is possible that the Anancus continued to coexist with *Archidiskodon* [Lebedeva, 1972]. Other taxa which persisted included *Dinofelis, Chasmoporthetes,* a Pliocene antelope, and several other Bovini known from the localities of Melaani, Kushkuna and Diliska. Among the small mammals, *Mimomys pliocaenicus* points to contemporaneity with the Villanyan complex of the west Mediterranean [Agusti, Moya-Sola, 1992] and the early Kchaprov fauna of Eastern Europe [Shevchenko, 1965; Alexeeva, 1977].

The end of Akchagylian and the beginning of Apsheronian phases was marked by further aridisation which caused a turnover in the mammalian fauna. It is characterized by the appearance of *Equus stenonis stenonis, Dicerorhinus etruscus etruscus, Archidiskodon meridionalis taribanensis* and several other species. In Eastern Europe this time corresponds to the Ferland and Domashkin horizons [Nikiforova, 1982] and in Western Europe a transition from the Villanyan to Biharian took place [Tchernov, 1982].

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საქართველოს ვილაფრანკული ხერხემლიანთა ფაუნა და მისი ბიოსტრატიგრაფიული მნიშვნელობა

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რეზიუმე

სტატიაში მოცემულია საქართველოს ვილაფრანკული ხერხემლიანთა ფაუნა, მისი გარემოს ბიოსტრატიგრაფიული მნიშვნელობა და პლიოცენური დროის პალეოგეოგრაფიული აღდგენის ცდა.

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EFFECT OF *CONIOTHYRIUM MINITANS* CONIDIAL SUSPENSION CONCENTRATIONS ON MORTALITY OF *SCLEROTINIA SCLEROTIORUM* SCLEROTIA UNDER CONTROLLED CONDITIONS

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Abstract

Coniothyrium minitans is a biological control agent of white mould causing plant fungal disease sclerotinios. Different concentrations of *C. minitans* were tested against sclerotia of *Sclerotinia sclerotium*. Increasing of the concentration of the mycoparasite caused increasing of sclerotia mortality. Very low concentration of *C. minitans* was enough to kill sclerotia, but for killing them in a shorter time it was more effective to use higher concentrations (10^7-10^8 sp/ml).

Keywords: soil-borne plant pathogen, biological control, mycoparasite, sclerotia.

Introduction

Sclerotinia sclerotiorum (Lib) de Bary is an important soil-borne plant pathogen of over 400 species of plants including a wide range of economically important crops world-wide and some glasshouse crops [Boland and Hall, 1994], and survives between crops in the soil as sclerotia [Coley-Smith and Cooke, 1971; Merriman, 1976]. These sclerotia may germinate myceliogenically to infect the plant directly or, more typically in glasshouse crops in the UK, carpogenically to produce great numbers of ascospores with the potential to infect plants over a wide area. Fungicide sprays can prevent infection by ascospores, however, due to difficulty in achieving spray penetration through the crop canopy, disease can still occur. Once the pathogen has become established in the soil, steam sterilisation or fumigation with methyl bromide can be used to kill the sclerotia. However, steam sterilisation is costly and the imminent withdrawal of methyl bromide over regular use of fungicides has promoted the search of biological control agents of *S. sclerotiorum* [Jonesa E., 2003].

Coniothyrium minitans is a mycoparasite of *S. sclerotiorum* and the addition of *C. minitans* to soil reduces sclerotial viability of the pathogen and can reduce disease incidence over time [Budge and Whipps, 1991; Gerlagh et al., 1999; Jones and Whipps, 2002]. However, although sclerotial numbers may be reduced in soil following the addition of *C. minitans,* the level of disease may not always be reduced significantly, particularly if the pathogen inoculum is high [Budge et al., 1995]. The combined use of soil pasteurisation and the application of the biological control agent *C. minitans* may be a strategy that is applicable for the control of Sclerotinia disease aimed at

destruction of sclerotia. Thus, *C. minitans* added to pasteurised soil has the potential to target any sclerotia that are unaffected by the heat treatment. Also, the biocontrol agent can survive and remain viable in the soil to parasitise any new sclerotia that fall onto the soil from plants infected by airborne ascospores, and thereby prevent a build-up of pathogen inoculum for future crops. Another potential benefit of the addition of a beneficial microorganism to pasteurised soil is that it may prevent or delay the reinvasion of the soil by other potentially deleterious microorganisms. Importantly, as freshly steamed soil can quickly be recolonised by a range of microorganisms [Rowe et al., 1977; Marois and Locke, 1985], it may be necessary to apply beneficial microorganisms directly to the soil immediately after the steam treatment to allow them to colonise the soil first, before other microorganisms reinvade and become established. Thus, application timing is an important consideration.

In this study, the relative efficacy of different concentrations of *C. minitans* inocula were tested in Petri dishes bioassays to evaluate their effect on mortality of sclerotia under controlled conditions (20-22°C).

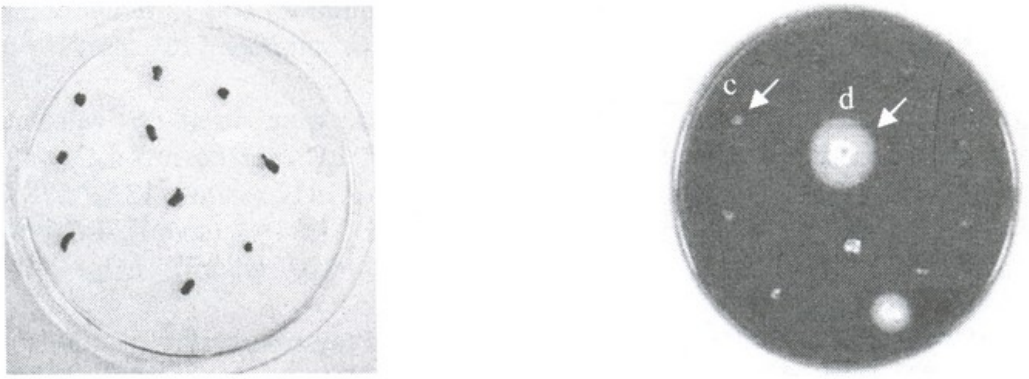
Materials and Methods

Source and Maintenance of Fungi. *C. minitans* was isolated from a Contans ® WG on potato dextrose agar (PDA). *S. sclerotiorum* was originally isolated from diseased carrot on PDA.

Bioassay with *C. minitans*. 2-3 ml sterilized water containing 0.15% fructose was added to a PDA plate with sporulating fungi and gently rub with sterilized spatula on the plate, to free spores. The concentration of the undiluted spore suspension was determined using a Thoma chamber and diluted to a concentration of $1 \cdot 10^2$ to $1 \cdot 10^8$ conidia/ml *C. minitans*.

3 weeks old sclerotia of *S. sclerotiorum* were placed in a plastic tube containing fungal spore suspension and leaved there for 20 min. After that sclerotia were removed by pincette and dried on the sterile filter paper and placed on the Petri dishes containing the moisturized circle of the filter paper. 200 µl ddH_2O was added every day during 4 weeks for moisturizing filter paper (for each variant 3 replicates were done and 10 sclerotia were treated per Petri dish). Assays were kept at 20-22°C temperature (optimal temperature of *C. minitans*) for 4 weeks.

Mortality of sclerotia was checked weekly on the bromcresol green Agar media (the blue plate test): sclerotia were placed by pincette on bromcresol green agar media and incubated during 6-7 days at 20-22°C. Sclerotia was considered as dead when medium around sclerotium was not changed color, when it turned blue to yellow sclerotium was considered as alive. Living sclerotia exude drops of oxalic acid that is changing color of indicator (Fig.1).



a) b)

Fig. 1. a) treated sclerotia placed on the moisten filter paper in a Petri dish; b) the "blue plate" test; treated sclerotia placed on the bromcresol green agar media: c – alive sclerotium, d - dead sclerotium.

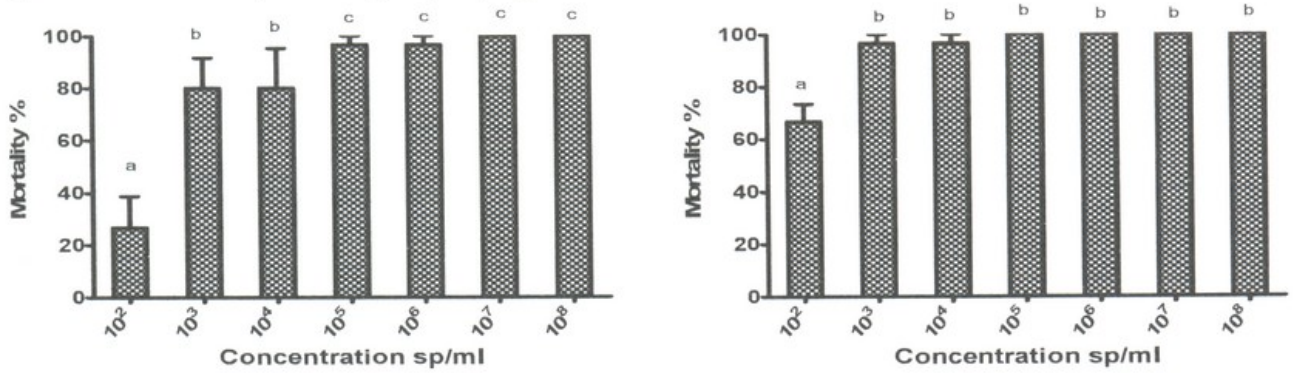
Statistical analyses. Statistical analyses were done using Graph Pad Prism 5 software. One-way Anova and Tukey's post-test were used for comparison of effects caused with different concentrations of spore suspensions.

For calculation of lethal concentrations Polo plus software (Version 1, copyright © 2002-2009 LeOra software) was used.

Results and Discussion

Tukey's post-test comparison showed that after 1 week mortality caused with concentration (10^2 sp/ml) was significantly low (27%) than mortality caused by using other concentrations ($P<0.05$). Mortality percentage was increasing (from 80% to100%) as concentration of spore suspension was growing (from 10^3 to 10^8 sp/ml), but statistically these results were not significantly different ($P>0.05$).

It is clear that after 1 week mortality of sclerotia is enough high to reduce number of resting structures of the pathogen even at low concentrations of *C. minitans* (10^2-10^4 sp/ml). At high concentrations (10^5-10^8 sp/ml) mycoparasite can kill almost 100 % of sclerotia.



a) b)

Fig 7. Bioassay with *C. minitans*. Mortality (%) of sclerotia of *Sclerotinia sclerotiorum* (a) after 1 week; (b) after 4 weeks at different concentrations of *C. minitans*

After 4 weeks mortality caused with concentration 10^2 was significantly low (67%) than mortality caused by using other concentrations ($P<0.05$). Mortality percentage was increasing as concentration of spore suspension was growing (from 91% to 100%), but statistically this results were not significantly different ($P>0.05$).

After 4 weeks mortality caused with all tested concentrations was sufficiently high to kill almost all treated sclerotia. Even very low concentration of *C. minitans* (10^2 sp/ml) was able to destroy more than 60% of sclerotia during 4 weeks.

Probit regression analyses of dose-response data were used to calculate lethal concentrations of *C. minitans*: a) after 1 week: LC_{10}=4 (sp/ml), LC_{20}=16 (sp/ml), LC_{30}=49 (sp/ml), LC_{40}=127 (sp/ml), LC_{50}=307 (sp/ml), LC_{60}=743 (sp/ml), LC_{70}=1912 (sp/ml), LC_{80}=5780 (sp/ml), LC_{90}=26795 (sp/ml) and b) after 4 weeks: LC_{10}=1 (sp/ml), LC_{20}=3 (sp/ml), LC_{30}=6 (sp/ml), LC_{40}=13 (sp/ml), LC_{50}=25 (sp/ml), LC_{60}=50 (sp/ml), LC_{70}=103 (sp/ml), LC_{80}=239 (sp/ml), LC_{90}=768 (sp/ml).

It is known, that even one *C. minitans* conidia can cause mortality of whole sclerotia. [Williams et al., 1998; Gerlagh et al., 2003]. As it is clear from the results, for killing sclerotia after 1 week more concentration of *C. minitans* is needed than after 4 weeks. If we take into account, that one sclerotium produces lots of ascospores which can infect many plants simultaneously it

should be more effective to use higher concentrations of the mycoparasite for killing resting bodies of the plant pathogenic fungus in the short time.

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Coniothyrium minitans–ის სპოროვანი სუსპენზიის კონცენტრაციის გავლენა *Sclerotinia sclerotiorum*–ის სკლეროციების სიკვდილიანობაზე კონტროლირებად პირობებში

ბიწაძე ნ.

ყანჩაველის მცენარეთა დაცვის ინსტიტუტი

(მიღებულია 02.06.2009)

რეზიუმე

სოკო *Coniothyrium minitans*-ი მცენარეთა სოკოვანი დაავადების, თეთრი სიდამპლის, ანუ სკლეროტინიოზის ბიოლოგიური კონტროლის აგენტია. *Sclerotinia sclerotium*-ის სკლეროციების მიმართ *C. minitans*-ის სპოროვანი სუსპენზიების სხვადასხვა კონცენტრაციები იქნა გამოცდილი. მიკოპარაზიტის სპოროვანი სუსპენზიის კონცენტრაციის ზრდა იწვევდა სკლეროციების სიკვდილიანობის პროცენტის ზრდას. აღსანიშნავია, რომ *C. minitans*-ის მცირე კონცენტრაციაც კი საკმარისი იყო სკლეროციების გასანადგურებლად, თუმცა მათი სწრაფი განადგურებისთვის უფრო მაღალი კონცენტრაციების (10^7-10^8 კონიდიუმი/მლ) გამოყენება აღმოჩნდა ეფექტური.

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REPRODUCTIVE POTENTIAL OF ENTOMOPATHOGENIC NEMATODE *STEINERNEMA FELTIAE* ON DIFFERENT HOSTS (*GALLERIA MELLONELLA, HYPHANTRIA CUNEA* AND *LEPTINOTARSA DECEMLINEATA*)

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Abstract

The study deals with the efficiency of comparative reproduction of entomopathogenic nematode *Steinernema feltiae* larvae in the following insects: the wax moth - *Galleria mellonella*, the fall webworm - *Hyphantria cunea* and Colorado potato beetle imago - *Leptinotarsa decemlineata*. An emphasis is placed on the control of parasitic insects number by means of entomopathogenic nematode *S. Feltiae*. New nutrient media for cultivation of nematode *S. Feltiae* have been revealed. High indices of *S. Feltiae* reproduction occur at the 7th day from the invasion on larvae *H. cunea* - 94.5 %, on *L. decemlineata* - 98.4 %, on *G. Mellonella* - 91.8 %. The obtained data make it possible to cultivate *S. Feltiae* against pests of agricultural crops.

Key words: *Galleria mellonella, Leptinotarsa decemlineata, Hyphantria cunea, Steinernema feltiae.*

Introduction

The fall webworm - *H. cunea* and Colorado potato beetle – *L. decemlineata* are the most important damage causing pests in Georgia today [Chkhubianishvili, 2004].

For the cultivation of entomopathogenic nematode (EPN) *S. feltiae* we have applied larvae *H. cunea* and imago *L. decemlineata.* Besides, there was used known method with larvae *G. mellonella.* The fall webworm *H. cunea* Drury *(Lepidoptera: Arctiidae)* is an important pest in Georgia, this insect damages more than 600 species of plants: forest and bush plants, orchard and berry plants, field and vegetable cultures, ornamental trees, herbs, etc. [Weizer J. 1977].

At present the pest is widely spread in West Georgia, including the Black Sea basin of Adjara region. The pest mainly inhabits the populated areas, parks and underwood belt, in the places of the mass resort. *H. cunea* produces two generations. Its range of population extends gradually [Chkhubianishvili, 2000].

In Georgia *L. decemlineata* is also an important pest of potato crops damaging potato, tomato, eggplant, pepper, tobacco and other salacious plants. In Georgia the insecticides are often ineffective due to the beetles resistance to toxins. Adults become active in May and start laying eggs as soon as host plants are found. Larvae and adult *L. decemlineata* feed on the foliage of the

host plants and can cause extensive damage if populations are big. Feeding occurs within two weeks of flowering peak [Giliarov, 1959].

For the cultivation of *S. feltiae* larvae of *G. mellonella*, the greater wax moth (*Lepidoptera: Galleridae*) was also used. *G. mellonella*, is a lepidopterous insect that destroys combs at its larval stage. It does not attack adult bees but may begin destruction of combs of a weak colony long before the bees have gone. It can also destroy stored combs of honey. When the larvae are ready to pupate, they often eat out a place to spin their cocoons in the soft wood of the beehive.

The goal of our study was to determine reproductive potential and the efficacy of EPN *S.feltiae* against various important pests in Georgia. These pests are distributed almost on the whole territory of Georgia and do great damage to national economy. Therefore, it is necessary to protect plants from pest insects by environmentally safe means, such as biological control agents, i.e. EPNs.

Materials and Methods

In the present study the important insect pests - *H. cunea* and *L. decemlineata* have been researched. Both pests were obtained from the infested plants. The experiments were conducted in laboratory conditions. Dead larva of *H. cunea* was delivered from the Western Georgia (Zugdidi city), the imago of *L. decemlineata* was collected from potato plants (Tserovani, Mtskheta region) in Eastern Georgia for the experiments on vegetable cultures in laboratory conditions. The mortality percentage of individuals was determined by Abbott formula [Abbott, 1925]. The experiment was repeated three times. *H. cunea* and *L. decemlineata* larva were invaded by means of *S. feltiae* Israel strain - SFG. The infestation of larva pests with SFG took every 24 hr, within 72 hr. The nematodes were cultivated at 25°C in the last-instars of the greater wax moth *G. mellonella* according to the method of Kaya & Stock, (1997). After storage at 5-6°C for 1 week, they were adopted at 21-23°C for 24h before using in different assays [Lacey, 1997].

These experiments were performed on pests in laboratory conditions at 24-25^{0}C and 50-60% relative humidity. For infection of some insects with nematode *S.feltiae* 150 insects were put on 15 Petri dishes, in each cup 10 specimens of insects. Each insect species placed on 5 Petri dishes were sprayed with nematode suspension of the following concentrations: I cup contained 10 nematode/ml, II cup - 20 nematode/ml, III - 50 nematode/ml, IV cup - 100 nematode/ml, V cup - 120 nematode/ml. Those concentrations of nematode suspension were applied for each insect species. Destruction of insects happened in 48 hours. After 48 hours the dead insects were placed on a white trap and nematodes started reproduction on them. The invasive larvae of nematodes from the dead insects started to transfer into the distilled water from *H. cunea* after 11 days, from *L. decemlineata* after 14 days and from *G. mellonella* after 12 days from infestation [Mayra de la Torre, 2003].

Results and Discussion

The study of the reproductive potential of *S. feltiae* [Oguzoglu & Ozer, 2003] concerning *H. cunea* which took place during 11 days after infestation showed that nematodes of various concentrations came out within 7 days from all 5 Petri dishes. The obtained results of reproduction of *S. feltiae* on *H. cunea* are shown in Fig. 1. The reproductive potential of *S. feltiae* on *L. decemlineata* imago which happened during 14 days after the infestation is shown on Fig.2. The output of *S. feltiae* from *L. decemlineata* occurred within 7 days from all 5 Petri dishes with the similar concentrations of nematodes as on *H. cunea*. The obtained results of reproduction of *S.*

feltiae on *G. mellonella* larvae which lasted 12 days after infestation are shown in Fig. 3. The output of nematodes *S. feltiae* from *G. mellonella* larvae occurs also within 7 days with the same concentrations of nematodes as on *H. cunea* and *L. decemlineata*. The indexes of reproduction of *S. feltiae* on larvae *H. cunea* are high and make 68%, 75% and 94.5% on 5th, 6th and 7th days from the beginning of reproduction accordingly (Fig.1). The indices of reproduction of *S. feltiae* on imago *L. decemlineata* are 72%, 78% and 98.4 % on 5th, 6th and 7th days from the beginning of reproduction (Fig. 2). As for reproduction indices of *S.feltiae* on larvae *G. mellonella* they are as follows: 75% and 91.8% on 6th and 7th days from the beginning of reproduction (Fig. 3).

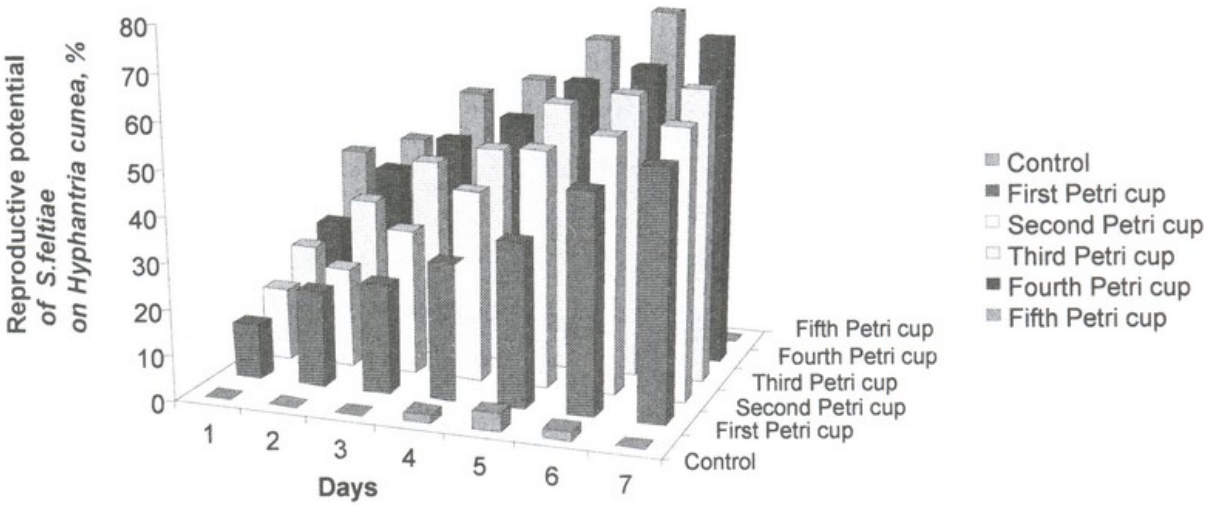

Fig. 1. Reproductive index of *S.feltiae* on larvae of *Hyphantria cunea*

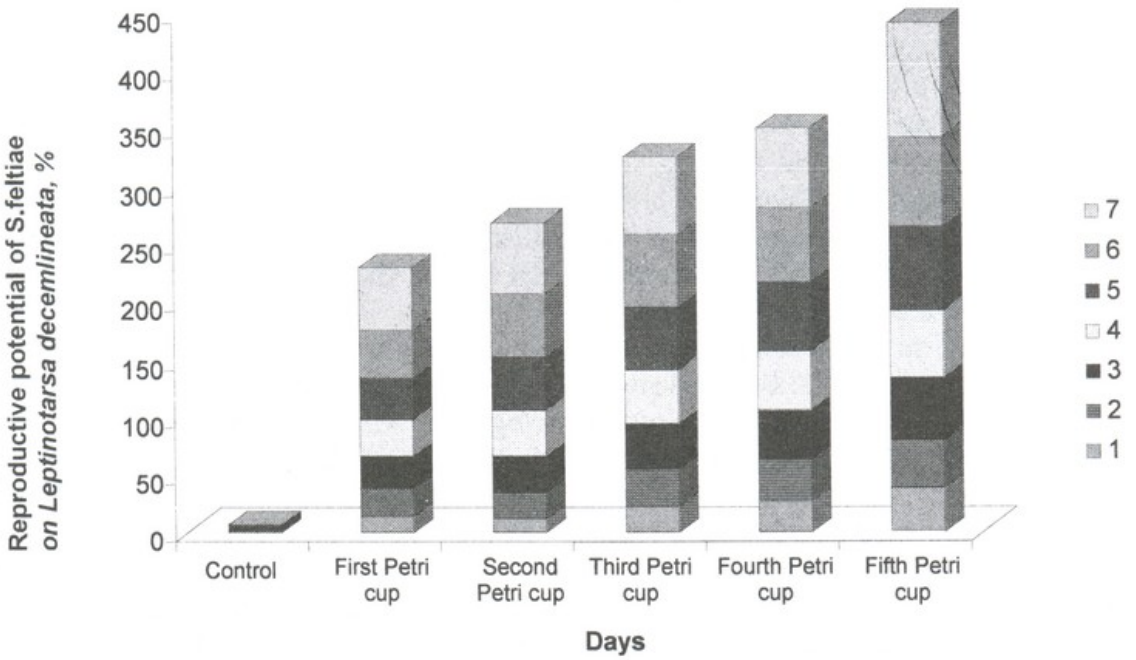

Fig. 2. Reproductive index of *S.feltiae* on imago of *Leptinotarsa decemlineata*

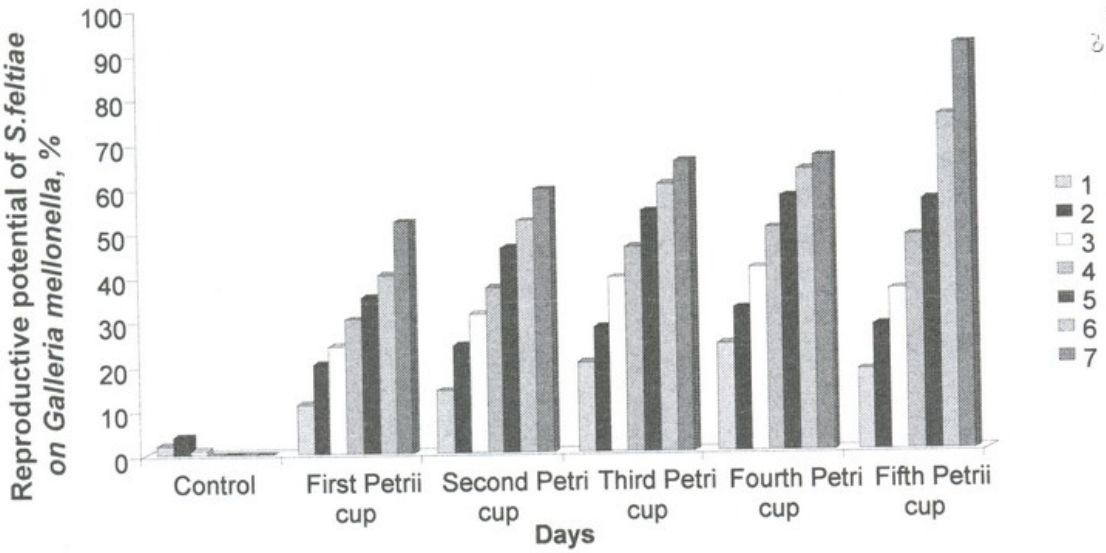

Fig. 3. Reproductive index of *S.feltiae* on larvae of *Galleria mellonella*

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ენტომოპათოგენური ნემატოდა *Steinernema feltiae*-ს რეპროდუქციული პოტენციალი სხვადასხვა მავნებლებზე (*Galleria mellonella, Hyphantria cunea* და *Leptinotarsa decemlineata*)

მიქაია ნ.

ყანჩაველის მცენარეთა დაცვის ინსტიტუტი

(მიღებულია 06.07.2009)

რეზიუმე

შესწავლილია ენტომოპათოგენური ნემატოდა *Steinernema feltiae*-ს რეპროდუქციის ეფექტურობა შემდეგ მწერებში: თაფლის ფიჭას ჩრჩილის (*Galleria mellonella*), ამერიკული თეთრი პეპელას (*Hyphantria cunea*) მატლებზე და კოლორადოს ხოჭოს (*Leptinotarsa decemlineata*) იმაგოზე. კვლევის მიზანია მავნე მწერების რიცხოვნობის რეგულირება *S. Feltiae*-ს საშუალებით. მიღებულია ახალი საკვები არეები *S. Feltiae*-ს კულტივირებისათვის. ნაჩვენებია, რომ *S. Feltiae*-ს რეპროდუქციის მაღალი მაჩვენებლები ვლინდება დაინვაზირებიდან მე-7 დღეს *H. cunea*-ზე – 94.5%, *L. decemlineata*-ზე – 98.4%, *G. Mellonella*-ს მატლებზე – 91.8 %. კვლევის შედეგად მიღებული მონაცემები მნიშვნელოვანია იმით, რომ მომავალში შესაძლებელია *S. Feltiae*-ს კულტივირება მავნე მწერების *H. cunea*-ს და *L. decemlineata* -ს წინააღმდეგ.

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STUDY OF BIODIVERSITY OF SVANETI (GEORGIA) NEMATODES

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Abstract

In order to study nematode inhabitants of Svaneti region several natural ecosystems were investigated. 45 forms of nematodes were recorded. Among them *Mesodorylaimus paetzoldi* Altherr, 1965 is new for the fauna of Georgia. Obtained results were compared with the existed data.

Key words: Svaneti, nematode, agrocenosis, natural ecosystems

Introduction

Determination of biodiversity of animals of Georgia is of great importance in the study of animal inhabitants of alpine regions. Svaneti region is interesting from the viewpoint of diversity of natural conditions. Several types of soils are formed here: mountain-meadow soils of sub-alpine and alpine zone, secondary meadow soils of the forest-mountain zone, black earth soils of the mountain-forest zone, and soils of mountain gorge, soils of humus-carbonate and unsuitable soils. Forests take a great place. Diversity of soils and growth stipulates diversity of soil invertebrates, and among them nematodes. As nematodes are inhabitants of any ecosystems, studies of their faunistic structure and ecological groups give a certain opinion about condition and maturity of one or the other soil.

Soil nematodes unite both free living and phytoparasitic forms. Free-living nematodes use such resources of soil as bacteria, fungi, algae, small invertebrates and nematodes of other groups. Herewith a big group of nematodes is related with plant tissues by ontogenesis, they often parasitize on plants and use them as habitat, or they are common non-parasitic phytobionts and feed at the expense of bacteria and fungi invaded in plant or by the decomposition products of plant tissues.

Materials and Methods

Material was collected by route method in upper Svaneti in August of 2007 (soil samples were provided by G. Arabuli).

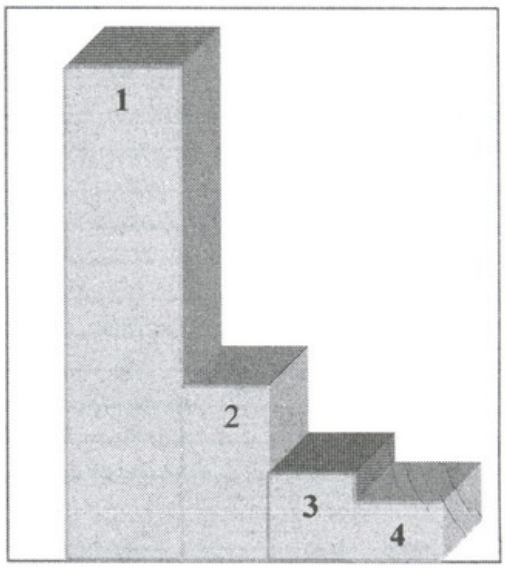
Four ecosystems were studied: 1. at the estuary of Mestiachala and Inguri (1300m above sea level, secondary meadow of the forest zone, pasture), 2. Vil. Lakhushti environs (1400m a. s. l., right slope of the river Inguri, oak-wood with azalea and nut-tree sub forest), northern slope of the Svaneti Range, left side of the river Inguri (1600m a. s. l., mixed deciduous forest-dark coniferous

forest with azalea and nut-tree sub forest), 4. Vil. Latali environs (1500m a. s. l., bushes with dominance of nut and barberries). Samples were collected only from the topsoil layer to 15cm depth. This is the layer of soil which unites **A** and **B** horizons and where main part of the soil nematodes are accumulated. Here are all resources for nematodes nutrition and airing is quite favorable for soil nematodes, great number of which is reflected well oxophils.

Extraction of nematodes from soil, fixation and identification was carried out by methods accepted in nematology in the Laboratory of Nematology of the Institute of Zoology.

Results and Discussion

First attempt to study free living and phytobiont nematodes in Svaneti was in 1959 [Eliava, 1961]. Several samples were taken in agrocenosis (wheaten field) in Mestia region (1479m a. s. l.) and in village Kalashi (2000 m a. s. l.). Nematodes were extracted from plant tissues (root, stem, leaf, wheat-ear) and soil rhizosphere. As it was expected the most inhabited with nematodes from plant tissues were roots (18 forms) and then stems (5 forms). Leaves and wheat-ear practically were free from nematodes (accordingly 2 and 1 forms) (Fig. 1).



1. Roots (18forms)
2. Stem (5 form)
3. Leaf (2 form)
4. Wheat ear (1 form)

Fig.1. Distribution of nematodes in wheat tissues

It is significant that 14 specimens of devisaprobiont (non-parasitic phytobiont) *Panagrolaimus rigidus* were determined in wheat stem. The same species dominated in roots also. The similar picture was in the material from the Moscow district [Baranovskaia, 1961], which enables us to suggest that *P. rigidus* finds favorable living conditions in stem and root of the corn. Phytohelminths (10 forms) and devisaprobionts or common non-parasitic phytobionts (10 forms) were represented from ecological groups in general.

Soil nematodofauna of upper Svaneti was studied by N. Iashvili during 1977-89 [Iashvili, 1987]. Studies determined 72 species of 6 orders, among which 20 species belonged to the order Dorylaimida and 19 to Tylenchida. Geographical and ecological ubicvists *Cephalobus persegnis* and *Aphelenchus avenae* dominated (list of nematodes is not provided).

As for the material of 2007, in collected samples 45 forms of nematodes occurred, among which 25 were determined up to species (table 1).

Table 1. Nematodes of Svaneti soil

	NEMATODES	AGROCENOSIS 1961 Y.	NATURAL ECOSYSTEMS 2007 Y.
1	*Alaimus primitivus*	———	+
2	*Tripylina arenicola*	———	+
3	*Tripylina* sp.	———	+
4	*Tripyla glomerans*	———	+
5	*T. papilata*	———	+
6	*Tripyla* sp.	———	+
7	*Tripylidae* g. sp.	———	+
8	*Monhystera* sp.	+	———
9	*Plectus elongatus*	———	+
10	*P. longicaudatus*	+	———
11	*P. parietinus*		+
12	*Plectus* sp.	+	+
13	*Anaplectus granulosus*	+	+
14	*Clarcus* sp.	———	+
15	*Prionchulus muscorum*	———	+
16	*Prionchulus* sp.	———	+
17	*Mylonculus subsimilis*	———	+
18	*Mylonculus* sp.	———	+
19	*Dorylaimus* sp.	———	+
20	*Mesodorylaimus bastiani*	+	+
21	*M. flagellatus*	———	+
22	*M. paetzoldi**	———	+
23	*Mesodorylaimus* sp.	———	+
24	*Thornenema* sp.	———	+
25	*Eudorylaimus acutus*	———	+
26	*E. carteri*	———	+
27	*E. centrocercus*	———	+
28	*E. leuckarti*	———	+
29	*E. lindbergi*	———	+
30	*E. iners*	———	+
31	*E. irritans*	———	+
32	*E. holdemani*	———	+
33	*Eudorylaimus* sp.	———	+
34	*Ecumenicus monhystera*	+	———
35	*Takamangai* sp.	———	+
36	*Qudsianematidae* g. sp.	———	+
37	*Aporcelaimellus adriani*	———	+
38	*A.stilus*	———	+
39	*A. obtusicaudatus*	———	+
40	*A. paraobtusicaudatus*	———	+
41	*Aporcelaimellus* sp.	———	+
42	*Dorydorella pratensis*	+	———
43	*Longidorella* sp.	———	+
44	*Pungentus* sp.	———	+
45	*Actinolaimidae* g. sp.	———	+
46	*Rhabditis brevispina*	+	+

47	*Rh. aspera*	+	———
48	*Rhabditis* sp.	+	+
49	*Rhabditidae* g. sp.	———	+
50	*Panagrolaimus rigidus*	+	———
51	*Cephalobus persegnis*	+	———
52	*Heterocephalobus elongatus*	+	———
53	*H. striatus*	+	———
54	*Chiloplacus simmetricus*	+	———
55	*Acrobeles ciliatus*	+	———
56	*Cephalobidae* g. sp	+	———
57	*Tylenchus filiformis*	+	———
58	*Tylenchus* sp.	+	———
59	*Tylenchorhynchus dubius*	+	———
60	*Tylenchorhynchus* sp.	———	+
61	*Ditylenchus* sp.a	+	———
62	*Ditylenchus* sp.b	+	———
63	*Pratylenchus pratensis*	+	———
64	*Aphelenchus avenae*	+	———
65	*Paraphelenchus tritici*	+	———
66	*Aphelenchoides parietinus*	+	———
67	*Aph. tenuicaudatus*	+	———
68	*Aphelenchoides* sp.	+	———

Nematodes of the accessible material belong to 7 orders and 19 families in total. One species *Mesodorylaimus paetzoldi* Altherr, 1965 was recorded for the first time for the fauna of Georgia. The order Dorylaimida was the most diverse in natural ecosystem which is typical, especially for the forests. According to trophic structure polyphaga dominate also in every natural ecosystem, bacteriotropes are on the second place. They are left behind by predators and phytoparasits. Typical saprobiotic forms and mycohelminths are presented by single species, but common non-parasitic phytobionts-devisaprobionts belong to the sub-order *Cephalobina* (7 forms).

As it is evident from table 1, in the material of agroecosystem 28 forms of nematodes occurred (among them 19 in soil), but in the material of the natural ecosystem soil - 45 forms.

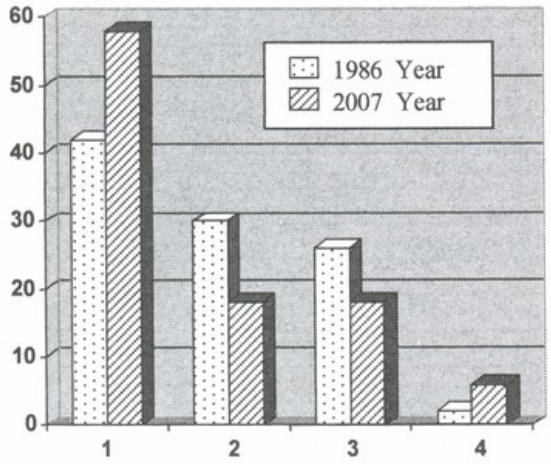
We analyzed material of ecological groups of the early period and 2007 (according to Paramonov classification). It appeared that almost all ecological groups were represented in agrocenosis as well as in natural ecosystems, though in different percentage. Phytohelminths prevail in agrocenosis, but in natural ecosystems - pararhizobionts, typical forms of the soil (Fig.2).

It is necessary to note, that typical edaphobionts are generally registered in the material of natural ecosystems, phytoparasits practically not present.

Such forms of soil, which occur in rhizosphere, but often invade in roots of plants (representatives of the family *Plectidae*) are registered in the material of rhizosphere in agroecosystems.

Comparison of the material collected in the early period and in 2007 persuades that nematode inhabitants of agroecosystems and natural ecosystems taxonomically noticeably differ. 5 species are common to all these ecosystems, though the coefficient is only 0.07 according to Jakard. It indicates that those nematodes which can use tissues of the plants as habitat and feed on these tissues or on their hydrolysates and at the expense of bacteria and fungi invaded in plants are in trophic relation with plant tissues. These nematodes have specific adaptive signs that ecologically and biologically differs them from typical edaphobionts. As it is evident from their morphology on the one hand these adaptations are related with significant transformation of the

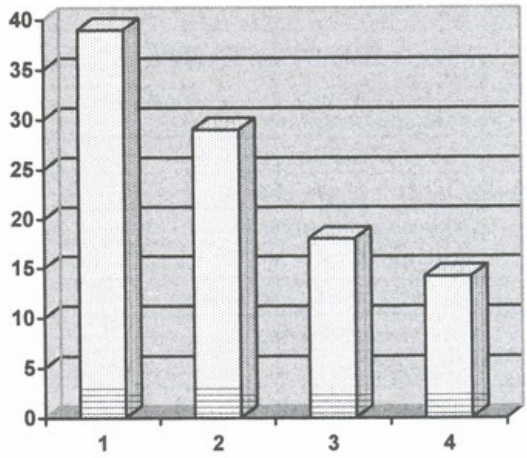
digestive system, which first of all is reflected on intensification of ezophagial glands and on the other hand on the enhancement of barrier function of the cuticle, which gives them possibility to exist in the condition of different chemism in the plant tissues.



Natural ecosystems

1. Pararhizobionts 3. Phytohelminths
2. Devisaprobionts 4. Eusaprobionts

A



Agrocenosis

1. Phytohelminths 3. Eusaprobionts
2. Devisaprobionts 4. Pararhizobionts

B

Fig.2. Ecological groups of nematodes (A - natural ecosystems; B - agrocenosis)

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სვანეთის რეგიონის ნემატოდების ბიომრავალფეროვნების შესწავლა

ილია ჭავჭავაძის თბილისის სახელმწიფო უნივერსიტეტი, ზოოლოგიის ინსტიტუტი

ცქიტიშვილი ე., კუჭავა მ., ცქიტიშვილი თ.

(მიღებულია 22.06.2009)

რეზიუმე

სვანეთის რეგიონის ნემატოდური მოსახლეობის შესწავლის მიზნით გამოკვლეულ იქნა რამოდენიმე განსხვავებული ბუნებრივი ეკოსისტემა, სადაც სულ აღინიშნა ნემატოდების 45 ფორმა, აქედან ერთი სახეობა *Mesodorylaimus paetzoldi* Altherr, 1965 პირველად იქნა რეგისტრირებული საქართველოს ფაუნისათვის. მიღებული შედეგები შეჯერებულ იქნა არსებულ მონაცემებთან.

Proc. Georgian Acad. Sci., Biol. Ser. B
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Letter to Editor
Botany

NEW LICHEN SPECIES FOR GEORGIA FROM ALGETI NATIONAL PARK (EAST GEORGIA)

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(Received July 6, 2009)

Abstract

The paper presents descriptions of 3 species new to the lichen flora of Georgia recorded in Algeti National Park: *Flavopunctelia flaventior* (Stirt) Hale, *Hypotrachina revoluta* (Florke) Hale, *Hypotrachina sinuosa* (Sm.) Hale. The work serves for revealing specific diversity of lichen flora of Georgia and distribution ranges of the species.

Key words: lichens, new species, Algeti National Park, Georgia.

Description and distribution areas of 3 species new to the lichen flora of Georgia and recorded in Algeti National Park are given below:

***Flavopunctelia flaventior* (Stirt.) Hale**

Syn. *Parmelia flaventior* Stirt

Thallus irregularly rosette-like, up to 20 cm in diam., upper surface smooth, yellow, yellow-green [Rassadina K.A., et al., 1971]. Lobes rounded, 4-8 mm wide. Soredia mainly in soralia formed on surface of lobes, few on margins. Relatively conspicuous pseudocyphellae present on lobe tips. Lower surface black, with a brown edge, rhizines black, rather sparse [Brodo Irwin M., et al., 2001] Cortex K, C-, medulla K-, C+ red.

Algeti National Park, Manglisi, pine forest (*Pinus sosnowskyi* Nakai).
Djvriskhevi, spruce (*Picea orientalis* (L.) Link.) – beech (*Fagus orientalis* Lipsky) forest, on the ground and fallen trees.

***Hypotrachyna revoluta* (Florke) Hale**

Syn. *Parmelia revoluta* Florke.

Thallus unclearly rosette-like, 4-10 cm in diam., pale gray or greenish gray; lobes relatively short, 1-4 mm wide, margins curled, downward, sometimes almost forming tubes at lobe tips, granular. Soralia dark grey, brown or olive-green. [Rassadina K.A., et al., 1971]. Soredia formed near lobe tips and sometimes on lobe surface either from coarse pustules or by erosion of

the thallus upper layers; rhizines sparse or abundant, short or long, usually unbranched. [Brodo Irwin M., et al., 2001]. Cortex K+ yellow, medulla K-.

Algeti National Park, Manglisi, pine forest (*Pinus sosnowskyi* Nakai).

Djvriskhevi, spruce (*Picea orientalis* (L.) Link.) – beech (*Fagus orientalis* Lipsky) forest, on the ground and fallen trees.

***Hypotrachyna sinuosa* (Sm.) Hale**

Syn. *Lichen sinuosus* Sm.

Thallus of irregular shape, sometimes rosette-like or composed of separate lobes, with wide rounded axils at branching points. [Rassadina K.A., et al., 1971]. Lobes rarely wider than 1.5 mm or longer than 3 mm, dichotomously branched, yellowish to yellow-green, smooth, slightly shiny especially at lobe edges. Fringes of black, forked rhizines mainly confined to marginal areas but without true cilia; puffs of powdery soredia formed on older lobe tips [Brodo Irwin M., et al., 2001]. Cortex K-, medulla K+ yellow becoming red.

Algeti National Park, Namtvriana, 1500 m a.s.l., spruce (*Picea orientalis* (L.) Link.) – beech (*Fagus orientalis* Lipsky) forest.

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საქართველოს ლიქენების ახალი სახეობები ალგეთის ეროვნული პარკიდან

კუპრაძე ი.

თბილისის ბოტანიკური ბაღი და ბოტანიკის ინსტიტუტი

(მიღებულია 06.07.2009)

რეზიუმე

სტატიაში აღწერილია საქართველოს ლიქენოფლორისთვის 3 ახალი სახეობა ალგეთის ეროვნული პარკიდან: *Flavopunctelia flaventior* (Stirt) Hale, *Hypotrachyna revoluta* (Florke) Hale, *Hypotrachyna sinuosa* (Sm.) Hale. ნაშრომი ემსახურება საქართველოს ლიქენოფლორის სახეობრივი მრავალფეროვნების გამდიდრებას, ასევე ამ სახეობების გავრცელების არეალის გაფართოებას.

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Letter to Editor
Mycology

SOME NOTES ON FUNGUS *STIGMINA OBTECTA* (PETRAK & ESFANDIARI) M.B. ELLIS ASSOCIATED WITH DEAD TWIGS OF CULTIVATED AND WILD WOODY PLANTS IN GEORGIA

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(Received July 10, 2009)

Abstract

The paper deals with nomenclature and host range of *Stigmina obtecta* associated with dead stems and twigs of different woody plants including mulberry and fig trees.

Key words: *Stigmina obtecta,* woody plants, Georgia

Stigmina obtecta was first described as *Thyrostroma obtecta* Petrak and Esfandiari in 1941 and then as *Stegonosporium fici* Ahmad in 1961. It occurs on dead branches of Ficus, also on Juglans, Morus, Populus and Sophora in Iran and Pakistan.

Stromata up to 400μ wide. Conidiophores up to 40 x 3-8 μ, occasionally with 1-2 annellations. Conidia golden brown, smooth, with 3-7 transverse and often 1 or more longitudinal or oblique septa, 23-59 x 10-24 μ. [Ellis, 1976].

Materials investigated include dry collections gathered in different regions of Georgia. Routine light microscopic methods of studying the structural-morphological features have been used for analyzing and identification.

According to the available materials and published data [Dekanoidze,1984; Svanidze, 1984; Gvritishvili, et al., 2008; Gotsadze, 2005] until now in Georgia (Kartli region, mainly Tbilisi environs) *Stigmina obtecta* is known to occur on dead stems (including fire injured ones) of different woody plants as follow: Albizia julibrissin, Amygdalus communis, Celtis caucasica, Celtis glabrata, Ephedra procera, Ficus carica, Lycium barbarum, Maclura pomifera, Morus alba, Populus sp., Robinia pseudoacacia, Solanum persicum, Sophora japonica (Styphnolobium japonicum), Ulmus minor (U. carpinifolia).

It is interesting to note that as long ago as 1930 L.A. Kantchaveli reported about *Thyrococcum sirakoffii* Bubak found on Morus alba, Ficus carica and Maclura pomifera in Tbilisi Botanic Garden [Kanchaveli, 1936; 1945]. Description and figure of *T. sirakoffii* indicate similarity (identity) of this fungus with *Stigmina obtecta* (see also Ellis, 1976; fig. 87 E). In other Georgian mycological and phytopathological sources [Kakuliya, 1951; Chanturia, 1963] this fungus is referred to *Thyrostroma kossaroffii* (Briosi) Bubák causing decline of Morus alba, Ficus carica and Maclura pomifera (M. aurantiaca), belonging to Moraceae family. However, as mentioned by B.C.

Sutton (1975) Ellis (1959) regarded *Thyrostroma* as a later name for *Stigmina* Sacc. and proposed the binominal *Stigmina kosaroffii* (Briosi) Sutton [Sutton, 1977].

Moreover, taking into account the similarity in conidial size and morphology and diversity in host range Sutton considered that *Coryneum mori* Nomura (1904), *Stegonsporium mori* (Nomura) Sacc. & Trotter (1913), *Thyrococcum mori* (Nomura) Bubak (1911), and *Thyrostroma mori* (Nomura) Höhn. (1911) will provide an earlier epithet for the fungus at present known as *Stigmina obtecta.*

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მონაცემები კულტურულ და ველურ მერქნიან მცენარეებთან ასოცირებული სოკოს ***Stigmina obtecta*** **(Petrak & Esfandiari) M.B. Ellis** შესახებ

გვრიტიშვილი მ.[1], ყაჭეიშვილი-თავართქილაძე ქ.[1], ჭურღულია-შურღაია ნ.[2], გოცაძე ნ.[1]

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[2]სოხუმის უნივერსიტეტი

(მიღებულია 10.07.2009)

რეზიუმე

შრომაში განხილულია საკვლევი სოკოს - *Stigmina obtecta* (Petrak & Esfandiari) M.B. Ellis ნომენკლატურის საკითხები და დადგენილია მცენარეთა უფრო ფართო და ჰეტეროგენული სპექტრი, ვიდრე აქამდე იყო ცნობილი.

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Vol. 7, No. 3-4, 2009.

ევროპული დეკლარაცია კვების პროდუქტების, ტექნოლოგიებისა და კვების შესახებ

2008 წლის 4-9 ნოემბერს ლუბლიანაში ჩატარდა კვების პროდუქტებზე პირველი ევროპული კონგრესი, სადაც მომზადდა დეკლარაცია კვების პროდუქტების, ტექნოლოგიებისა და კვების შესახებ. დეკლარაცია ხელმოწერილია საკვების შესახებ პირველი ევროპული კონგრესის პრეზიდენტის, პროფესორ პიტერ რასპორის და 41 ევროპული ქვეყნის წარმომადგენლის, მათ შორის საქართველოდან აკადემიკოს გ. კვესიტაძის მიერ.

დეკლარაციაში ყურადღება გამახვილებულია საკვებ პროდუქტებსა და კვების საკითხებზე, რომლებიც ეფუძნება კვების პროდუქტების შესახებ ევროპულ მეცნიერებას, ტექნოლოგიასა და კვების სტატუსს და ხელს უწყობს კვების სფეროში ყველაზე მნიშვნელოვან საკითხებზე ევროპული კვების მეცნიერებისა და ტექნოლოგიის ერთიანობას; ამასთანავე, მოითხოვს მთავარი პრინციპების უზრუნველყოფასა და ხელშეწყობას.

დეკლარაციის მიზანია ევროპელ მეცნიერებს, კვების ტექნოლოგებსა და დიეტოლოგებს შორის დებატების ინიცირება, რათა შემუშავებულ იქნეს მექანიზმები, რომელთა საშუალებითაც მომავლში მიღწეული იქნება ევროპული კვების მრეწველობის, საკვების ხარისხისა და უსაფრთხო სტანდარტების ჰარმონიზაციის ინტეგრაცია.

Acta Alimentaria, Vol. 38 (1), pp. 3–7 (2009)
DOI: 10.1556/AAlim.38.2009.1.2

THE EUROPEAN DECLARATION ON FOOD, TECHNOLOGY AND NUTRITION

The term 'declaration' is used for various international instruments. International declarations are not legally binding. However, the term is often deliberately chosen to indicate that the parties do not intend to create binding obligations but merely want to declare certain aspirations to develop or introduce systems of values that should berespected not just by professionals and policy makers but also by the general population, which we understand today as consumers.

The development of food making/processing along the history gives us many opportunities to develop new concepts how to look at this basic ingredient of life. Along different approaches a lot of effort has been done to improve it. We were successful on many different levels. But now it is time to connect and interact and integrate all theseactivities. The Congress on Food and Nutrition which took place in Ljubljana, inspired the ambassadors of this movement who were enthusiastic enough and capable to prepare in one year the declaration which was signed in Ljubljana in City hall at 7th of November 2008. This event was part of the First European Food

Congress. The congress was taking place from 4th to 9th of November 2008 in Ljubljana and was organized with the essential impact of the Slovenian Nutritional Society. Invited distinguished experts delivered 18 plenary lectures, which were accompanied by more than 200 oral presentations in more than 30 symposia together with workshops, poster and round table sessions in the scientific program. The congress will stay in our memories for several reasons. First "pre-congress event" was introduced which enabled dissemination of COST, NATO and EU programs and projects of framework FW6/7.

This was the first European congress, which introduced a "country day" where one could enjoy presentations of scientists and professionals on the state of the art in food and nutrition of the host country. Ambassadors were glad to deliver the European Declaration on Food, Technology and Nutrition as completed document to the participants of the Congress at the Closing ceremony. The Declaration was accepted and signed after intensive discussion, voting, and addition of a few amendments to the draft document. The declaration is addressing food and nutrition issues based on European food science, technology and nutrition status and promotes cohesion of European food science, technology and nutrition within top issues in food area and asks for basic principles to be maintained and promoted, and declares key actions to bepromoted in the future.

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Congress ambassadors were questioned many times why we in Europe need this type of declaration. The answer matured with the time elapsed is because we have to:

Enhance awareness about food,
Formulate relevant knowledge and skills about it,
Fertilize this knowledge at all levels of society,
organize societies in food and nutrition area in geographical Europe,
Stabilize activities in this area and
Transfer all experiences we gain to daily practice.

We trust that the spirit of the declaration will enhance activities at different levels and will bring food to proper understanding to all stakeholders on relent levels not just in Europe but also in the rest of the world.

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President of EFFoST
(European Federation of Food Science and Technology)

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The Declaration was prepared by the Congress Ambassadors Committee from European countries and signed in Ljubljana City Hall on 7th November 2008

The European Declaration on FOOD, TECHNOLOGY and NUTRITION

Preamble

We participants of the First European Food Congress (Ljubljana, Slovenia 2008) endorse the FAO/WHO Declaration of Nutrition (Rome 1992) and the WHO Declaration on Food Safety (Beijing 2007). Through this Declaration we would like to emphasize issues related to food, technology and nutrition that are of importance to Europe

TARGET AUDIENCE

The European Declaration on FOOD, TECHNOLOGY and NUTRITION is targeted at the European Consumers, Policy Makers, Scientists, European Food Industry, and other Stakeholders.

FOOD AND NUTRITION

Food is necessary for human growth, development and the functions of the body. Good nutrition demands a well-balanced diet that provides an adequate daily amount of all nutrient classes and optimal intake of energy for the human body. Food must be safe, nutritious and provided in a sustainable way that maintains consumer's dignity and cultural identity.

EUROPEAN FOOD SCIENCE, TECHNOLOGY AND NUTRITION

European professionals in food science, technology and nutrition continuously contribute to important advances in food production, conservation, processing, storage and distribution, as well as in nutrition and human well-being by implementing basic sciences to these applying research fields. They have also organised themselves into many scientific and professional associations.

These are normally based in single countries but also in clusters of regions with the same eating habits and may be either general or organised into single science- and vocational-based disciplines such as biology, chemistry, biochemistry, biotechnology, microbiology, engineering, nutritional sciences, medical sciences, and other related sciences.

It is the aim of this Declaration to provide a focus for all these disciplines and serve the general population of Europe as well as the community of scientists, politicians, regulators and industry in the most optimal way. This cohesion will enable Europe to compete successfully in the international market, to increase the availability of healthy food, and to lead the world in standards of scientific rigour and integrity.

COHESION OF EUROPEAN FOOD SCIENCE, TECHNOLOGY AND NUTRITION

This Declaration aims to initiate debate amongst European food scientists, food technologists and nutrition professionals to establish mechanisms through which, hopefully, future integration can be achieved. A second objective is to assist the harmonisation of the European food industry and food quality and safety standards. All of these initiatives can help to promote the expanding influence that the food science, technology and nutrition professions should have on technological, scientific, political, environmental, social and cultural thought in Europe. Of course, this must be done without harming nutrition and regional specificities of food and diets in Europe since it is this diversity, which will generate future discoveries and innovations.

TOP ISSUES

This Declaration should specifically stimulate debate on the following issues:

- Food is a very important strategic and political issue;
- Contamination of food with microbiological, chemical and physical agents or food allergen remains a key public health concern;
- Local food production influences regional culture, preserves biological diversity, and contributes to regional social and economic stability;
- Long-distance transportation chains may reduce food quality and may have an adverse effect on the environment;
- Food has a key role in establishing and maintaining good dietary habits as part of a healthy lifestyle along the whole human life span;
- Increasing incidence of obesity and diet-related chronic non-infectious diseases are among major public health concerns and causes of social security expenditure;
- Education levels and current knowledge transfer in the field are not adequate for consumers to make informed choices;
- Training and education of stakeholders of the food chain constitute a huge challenge.

BASIC PRINCIPLES TO BE MAINTAINED AND PROMOTED

A nutritionally adequate supply of safe food is a basic human right of every consumer:

- Everyone has the right to reliable information on foods, diet and their health impacts;
- Food production, processing, transport and distribution must be carried out in a sustainable manner taking into consideration environmental, societal and ethical consequences as well as regulatory issues including traceability;
- Technological achievements in developments of food that is suitable for targeted groups with special nutrition requirements should be welcomed;
- Honest and appropriate labelling of foods in cooperation with food industry and official legislation authorities has to be obeyed;
- Understandable and scientifically evidence-based media communication is recognized as an essential requirement;
- Adequate nutrition and healthy lifestyles that have a key roles in prevention and reduction of diseases should be promoted;
- Everyone has the right to get early education about food and nutrition to be able to take personal responsibility for own health and apt food choices;
- Managers of food industry must recognise the need for expertises in nutrition and food safety and ensure that resources necessary for food safety assurance are adequate.

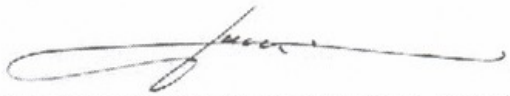
KEY ACTIONS

This Declaration promotes the following Key Actions:

- Food producers, processors and retailers should consider, in addition to regulation, ethical measures in all processes in the food chain;
- Experts should work in accordance with professional and ethical guidelines;
- Governments and other responsible authorities and institutions should harmonise regulations between national food policies so as to adapt more readily and efficiently to future global changes;
- Governments and other responsible authorities and institutions should develop national food policies that take into account social and cultural differences, experiences and specific needs;
- All food science, nutrition and technology professionals should campaign for the establishment of educational systems that will result in better-informed consumers by effective knowledge transfer;
- Everyone should be able to take responsibility for own health and apt food choice;
- Signatories will do all that is in their power to ensure that the principles enshrined in this Declaration will be achieved.

Signed by

Professor Peter Raspor, President of the First European Food Congress on behalf of the Congress Ambassadors Committee from 41 European countries.



Ilirjana Boci represented by Haxhi Allmuca, Albania; Ashot Saghiyan, Armenia, Wolfgang Kniefel represented by Gerhard Schleining, Austria; Koen Dewettinck represented by Bart Heyman, Belgium; Faruk Čaklovica, Bosnia and Hercegovina; Iordanka Alexieva represented by Laska Rangelova, Bulgaria; Mate Bilić replaced by Martina Piasek, Croatia; Athina Panayiotou, Cyprus; Jana Hajšlovб, Czech Republic, Henning Otte Hansen, Denmark; Toomas Paalme, Estonia; Anu Kaukovirta-Norja represented by Helena Pastell, Finland; Paul Colonna represented by Michel Franck, France; George Kalantzopoulos replaced by Ioannis Samelis, Greece; Giorgi Kvesitadze replaced by Tinatin Sadunishvili, Georgia; Sabine Kulling, Germany; Andrбs Salgy, Hungary; Inga Thorsdottir, Iceland; Francis Butler represented by Brian McKenna, Ireland; Rosangela Marchelli, Italy; Daina Karklina replaced by Aija Mengaile, Latvia; Rimantas Venskutonis, Lithuania; Torsten Bohn, Luxembourg; Vladimir Kakurinov, Macedonia; Anna McElhatton, Malta; Slavko Mirecki, Montenegro; Bernd van der Meulen represented by Sarah De Vito, Nederland, Wenche Frшlich, Norway; Włodzimierz Grajek, Poland; Xavier Malcata represented by Ana Oliveira Madsen, Portugal; Mona Popa, Romania; Iosif Rogov, Russia; Miomir Nikšić, Serbia; Peter Šimko represented by Norbert Bomba, Slovakia; Božidar Žlender, Slovenia; Manuel Vбzquez, replaced by Ascension Marcos, Spain; Anne-Marie Hermansson, Sweden; Klaus Zimmermann replaced by Yasmine Motarjemi, Switzerland; Fatih Yildiz, Turkey; Sergey Fedosov, Ukraine; David White, UK.

სამეცნიერო ნაშრომი გამოიცემა ინგლისურ ენაზე, მას უნდა დაერთოს რეზიუმე ინგლისურ და ქართულ ენაზე, სამეცნიერო მიმართულება, სათაური, ავტორთა გვარები და მათი სამუშაო დაწესებულების დასახელება, საკვანძო სიტყვათა მოკლე (4-6) სია.

წერილის მოცულობა არ უნდა იყოს 5 გვერდზე ნაკლები და 12 გვერდზე მეტი. წერილი უნდა გაფორმდეს შემდეგი რუბრიკაციით: შესავალი და მიზნები (Introduction), მასალა და მეთოდები (Materials and Methods), შედეგები და მათი განხილვა (Results and Discussion), დამოწმებული ლიტერატურა. უკანასკნელი უნდა იყოს დალაგებული ანბანის მიხედვით, ხოლო ტექსტში წყაროების მითითება უნდა ხდებოდეს ფრჩხილებში ჩასმული ავტორის გვარითა და წლით [Lernmark, Hagglof 1981].

მითითებული ლიტერატურა წარმოდგენილი უნდა იყოს შემდეგნაირად:

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Brush S. *Flowing waters or teeming crowds*. In: Mental Models. D. Gentner (Ed.), Chicago IL., Chicago Press, 865-900, 2001.

მასალა რედაქციაში წარმოდგენილი უნდა იყოს ქაღალდზე ამობეჭდილი და დისკეტით (ან CD-ით). წერილი ერთი ფაილით უნდა იყოს შენახული (ცალკე ფაილად შეიძლება ილუსტრაციების წარმოდგენა), ხოლო ფაილის სახელწოდება წერილის პირველი ავტორის გვარს უნდა ატარებდეს.

ქართული ტექსტისთვის ოპტიმალური ფონტებია AcadNusx და AcadMtavr, ინგლისური ტექსტებისთვის - Times New Roman. შრიფტის ზომა - 12 პუნქტი, ინტერვალი - 1,5. ცხრილებში დასაშვებია უფრო მცირე ზომის შრიფტები. წერილი უნდა დაიბეჭდოს A4 ფორმატით, ზევით და ქვევით - 2,5 სმ., მარცხნივ – 3 სმ. და მარჯვნივ – 2სმ. დაშორებით. ცხრილები, გრაფიკები და დიაგრამები (მხოლოდ შავ-თეთრი) შესაძლებელია დამზადდეს როგორც Microsoft Word-ში, ისე Excel-ში, ფოტოსურათები მიიღება აგრეთვე ორიგინალების (არაელექტრონული) სახითაც.

ჟურნალის გამოცემა ავტორთა ხარჯებით ხორციელდება. თანხა რედაქციაში უნდა შემოვიდეს ნაშრომზე დადებითი რეცენზიის მიღებისთანავე. ნაშრომის რეცენზირება ანონიმურია და ავტორს აქვს უფლება მიიღოს ან არ მიიღოს რეცენზენტის შენიშვნები. უკანასკნელ შემთხვევაში ნაშრომი, დამატებით გაეგზავნება სარედაქციო საბჭოს ერთ-ერთ წევრს. მეორე უარყოფითი დასკვნის შემთხვევაში, ნაშრომი არ გამოქვეყნდება.

ნაშრომის ჩაბარება შეიძლება სამუშაო დღეებში, 12-დან 16 საათამდე, შემდეგ მისამართზე: თბილისი, რუსთაველის გამზირი 52, საქართველოს მეცნიერებათა აკადემია, ბიოლოგიის განყოფილება, IV სართული, 429 ოთახი, ტელ: 93-58-92, პასუხისმგებელი მდივანი – მაია გრიგოლავა.