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Aims and Scope

The aim of “Annals of Agrarian Science” is to overview problems of the following main disciplines and subjects: Agricultural and Biological Sciences, Biochemistry, Genetics and Molecular Biology, Engineering, Environmental Science. The Journal will publish research papers, review articles, book reviews and conference reports for the above mentioned subjects.

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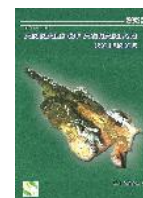
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Determination of Cr (III) by Means of Atom Absorption Spectrometry

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ABSTRACT

The atomic absorption (AA) method is proposed for determination of trivalent chromium [Cr(III)] concentration in the samples containing the mixture of Cr (III) and Cr (VI). It was found that addition of chloric acid (HClO₄) to Cr (III) solution substantially increases AA spectrometric signal. However, with the addition of HClO₄ to Cr (VI) solution the signal remains unchanged. The addition of chloric acid to the mixture of chromium ions causes the increase of the signal of absorption that is related to the presence of Cr (III) ions. This fact allows quick and selective determination of Cr (III) concentration in the samples without prior separation of chromium compounds with different valences. The proposed method can be used for both atomic absorption spectrometry and atomic fluorescence spectrometry. Possibility of experimental application of the method is shown on the example of examination of interaction between Cr (VI) ions and chromium bacteria *Arthrobacter oxydans*.

Key words: Atomic absorption spectrometry, Chromium, Cr (III), Chloric Acid

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Introduction

Chromium is one of the most problematic metals with both toxic and vitally important properties [1, 2]. Of several possible oxidation states from Cr (II) to Cr (VI), two states such as trivalent Cr (III) and hexavalent Cr (VI) are characterized by stability in natural and biological systems. Cr (VI) compounds are readily soluble, highly toxic and carcinogenic, while Cr (III) compounds are less soluble and less toxic. Moreover, Cr (III) is a trace element vital for functioning of living organisms. Through various processes (chemical and / or biological), the toxic form of Cr (VI) can be transformed into non-toxic form of Cr (III). Therefore, the express information on the chromium content in the environment is essential for assessing and prevention its harmful effects.

Currently, various methods are used to determine the chromium content, such as electron spin resonance spectrometry, ion chromatography, high-pressure liquid chromatography, inductively coupled plasma mass spectrometry, neutron activation analysis, etc. Atomic absorption spectrometry (AAS) is the simplest and cheapest method used as the main technique for determining the chromium content [3, 4].

However, for a quantitative determination of Cr (VI) or Cr (III) concentrations, the preliminary separation and concentration procedures are necessary, which require additional time and costs. Besides, the process of chemical separation can cause errors in determining the concentration [3].

In recent years, Gaspar et al. carried out researches to improve the efficiency of Cr (VI) and Cr (III) concentration determination by means of

preliminary separation of Cr ions in biological and environmental samples using high-pressure liquid chromatography and AAS [5-8].

In our previous studies, we had an attempt to simplify the determination of Cr (VI) and Cr (III) concentrations by means of AAS method without their preliminary separation in the test solutions. Two well-known facts, which are essential in the atomic absorption analysis of chromium, were taken as the basis. First, in some test conditions (using C_2H_2 -air flame in atomic absorption or atomic emission spectrometry) Cr (III) detection sensitivity is somewhat better (up to 10%) than that of Cr (VI) [9, 10]. Similar results were obtained in the case of atomic fluorescence spectrometry (AFS) [11]. We found that Cr (VI) detection sensitivity becomes twice greater than that of Cr (III) when the mixed propane-butane-air flame is used in AAS and AFS [11, 12]. Second, it is known that the presence of some special “additives” in the sample can change the signal of the element during AAS or AFS [3]. Based on this, we examined the effect of various chemical reagents (H_2SO_4 , H_2O_2 , HCl, H_3PO_4 , KOH, $FeSO_4$) on the magnitude of the atomic absorption and atomic fluorescence signals of chromium upon spraying Cr (VI) and Cr (III) solutions [13]. Experiments showed that hydrogen peroxide (H_2O_2) is most suitable for determination of Cr (VI) concentration in all test compounds. The addition of H_2O_2 to the sample solutions had practically no effect on the atomic absorption (atomic fluorescence) signal of Cr (III), while the signal of Cr (VI) was halved.

However, due to the fact that in atomic absorption analysis the samples are often preashed (wet ashing) with nitric acid (HNO_3), the use of H_2O_2 does not always work in practice, since the presence of HNO_3 in the sample reduces the effect of hydrogen peroxide and complicates the selective determination of Cr (III).

The paper presents the results of further investigations of effective reagents for detection of Cr (III) by means of AAS or AFS. When chloric acid was used as a reagent in the experiment, the presence of HNO_3 had no effect on the results.

Objectives and Methods

Equipment used. In the experiments, we used a double-beam atomic absorption spectrometer Beckman-495 with a double-slit burner for H_2O_2 -

air flame. To obtain the flame of the mixed propane-butane-air a forced mode of burning gas and reduced air flow ($P \approx 18$ psig) ($1 \text{ psig} \approx 1/15 \text{ atm}$) were used. The distance between the transmission beam and the burner nozzle was 10 mm. A spectral lamp with a hollow cathode of “ЖСП-1” type (Russia) was used as a source of primary radiation. The lamp current for Cr was 25 mA (continuous). Chromium resonance line of 357.9 nm wavelength and 0.5 nm slit width was used.

In the experiments the atomic fluorescence spectrometer described in [14] was also used. For excitation of the chromium atoms the “ЖСП-1” hollow cathode lamp was used. The lamp was powered by “ИИЦЛ-2” device (Russia) in the external modulation mode with the supply current of 23 mA, on average. Modulation was carried out at frequency of 350 hertz using “3Г-10” generator (Russia). The photomultiplier “ФЭУ-71” (Russia) was used as a photodetector. From the photomultiplier the electronic signal was transmitted to a narrow-band amplifier tuned to the frequency of 350 hertz, and then to a synchronous detector “СД-9” (Russia). The reference signal was transmitted from the generator “3Г-10”. The outgoing signal from “СД-9” was recorded by means of a potentiometric recorder “ЖК-3” (Russia).

Sample Preparation

Initial solutions of Cr (VI) and Cr (III) were prepared with concentrations of $C = 4 \text{ mg / ml}$ each. For preparation of Cr (VI) solution we placed $m = 1.1315 \text{ g}$ of compound $K_2Cr_2O_4$ in 100 ml flask and filled it with bidistilled water. For preparation of Cr (III) solution, we placed $m = 1.9207 \text{ g}$ of compound $CrK(SO_4)_3 \cdot 12H_2O$ in 50 ml flask and filled it with bidistilled water. Then, 0.1 ml of initial solution of Cr (VI) was transferred into eight test tubes. The test tubes were added by solution of $HClO_4$ ($d = 1.25 \text{ kg / L}$) in the following amounts: 0 ml, 0.03 ml, 0.06 ml, 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.6 ml, respectively. All tubes were filled with bidistilled water up to 8 ml, so that the concentration of Cr (VI) in each tube was $50 \mu\text{g/ml}$ (first series). Another eight tubes prepared in the same way were added by extra 0.2 ml of HNO_3 (73%) per tube (second series). In the third series each tube prepared in the same way was added by 0.4 ml of HNO_3 .

The mixtures were prepared from the initial

solution of Cr (III) in the same way. Concentration of Cr (III) in each tube was also $50 \mu\text{g} / \text{ml}$. The eight test tubes of the first series were added by HClO_4 in the amount of 0 ml, 0.03 ml, 0.06 ml, 0.1ml, 0.2ml, 0.3ml, 0.4 ml, 0.6 ml, respectively. The test tubes of the second series prepared in the same way as those in the first series were added by an extra 0.2 ml of HNO_3 per test tube, and those of the third series were added by 0.4 ml.

The signals of atomic absorption (AA) and atomic fluorescence (AF) of chromium were measured by spraying the obtained solutions. As far as the results obtained in AA and AF coincided, only the data of AA measurements are presented below.

Results and Discussion

It turned out that the absorption signal remained practically unchanged when about 0.6 ml of HClO_4 was added to 8 ml of Cr (VI) solutions (Fig. 1a). Addition of a small amount of HClO_4 to Cr (III) solutions (0.03 ml per 8 ml) increases the atomic absorption signal approximately twice. Further increase of the amount of added HClO_4 practically does not cause any more changes in the absorption signal. The dependences will be similar if extra amount 0.2 ml of HNO_3 (Fig. 1b) or 0.4 ml of HNO_3 (Fig. 1c) is added to Cr (VI) and Cr (III) solutions.

The results obtained show that if a certain amount of HClO_4 is added to a mixture of Cr (VI) and Cr (III) solutions, the atomic absorption signal increases by ΔA (absorption jump) that is caused exclusively by the presence of trivalent chromium in the test solution. The magnitude of the atomic absorption signal ΔA depends only on the concentration of Cr (III) in the test solution.

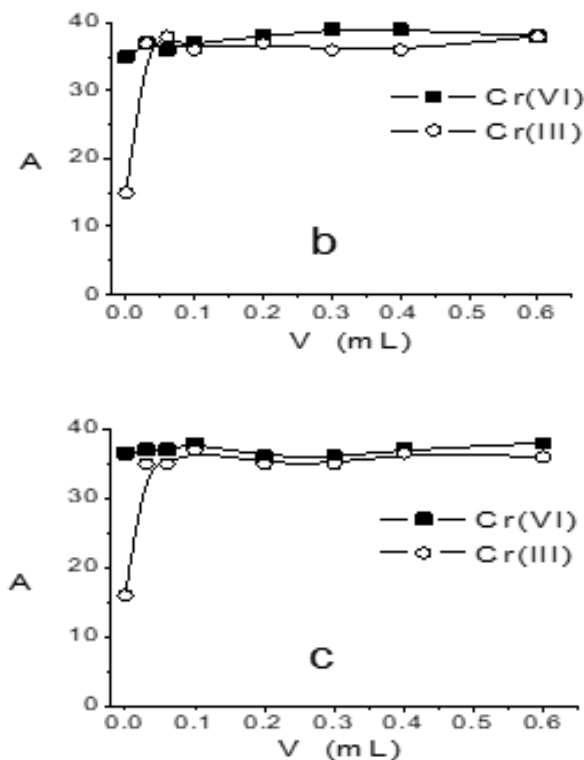
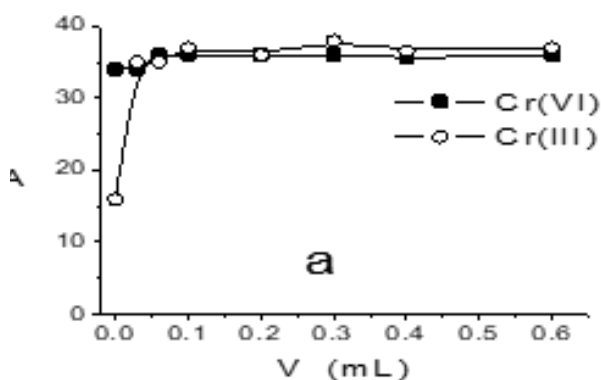


Fig. 1.

Dependence of the atomic absorption signal (A) of chromium ($C = 50 \mu\text{g} / \text{ml}$) on the volume V (ml) of HClO_4 added to the test solutions. The volume of the test solutions is 8 ml. Flame: propane + butane - air.

■ - Cr (VI) solutions, ○ - Cr (III) solutions
 a - without adding HNO_3 to the test solutions.
 b - added by 0.2 ml of HNO_3 (70%) to all tubes.
 c - added by 0.4 ml of HNO_3 .

To establish the dependence of the absorption jump ΔA on the concentration of Cr (III), we prepared standard aqueous solutions of trivalent chromium with concentrations of $3.2 \mu\text{g} / \text{ml}$, $6.4 \mu\text{g} / \text{ml}$, $12.8 \mu\text{g} / \text{ml}$ and $25.6 \mu\text{g} / \text{ml}$ in 25 ml flasks. The content of each flask was divided into two halves. One of them was added by 200 μL of HClO_4 . Thus, by adding HClO_4 and without its adding we obtained two types of solutions with different concentrations of Cr (III). After measuring the atomic absorption signals, calibration curves were plotted (Fig. 2). As Fig. 2 shows, the addition of HClO_4 to the standard solutions of Cr (III) sharply increases the slope of the calibration curve.

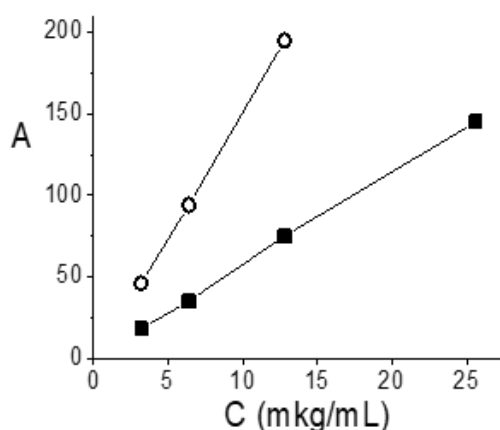


Fig. 2.

Dependence of the atomic absorption signal A on the concentration of Cr (III) .
 ■ - without HClO_4
 ○ - with $200 \mu\text{l}$ of HClO_4 ($d = 1.25 \text{ g/ml}$) per 12 ml solution

For the same concentration of Cr (III) , we calculated the difference ΔA between the absorption signals obtained by HClO_4 addition (A') and without its addition (A):

$\Delta A = A' - A$. The dependence of the absorption jump ΔA of Cr (III) concentration is shown in Fig. 3. As Fig. 3 shows, the dependence of the absorption jump ΔA of Cr (III) concentration is linear. The dependence does not change when extra 0.2 ml or 0.4 ml of HNO_3 is added to the sprays.

Taking into consideration that addition of 0.4 ml of HNO_3 to the solutions of Cr (III) and Cr (VI) does not change the effect of HClO_4 on the atomic absorption signal, the proposed method can be used for the analysis of such samples where HNO_3 is present, such as the biological samples ashed by means of concentrated HNO_3 .

The scheme for determining the concentration of Cr (III) is as follows: After ashing the sample, its volume is increased up to 6 ml with bidistilled water (the volume should be sufficient for two atomic absorption measurements). The sample is divided into two approximately equal halves ($\sim 3 \text{ ml}$), one part of which is added by $30 \mu\text{l}$ of HClO_4 . The same amount of bidistilled water is added to the second part of the sample. The solutions obtained are sprayed, the corresponding signals A' and A of atomic absorption of chromium are measured, and the

difference between them ΔA is calculated. The same procedures are carried out for the standard samples with known concentrations of Cr (III) . The desired concentration of Cr (III) is calculated by means of the obtained value ΔA and the calibration graph obtained by means of the above method (Fig. 3) [15].

The minimum effective amount of chloric acid used in the experiments was 0.1% of the total volume of the sample. In our opinion, within the investigated range of 0.1 - 10% the most effective amount of HClO_4 is within the range of 0.1 - 1%. The concentration of the main (added) solution of chloric acid is 35%.

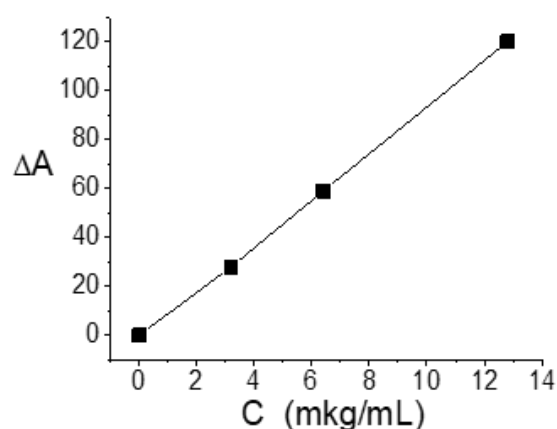


Fig.3.

Dependence of the absorption signal change ΔA (absorption jump) on Cr (III) concentration. $\Delta A = A' - A$, where A - atomic absorption signal obtained by spraying Cr (III) solutions without HClO_4 , A' - atomic absorption signal in case of adding $200 \mu\text{l}$ of HClO_4 per 12 ml of Cr (III) solution.

Examples of Application of The Method

The proposed method was used in our research to determine Cr (III) content. From this point of view, our focus was on the chromium-tolerant bacteria, which has the ability to reduce Cr (VI) to Cr (III) [16]. In our experiments, we used *Arthrobacter oxydans* (*A. oxydans*) as model bacteria.

The experiments carried out using Fourier transform synchrotron radiation and infrared spectroscopy showed that the Gram-positive bacteria *A. oxydans* isolated from the basalt samples collected in Columbia (USA) are Cr (VI) -tolerant, which has the ability to reduce Cr (VI) to Cr (III) [17]. The

mechanisms of Cr (VI) reduction by means of *A. oxydans* bacteria were considered in our previous studies [18-21].

Within the present work, general content of chromium as well as Cr (III) was determined in *A. oxydans* cells after their exposure to 35 mg /l Cr (VI) (in the form of K_2CrO_4) for one and seven days, respectively.

Bacterial cells were grown under the conditions described in [18]. The cells were separated by centrifugation (10,000 rpm 15 min, 40°C), washed three times with NaCl solution (0.15M, pH7), and analyzed by means of AAS.

To determine the concentration of Cr (III), dry bacterial samples were weighed, placed in test tubes and added by 0.4 ml of HNO_3 (70%). After ashing the cells, the test tubes were filled by 6 ml of distilled water. Then, each sample was divided into two equal parts, one was added by 30 μ l of distilled water, and the other by 30 μ l of chloric acid (0.1%).

The solutions were sprayed. Atomic absorption signals A_1 and A_2 of corresponding chromium were measured and the difference ΔA between them was calculated. Using this value, the concentration of Cr (III) was determined according to the calibration curve.

It was found that after the exposure to Cr (VI) for several days, the content of Cr (III) in *A. oxydans* cells reaches ~ 15% of the total amount of chromium. After seven days, Cr (III) fraction in the total amount of chromium accumulated by bacterial cells significantly increases (up to 70%), which coincides with the results obtained by us earlier [18, 21].

Thus, the proposed method of Cr (III) detection by means of AAS and APS significantly increases the possibility of their use in practice.

Conclusion

The possibility of Cr (III) concentration determination without its preliminary separation from the test solution is shown. For this purpose, by spraying Cr (III) and Cr (VI) in a propane + butane - air flame the effect of H_2SO_4 , H_2O_2 , $HClO_4$, HNO_3 , HCl , H_3PO_4 , KOH , and $FeSO_4$ on the signal of atomic absorption of Cr was studied. It was found that the effect of $HClO_4$ is convenient for determination of Cr (III) concentration. Addition of a small amount of $HClO_4$ to Cr (VI) solution does not change the atomic absorption signal, while addition of the same amount of $HClO_4$ to Cr (III) solution approximately

twice increases the absorption signal. An increase in the atomic absorption signal upon addition of $HClO_4$ to the test solutions is caused only by the presence of Cr (III) in the solution, and the magnitude of the absorption signal determines Cr (III) concentration. The effect of $HClO_4$ on the atomic absorption signal does not change when 0.4 ml of HNO_3 is added to Cr (III) and Cr (VI) solutions. This fact makes allows us to ash the preparations tested for Cr (III) content in HNO_3 .

Acknowledgement

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Technical means of small mechanization +1

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ABSTRACT

The article deals with the designs of technical means of small mechanization that are used in Georgia, namely agricultural machinery installed on motoblocks, the scale and advantages of their use in comparison with powerful equipment, and on the basis of a patent (No AP 2020 15395) a completely new combined machine for motoblock is proposed, which is currently in demand in the conditions of development of modern technologies for growing crops on the market. In particular, on small plots and greenhouse farms. The article offers a constructive description of the mentioned combined unit and the principles of its operation.

Key words: Agriculture, Mechanization, Walk-behind tractor, Unit, New technologies

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Introduction

Both mobile and small mechanization technical means, motoblocks and devices assembled on them are used to perform the operations necessary for the complex mechanization of maintenance and cultivation of agricultural crops. [1] Their use is particularly effective for regions where agricultural areas are small in contour and located in mountainous conditions. For these regions, proposes a combined resource-saving agricultural aggregate based on a motoblocks for furrow forming on soil and mulching.

The combined aggregate allows loosening of soil with powered tillage tools, forming furrow, laying a drip water system, laying plastic mulch, covering it with soil, and drilling laid plastic mulch with one pass through small energy consumption. The proposed aggregate is a significant innovation in terms of both scientific and practical use, it will complement the untapped market segment in terms of the use of equipment in the agricultural sector, where mobile equipment cannot be used and will be in high demand in small and closed ground (greenhouses), among the producers of agricultural crops,

growing vegetables, cucurbits and small-fruit crops, which are considered by the Georgian state a priority in the agricultural sector.

The highland regions of Georgia are distinguished by such features as the location of agricultural lands on the slopes, fragmented narrow contour plots, mountainous conditions, sloping terrain, high air humidity, solar radiation, duplex soils, and more.

Due to the mentioned circumstances, it is not possible to use mobile and heavy equipment in most of the arable lands, there is no mountainous equipment and therefore, the main works are done by hand and use of technical means of small mechanization.

According to the conducted research, the total volume of plots up to 1 hectare is 12.7% of the total arable land, and the volume of plots from 1 to 5 hectares is 19.3%. Just in these areas the smallholder farmers and peasants produce vegetables, cucurbits, and small-fruit crops, and the above-mentioned smallholder farmers and peasants are the main users of small-scale mechanization equipment.

Basic part

The machine for laying on the soil of plastic tape for mulching contains a frame, connected with the power tool, on which there are installed in sequence the tools for tillage and profiling of surface of furrows, a roll of plastic mulching tape, and soil fillers. The machine is additionally equipped with a drum with a drip irrigation pipe and a mulch tape perforator. Also, the drum with a drip irrigation pipe reeled on it is installed on the frame after the tool for profiling of furrow surface, which is a bow-shaped shield, and the soil-cultivating tool is made in the form of a rotary tiller with a horizontal shaft mounted on the front of the frame. Also, the power supply is a motoblocks, while the frame has supporting wheels, with the mulch tape perforator placed on the axle of support wheels and positioned between the mulch tape roll and the soil fillers. (Figures 1-2) shows the principle diagram of the proposed aggregate.

We proposed a completely new innovative, combined device for the motoblock, which is protected by the patent of Georgia (No AP 2020 15395). [2].

Authors of the patent: 1. Giorgi Kutelija; 2. Jemal katsitadze; 3. Vladimir Miruashvili; 4. Otar karchava.

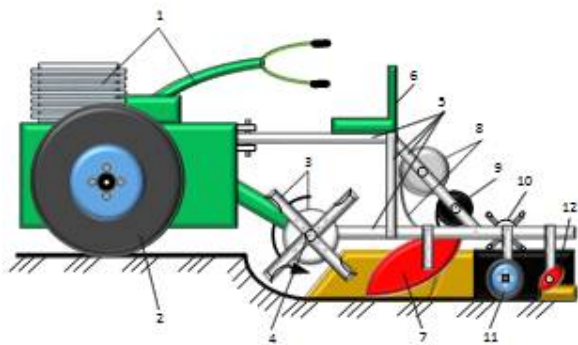


Fig-1

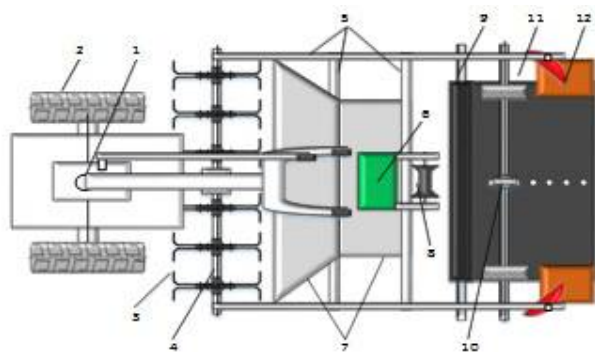


Fig-1

Innovative combined unit for mulching soil on the basis of a motoblocks (fig-1) side view (fig-2) top view.

The aggregate laying of plastic tape on soil for mulching contains the power tool - motoblocks 1 with drive wheels 2 to which frame 3 is connected. On the front part of frame 3, a soil cultivating tool is mounted, which is made in the form of rotary tiller 5 with horizontal shaft 4. The operator's seat 6 is attached to the upper side of frame 3. On the lower side of frame 3, after the rotary tiller 5, a tool for profiling the furrow surface is attached, which is a bow-shaped shield 7. After the mentioned tool 7 for profiling of furrow surface, a drum 8 with drip watering pipe reeled on it, the roll 9 of the mulching tape, the perforator of the mulching tape 10, and soil fillers 11 are mounted on the frame 3 in sequence. Frame 3 is equipped with support wheels 12. The perforator 10 of the mulching tape 9 is placed on the axle of the support wheels 12 and is located between the roll of mulch tape 9 and soil fillers 11.

The aggregate works as follows: after starting the engine of the motoblocks 1, the coupling (not shown in the drawings) of the drive wheels 2 and the rotary tiller 5 is turned on, as a result of which the two-wheel tractor 1 moves forward and the rotary tiller 5 starts working - cutting and loosening the soil layer. The layer of soil cut by the knives of the rotary tiller 5 is thrown back and meets the tool for profiling of furrow surface, which is a bow-shaped shield 7; its shape gives the soil layer cut by the rotary tiller 5 a certain direction and forms a furrow with a smooth surface. At the center of the profiles made by the rotary tiller 5 and the bow-shaped shield 7, a flexible drip irrigation pipe reeled on the drum is unreel, then the pipe is covered with plastic mulch tape 9 from the roll 9 for mulching. The support wheels 12 move on the edges of the mulch tape 9, and the perforator 10, rotating under the action of the axle of support wheels, in the same zone makes holes for planting of saplings, and the edges of the mulch tape 9 are covered by loose soil using soil fillers 11.

The proposed aggregate for laying on the soil of mulching plastic tape is of simple construction, is characterized by low energy consumption, and allows to loosen a soil through a single pass of the motoblocks, to make furrows, unreel flexible drip irrigation pipe, to lay plastic mulch tape on furrows, to make pits for planting of saplings and cover the edges of the mulch tape with soil.[3-4].

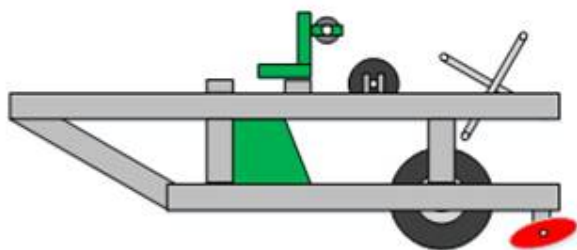


Fig-3 Aggregate, side view

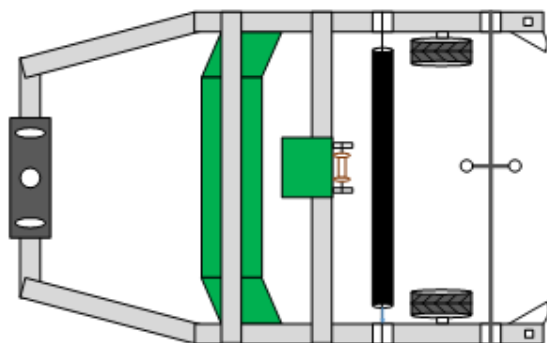


Fig-4 Aggregate, top view

The experimental unit will be mounted on a high clearance 7 hp two-wheel tractor. There is will be field tested on annual crops in 2023 in the scientific-Research Center for Agriculture of Georgia [5-6]. After field testing of this equipment, the data will be processed to improve the reliability of resource-saving technologies using the theory of similarity and dimensions.

Conclusions:

The proposed combined unit, allowing from one pass: loosen the soil, form a furrow, lay a drip water supply system, cover the furrow plastic mulch, cover the edges of the plastic mulch with earth and create holes for planting plants. In essence, the proposed unit is a unit of minimal tillage. With the help of the grant, an experimental innovative, combined device will be manufactured, which will undergo laboratory and field experiments 2023 years. This device to get a lot of consumption from peasants and small farmers.

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Georgian traditional soft cheese screening on existence of antibiotic resistant *Escherichia coli* and Coagulase-positive *Staphylococci* strains in Tbilisi, Georgia

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ABSTRACT

The research aims to fill the knowledge gap of antimicrobial resistance spread through the Georgian dairy food chain and support countries' sustainable development in facing the global threat. Forty-two traditional Georgian soft cheese samples were collected from major organized retail and open markets in the Tbilisi capital of Georgia. All samples were tested on the presence of food pathogens *Escherichia coli* (*E. coli*) and Coagulase-positive *Staphylococci* (*CoPS*), within limits set by the national regulation (№581:2015 and №301n:2001). Identified bacteria strains were tested on antimicrobial sensitivity for the top eight registered antibiotics used in veterinary.

Research showed alerting results, *E. coli* was detected in 92% of samples and *CoPS* in 42%. All strains of *E. coli* and *CoPS* show multidrug resistance toward different antibiotics.

Key words: Georgian Cheese, antimicrobial resistance, dairy sector, *E. coli*, *Staphylococcus aureus*, *Escherichia coli*, Coagulase-positive *Staphylococci*, food microbiology, food safety.

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Introduction

Safe food, free of pathogenic bacteria, is fundamental for food security. Providing nutrients necessary for body functioning and supporting sustainable development of United Nations (UN). Food security is endangered by the rapid development of antimicrobial resistance (AMR). Antimicrobial resistance is a silent pandemic threatening human health by endangering the ability to treat even modest infectious diseases [1]. Antibiotics in veterinary exceed usage in the health sector and are often misused [2]. However, more than 84% of countries do not have suitable legislation for veterinary drug

control[3]. The deviation between human health and animal agriculture remains the main challenge that requires multidisciplinary work of all sectors and global partnership as part of the One Health Approach [4]. AMR development in pathogens causes food poisoning outbreaks[5] and Antibiotic-resistant bacteria spread through the food chain. Combating antimicrobial resistance is essential for the attainment of the Sustainable Development Goals (SDG; United Nations) and relates closely to accomplishing multiple SDGs (Fig. 1) [6]–[8]. Since 2015 Georgia adopted Sustainable Development Goals and initiated a strategy to combat AMR [9].



Fig. 1. AMR reflection in SDGs

Research question. This research aims to fill the knowledge gap of antimicrobial resistance spread in food pathogenic bacteria in the dairy industry, on the example of Georgian traditional soft cheese food chain. The research aims to supports countries' sustainable development road map, as according to National Strategy for Combating Antimicrobial Resistance, data concerning antimicrobial resistance spread through the food chain is deficient [10].

To address emerging risks, we have selected common food pathogens *Escherichia coli* (*E. coli*) and Coagulase-positive *Staphylococci* (CoPS) as research objectives. Collect cheese from organized retail and open markets located in Tbilisi capital of Georgia. Analyze the presence of pathogenic bacteria within the regulation limits, and assess antimicrobial sensitivity of identified strains, toward the top eight antibiotics registered for cattle veterinary [11] [12].

***Escherichia coli* and Coagulase-positive *Staphylococci* in food microbiology regulations.** *Escherichia coli* and Coagulase-positive *Staphylococci* are considered as hygiene indicators of the food industry internationally [11]. Indicating poor production hygiene, process failure, or inadequate process (e.g., pasteurization) or post-process contamination of food [13].

E. coli is spread in the food industry due to fecal contamination of raw materials. Usage of *E. coli* as a hygiene indicator is affected by the low cost of analyses and the possibility to receive results within 48hr, compared to five to seven-day long analyses

necessary to identify other pathogens such as *Salmonella* [14]. Besides several types of *E. coli* are pathogenic by themselves, such as *E. coli* O157:H7, which has caused food poisoning outbreaks in different countries[14], [15].

Around 30% of the population is a carrier of CoPS [16]. CoPS causes different community acquired infections and produces heat-stable enterotoxins, which cause food poisoning[15]. Multi-drug-resistant *Staphylococci* is presenting a global threat to human health [17]. Both *E. coli* and CoPS are the common causes of bovine mastitis. Recent studies suggest that methicillin-resistant *Staphylococcus aureus* (MRSA) strain, which causes more deaths than homicide, AIDS/HIV, and Parkinson's disease, originated from dairy cattle farms, transmitting through the food chain from cattle to farm workers[17][18].

Two legal acts regulate food microbiology in Georgia: Order of the Minister of Georgia №301/n "On Approval of Sanitary Rules and Norms on the Quality and Safety of raw food ingredients and Foodstuffs" and Resolution №581 "On the approval of the technical regulation on microbiological criteria for food"[19].

Each regulation sets acceptable limits for food microbiological criteria. №581 limits the number of *E. Coli* up to 1000 cfu/g in Cheeses made from milk that has undergone heat treatment, while №301/n prohibits coliforms, the working group of *Enterobacteriaceae*, including *E. coli*, in 0.001 g/cm³ of cheese sample. Both regulations control fecal contamination risk. Limits for bacteria in the genus of *Staphylococci* also differ. №301/n allows up to 1000cfu/g *S. aureus* in cheese made with bacterial culture and prohibits presence in 0.1g/cm³ of home-made cheese. №581 controls the group of Coagulase-positive *Staphylococci* bacteria, including *S. aureus*, while limits vary according to cheese heat treatment methods from 10⁵ CFU/g for raw milk, 1000CFU/g for low heat treatment, and 100 CFU/g for pasteurized.

The difference between the two active regulations presents challenges for food business operators, who must comply with both. The historical development of food microbiology limits explains the difference. For example, at the end of the 20th century, coliform was replaced with *E. coli* as an indicator of food industry fecal contamination due to the higher accuracy of *E. coli* analyses [14][20]. № 301/n was published in 2001 using Soviet Sanitary rules and norms 2.3.2. [21] as a reference while №

581 is aligned with Commission Regulation (EC) No 2073 and is enforced since 2015. We can conclude that microbiological limits set by regulation № 581 are more recent than regulation №301/n, which follows older guidelines and specific requirements toward traditional cheese microbiological limits require revision.

Dairy industry food chain. Traditional Georgian cheese is in high demand and is an essential part of Georgian cuisine, consumed fresh or used in the traditional dishes. Dairy cattle are a central resource for peasants and contribute to household food security. Shares of family holdings in the dairy sector are at 97.5% second place after poultry, which is mainly owned by industrial farms, with 61% share[22]. The small household farms own 89% of cattle, 1 to 9 cows, while only 1% is owned the large holding with over 50 cattle heads [23]. There are 360 registered dairy business operators[24] working on raw milk collected from peasants and on importing milk powder, due to lack of milk supply or low price.

Peasants often use outdated practices and have low awareness of personnel hygiene, good hygiene practices, and veterinary control. Reasonable procedures such as hand washing, cattle hygiene before milking, equipment and utensils cleaning, milk pasteurization, storage temperature are rarely followed[25]. In addition, cattle in small farms do not receive regular veterinary control and depend on free vaccination by the Government[26]. Lack of veterinary control increases the risk of veterinary diseases and AMR development in the food chain, through incorrect usage of veterinary drugs, which can be purchased without prescription and monitoring.

A major part of cheese from all the regions ends up in Tbilisi capital of Georgia, supplying one-third of the country's population. Wholesales collecting the dairy products produced in small households deliver cheese in five main agrarian open market areas in Tbilisi: Gldani, Didube, Vagzali, Samgori, Varketili. The open market assessment during sample collection highlighted breaches of regulatory requirements and a lack of control. Which presents a direct risk for consumer health and an increased risk of pathogenic bacteria contamination[27]. Major areas for improvement are food handler hygiene and awareness, cleaning practices, good hygiene practices and cross-contamination risks, food storage temperatures, shelf-life control, food labeling. Most open markets are not provided with well-maintained handwashing facilities nearby[28][13]. Open markets are rarely equipped with water sources,

necessary cleaning equipment, and chemicals, leading to insufficient cleaning practices [28][30]. Dairy products are sold at ambient temperature directly from display tops or from old display units, which rarely maintain the temperature within safe storage limits [28, 31, 32]. Cross-contamination occurs between different product categories: Ready to eat, raw meat, dirty fruit, and vegetables, sold from the same display despite the separation of market areas[28]. Dairy products are sold without packaging or placed directly on dirty surfaces while the same knife, trays are used without regular cleaning[15]. Dairy products are sold without labels and shelf-life markings, indicating a lack of traceability[33][34]. In addition to open markets organized retail chains are highly concentrated in Tbilisi, with 28% of market share, expected to reach 41% by 2024[35]. Retail stores sell a major part of cheese produced by dairy factories. While big producers have a top share on market and are supplying several retails. Assessment during sample collection highlighted better follow-up of basic hygiene requirements, effected by frequent control from the National Food Agency [27][36]. Product storage temperature is followed[28][31][32], cross-contamination risk is reduced by keeping products in the self-service(70%) or in separated traditional displays(30%). Food labeling requirements are better followed and most products have a label with expiry dates [33][33].

Consumers awareness as a driver of the dairy industry food chain. Consumers' demand is the main driver for food business operators. The majority of the population purchase products on the open market, supporting continued trade, despite distinct unhygienic conditions. It puts an economical strain on retail stores and other food business operators who face additional expenses following the regulation. Additionally, the consumer's purchase traditions to touch and taste the product before purchase initiates practices of selling product on ambient temperature and increases cross-contamination.

Consumers' awareness is reflected in the accurate implementation of food labeling regulations as well. Regulated by the resolution №301 "Technical Regulation—on approval of the provision of food information to consumers" [19] and Resolution №152, "On the approval of the technical regulation on milk and dairy products" [33] [37]. Assessment done during samples collection highlighted that none of the open market merchants followed food labeling regulation, and only 40% had an inaccurate old label. While 100% of cheese sold in retail had labels, most

of them were missing some mandatory information. Food nutritional value indicated on labels was on average 20% higher than the ratio between proteins, carbohydrates, and fats [33]. There was no correlation between shelf life and packaging type, storage temperature, salt content, key extrinsic characteristics used for shelf-life detecting[38], which indicates lack of shelf-life study. Milk thermal treatment method was indicated only on 50% of labels, 30% of labels indicated usage of raw milk without information of the thermal treatment, while 20% of labels did not have any information. Information about milk pasteurization is vital for consumers to make conscious decisions concerning using unpasteurized dairy products with a high microbial load [37].

Low awareness of food labeling is misused in marketing, soft cheese is often sold next to its cheaper “alternatives” made from plant oil or milk powder. The violation of appellation of origin is also an example of consumers’ awareness. All cheeses collected in the retail chain were named “Imertariani” cheese when none was made in the Imereti region[39]. Following is affected by the tradition of calling soft cheese “Imeretiani” cheese, contradicting the regulation.

Material and methods

To assess the presence of food pathogens in Georgian traditional soft cheese, we collected

double cheese heads from 10 major organized retail chains[35] and 11 open markets in Tbilisi, 42 samples in total. Samples were selected randomly, avoiding collecting cheese from the same producer for diversity and when possible, prioritizing cheese from bigger cold display units on the open market.

All samples were delivered to the food microbiology laboratory and analyzed directly after purchase. Microbiological analyses methods were selected in compliance with Resolution N581[40] as follows: EN ISO 16649-2 -Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* and ISO 6888-1 Horizontal method for the enumeration of CoPS. The Kirby-Bauer disc diffusion method was used to detect bacteria strains’ antibiotic sensitivity[41][44]. Analyses are done in a laboratory having accreditation for all the above methods in compliance with EN ISO 17025[45].

Results of cheese microbial contamination load and antimicrobial-resistant development

Laboratory analyses showed alerting results. *Escherichia coli* was detected in 93% of samples, moreover, in 85% of samples, results were above 1000CFUs; Coagulase-positive *Staphylococci* results showed fewer colony-forming unit numbers from 300cfus up to above 300,000cfus. *CoPS* were detected in 62% of samples (Fig. 2).

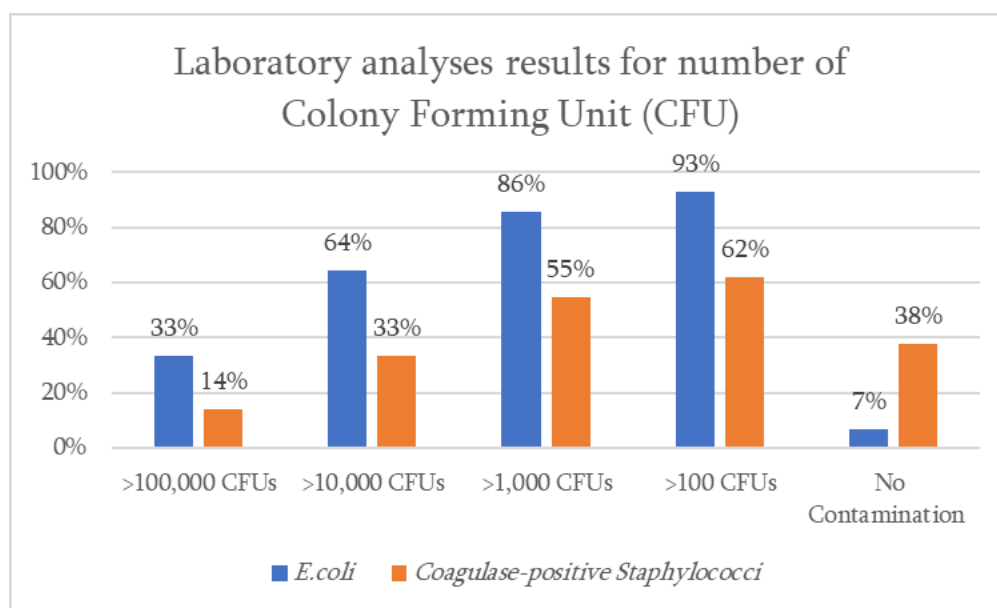


Fig. 2. Laboratory analysis results for *E. coli* and CoPS number of Colony Forming Unit (CFU), N=42.

These results can have several interpretations based on which regulations have been used for assessment. According to №301/n *E. coli* results directly violate limits, as coliforms are not allowed in 0.001g/cm³ of the sample. To assess compliance of genus of *Staphylococcus* bacteria load with №301/n, information of production technology is required; as up to 1000cfu/g *S. aureus* is allowed in cheese made with the addition of bacterial culture and is prohibited in 0.1g/cm³ of homemade cheese[46]. Nevertheless, 55% of laboratory analyses results showed higher colony-forming units of CoPS, than stated in either of the parameters.

Information concerning cheese thermal treatment is required to assess compliance of CoPS and *E. coli* results with №581. CoPS limits vary for raw milk, pasteurized, and low thermally treated cheeses. While *E. coli* limits are set only for Cheeses made from milk or whey that has undergone heat treatment [13], [40]. Without an indication of the pasteurization process on the label, we are unable

to give a more detailed assessment for regulatory compliance. Nevertheless, 14% of samples violated all parameter requirements. Out of 42 cheeses, only 23% (ten samples) collected from organized retail chains indicated using pasteurized milk; 100% of the which violated requirements for *E. coli* limits, and 40% violated CoPS limits.

Identified *E. coli* and CoPS strains were tested on antimicrobial sensitivity towards eight antibiotics: Oxytetracycline; Tylosin; Trimethoprim; Enrofloxacin; Amoxicillin; Penicillin G; Streptomycin; Colistin. At least one of them contain 80% of veterinary drugs registered in Georgia for dairy cattle contain [12].

CoPS strains showed high multidrug resistance to a minimum of 3 out of 6 test antibiotics. All strains show resistance to Amoxicillin, Penicillin, Enrofloxacin. At the same time, 42% of strains were resistant to Streptomycin and 35% to Trimethoprim. Oxytetracycline results were most promising, with only 19% resistance (Fig. 3).

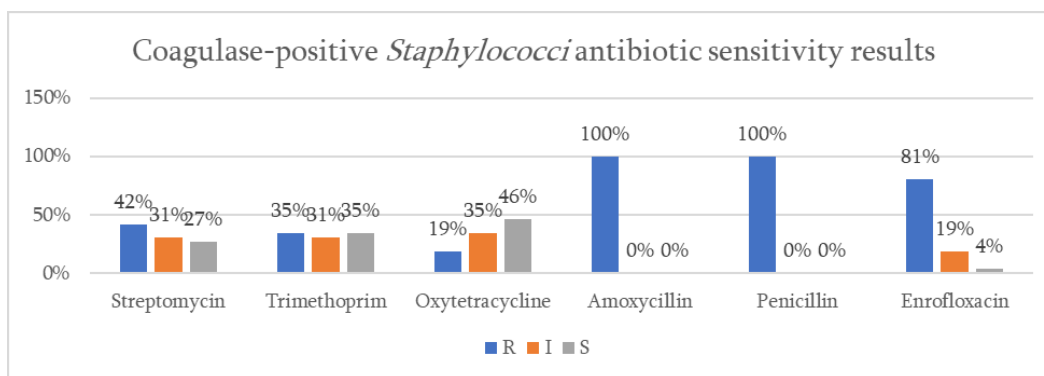


Fig. 3. Antibiotic sensitivity of CoPS (N=26).

E. coli strains showed multidrug resistance to three out of eight tested drugs. All strains show resistance to Amoxicillin, Penicillin, Tylosin. At the same time, 44% of strains were resistant to Streptomycin and 36% to Trimethoprim. Enrofloxacin, Oxytetracycline, and Colistin showed relatively low 8%, 15%, and 26% resistance. (Fig. 4)

tylosin and 36% to Trimethoprim. Enrofloxacin, Oxytetracycline, and Colistin showed relatively low 8%, 15%, and 26% resistance. (Fig. 4)

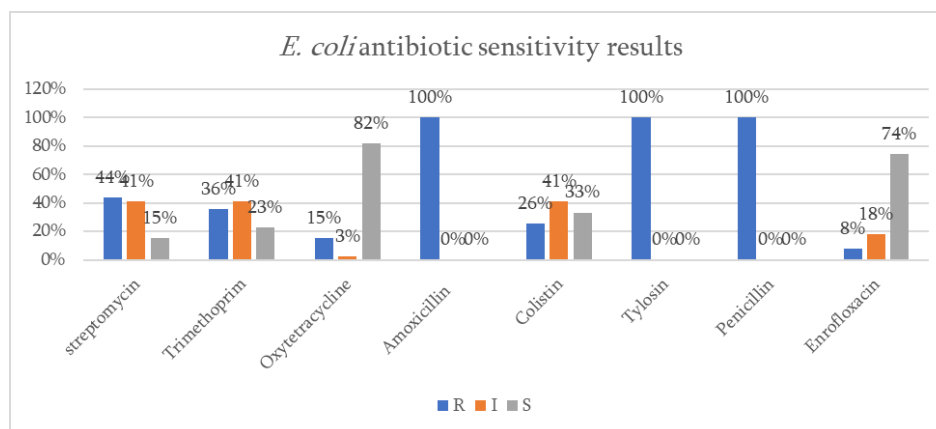


Fig. 4. Antibiotic sensitivity of *E. coli* (N=39).

Conclusion

Pathogenic bacteria contamination results and high antimicrobial resistance are alerting and indicate uncontrolled and incorrect usage of antibiotics in dairy farming and the need for hygiene and food safety requirement improvement along the full dairy food chain. In addition, microbiological limits set by the regulation and shelf-life study performance by food business operators require review. Findings are especially critical in times of rapid antimicrobial resistance, which can easily spread through the food chain to consumers. Research highlights that current agriculture, veterinary, food industry, and safety practices and regulatory controls are not sustainable for the future. The multi-sectoral approach is required to increase consumers' and farmers' awareness as key drivers of supply and market.

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Effect of microscopic fungi lysates on Ehrlich carcinoma growth

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ABSTRACT

The article presents the influence of biologically active substances NL-51, T1 and K1 (supernatants, isolated from microscopic fungi collected from different regions of Georgia) on Ehrlich's carcinoma growth in mice. Investigations have shown that T1 and K1 samples reveal inhibitory activity on proteolytic enzymes that supposedly explains their anticancer effects (increased lifespan of treated carcinoma-bearing mice compared to control mice). The opposite effect was observed in case of NL-51 treated mice. NL-51 did not show inhibitory properties on proteolytic enzymes and the life span of treated animals was not prolonged compared to the control group animals.

Key words: Cancer, Proteolytic enzyme, Enzyme inhibitors, Microscopic fungi.

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Introduction

Cancer is one of the leading causes of morbidity and mortality worldwide. According to the World Health Organization, the number of deaths from cancer in 2020 reached 10 million, and the number of new cases – 14 million. Annually increases the number of lung, liver, stomach, breast, and colon cancer cases [1].

At present, despite of wide range of anticancer treatment methods (surgical intervention, chemotherapy, radiotherapy, immunotherapy, hormone-based therapy, etc.), treatment still remains unsolved due to accompanying side effects (neuro-, hepato-, and cardiotoxicity, superinfections, etc.) [2-7].

For these reasons, scientists are actively involved in the development of anticancer drugs and methods that will be much more effective, safer, and less expensive. In this regard, the natural sources such as enzymes, their inhibitors, and biologically active compounds obtained from different microor-

ganisms, supposedly having antitumor (tumor cell growth inhibition and/or destruction) activity, could be used against malignant growth [8-13].

Studies have shown that proteases – the representatives of the class of hydrolases, play an important role in vitally important biological processes. In particular, they can regulate cell proliferation and apoptosis, participate in the synthesis of new bioactive substances, regulation of intercellular signaling pathways, etc. [14-16]. However, in addition to a positive feature, the variability of their activity may dramatically affect the living organism [17].

According to recent studies, in some types of tumors at an early stage of development, the level of proteolytic enzymes involved in the processes of cancer development (proliferation, angiogenesis, metastasis) is sharply increased [18]. Therefore, their inhibitors could be considered as one of the most powerful anticancer treatment strategies [19].

Most of the studied and known inhibitors are proteins, peptides, polysaccharides, polyphenols, glycerolipids, triterpenes, and low molecular weight

non-protein compounds [20]. At present interest in the role of inhibitors of proteolytic enzymes isolated from microscopic fungi has increased [21]. Recently, inhibitors of serine, cysteine, and several aspartic proteases have been discovered and studies are underway to determine their antitumor potential [22].

Serine peptidase inhibitors have been found in various representatives of ascomycetes and basidiomycetes [23, 24]. Cysteine peptidase inhibitors are found in basidiomycetes. Aspartic protease inhibitors have been found in yeast. Low molecular weight inhibitors of serine and cysteine proteases have also been found in actinomycetes and streptomycetes [25–28].

Based on the above discussed, we think that, microscopic fungi could have anticancer treatment potential. **Aim:** determine the presence of inhibitors of proteases in intracellular biologically active substances (biomass/lysate) isolated from microscopic fungi through solid-phase fermentation and investigate their antitumor effects on Ehrlich carcinoma growth in lab mice.

Materials and Methods

Agarose, salts for buffer solution Na_2HPO_4 , KH_2PO_4 , NaCl , and KCl were purchased from Alfa Chemical (India). Skimmed milk powder was purchased from LTD Biologica. All chemicals were of analytical grade. As a source of commercial protease Chymoral, produced by Golenikaa.d was used. Microscopic fungi's intracellular lysate, obtained through solid-phase fermentation was purchased from the scientific and Educational Center "Biomed", Technical University of Georgia.

The amount of total protein in the intracellular lysate was determined using the Bradford method [29]. 100 μl of analytical solutions were added to 1 ml of Bradford's reagent, the samples were placed for incubation in a thermostat at 25°C for 10 minutes. After incubation, the samples were measured using a spectrometer at 578 nm against the reagent blank. The protein concentration was determined on a calibration curve constructed with standard solutions of bovine serum albumin (0.03, 0.062, 0.125, 0.25, 0.5, 1, 2 mg/ml).

The gel diffusion method with slight modifications was used to determine the inhibitory properties of culture fluids on proteolytic enzymes [30]. 1% skim milk powder was taken as a substrate, which was polymerized in 1% agarose gel (in Petri dishes). After the gel was solidified, rings with a diame-

ter of 3 mm were cut and 20 μl samples were added (proteolytic enzyme was mixed to the test solutions in a ratio of 1:2. PBS and enzyme were taken in the same ratio as the control solution). Samples were incubated at 37°C for 18 hours. Inhibition can be visualized by the decrease in the diameter of the clear zone compared to the positive control generated diameter.

The Ehrlich ascites carcinoma (EAC) cells were provided by the Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology of the National Academy of Sciences of Ukraine, Department of Experimental Cell Systems, The Cell Line Bank (BCL) from Human and Animal Tissues (<https://iepor.org.ua/www.onconet.kiev.ua>).

The antitumor properties of biologically active compounds of lysates were tested on albino male mice with the weight range 20-25 g, purchased from the vivarium of the Alexander Natishvili Institute of Morphology (Tbilisi, Georgia. <https://www.tsu.ge/en>). After being placed in a laboratory (8 per cage) the animals were given a 7-day interval for acclimatization before the experiment. During this period, the animals were kept under constant environmental conditions with a light-dark cycle of 12/12 at a temperature of $23 \pm 2^\circ\text{C}$. They were fed a standard laboratory chow and given free access to water.

For in vivo modeling of the malignant tumor under brief ether anesthesia, each mouse was inoculated subcutaneously with a fixed number of viable cancer cells (2×10^6 cells/20 g body weight). Cells were counted by the hemocytometer. The viability of the EAC cells was 98% (by trypan blue exclusion assay) [31, 32].

For investigation of the effect of intracellular lysates on Ehrlich carcinoma growth the control group mice were injected with 100 μl of physiological solution, and the experimental groups were injected with 100 μl of the test solutions - T1, K1, NL-51 (protein concentration 2 mg/ml). The observation was carried out for 82 days.

SPSS (version 10.0) was used for analyzing data. Differences between tumor control and treated animals were determined by using the Independent-Samples T-test. The criterion for significance was set to $p < 0.05$.

Results and discussion

The protein concentration in the research samples was calculated from the linear area of the calibration curve (Table.1). As part of the study, we wanted to determine the total protein amount of in-

tracellular lysates, to standardize injection solutions by protein. This was necessary to administer the same amount of protein to the test mice.

Research samples	Protein concentration mg/ml
T1	5 ± 0.1
K1	7.9 ± 0.14
NL-51	5.8 ± 0.11

Detection of protease inhibitors and observation of their influence on mice

Since some proteases may have tumor-promoting effects, one of the ways of suppressing malignant growth is the use of substances containing proteases inhibitors. In the study's framework, the test samples' inhibitory activity was determined by the gel-diffusion method. The presence of clear rings on agar is an indicator of proteolytic activity and the absence of rings indicates the presence of an inhibitor.

After 18 hours of incubation of the samples, the diameter of the control ring was compared with the rings of the test solutions (Diameter of the transparent circle (cm): Control - 1.3 ± 0.05 , T1 - 1 ± 0.03 , K1 - 0.6 ± 0.01 , NL-51 - 1.3 ± 0.05). The presence of the inhibitor of proteolytic enzymes was clearly observed on the K1 sample, and weakly on the T1, as

for the samples - NL-51 the presence of the inhibitor was not observed (Fig. 1).



Fig. 1 Determination of inhibitors of proteolytic enzymes in research samples by gel diffusion method.

Lifespan of Ehrlich's carcinoma bearing untreated and treated with K1, T1 and NL 51 mice.

After the injection of the test solutions in an in vivo model of Ehrlich's carcinoma, different results were obtained. As a result of exposure to K1 and T1 solutions, the viability of mice increased compared to the control group. The opposite effect was observed in the NL-51 sample (Fig. 2).

In T1 treated mice the lifespan was prolonged

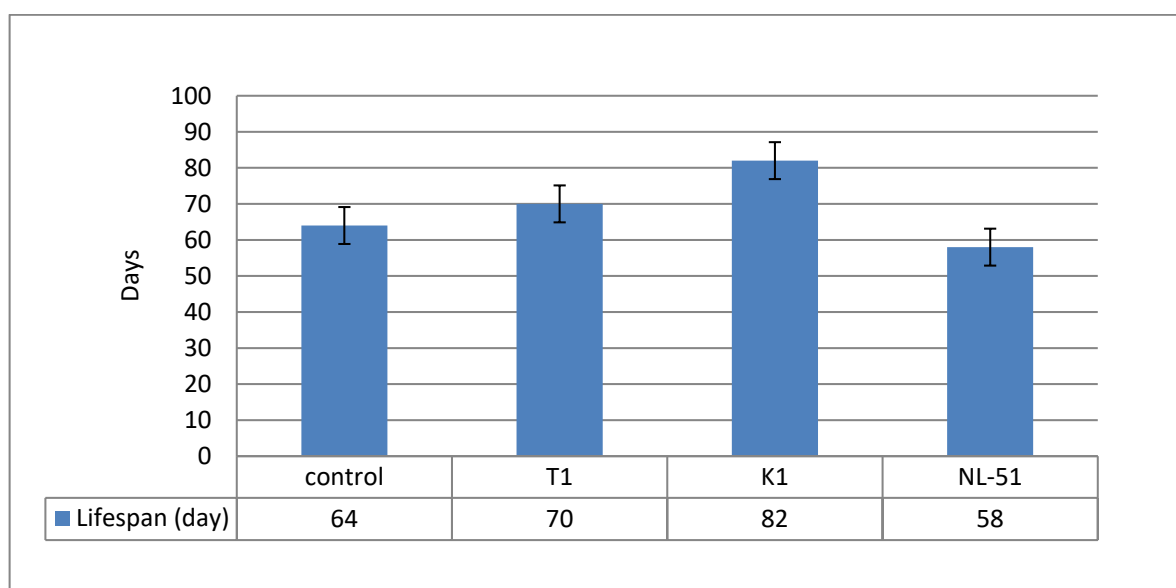


Fig. 2 Lifespan of mice with Ehrlich carcinoma after exposure to K1, T1, and NL 51.

by 11% ($p < 0.01$), in K1 treated mice – by 28%, and in NL-51 treated mice, the lifespan was less by 9%

compared to the control respectively (Fig. 3,4).

Conclusion

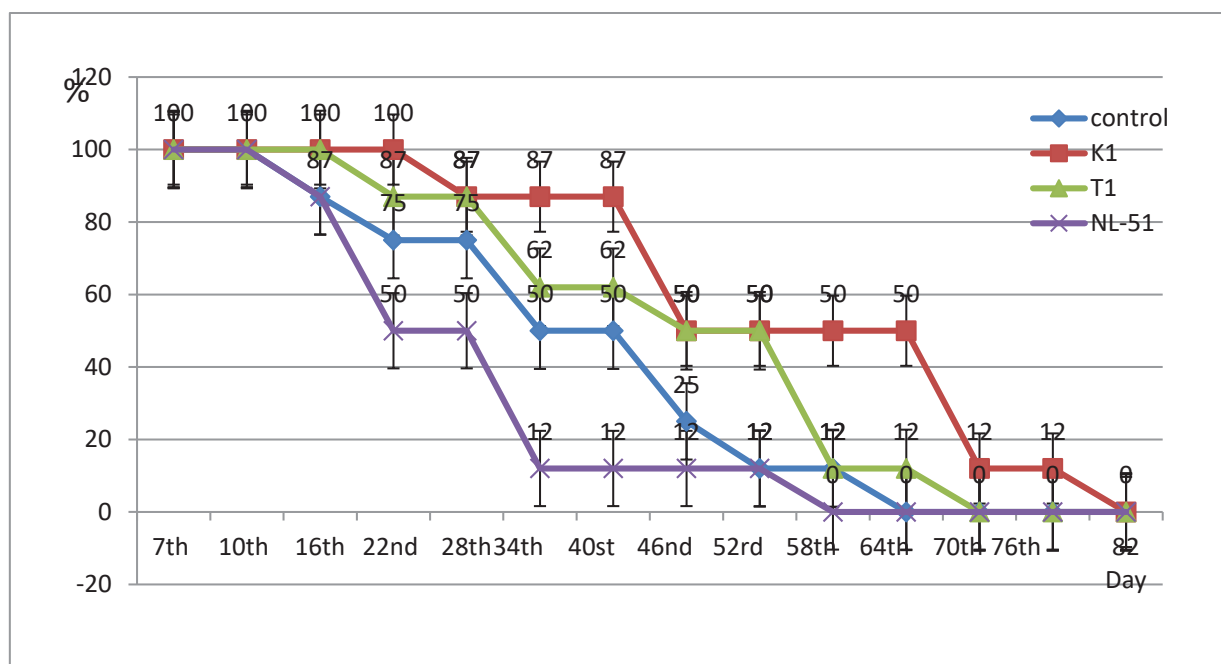


Fig. 3 Lifespan and Percent of Survived mice with Ehrlich carcinoma (control) and after exposure to K1, T1, and NL 51.

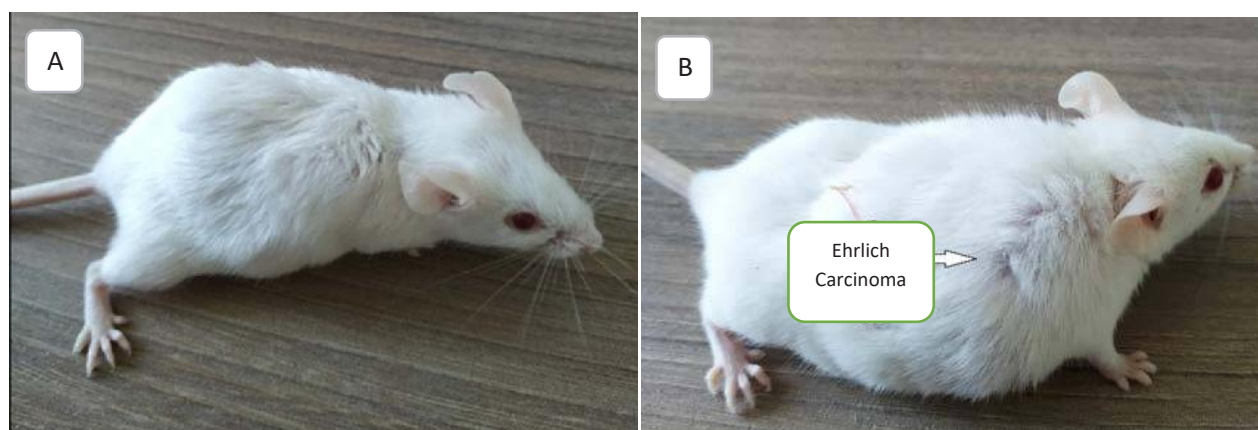


Fig. 4 (A) mouse with Ehrlich's carcinoma after treatment with K1 (B) control mouse (untreated, cancer bearing) 58th day of Ehrlich carcinoma growth.

Could be concluded that proteases inhibitors detected in K1 and T1 samples reveal antitumor effects. The study conducted provides preliminary evidence to support this claim. However, it is important to note that further research is needed to confirm the exact mechanism of action of the protease inhibitors and their potential as a therapeutic agent for cancer treatment. Therefore, it can be concluded that while the current study is promising, further investigation is necessary to fully understand the potential of protease inhibitors in the fight against cancer.

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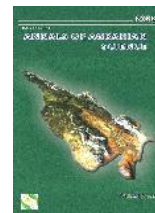
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Sawflies (Hymenoptera: Symphyta) from the high altitudes of Kintrishi National Park, south-west Georgia (Sakartvelo) (part II)

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ABSTRACT

Sixty-four species of 513 specimens were collected in the montane and submontane altitudes of Kintrishi Nature Reserve. Twentythree species are country new records. Zoogeographic analysis and description of the flight periods are provided.

Key words: Caucasus, Hymenoptera, Symphyta, faunistics, ecology

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Introduction

The Kintrishi Protected Areas were established to preserve unique flora and fauna of the local Colchis forest. Additionally, archeological excavations revealed pre-Christian monuments in this areas. About 80% of Kintrishi's protected areas are covered by forest. We must note, the forest covered areas occupy the largest territories among all protected regions of Georgia. [1]. Dominant trees of these protected forests are Beech (*Fagus orientalis*) and Chestnut (*Castanea sativa*), mixed with Ponto Oak (*Quercus pontica*), Birch (*Betula medwedewi*, *B. litwinowii*), Bladdernut (*Staphylea colchica*) and Box (*Buxus colchica*).

Sawflies of lower altitudes of KNP were published by Japoshvili and Haris [2] and 1703 specimens of 42 species were listed including high number of first sawfly records for the country. The present paper is dealing with the high alpine altitudes of KNP. It is included in the CaBOL (Caucasus Barcode of Life) project, to explore the biodiversity of the Caucasus.

Methods and material

The sawfly material was collected by using Malaise traps located at 1 634 (Fig. 1A), 2 280 (Fig. 1B) and 2 465 m (Fig. 1C, D) altitudes above sea level, at coordinates: N 41° 44' 53.5308, E 42° 5' 38.4144 (approximately 7.03 km E of Didvake village); N 41° 45' 19.3680, E 42° 06' 46.6920 (approximately 300 meters West from Kintrishi River and 8.5 km South West from Gomismta village) and N 41.76246, E 42.11571 (approximately 8.65 km NE of Didvake village). These altitudes (1 634, 2 280 and 2 465 m above sea level) are the zones from the higher subalpine Colchis forest belts up to the alpine meadow belt of the Lesser Caucasus vegetation and the 2 465 m is the highest altitude where Malaise trap worked. There is no other data in the sawfly literature to work a Malaise trap in so high altitude ever (only below 1 800 meters asl. see Roller [3] and Savina et al. [4]). The Malaise traps were installed from 20. 04. 2018 and the collecting lasted

until 8th of September 2018. During this time, Malaise traps were checked biweekly.

The traps were installed in the intermediary zone between the sub-alpine forest from 1500 to 2 500 m alt. above the sea level and the alpine meadows generally between 2 400 and 3 000 meters. These altitudes are characterized by moderately humid climate with cold winters and long, cool summers.

At an altitude of 1,000-1,800 m above sea level, the average temperature is 8.5-9°C. The coldest month is January - with an average temperature of 0.5°C. The absolute minimum temperature ranges from -16-17°C. The warmest month is August, with an average temperature of 15-15.5°C. The annual amount of precipitation is 3000 mm.

The beech forest belt is spread at an altitude of 1,000-2,100 m. Common chestnut (*Castanea sativa*), Caucasian hornbeam (*Carpinus caucasica*), Caucasian linden (*Tilia caucasica*), and Kolkhi undergrowth consisting of Pontic rhododendron (*Rhododendron ponticum*), Caucasian whortleberry (*Vaccinium arctostaphylos*), Yellow azalea (*Rhododendron luteum*), *Rhododendron ungueri*, Georgian snow rose (*Rhododendron caucasicum*), and other species. On the upper border, oriental beech and Caucasian fir (*Abies nordmanniana*) may be present together with individual species of oriental spruce (*Picea orientalis*).

At the altitude ranges of 1,800-2,400 m summers are short and winters are long. The vegetation period lasts 3.5-4.5 months. Snow cover is common in late spring. Subalpine temperate forest, shrubland and subalpine meadows are common at an altitude of 1,800-2,400 m. Along with the typical temperate forests of oriental beech (*Fagus orientalis*), there are warty birch, Litvinov's birch (*Betula litwinowii*), as well as endemic and relict species: Medvedev's birch (*Betula medwedewii*), Ponto's oak (*Quercus pontica*), *Rhamnus imeretina* and mountain currant (*Ribes biebersteinii*). The shrubby understory is rich in plant species, including georgian snow rose (*Rhododendron caucasicum*), cherry laurel (*Laurocerasus officinalis*), Colchis holly (*Ilex colchica*), *Daphne elbowviana*, and others.

For identification, and for host-plant data, Zhelochovtsev's work on the sawflies of the European part of the former USSR [5], the handbook of Lacourt on the identification of the European Symphytans [6], the monograph of Robert Benson on the Turkish sawflies fauna [7], and Gussakovskij's monographs on the sawflies of the former USSR [8,9] were used. We also consulted recent revisions [10, 11, 12, 13] to confirm the identifications of particular taxa. The general dis-

tribution of species are reported based on Roller and Haris [14], Taeger et al. [15], Sundukov [16]. Further, reference material was studied in the Budapest collection in the Hungarian Natural History Museum. The nomenclature used in this paper, follows the latest monograph of European sawflies [6] with special concern for the subfamily Nematinae to address the conclusions of Prous et al. [17]. The higher classification of Symphyta, applied in this work, follows the Hymenoptera part of Fauna Europaea [18]. Host plant records are given according to Macek et al. [19].

Results

Family - Pamphiliidae

Genus *Pamphilius* Latreille, 1803

Pamphilius sylvaticus (Linné, 1758)*: 02-06. 06. 2018, 2 280 m, 1 female. Frequent, West Palaearctic species. Larva on *Sorbus aucuparia*, *Prunus* spp. like *Prunus padus* and *Crataegus* spp.

Family - Diprionidae

Genus *Monoctenus* Dahlbom, 1835

Monoctenus juniperi (Linné, 1758)*: 15-29. 06. 2018, 2 465 m, 1 male. Sporadic, West Palaearctic species. Larva on *Juniperus communis*.

Family - Tenthredinidae

Subfamily - Allantinae

Genus *Allantus* Panzer, 1801

Allantus (Emphytus) cinctus (Linné, 1758): 19. 05. - 02. 06. 2018, 2 280 m, 1 female, 16-30. 06. 2018, 2 280 m, 1 male, 30. 06. - 24. 07. 2018, 2 280 m, 2 females, 7 males, 24-28. 07. 2018, 2 280 m, 1 male, 05-20. 05. 2018, 1 634 m, 1 male, 20. 05. - 01. 06. 2018, 1 634 m, 1 female, 1 male, 01-15. 06. 2018, 1 634 m, 1 female, 1 male, 15-29. 06. 2018, 1634 m, 1 male. Common. Host plants: *Rosa* and *Fragaria* spp. Holarctic.

A. (Emphytus) laticinctus (Serville, 1823): 16-30. 06. 2018, 2 280 m, 1 female. Sporadic, West Palaearctic species. Larva on *Rosa* spp.

Genus *Ametastegia* Costa, 1882

Ametastegia (Protemphytus) carpini (Hartig, 1837): 16-30. 06. 2018, 2 280 m, 2 females, 1 male, 30. 06. - 24. 07. 2018, 2 280 m, 2 females, 2 males, 15-29. 06. 2018, 2 465 m, 2 females, 29-06. -13. 07. 2018, 2 465 m, 1 female. Frequent. Holarctic. Host plants: *Geranium* spp.

A. (Protemphytus) perla (Klug, 1818): 02-06. 06. 2018, 2 280 m, 1 female. West-Palaearctic species. Sporadic. Host plants: *Salix* sp., it is also recorded from *Quercus* and *Populus* spp.

A. (Protemphytus) tenera (Fallén, 1808): 15-29. 06. 2018, 2 465 m, 1 female, 3 males, 29-06. -13. 07. 2018, 2 465 m, 1 male. Holarctic. Frequent. Larva on *Rumex* spp.

Genus *Athalia* Leach, 1817

Athalia circularis (Klug, 1815): 02-06. 06. 2018, 2 280 m, 1 female, 30. 06. - 24. 07. 2018, 2 280 m, 1 female, 2 males, 29-06. -13. 07. 2018, 2 465 m, 1 female, 20. 04. - 05. 05. 2018, 1 634 m, 1 male, 01-15. 06. 2018, 1 634 m, 1 female, 1 male, 15-29. 06. 2018, 1634 m, 1 male, 29. 06. - 13. 07. 2018, 1 634 m, 2 males, 13-27. 07. 2018, 1 634 m, 3 males. Frequent. Host plants: *Arctium lappa*, *Ajuga reptans*, *Veronica beccabunga*, *V. longifolia*, *V. officinalis*, *Alliaria petiolata*, *Glechoma hederacea*, *Melampyrum*, *Capsella* and *Lycopus* spp. Palaearctic.

A. cornubiae Benson, 1931*: 05-20. 05. 2018, 1 634 m, 1 female. Sporadic West Palaearctic species. Larva on *Sedum album*.

A. liberta (Klug, 1815): 02-06. 06. 2018, 2 280 m, 1 female, 30. 06. - 24. 07. 2018, 2 280 m, 1 female, 24-28. 07. 2018, 2 280 m, 1 female, 1 male, 01-15. 06. 2018, 1 634 m, 1 female, 15-29. 06. 2018, 1634 m, 1 female, 29. 06. - 13. 07. 2018, 1 634 m, 1 male, 13-27. 07. 2018, 1 634 m, 1 male. Frequent, West Palaearctic species. Feeding on *Alliaria petiolata*, *Arabidopsis thaliana*, *Cardamine hirsuta* and *Sisymbrium officinale*.

A. lugens (Klug, 1815): 20. 05. - 01. 06. 2018, 1 634 m, 1 female, 29. 06. - 13. 07. 2018, 1 634 m, 1 male. Sporadic, Palaearctic species. Feeding on various Cruciferae: *Brassica* spp., *Cardamine* spp., *Raphanus* spp. and *Lepidium sativum*.

Genus *Empria* Lepeletier & Serville, 1828

Empria liturata (Gmelin, 1790)*: 15-29. 06. 2018, 2 465 m, 3 males. Frequent Palaearctic species. Host plants: *Fragaria* and *Geum* spp.

Comment: It's erroneously indicated as distributed in Georgia [20] based on Zhelekhovtsev, Zinovjev [21], page p. 366.

E. longicornis (Thomson, 1871): 19. 05. - 02. 06. 2018, 2 280 m, 1 male, 02-06. 06. 2018, 2 280 m, 2 females, 25 males, 16-30. 06. 2018, 2 280 m, 4 males, 30. 06. - 24. 07. 2018, 2 280 m, 2 females, 3 males, 29-06. -13. 07. 2018, 2 465 m, 1 female, 20. 04. - 05. 05. 2018, 1 634 m, 3 males, 05-20. 05. 2018, 1 634 m, 3 males, 20. 05. - 01. 06. 2018, 1 634 m, 1 male. Frequent, West Palaearctic species. Larva on *Rubus idaeus*.

E. tridens (Konow, 1896): 15-29. 06. 2018, 2 465 m, 1 female, 20. 05. - 01. 06. 2018, 1 634 m, 1 female. Palaearctic. Frequent. Host plants: *Geum* spp. and *Rubus idaeus*.

Subfamily – Blennocampinae

Genus *Claremontia* Rohwer, 1909

Claremontia alternipes (Klug, 1816): 20. 04. - 05. 05. 2018, 1 634 m, 5 males, 05-20. 05. 2018, 1 634 m, 2 males. Sporadic. Hostplant: *Rubus idaeus*. West Palaearctic.

Genus *Eurhadinoceraea* Enslin, 1920

Eurhadinoceraea fulviventrus (Scopoli, 1763): 02-06. 06. 2018, 2 280 m, 1 male. Locally common. Hostplant unknown. Southern part of the Palaearctic region.

Genus *Eutomostethus* Enslin, 1914

Eutomostethus ephippium (Konow, 1899): 01-15. 06. 2018, 1 634 m, 1 male. Ponto-Caspian species. Common. Hostplants: Poaceae.

Family - Selandrinae

Genus *Aneugmenus* Hartig, 1837

Aneugmenus coronatus (Klug, 1818): 30. 06. - 24. 07. 2018, 2 280 m, 14 females, 1 male, 16-30. 06. 2018, 5 females, 24-28. 07. 2018, 2 280 m, 3 females, 29-06. -13. 07. 2018, 2 465 m, 2 females, 29. 06. - 13. 07. 2018, 1 634 m, 1 female. Sporadic, Palaearctic species. Larva on *Dryopteris filix-mas*, *Aspidium* sp., *Athyrium filix-femina* and *Pteridium aquilinum*.

Genus *Birka* Malaise, 1944

Birka (Birka) catellata (Konow, 1900): 05-20. 05. 2018, 1 634 m, 1 female, 20. 05. - 01. 06. 2018, 1 634 m, 1 female. Ponto-Caspian and Turanian. Common species. Host plant unknown.

Genus *Strongylogaster* Dahlbom, 1835

Strongylogaster caucasica Schaposchnikov, 1885: 02-06. 06. 2018, 2 280 m, 12 females, 1 male, 16-30. 06. 2018, 2 280 m, 1 male, 19. 05. - 02. 06. 2018, 2 280 m, 1 female, 1 male, 20. 04. - 05. 05. 2018, 1 634 m, 4 females, 2 males, 05-20. 05. 2018, 1 634 m, 2 females, 01-15. 06. 2018, 1 634 m, 1 female, 15-29. 06. 2018, 1634 m, 1 female. Sporadic. Hostplants unknown. Ponto-Caspian.

Subfamily - Heterathrinae

Genus *Caliroa* Costa, 1859

Caliroa cerasi (Linné 1758): 24-28. 07. 2018, 2 280 m, 1 male, 13-27. 07. 2018, 1 634 m, 1 female. Cosmopolitan. Frequent. Host plants: *Pyrus*, *Malus*, *Prunus*, *Crataegus*, *Sorbus*, *Rosa*, *Cydonia*, *Mespilus*, *Rubus*, *Amygdalus*, *Cerasus*, *Amelanchier*, *Pyracantha*, *Cotoneaster* rarely *Quercus*, *Salix* spp.

C. tremulae Chevin, 1974: 16-30. 06. 2018, 2 280 m, 1 female. Sporadic. Hostplant: *Populus tremula*. West Palaearctic.

Genus *Endelomyia* Ashmead, 1898

Endelomyia aethiops (Gmelin, 1790): 15-29. 06. 2018, 2 465 m, 1 female, 29-06. -13. 07. 2018, 2 465 m, 1 male. Frequent, Holarctic species. Larva on *Rosa* spp.

Genus *Metallus* Forbes, 1885

Metallus pumilus (Klug, 1816): 30. 06. - 24. 07. 2018, 2 280 m, 1 male, 24-28. 07. 2018, 2 280 m, 1 male, 15-29. 06. 2018, 2 465 m, 2 males. Common Palaearctic species. Larva inside the leaves of *Rubus caesius* and *Rubus idaeus*.

Subfamily - Nematinae

(The nomenclature of this part follows the proposed changes in nomenclature of the last monograph on sawflies written by Lacourt, 2020)

Genus *Cladius* Illiger, 1807

Cladius pectinicornis (Geoffroy, 1785): 15-29. 06. 2018, 2 465 m, 4 males, 29-06. -13. 07. 2018, 2 465 m, 1 male, 10-24. 08. 2018, 2 465 m, 1 male. Holarctic. Common. Host plants: *Alchemilla*, *Filipendula*, *Fragaria*, *Potentilla*, *Sanguisorba*, *Rosa* and *Rubus* spp.

Genus *Dineura* Dahlbom, 1835

Dineura testaceipes (Klug, 1816)* : 02-06. 06. 2018, 2 280 m, 1 female. Sporadic, West Palaearctic species. Host plants: *Crataegus* spp. and *Sorbus aucuparia*.

Genus *Epicenematus* Lacourt, 1998

Epicenematus montanus (Zaddach, 1883)* : 30. 06. - 24. 07. 2018, 2 280 m, 1 male. Sporadic, West Palaearctic species. Larva on *Picea* spp.

Genus *Hoplocampa* Hartig, 1837

Hoplocampa alpina (Zetterstedt, 1838)* : 29-06. -13. 07. 2018, 2 465 m, 1 female. Sporadic, Palaearctic species. Larva on *Sorbus aucuparia*.

Genus *Priophorus* Dahlbom, 1835

Priophorus compressicornis (Fabricius, 1804): 02-06. 06. 2018, 2 280 m, 2 males, 30. 06. - 24. 07. 2018, 2 280 m, 5 females, 14 males, 16-30. 06. 2018, 2 280 m, 6 females, 12 males, 24-28. 07. 2018, 2 280 m, 1 male, 11-25. 08. 2018, 2 280 m, 1 male, 25. 08 - 08. 09. 2018, 2 280 m, 1 female. Frequent pest. Hostplants: *Betula*, *Cotoneaster*, *Prunus*, *Rubus*, *Sorbus*, *Fragaria*, *Crataegus*, *Corylus* and *Rosa* spp. Holarctic.

Genus *Nematus* Panzer, 1801

Nematus lucidus (Panzer, 1801): 02-06. 06. 2018, 2 280 m, 1 male, 30. 06. - 24. 07. 2018, 2 280 m, 10 males, 24-28. 07. 2018, 2 280 m, 2 males, 16-

30. 06. 2018, 2 280 m, 2 males, 01-15. 06. 2018, 1 634 m, 4 males, 29. 06. - 13. 07. 2018, 1 634 m, 1 male. Frequent. Larva on *Crataegus*, *Rosa* spp. and *Prunus spinosa*. Palaearctic.

N. wahlbergi Thomson, 1871: 16-30. 06. 2018, 2 280 m, 1 male. Sporadic, West-Palaearctic species, larva on *Symphoricarpos albus* and *Lonicera* spp.

Genus *Pachynematus* Konow, 1890

Pachynematus clitellatus (Serville, 1823)*: 01-15. 06. 2018, 1 634 m, 1 male. Palaearctic species introduced to North America. Frequent. Host plants: *Gramineae*, *Carex* and *Juncus* spp.

Genus *Phyllocolpa* Benson, 1960

Phyllocolpa leucapsis (Tischbein, 1846)*: 16-30. 06. 2018, 2 280 m, 1 female, 15-29. 06. 2018, 1 634 m, 1 female. Frequent, Holarctic species. Larvae make galls on leaves of *Salix cinerea*, *S. aurita* and *S. silesiaca*.

Ph. leucosticta (Hartig, 1837): 02-06. 06. 2018, 2 280 m, 1 female. Frequent, West Palaearctic species. Larva makes gall on leaves of *Salix caprea*, *S. aurita* and *S. cinerea*.

Genus *Polynematus* Zhelochovtsev, 1988

Polynematus annulatus (Gimmerthal, 1834)*: 15-29. 06. 2018, 2 465 m, 1 female, 1 male. Larva on *Rumex* spp. Frequent, Holarctic species.

P. punctifrons (Malaise, 1921)*: 02-06. 06. 2018, 2 280 m, 1 male. Sporadic, West Palaearctic species. Hostplant unknown.

Genus *Pristiphora* Latreille, 1810

Pristiphora acutidens Lindqvist, 1977*: 02-06. 06. 2018, 2 280 m, 1 female, 16-30. 06. 2018, 2 280 m, 1 female, 24-28. 07. 2018, 2 280 m, 1 female, 11-25. 08. 2018, 2 280 m, 1 female. Sporadic, Ponto-Caspian-Siberian. Hostplant unknown.

P. aphantoneura (Förster, 1854)*: 02-06. 06. 2018, 2 280 m, 1 female. Sporadic, Palaearctic species. Parthenogenetic. Larva on *Lathyrus pratensis*.

P. appendiculata (Hartig, 1837)*: 16-30. 06. 2018, 2 280 m, 1 male, 30. 06. - 24. 07. 2018, 2 280 m, 1 female. Sporadic, Holarctic species. Host plants: *Ribes* spp. like *Ribes alpinum*, *R. aureum*, *R. nigrum*, *R. rubrum* and *R. uva-crispa*.

P. armata (Thomson, 1863): 02-06. 06. 2018, 2 280 m, 2 males, 30. 06. - 24. 07. 2018, 2 280 m, 13 males, 16-30. 06. 2018, 2 280 m, 4 males, 24-28. 07. 2018, 2 280 m, 2 males, 25. 08 - 08. 09. 2018, 2 280 m, 1 male, 15-29. 06. 2018, 2 465 m, 1 male, 29-06. -13. 07. 2018, 2 465 m, 1 female, 1 male. Frequent, Palaearctic species. Larva on *Crataegus* spp.

P. cincta Newman, 1837*: 30. 06. - 24. 07. 2018, 2 280 m, 5 males, 24-28. 07. 2018, 2 280 m, 1 female, 1 male, 6-30. 06. 2018, 1 male, 13-27. 07. 2018, 1 634 m, 1 male. Larva on *Betula* spp. and *Vaccinium myrtillus*. Sporadic, Holarctic species.

P. leucopus (Hellén, 1948): 19. 05. - 02. 06. 2018, 2 280 m, 1 female, 02-06. 06. 2018, 2 280 m, 1 female, 30. 06. - 24. 07. 2018, 2 280 m, 3 females, 10 males, 16-30. 06. 2018, 2 280 m, 1 female, 12 males, 24-28. 07. 2018, 2 280 m, 9 males, 28. 07.-11. 08. 2018, 2 280 m, 1 male, 11-25. 08. 2018, 2 280 m, 1 male, 25. 08 - 08. 09. 2018, 1 male, 15-29. 06. 2018, 2 465 m, 1 male, 29-06. -13. 07. 2018, 2 465 m, 1 female, 20. 05. - 01. 06. 2018, 1 634 m, 2 males, 01-15. 06. 2018, 1 634 m, 3 males, 15-29. 06. 2018, 1634 m, 1 male, 29. 06. - 13. 07. 2018, 1 634 m, 1 male, 13-27. 07. 2018, 1 634 m, 1 female, 1 male, 27. 07. - 10. 08. 2018, 1 634 m, 1 male. Frequent, West-Palaeartic species. Larva on *Tilia* spp.

P. pallidiventris (Fallén, 1808): 24-28. 07. 2018, 2 280 m, 1 female, 29. 06. - 13. 07. 2018, 1 634 m, 1 female. Frequent. Larva on *Geum*, *Potentilla*, *Rubus* and *Filipendula* spp. Holarctic.

P. thalictri (Kriechbaumer, 1884)*: 20. 04. - 05. 05. 2018, 1 634 m, 1 male. Host plants: *Thalictrum aquilegifolium* and *T. minus*. Sporadic, Palaeartic species.

Genus *Pteronidea* Rohwer, 1911

Pteronidea glutinosae (Cameron, 1882)*: 01-15. 06. 2018, 1 634 m, 1 male. Sporadic, West Palaeartic species. Hostplant: *Alnus* spp.

P. miliaris (Panzer, 1797): 02-06. 06. 2018, 2 280 m, 1 female, 30. 06. - 24. 07. 2018, 2 280 m, 3 males, 16-30. 06. 2018, 2 280 m, 2 males, 24-28. 07. 2018, 8 males, 15-29. 06. 2018, 2 465 m, 1 male. Larva on *Salix aurita* and *S. capreae*. Sporadic, West Palaeartic species.

P. myosotidis (Fabricius, 1804): 24-28. 07. 2018, 2 280 m, 1 male. Common. Larval hosts: *Onobrychis*, *Vicia* and *Trifolium* spp. also *Lathyrus pratensis*. Palaeartic.

P. oligospila (Förster, 1854): 30. 06. - 24. 07. 2018, 2 280 m, 2 females, 1 male, 24-28. 07. 2018, 2 280 m, 1 female, 16-30. 06. 2018, 2 280 m, 1 female. Originally Palaeartic species, introduced globally, now cosmopolitan. Frequent. Host plants: *Salix* spp. It is also reported from *Populus* spp.

P. pavidus (Serville, 1823)*: 15-29. 06. 2018, 2 465 m, 1 male. Larva on *Salix* spp. and *Populus tremula*. Sporadic, West Palaeartic species. Larva on *Salix* spp: *S. cinerea*, *S. caprea* and *Populus* spp: *Populus tremula* and *P. nigra*.

P. ribesii (Scopoli, 1763)* : 02-06. 06. 2018, 2 280 m, 1 male, 16-30. 06. 2018, 2 280 m, 1 male. Host plants: *Ribes rubrum* and *Ribes uva-crispa*. Larvae are gregarious and in last instar stadium change to solitary life way. Frequent, Palaeartic species.

P. sylvestris (Cameron, 1884)* : 13-27. 07. 2018, 1 634 m, 1 female. Sporadic, Palaeartic species. Larva on *Salix caprea*, *S. pentandra* and *S. phylicifolia*.

Genus *Sharliphora* Wong, 1969

Sharliphora nigella (Förster, 1854): 20. 04. - 05. 05. 2018, 1 634 m, 5 females, 8 males. Frequent, West Palaeartic species. Larva on *Picea* spp.

Subfamily - Tenthredininae

Genus *Macrophya* Dahlbom, 1835

Macrophya (Macrophya) hamata ssp. *caucasicola* Mucche, 1969: 01-15. 06. 2018, 1 634 m, 1 male. Frequent, Ponto-Caspian subspecies. Host plant unknown.

Genus *Pachyprotasis* Hartig, 1837

Pachyprotasis rapae (Linné, 1767): 16-30. 06. 2018, 2 280 m, 1 male, 15-29. 06. 2018, 2 465 m, 2 females, 8 males, 29-06. -13. 07. 2018, 2 465 m, 3 females, 2 males. Common. Holarctic species. Host-plants: *Solanum tuberosum*, *Pedicularis palustris*, *Angelica sylvestris*, *Veronica beccabunga*, *Betonica officinalis*, *Corylus avellana*, *Salix caprea*, *Fraxinus excelsior*, *Tussilago farfara*, *Symphoricarpos albus*, *Scrophularia*, *Solanum*, *Solidago virgaurea*, *Verbascum*, *Origanum vulgare*, *Atropa belladonna*, *Sarothamnus*, *Senecio*, *Polygonum*, *Lamium*, *Aspidium*, *Epilobium*, *Hypericum*, *Galeopsis*, *Glechoma*, *Mentha*, *Polystichum*, *Plantago*, *Misopates*, *Veronica*, *Quercus* and *Stachys* spp.

Genus *Tenthredo* Linnaeus, 1758

Tenthredo (Tenthredella) atra Linné, 1758*: 16-30. 06. 2018, 2 280 m, 1 female. Frequent, Palaeartic species. Host plants: *Alchemilla* spp., *Betula* spp., *Cirsium* spp., *Gentiana asclepiadea*, *Lamium* spp., *Silene* spp., *Stachys* spp., *Solanum* spp., *Plantago* spp., *Mentha* spp., *Scabiosa* spp., *Succisa pratensis* and *Rubus idaeus*.

T. (Tenthredella) ferruginea Schrank, 1776: 02-06. 06. 2018, 2 280 m, 1 female, 15-29. 06. 2018, 1634 m, 1 male. Sporadic, Palaeartic species. Host plants: *Alnus glutinosa*, *Atropa belladonna*, *Filipendula* spp., *Geranium* spp., *Ranunculus* spp., *Pteridium aquilinum*, *Rubus* spp., *Salix* spp., *Senecio* spp., *Sorbus aucuparia*, *Spiraea* spp. and *Vaccinium myrtillus*.

T. (Tenthredo) liturata (Mocsáry, 1886): 16-30.

06. 2018, 2 280 m, 1 female, 24-28. 07. 2018, 2 280 m, 1 female; 01-15. 06. 2018, 1 634 m, 1 female. Sporadic, Ponto-Caspian-Anatolian species. Host-plant unknown.

T. (Tenthredella) livida Linné, 1758: 02-06. 06. 2018, 2 280 m, 1 female, 30. 06. - 24. 07. 2018, 2 280 m, 1 male, 16-30. 06. 2018, 2 280 m, 1 female, 15-29. 06. 2018, 2 465 m, 1 female, 1 male. Frequent, Palearctic species. Host plants: *Aruncus dioicus*, *Athyrium* spp., *Corylus avellana*, *Epilobium* spp., *Lamium* spp., *Melampyrum* spp., *Melissa* spp., *Pteridium aquilinum*, *Rosa* spp., *Rubus idaeus*, *Salix* spp., *Sorbus aucuparia*, *Symphoricarpos albus*, *Tilia* spp., *Vaccinium myrtillus* and *Viburnum lantana*. The male specimen belongs to the color variation formerly called var. *clara* Enslin.

T. (Eurogaster) mesomela Linné, 1758: 30. 06. - 24. 07. 2018, 2 280 m, 1 female, 15-29. 06. 2018, 2 465 m, 1 male. Frequent, Palearctic species. Host plants: *Ranunculus repens*, *Plantago media*, *Aruncus sylvestris*, *Senecio* spp., *Persicaria* spp. *Arctium lappa*, *Rubus idaeus*, *Salix caprea*, *Epilobium* and *Rumex* spp.

T. (Elinora) radoszkowskii (André, 1881)* : 24-28. 07. 2018, 1 female; 20. 05. - 01. 06. 2018, 1 634 m, 1 female, 13-27. 07. 2018, 1 634 m, 1 male. Sporadic, Ponto-Caspian-Persian species. Hostplant unknown.

T. (Temuledo) temula Scopoli, 1763: 02-06. 06. 2018, 2 280 m, 2 females. Frequent, Palearctic species. Host plants: *Melampyrum*, *Ligustrum*, *Rosa*, *Sorbus* spp. and *Prunus spinosa*. In the Caucasus, a special colour form lives (the former var. *xanthaspis* Enslin).

Genus *Tenthredopsis* Costa, 1859

Tenthredopsis ornatrix Konow, 1890: 30. 06. - 24. 07. 2018, 2 280 m, 1 male, 15-29. 06. 2018, 2 465 m, 3 males, 29-06. -13. 07. 2018, 2 465 m, 1 female. Ponto-Caspian species. Host plant unknown.

T. tessellata (Klug, 1817)*: 15-29. 06. 2018, 2 465 m, 1 female, 1 male. Frequent, West Palearctic species. Sporadic. Larva on *Deschampsia*, *Dactylis*, *Aira* and *Lolium* spp.

T. viridis Zhelochovtsev, 1941: 02-06. 06. 2018, 2 280 m, 1 male, 20. 05. - 01. 06. 2018, 1 634 m, 1 male. Ponto-Caspian species. Sporadic. Host plant unknown.

Discussion

Species richness

Sixty-four sawfly species were identified from a total of 513 specimens collected in Kintrishi National Park at three different altitudes using Malaise traps. Twenty three species were recorded from Georgia (Sakartvelo) for the first time. This brings the number of Symphyta species recorded in Georgia to 230 [22, 23, 24]. We believe that this number will be double in our future studies at least.

Sawfly density and species richness in correlation with altitudes

In our previous study [2], abundance of sawflies and their species richness were measured at 3 different altitudes, namely 404 meters, 1,020 meters and 1,284 meters. The highest diversity and abundance, 83.1% of sawflies (1,416 specimens), were collected at the altitude of 404 meters (Zeraboseli: Kintrishi River). 172 specimens (10.1%) were collected at 1,020 meters and only 115 specimens (6.8%) in 1,264 meters. In the present study, 140 specimens were captured at 1 640 m, 311 specimens at 2 280 m and 62 specimens at 2 465 m altitude above sea level. Unfortunately, the present study cannot be comparable with the other altitudes, since in this high altitude, we were succeeding to investigate, the whole flight period, while in the lower altitude the period with the highest population density was missing. It is also true for the species richness either.

Frequent species

The most frequent species is *Pristiphora leucopus* (Hellén, 1948) with 54 collected specimens. Other frequent species are *Empria longicornis* (Thomson, 1871) with 47, *Priophorus compressicornis* (Fabricius, 1804) with 42, *Strongylogaster caucasica* Schaposchnikov, 1885 with 26, *Pristiphora armata* (Thomson, 1863) with 25, *Aneugmenus coronatus* (Klug, 1818) with 18, *Allantus cinctus* (Linné, 1758) with 18 and *Nematus lucidus* (Panzer, 1801) with 17 collected specimens. These 8 species make 48% of the total collected material.

Zoogeographical area	Number of species	%
Ponto-Caspian-Persian	1	1.6%
Ponto-Caspian	6	9.3%
Ponto-Caspian-Anatolian	1	1.6%
Ponto-Caspian-Siberian	1	1.6%
West-Palearctic	17	26.5%

Palearctic	24	37.5%
Southern Palearctic	1	1.6%
Holarctic	12	18.7%
Cosmopolitan	1	1.6%

The zoogeographic distribution of the collected sawflies was evaluated (Table 1). Most of the species have wide geographic distribution, i.e. Holarctic, Palearctic, West Palearctic, South Palearctic, Ponto-Caspian-Siberian and Cosmopolitan; their proportion is 87%. The so called characteristic components are the species with limited distribution areas: Ponto-Caspian, Ponto-Caspian-Anatolian and Ponto-Caspian-Persian. These species are: *Strongylogaster caucasica* Schaposchnikov, 1885, *Birka catellata* (Konow, 1900), *Eutomostethus ephippium* ssp. *vopiscus* (Konow, 1899), *Macrophya hamata* ssp. *caucasicola* Muche, 1969, *Tenthredo liturata* (Mocsáry, 1886), *Tenthredo radoszkowskii* (André, 1881), *Tenthredopsis ornatrix* Konow, 1890 and *Tenthredopsis viridis* Zhelochovtsev, 1941. Their proportion is 13%. Similar proportions (12-13%) were experienced during our investigations in the different regions of Caucasus (Japoshvili and Haris, 2022 b, c and d). Some species, like: *Epicenematus montanus* (Zaddach, 1883), *Polynematus punctifrons* (Malaise, 1921), *Tenthredopsis viridis* Zhelochovtsev, 1941, *Nematus wahlbergi* Thomson, 1871 reaching the Southern border of their distribution in this area.

Flight period of sawflies

In the highest altitudes, flight period starts at last decade of May or first decade of June. Soon after, with sudden and strong eruption, the flight period is culminated in the second decade of June. In the sub-alpine region of the Caucasus, it erupts in the last decade of April, similarly to lower altitudes, and probably starts in early March. It collapsed for the last decade of August or first decade of September in the alpine region (above 2 000 m alt.asl.) and for the first decade of August in the sub-alpine region (this data shall be confirmed or refined with further researches). Opposite of the lower altitude [2], in the 2 high altitudes (2 280 and 2 465 m), the annual flight activity consists of only one curve instead of 2 curves model as it figured in Japoshvili and Haris [2]. The culmination point is not emerged, the shape of activity curve flat.

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Fig. 1. Habitats of trap locations in KNP: A – Trap location at 1634 m altitude; B - Trap location at 2280 m altitude; C,D – Trap location at 2465 m altitude.



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New localities of *Montagnea arenaria* (DC.) Zeller in Georgia

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ABSTRACT

Montagnea arenaria (DC.) Zeller one of the rare species in Georgia, was found in a new locality in semi-arid habitats of Kvernakebi low range (Eastern Georgia). The article provides a brief taxonomic description, habitat, ecology, geographic distribution and original illustrations.

Key words: Agaricoid fungi, *Montagnea arenaria*, semi-arid habitats, distribution, Georgia.

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Introduction

Montagnea arenaria (DC.) Zeller is a secutioid gastroid fungus adapted to arid, steppes, desert and semidesert regions and distributed worldwide [1-4]. It is a humus saprotroph, decomposer on organic matter, dead desert plants and grass, sometimes associated with juniper and shrubs [1].

According to Index Fungorum, the genus *Montagnea* belongs to the class Agaricomycetes, order Agaricales and family Agaricaceae. *Montagnea arenaria* (DC.) Zeller was first diagnosed in 1815 by Augustin Pyramus de Candolle, naming it *Agaricus arenarius*. Its current name, recognized by Index Fungorum, was given to it by S. M. Zeller in 1943 [5].

In Georgia, *Montagnea arenaria* was first found in 1955 by I. Nakhutsrishvili [6] in the Vashlovani State Reserve, where it grew in the steppe community, under juniper, in a form of scattered individual specimens. Later, in 1973 and 1988, I. Nakhutsrishvili found the species again in the Vashlovani State

Reserve, in 1969, I. Nakhutsrishvili collected *M. arenaria* in Tusheti, on Mount Samekhe, at 2000 m a.s.l., in the *Artemisia-Bothriochloa* meadow. The species, was last seen in 2008 in the Gareji semi-desert, one carpophore [7]. Until 2021, all specimens were collected only in eastern Georgia, in the Kakheti region. All specimens except one were collected in typical arid and semiarid habitats, steppe formations, at the elevation of 300 - 600 m. Only one sample was taken in Tusheti, an atypical arid habitat, at the 2000 m. *M. arenaria* is recorded at 2000 m in Kazakhstan, on the stepped slopes [8].

The new specimen was found in the Shida Kartli region of eastern Georgia, on the Kvernakebi ridge. The Kvernakebi ridge is characterized by arid habitats, scrub constituted by various hemixerophilic species with predominance of *Paliurus spina-christi* Mill., various steppe communities mostly made up of *Bothriochloa ischaemum* (L.) Keng, semi-desert vegetation dominated by *Artemisia lerchiana* Weber and a number of transitional and mixed communities [9].

Materials & Methods

The study materials were collected in 2021 during a field survey of the Kvernakebi low range. Carpophores and habitats were photographed using Nikon Coolpix L830. Geographic coordinates were recorded by Garmin Etrex 20. After collection, the fruit bodies were put in paper boxes and transferred to the Fungarium. Species were identified and

stored at the Herbarium of the Department of spore-producing plants and fungi of the Institute of Botany of Ilia State University (at the Cryptogam Herbarium, which is a part of the National Herbarium of Georgia, TBI). Melzer's iodine reagent or 5% KOH was used for treatment of small sections of gleba for microscopical examination with Olympus CX31RBSF binocular microscope. Various keys were used for species identification [8, 10,11].

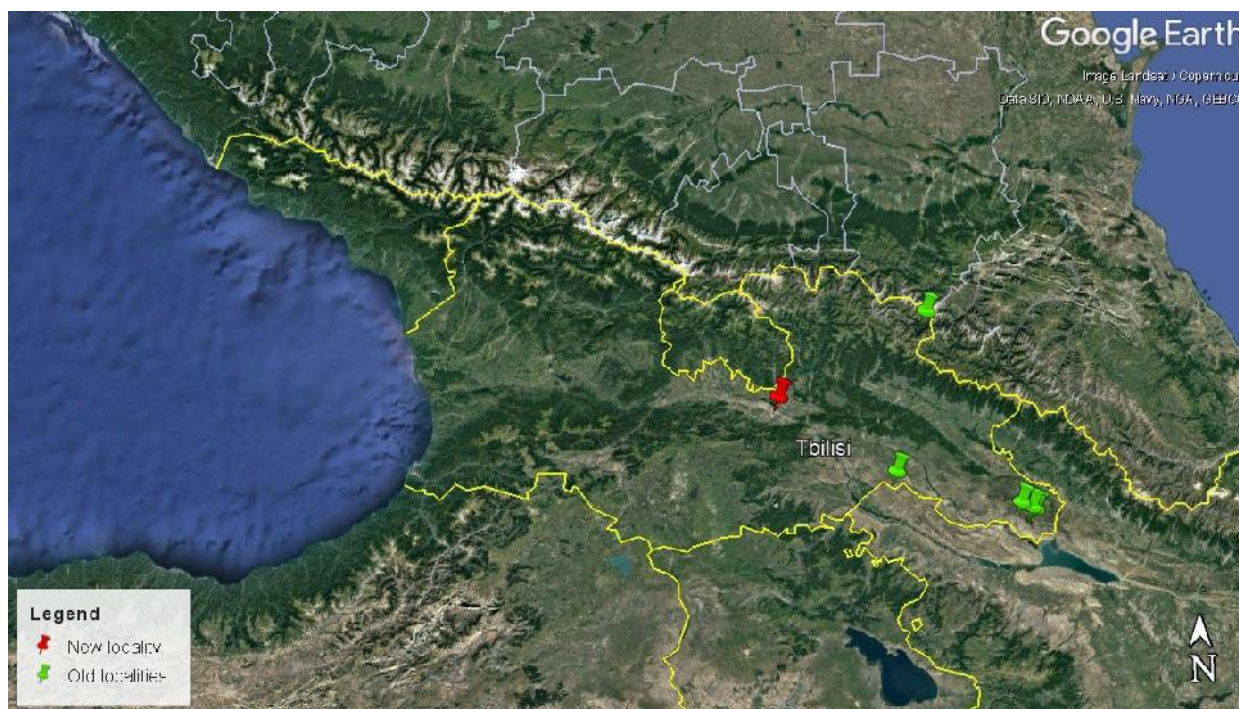


Fig. 1. Distribution of *Montagnea arenaria* in Georgia



Fig. 2. Landscape images from Kvernakebi low range



Fig. 3. Basidiocarps and basidiospores of *Montagnea arenaria*

Results & Discussion

The morphological description of *M. arenaria* is based on a recently collected specimens in Georgia.

***Montagnea arenaria* (DC.) Zeller**, Mycologia 35(4): 418 (1943). – Syn.: *Agaricus arenarius* DC., Flore Franc.6, 45, 1815. – *Montagnites arenarius* (DC.) Morse, Mycologia 40(2): 256 (1948). – *Montagnea delilei* Fr., Fl. Scan.: 339 (1836). – *Montagnites pallasii* Fr., Epicr. syst. mycol. (Upsaliae): 241 (1838). – *Montagnea pallasii* (Fr.) Mont., Annls Sci. Nat., Bot., sér. 2 20: 77 (1843); – *Montagnea arenaria* var. *macrospora* D.A. Reid & Eicker, S. Afr. J. Bot. 57(3): 166 (1991)

Fruiting body semi-subterranean at first, egg-shaped, up to 3 cm across. Mature basidiocarp coprinoid, with a woody stipe. **Stem** 4 – 8 x 0.4 – 0.6 cm, cylindrical, often tapering towards the base, at first whitish, fleshy, soft, full, smooth, later whitish-yellowish, hollow, woody, pitted, fibrous-scaly, at the base of the leg has an elongated whitish-yellowish volva, completely hidden in the substrate, which is often absent with age. Top of stem disc-like cap. **Pileus** 1– 3.5 cm across, grayish, yellowish-brown, slightly depressed, with a dry wrinkled surface, brittle, wavy edges, later the edges crack radially and pitch black plates appear. **Lamellae** free, frequent, wavy, blackish-brown to black, not deliquescent, fragile. **Spores** dark brown (spore powder black), oblong-ovoid, smooth, with a germinal pore, 16–17 x 8–10 µm. (Fig. 3)

Habitat. Humus saprotroph. Grows solitary, scattered, on dry sandy soil on pastures, steppe community. (Fig. 2)

New records. Georgia, Shida Kartli, Kaspi Municipality, Kavtiskhevi Railway Station, Farm, Kvernakebi low range, N41.914582 E44.466362, alt. 530 m, 10 June 2021, leg. Angelina Jorjadze (TBI50225282). (Fig. 1, 2)

Previous collections in Georgia. Kakheti: Akhmeta Municipality, Tusheti, Shenako, Samekhe Mountain, *Artemisia-Bothriochloa* meadow, soil, 07.09.1969, I. Nakhutsrishvili (TBI5009217); Dedoplistskaro Municipality, Vashlovani Strict Nature Reserve, Pantishara Canyon, Lekistskali, steppe community, under juniper, sandy soil, solitary, scattered, 26.04.1955, I. Nakhutsrishvili (TBI5009215); Vashlovani Strict Nature Reserve, Chigont-khevi, *Artemisia-Bothriochloa* meadow, soil, 04.11.1973, I. Nakhutsrishvili (TBI5009218); Vashlovani Strict Nature Reserve, soil, 14-16.04.1988, I. Nakhutsrishvili (TBI5009216; TBI5009219); Sagarejo Municipality, David Gareja, steppe community, sandy soil, 27.08.2008, A. Jorjadze (TBI5009220) [7]. (Fig. 3).

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