

2021

ISSN 1512-1887

# ANNALS OF AGRARIAN SCIENCE



Vol. 19 No. 4

# EDITORIAL BOARD

## Editor-in-Chief :

**T. Urushadze**

*Agricultural University of Georgia, Tbilisi, Georgia*

## Associate Editor:

**H. Hidaka**

*Meisei University, Tokyo, Japan*

## Co-editors:

**A. Babayev**

*Azerbaijan State Agrarian University, Ganja,  
Republic of Azerbaijan*

**J. Dorner**

*Austral University of Chile, Valdivia, Chile*

**O. Nagorniuk**

*Institute of Agroecology and Environment  
Management Kyiv, Ukraine*

**D. Petrosyan**

*National Agrarian University of Armenia,  
Yerevan, Republic of Armenia*

**A. Ploeger**

*University of Kassel, Kassel, Germany*

## Executive Secretary:

**I. Pipia**

*Agricultural University of Georgia, Tbilisi, Georgia*

## Editorial Board Members:

**V. Abrahamyan**

*National Agrarian University of Armenia, Yerevan,  
Republic of Armenia*

**R. Agabeyli**

*Institute of Botany, Azerbaijan National Academy  
of Sciences, Baku, Republic of Azerbaijan*

**U. Alekperov**

*Academy of Public Administration under the  
President of the Republic of Azerbaijan, Baku,  
Republic of Azerbaijan*

**T. Altan**

*Cukurova University, Adana, Turkey*

**M. Babayev**

*Institute of Science and Agrochemistry of ANAS,  
Baku, Republic of Azerbaijan*

**V. Babayev**

*Ganja Agribusiness Association, Ganja,  
Republic of Azerbaijan*

**J. Bech**

*Universitat de Barcelona, Barcelona, Spain*

**R. Beglaryan**

*National Agrarian University of Armenia, Yerevan,  
Republic of Armenia*

**W. Blum**

*University of Natural Resources and Life Sciences,  
Vienna, Austria*

**A. Didebulidze**

*Agricultural University of Georgia, Tbilisi, Georgia*

**P. Dlapa**

*Comenius University in Bratislava, Slovakia*

**K. H. Erdmann**

*Federal Agency for Nature Conservation of  
Germany, Bonn, Germany*

**P. Felix-Henningsen**

*Justus-Liebig University, Giessen, Germany*

**O. Furdychko**

*Institute of Agroecology and Environment  
Management Kyiv, Ukraine*

**M. Gerzabek**

*University of Natural Resources and Life Sciences,  
Vienna, Austria*

**T. Gokturk**

*Artvin Coruh University, Artvin, Turkey*

**R. Gracheva**

*Institute of Geography, Moscow, Russia*

**I. Ibatullin**

*National University of Life and Environment  
Sciences of Ukraine, Kyiv, Ukraine*

**G. Japoshvili**

*Agricultural University of Georgia, Tbilisi, Georgia*

**G. Javakhishvili**

*Georgian Technical University, Tbilisi, Georgia*

**N. Karkashadze**

*Academy of Agricultural Sciences, Tbilisi, Georgia*

**A. Korakhashvili**

*Agricultural University of Georgia, Tbilisi, Georgia*

**V. Kuznetsov**

*Russian Academy of Sciences, Moscow, Russia*

**G. Kvesitadze**

*Georgian National Academy of Sciences,  
Agricultural University of Georgia,  
Tbilisi, Georgia*

**W. Lawrence**

*Organic Research Centre, Hamstead Marshall, UK*

**N. Makarenko**

*National University of Life and Environment  
Sciences of Ukraine, Kyiv, Ukraine*

**G. Mammadov**

*Baku State University, Baku, Republic  
of Azerbaijan*

**Y. Marmaryan**

*National Agrarian University of Armenia, Yerevan,  
Republic of Armenia*

**A. Melikyan**

*National Agrarian University of Armenia, Yerevan,  
Republic of Armenia*

**A. Mohammad**

*Aligarh Muslim University, Aligarh, India*

**L. Montanarella**

*European Commission, Ispra, Italy*

**A. Otte**

*Justus-Liebig-University, Giessen, Germany*

**C. Quezada**

*Universidad de Concepcion-Chile, Chillan, Chile*

**T. Sadunishvili**

*Agricultural University of Georgia,  
Tbilisi, Georgia*

**P. Schmidt**

*Dresden University of Technology,  
Dresden, Germany*

**N. Senesi**

*University of Bari, Bari, Italy*

**K. Stahr**

*University of Hohenheim,  
Stuttgart, Germany*

**W. Stepniewski**

*Lublin University of Technology, Lublin, Poland*

**A. Tarverdyan**

*National Agrarian University of Armenia, Yerevan,  
Republic of Armenia*

**L. Vasa**

*Institute for Foreign Affairs and Trade,  
Budapest, Hungary*

**Y. Vodyanitskii**

*Lomonosov Moscow State University,  
Moscow, Russia*

**H. Vogtmann**

*University of Kassel, Kassel, Germany*

**A. Voskanyan**

*National Agrarian University of Armenia,  
Yerevan, Republic of Armenia*

**V. Yavruyan**

*National Agrarian University of Armenia, Yerevan,  
Republic of Armenia*

# **Annals of Agrarian Science**

**Volume 19, Number 4, December 2021**

# **Aims and Scope**

*The issue is dedicated to the memory of Professor Tengiz (Gizo) Urushadze - the internationally renowned Georgian soil scientist, the member of Georgian National Academy of Sciences, Director of Michail Sabashvili Institute of Soil Science, Agrochemistry and Melioration of Agricultural University of Georgia, the founder of this journal and our beloved Editor-in-Chief.*

# Annals of Agrarian Science

---

Volume 19, Number 4

December 2021

Wasps of the families Ampulicidae, Bembicidae, Bethylidae, Chrysididae, Crabronidae, Evanidae, Gasteruptidae, Heloridae, Pemphredonidae, Pompilidae, Psenidae, Sphecidae and Vespidae (Hymenoptera) of Lagodekhi protected areas, from Georgia (Sakartvelo)

G. Japoshvili, T. Ljubomirov ..... 269-287

Role of the subalpine forest ecosystems (on example of south slope of Great Caucasus)

L. Dolidze, M. Bachilava ..... 288-292

Survey Results of Sanitary-Hygienic Conditions and Landscaping of Urban Forest Parks in the Republic of Armenia

M.H. Galstyan, H.Ya.Sayadyan\*, K.Sh. Sargsyan, Z.H. Hoveyan, I.L.Hakobjanyan ..... 293-297

Determining the Likelihood of Emerging Illegal Dumpsite on the Example of Georgia

N. Dzebisashvili, N. Buachidze, G. Berechikidze ..... 298-304

Farming according to the Grand Defter of Gurjistan Vilayet of 1595

D. Nikolaishvili, D. Sartania, A. Ujmajuridze ..... 305-312

New probiotic produced from spore forming Bacillus cultivated on local agro-industrial wastes substituting antibiotic in broiler nutrition

A. Chkuaseli, A. Chagelishvili, M. Khutsishvili-Maisuradze, V. Elisashvili, T. Khardziani, T. Lashkarashvili, G. Chagelishvili, R. Lapachi, K.Samkurashvili, V.Berikashvili ..... 313-323

Development and laboratory and field testing of new combined insecticides against the resistant and non-resistant populations of the Brown Marmorated Stink Bug (Halyomorpha Halys)

B. A. Chirakadze, N. Mitagvaria, N. Lomidze, M. Ambokadze, A. Gigineishvili, C. Z. Buachidze, T. Chichua, A. Jishiashvili, K. Chigogidze, Z. Chekurashvili, Z. Lipartia, D. N. Meskhi, L. Zazadze ..... 324-345

Arsenic contamination in Racha and Lower Svaneti district of Georgia, consequences and remediation strategies

E. Bakradze, T. Butkhuzi, S. Khmiadashvili, E. Bunin, K. Didebulidze, A. Gujabidze, N. Dumbadze, T. Glurjidze, A. Giorgadze ..... 346-353

Bover-Ge – mycopesticides for the control of Brown marmorated stink bug - <i>Halyomorpha halys</i> (Stål) (Hemiptera: Pentatomidae)	
M. Burjanadze, N. Kharabadze, M. Arjevanidze .....	354-359
Microorganisms and Plants as Tools for Phytoremediation of Soil Polluted with Different Forms of Arsenic	
N. Asatiani, E. Bakradze, T. Butkhuzic, K. Didebulidze, A. Gujabidze, T. Kartvelishvilia, G. Khatishvili, S. Khmiadashvili, M. Kurashvili*, M. Pruidze, D. Razmadze, N. Sapojnikova, T. Varazic.....	360-363
Estimation of biomass and carbon stock of components of subalpine birch forest of Georgia	
G. Vachnadze*, Z. Tiginashvili*, G. Tsereteli**, B. Aptsiauri* .....	364-376
Effectiveness Of Pest Management Options For Management Of Brown Marmorated Stink Bug <i>Halyomorpha Halys</i> (Hemiptera: Pentatomidae) In Hazelnut Orchards In The Republic Of Georgia	
M. Murvanidze <sup>1</sup> , G. Krawczyk <sup>2</sup> , N. Inasaridze <sup>3</sup> , M. Kodua <sup>4</sup> , N. Samsonidze <sup>3</sup> , E. Alimbarashvili <sup>3</sup> , D. Lipartia <sup>4</sup> , R. Shonia <sup>5</sup> , N. Archvadze <sup>3</sup> .....	377-391
Aromas of Grape and Wine	
I. Pipia, T. Nozadze.....	392-398



## Annals of Agrarian Science

Journal homepage: <http://journals.org.ge/index.php>



### Wasps of the families Ampulicidae, Bembicidae, Bethylidae, Chrysididae, Crabronidae, Evanidae, Gasteruptidae, Heloridae, Pemphredonidae, Pompilidae, Psenidae, Sphecidae and Vespidae (Hymenoptera) of Lagodekhi protected areas, from Georgia (Sakartvelo)

G. Japoshvili,<sup>a,b\*</sup> T. Ljubomirov<sup>c</sup>

<sup>a</sup>*Invertebrate Research Center, Tetrtsklebi, Telavi, e-mail: [giorgij70@yahoo.com](mailto:giorgij70@yahoo.com)*

<sup>b</sup>*Institute of Entomology, Agricultural University of Georgia, e-mail: [g.japoshvili@agruni.edu.ge](mailto:g.japoshvili@agruni.edu.ge)*

<sup>c</sup>*Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1, Tzar Osvoboditel boulevard, Sofia, 1000, Bulgaria, e-mail: [toshkoljubomirov@gmail.com](mailto:toshkoljubomirov@gmail.com)*

Received: 01 September 2021; Accepted: 18 October 2021

#### ABSTRACT

The wasp species composition of 13 families (Ampulicidae, Bembicidae, Bethylidae, Chrysididae, Crabronidae, Evanidae, Gasteruptidae, Heloridae, Pemphredonidae, Pompilidae, Psenidae, Sphecidae and Vespidae) was studied in the Lagodekhi Protected Areas. Collections were performed from April to November 2014. The collection method included the use of flight intercept trapping by Malaise traps. In total, 1797 individuals belonging to 94 species were collected. For the sake of outlining the newly established taxa for the country, a full reference to the previous reports on thirteen families at species level for Georgia is made. Three families, 14 genera and 57 species are new records for Georgia (Sakartvelo). The study underscores the importance of including the protected areas studied in the conservation of wasp diversity and in addition, suggests that insect diversity should be studied separately, as it does not necessarily follow the same patterns as other groups of organisms (e. g. vertebrates or plants).

**Keywords:** Insects, Malaise trap, new records, biodiversity.

\*Corresponding Author: George Japoshvili, E-mail address: [g.japoshvili@agruni.edu.ge](mailto:g.japoshvili@agruni.edu.ge)

#### Introduction

Biodiversity is a crucial element in national and international legislation for the selection of territories with conservation status. A common practice, worldwide, is to limit the knowledge of the biodiversity of most protected areas, focusing primarily on plants and vertebrates. Thus, the most noticeable gap in knowledge is with respect to arthropod and in particular, to insect diversity. The vast majority of the almost 17 000 newly described species per year belongs to insects [1]. More than 1.3 million species have been identified so far. Considering this large diversity of insects and the

current rate of species loss [2,3], the identification of areas with great entomological diversity and with an abundance of endemic and/or rare species has high priority [4-6].

The Lagodekhi Protected Areas (LPA) is located on the southern slopes in the eastern part of the Greater Caucasus, the major mountain range of the Caucasus Mountain, within the northeastern limits of Georgia (figure 1). The uniqueness of the landscape of LPA, represented by a series of gorges on the mountain slope of the Greater Caucasus extending from 590 to 3 500 meters above sea level justified the creation of the protected area back in

1912 [7]. The area of the protected territory being initially of 3 500 ha increased through the time as follows: at 10 000 ha in 1935, at 14 000 ha in 1970, at 17 818 ha in 1990 until recently at a total area of 24 451 ha.

The knowledge of the wasp fauna of the LPA is fragmentary, but it dates back from the very end of XIX century. It was the Polish explorer and naturalist Ludwik Młokosiewicz who collected widely and assiduously insects from the Lagodekhi gorge at that time. It is generally known that he supplied foreign museums with botanical and zoological material gathered by himself in Lagodekhi where he stayed between 1853 and 1860 and between 1880 and 1909. Certain material of Hymenoptera gathered by Młokosiewicz in Lagodekhi had been studied at that time mostly by Octavius Radszkowsky, Ferdinand Morawitz and Andrey Semenov. As a result, a number of new wasp taxa had been described (*Chrysis analis caucasicola*; *Chrysis glycera*; *Chrysis lagodechii*; *Chrysis Sappho*; *Chrysis verae*; *Euchrum gemma*; *Psen caucasicus* among others). Unfortunately, at that period, it was not customary to label all or any individual specimens with full details of places, dates and/or collectors. Thus, much of the material gathered by Młokosiewicz had left obscure regarding the strict locations and data of origin. Due to the poor labelling, very frequently in shape of “Caucasus” or “Mlok.”, or “1893” with no plain evidence that they come from Lagodekhi these newly described or just established wasps now are considered to come from unknown place in Caucasus.

## Material and Methods

### *Study area, sampling and specimen depositories*

The study was conducted in the Kudigora mountain range on elevational transect located in the central part of Lagodekhi Protected Areas, Georgia. The transect covered a wide variety of different habitat types both of the Managed Reserve as well as the Strict Nature Reserve (figure 1). The climate of Lagodekhi district is considered as a humid subtropical climate, with significant rainfall even in the driest month. The climate here is classified as Cfa by the Köppen-Geiger system [8].

Following the standing point that the most effective means of obtaining specimens, and temporal and geographic distributional data,

particularly for active flying insects, is the use of passive collecting methods we used flight interception traps (in particular, the Malaise traps) to collect the material for study. Seven Malaise traps were located along the transect at the following points:

H1: mountain low zone – mixed mountain forest with dominant *Fageto-Carpinetum-Festucosum* (41°51.149'N; 46° 17.266'E; 666 m);

H2: mountain middle zone – mixed mountain forest with dominant *Fagetum-Nudum* (N41°51.351'N; 46°17.564'E 847 m);

H3: mountain middle zone – mixed mountain forest, with dominant *Fageto-Galiosum* (41°52.288'N; 46°18.692'E; 1351 m);

H4: mountain upper zone – broad-leaved upper montane forest, with dominant *Fagetum-Acereto-Rubosum* (41°52.964'N; 46°19.311'E; 1841 m);

H5: mountain upper zone – broad-leaved upper montane forest, with dominant *Acereto-Rubosum* (41°53.135'N; 46°19.447'E; 1902 m);

H6: subalpine zone – with dominant *Juniperetum*, (41°53.883'N; 46°20.033'E; 2230 m);

H7: alpine zone – mainly grassland with dominant *Alchemilletum* (41°54.371'N; 46°20.004'E; 2559 m alt. (see figure 1).

In forest areas the traps were positioned at small naturally open areas/forest edges with dense herbaceous vegetation. The sampling began on April 2<sup>nd</sup> 2014 and lasted in November 7<sup>th</sup> 2014, although in alpine and subalpine areas collecting began later (for the subalpine area the alpine area in May 5<sup>th</sup> and 23<sup>rd</sup>, respectively) and completed earlier (in October), due to climate conditions and altitude. Containers were filled with 80% ethanol, material was collected every 10 (±2) days and placed at first in 96% ethanol, then it was sorted, dried and pinned up. All wasp specimens collected were stored in alcohol and identified to the species levels. Study design and collecting was conducted by second author.

### *Recording standards for previously published records for Georgia*

In order to highlight the new species of the respective families for the fauna of Georgia, we have made a full reference to the previous reports on them. It is presented into ten tables concerning those families being previously recorded for the territory of Georgia. The tables were developed from original articles and do not include any web-based lists or articles giving generic names only for

the respective wasp family. An important element in the information into the tables is the quotation of the strict locations (some in Cyrillic) according to how they are mentioned in the original source. The wide range of publications on Hymenoptera from the Caucasus region often provide distribution ranges rather than explicit local records. Therefore, the presence or absence of certain species in Georgia is not always clear. In the absence of other explicit literature records the following standard was adopted for interpreting distribution ranges: species with data entries like “Caucase”, “Caucasus”, “Кавказусбеbiet”, “Transkaukasus”, “Закавказья”, “Кавказкое побережье Черного моря” were treated as unrecorded for Georgia because Georgia merely is a part of Caucasus Mountains and East Black Sea coast; in addition, it is well generally known that abundant material gathered in the past being simply published as coming from Caucasus is actually coming from Azerbaijan (e. g. from “Helenendorf”). Taxa pointed out solely with “Georgia” or “Грузия” for their range only, also are avoided because their inclusion would in many cases have been subjective (they are not supported by strict locations); thus, it is not possible to incorporate them for Georgia meaningfully. New recorded families, genera and species for Georgia are marked with an asterisk into the text.

#### **Data analysis**

We chose this ecotone in order to increase efficiency of sampling species associated exclusively with forests and meadows. At each location, a single Malaise trap was set up from early spring to the end of the vegetation season to collect Encyrtidae. Samples we collected every 10 days resulted in a total of 1080 trap-days, with 190 trap-days at the lowest (665 m) and 130 trap-days at the highest elevation (2559 m) [9].

In each plot, we assessed the richness of vascular plants using total counts in four randomly located 10×10 m<sup>2</sup> plots within an area of 2500m<sup>2</sup> around each Malaise trap. Plant inventories were performed in May, June, and September. The Hymenopterans were identified mostly according original descriptions, redescription or diagnosis of the species. All identification except Vespidae was done by first author. Members of the family Vespidae are kindly identified by Josef Gusenleitner.

## **Results**

During the seven months of the study 1797 Hymenoptera Aculeata specimens were collected, belonging to 94 species, 46 genera, and 13 families. The hymenopteran families represented are as follows: Heloridae, Evaniidae, Gasteruptiidae, Bethyridae, Chrysididae, Pompilidae, Ampulicidae, Bembicidae, Crabronidae, Pemphredonidae, Psenidae, Sphecidae and Vespidae.

### **Family Heloridae\***

The family Heloridae embraces proctotrupoid wasps which develop as solitary larval endoparasitoids in neuropterans of the family Chrysopidae; adults emerge from the host cocoon. The single genus in the family, *Helorus* Latreille, 1802 is represented by 13 species [10]. Two species are established in LPA.

#### **Genus *Helorus* Latreille, 1802\***

*Helorus anomalipes* (Panzer, 1798)\*

Material: H3: 1♀1♂, 25.06-5.07.2014.

*H. ruficornis* A. Förster, 1856\*

Material: H1: 1♂, 4-14.06.2014; 1♀, 5-15.08; H3: 113♂♂, 25.06-5.07.2014; 5♀♀, 98♂♂, 5-15.07.2014.

#### **Species previously recorded from Georgia:**

There are no previous records of family Heloridae for Georgia.

### **Family Evaniidae**

The Evaniidae (ensign wasps) is a specialized group of parasitoids in cockroach oothecae. Twenty genera are known in the group with about 430 species. One species is established in LPA.

#### **Genus *Brachygaster* Leach, 18153.**

*Brachygaster minuta* (Oliver, 1791)

Material: H1: 1♀, 5-15.07.2014; H3: 8♂♂, 25.06-5.07.2014; 18♂♂, 5-15.07.2014; 1♀9♂♂, 25.07-5.08.2014; 5♂♂, 25.08-4.09.2014.

#### **Species previously recorded from Georgia:**

Two insign wasp species have been reported previously from Georgia: *Brachygaster minuta* (Oliver, 1791) recorded from Akhaltsikhe, Sapari, Borjomi, Batumi, Mtsvane Kontskhi, Lagodekhi, Pitsundo-Miussersa Nature Reserve and *Prosevania fuscipes* (Illiger, 1807) recorded from Gulripsh, Sukhumi, Pitsunda [11].

## Family Gasteruptiidae\*

The Gasteruptiidae (carrot wasps) are medium-sized family with nearly worldwide distribution which contain about 550 described species. Carrot wasps, in their larval stage are predators on eggs and larvae of wild bees; once after the wild bee larva being consumed, the carrot wasp larva continues with the stored food in the nest. Two species are established in LPA.

### Genus *Gasteruption* Latreille, 1796\*

*Gasteruption assectator* (Linnaeus, 1758)\*

Material: H5: 1♀, 15-25.07.2014.

*G. diversipes* (Abeille de Perrin, 1879)\*

Material: H1: 1♀, 15-25.06.2014.

Species previously recorded from Georgia:

*Gasteruption merceti* Kieffer, 1904 has been mentioned for Lagodekhi under the name “*Gasteruption pyrenaicum*” and *Gasteruption caucasicum* (Guérin-Méneville, 1844) has been mentioned for Abasumani under the name “*Gasteruption pedemontanum*” [82].

## Family Bethylidae\*

The family Bethylidae (flat wasps) is a moderately large group of about 2 920 described species [12]. Bethylids are recognized mainly as gregarious ectoparasitoids developing on larvae of Coleoptera and Lepidoptera. Five species of flat wasps are known in LPA.

### Genus *Bethylus* Latreille, 1802\*

*Bethylus fuscicornis* (Jurine, 1807)\*

Material: H3: 1♀, 5-15.05.2014; 1♀, 15-25.07.2014.

### Genus *Epyris* Westwood, 1874\*

*Epyris bilineatus* Thomson, 1862\*

Material: H2: 1♂, 15-25.08.2014; 1♂, 25.08-4.09.2014; H3: 1♀, 25.08-4.09.2014.

*E. inermis* Kieffer, 1906\*

Material: H1: 1♀, 5-15.08.2014; H3: 1♀, 4-14.06.2014; 1♀, 5-15.08.2014; H4: 1♀, 15-25.07.2014; 1♀, 5-15.08.2014.

### Genus *Laelius* Ashmead, 1893\*

*Laelius fulvipes* Kieffer, 1906\*

Material: H1: 1♂, 4-14.09.2014.

### Genus *Pseudisobrachium* Kieffer, 1904\*

*Pseudisobrachium subcyaneum* (Halyday, 1838)\*

Material: H2: 6♂♂, 25.08-4.09.2014; 22♂♂, 4-14.09.2014; 1♂, 15-27.09.2014; 9♂♂, 27.09-6.10.2014.

### Species previously recorded from Georgia:

There are no previous records of family Bethylidae for Georgia.

## Family Chrysididae

The family Chrysididae (cuckoo wasps) contains more than 3 200 species worldwide. Cuckoo wasps are parasitoids of the eggs or of the mature larvae or are cleptoparasites in the nests of various insects from the orders Phasmatodea, Lepidoptera and Hymenoptera.

Chrysididae are one of the families of Hymenoptera with high number of species reported from Georgia. The group of cuckoo wasps is one of the earliest detected Hymenoptera Aculeata in that country [13]. A notable example for a large species spectrum at local level is the posthumous paper of Andrey Petrovich Semenov [14], where he described 16 new species of Chrysididae from Georgia. The previous studies of the cuckoo wasps provide up to now 54 established species. Eight species were recorded during survey LPA.

### Genus *Chrysis* Linnaeus, 1767

*Chrysis fasciata* Olivier, 1790\*

Material: H1: 1♀, 24.05-4.06.2014; 1♀, 25.06-5.07.2014; H2: 1♀, 4-14.06.2014; 1♀, 5-15.07.2014; 1♀, 15-25.07.2014.

*C. ignita* (Linnaeus, 1758)

Material: H1: 2♀♀, 2-12.04.2014; 3♀♀, 12-23.04.2014; 3♀♀, 23.04-4.05.2014; 3♀♀, 5-15.05.2014; 1♀, 15-24.05.2014; 1♀, 24.05-4.06.2014; 1♀, 15-25.06.2014; H2: 1♀, 12-23.04.2014; 2♀♀, 4-14.06.2014; 2♀♀, 4-14.09.2014; H3: 3♀♀, 5-15.05.2014; 4♀♀, 15-24.05.2014; 1♀, 24.05-4.06.2014; 1♀, 25.06-5.07.2014; 2♀♀, 5-15.08.2014; 1♀♀, 25.08-4.09.2014; 2♀♀, 4-14.09.2014; H4: 1♀, 15-25.07.2014; H5: 1♀, 25.06-5.07.2014; H5: 3♀♀, 5-15.08.2014; 1♀, 25.08-4.09.2014; H6: 1♀, 24.05-4.06.2014.

*C. hungarica* Scopoli, 1770\*

Material: H2: 1♀, 25.08-4.09.2014.

### Genus *Cleptes* Latreille, 1802

*Cleptes semiauratus* (Linnaeus, 1761)\*

Material: H2: 1♂, 4-14.06.2014; H3: 2♂♂, 4-14.06.2014; 4♂♂, 15-25.06.2014; 1♂, 25.06-5.07.2014; 2♂♂, 5-15.07.2014; H6: 1♂, 15-25.06.2014.

### Genus *Pseudomalus* Ashmead, 1902

*Pseudomalus auratus* (Linnaeus, 1758)

Material: H1: 1♀, 5-15.05.2014; H3: 1♀  
4-14.06.2014; 1♀, 15-25.06.2014.

*P. pusillus* (Fabricius, 1804)

Material: H1: 1♀, 5-15.05.2014; 1♀, 24.05-  
4.06.2014; 1♂, 4-14.09.2014.

*P. violaceus* (Scopoli, 1763)

Material: H3: 1♀, 23.04-4.05.2014.

### Genus *Trichrysis* Lichteinsein, 1876

*Trichrysis cyanea* (Linnaeus, 1758)

Material: H1: 1♀, 23.04-4.05.2014; 2♀♀,  
5-15.05.2014; 10♀♀, 15-24.05.2014; 8♀♀,  
24.05-4.06.2014; 4♀♀, 15-25.06.2014; 4♀♀,  
25.06-5.07.2014; 5♀♀, 5-15.07.2014; 2♀♀,  
15-25.07.2014; 1♀, 25.07-5.08.2014; 2♀♀,  
5-15.08.2014; 3♀♀, 4-14.09.2014; H2: 2♀♀,  
5-15.05.2014; 1♀, 15-24.05.2014; 4♀♀, 24.05-  
4.06.2014; 1♀, 4-14.06.2014; 4♀♀, 15-25.06.2014;  
3♀♀, 5-15.07.2014; 5♀♀, 15-25.07.2014; 1♀,  
15-25.08.2014; 5♀♀, 25.08-4.09.2014; 4♀♀,  
4-14.09.2014; 2♀♀, 15-27.09.2014; H3: 1♂,  
5-15.07.2014; 2♀♀, 15-24.05.2014; 4♀♀, 24.05-  
4.06.2014; 1♀, 4-14.06.2014; 3♀♀, 15-25.06.2014;  
1♀, 25.06-5.07.2014; 2♀♀, 5-15.07.2014; 3♀♀,  
25.07-5.08.2014; 1♀, 5-15.08.2014; 9♀♀,  
25.08-4.09.2014; 2♀♀, 4-14.09.2014; H4 1♀,  
5-15.08.2014; H5: 1♀, 25.06-5.07.2014; 2♀♀,  
15-25.07.2014; 1♀, 5-15.08.2014; H7: 1♀, 25.07-  
5.08.2014.

### *Species previously recorded from Georgia:*

*Chrysis albanica* W. Trautmann, 1927, recorded  
from Kojori [15,16]; *C. angustifrons* Abeille de  
Perrin, 1878, recorded from Lagodekhi [14,17],  
Kojori [14]; *C. bicolor* Lepeletier de Saint-Fargeau,  
1806, recorded from Kojori [16]; *C. caucasicola*  
Balthasar, 1953, recorded from Kojori and  
Lagodekhi [14]; *C. cingulicornis* A. Förster, 1853,  
recorded from Lagodekhi [16]; *C. coeruleiventris*  
Abeille de Perrin, 1878, recorded from Lagodekhi  
[14] (Semenov-Tian-Shanskij 1967); *C. comparata*  
Lepeletier de Saint-Fargeau, 1806, recorded from  
Gudauri, Pasanauri [13]; *C. distincta* Mocsáry,  
1887, recorded from Alazani valley [16]; *C. eldari*  
[18], recorded from Eldari [18]; *C. erivanesis*  
Radoszkowski, 1879, recorded from Kazbegi  
[17,19]; *C. frivaldszkyi* Mocsáry, 1882, recorded  
from Tbilisi [16]; *C. glasunovi* Semenov, 1967,  
recorded from Lagodekhi [16]; *C. graelsii* Guérin-  
Méneville, 1842, recorded from Tbilisi [19]; *C.*  
*ignita* (Linnaeus, 1758), recorded from Borjomi  
[20]; *C. indigotea* Dufour & Perris, 1840, recorded  
from Telavi [14]; *C. kolazyi* Mocsáry, 1889, recorded

from Tbilisi [13]; *C. lyda* Rosa, 2017, recorded  
from Pitsunda [15]; *C. mirabilis* Radoszkowski,  
1877, recorded from Lagodekhi [14], Khando,  
Akhalkalaki [16]; *C. mokrousovi* Rosa, 2017,  
recorded from Akhali Atoni [15]; *C. mutabilis* Du  
Buysson, 1887, recorded from Lagodekhi [16]; *C.*  
*placida* Mocsáry, 1879, recorded from Lagodekhi  
[21]; *C. rutilans* Olivier, 1790, recorded from  
Tbilisi [22]; *C. soror* Dahlbom, 1854, recorded from  
Tbilisi [14]; *C. subsinuata* Marquet, 1879, recorded  
from Tbilisi [16]; *Chrysuria cuprea* (Rossi, 1790),  
recorded from Abkhazeti [23] (Radoszkowsky  
1866); *C. dichroa* (Dahlbom, 1854), recorded from  
Tbilisi [20] (Radde 1899); *C. ignifrons* (Brullé,  
1833), recorded from Borjomi [14] (Semenov-Tian-  
Shanskij 1967); *C. lampa* (Semenov-Tian-Shanskij,  
1967), recorded from Lagodekhi, Tbilisi [14]; *C.*  
*nikolaji* Rosa, 2017, recorded from Pitsunda [14];  
*C. radians* (Harris, 1776), recorded from Tbilisi  
[20]; *C. simplex* (Dahlbom, 1854), recorded from  
Lomis mta [20]; *C. varicornis* (Spinola, 1838),  
recorded from Tbilisi [19]; *Cleptes ignidorsum*  
Móczár, 1998, recorded from Lagodekhi [24];  
*C. radoszkowskii* Mocsáry, 1889, recorded from  
Lagodekhi [22]; *C. splendidus* (Fabricius, 1794),  
recorded from Kojori [16,22,25]; *Elampus pliginskii*  
(Semenov-Tian-Shanskij, 1967), recorded from  
Tbilisi surroundings [14]; *E. spina* (Lepeletier de  
Saint-Fargeau, 1806), recorded from Lagodekhi  
[16]; *Hedychridium dzhanelidzei* Semenov-Tian-  
Shanskij, 1967, recorded from Tbilisi [16], Tbilisi  
surroundings [14]; *H. gemma* (Semenov-Tian-  
Shanskij, 1967), recorded from Lagodekhi [14];  
*H. roseum* (Rossi, 1790), recorded from Lagodekhi  
[14]; *H. satunini* Semenov-Tian-Shanskij, 1967,  
recorded from Tbilisi surroundings [14]; *H. satunini*  
Semenov-Tian-Shanskij, 1967, recorded from  
Tbilisi [16]; *Hedychrum luculentum* A. Förster,  
1853, recorded from Kojori, Tbilisi surroundings  
[14]; *H. gerstaeckeri* Chevriér, 1869, recorded from  
Kojori, Lagodekhi [16]; *H. rutilans* Dahlbom, 1854,  
recorded from Lagodekhi [16]; *H. virens* Dahlbom,  
1854, recorded from Lagodekhi [16]; *Holopyga*  
*raziborskii* Semenov-Tian-Shanskij, 1967, recorded  
from Kojori, Tbilisi, Lagodekhi [14]; *Omalus*  
*aeneus* (Fabricius, 1787), recorded from Gomi  
(Gori), Lagodekhi [16]; *Philoctetes bogdanovii*  
(Radoszkowski, 1877), recorded from Tbilisi [20];  
*Ph. kuznetsovi* (Semenov, 1932), recorded from  
Kojori [26]; *Pseudomalus auratus* (Linnaeus,  
1758), recorded from Tbilisi [20], Borjomi [27];  
*P. pusillus* (Fabricius, 1804), recorded from Tbilisi

[20]; *P. violaceus* (Scopoli, 1763), recorded from Atskuri [16](Rosa et al. 2019); *Stilbum calens* (Fabricius, 1781), recorded from Samegrelo, Tetra aragvi shores, Kazbegi mountain [23]; *Trichrysis cyanea* (Linnaeus, 1758), recorded from Mtirala National Park [28].

## Family Pompilidae

Family Pompilidae (spider wasps) is a cosmopolitan (predominantly tropical) group of wasps containing about 4 440 described species. Spider wasps are solitary predators on arachnids (predominantly Araneae); the spider wasp larva develops upon a single paralyzed prey in a concealed environment. Previous studies (from four sources only) of the spider wasps provide up to now 20 species to occur in Georgia. Twenty-five spider wasp species are established in LPA.

### Genus *Agenioideus* Ashmead, 1902

*Agenioideus cincitellus* (Spinola, 1807)\*

Material: H1: 3♂♂, 15-24.05.2014; 21♂♂, 23.05.-4.06.2014; 7♂♂, 4-14.06.2014; 4♂♂, 15-25.06.2014; 4♂♂, 25.06-5.07.2014; 2♂♂, 15-25.07.2014; 6♂♂, 25.07-5.08.2014; H2: 10♂♂, 25.05-4.06.2014; 9♂♂, 15-25.07.2014; 10♂♂, 15-25.08.2014; 17♂♂, 15-25.06.2014; 15♂♂, 5-15.07.2014; 1♂, 15-25.06.2014; 5♂♂, 4-14.06.2014; 2♂♂, 25.08-4.09.2014; H3: 1♂, 23.05-4.06.2014; H4: 7♂♂, 5-15.08.2014.

*A. sericeus* (Vander Linden, 1827)\*

Material: H2: 1♀, 25.05-4.06.2014; 2♀♀, 2♂♂, 4-14.06.2014; 1♂, 15-25.06.2014; 1♀, 5-15.07.2014; 1♀, 5-15.08.2014; 1♂, 15-25.08.2014.

*A. usurarius* (Tournier, 1889)\*

Material: H2: 1♂ 15-25.06.2014.

### Genus *Anoplius* Dufour, 1834

*Anoplius nigerrimus* (Scopoli, 1763)\*

Material: H1: 2♂♂, 5-15.05.2014; 5♂♂, 15-25.05.2014; 2♀♀, 11♂♂, 23.05-4.06.2014; 2♂♂, 4-14.06.2014; 1♀, 3♂♂, 15-25.06.2014; 2♂♂, 15-25.07.2014; 1♀, 25.07-5.08.2014; 2♀♀, 2♂♂, 15-25.08.2014; H2: 10♂♂, 15-25.05.2014; 1♀, 23♂♂, 25.05-4.06.2014; 6♂♂, 4-14.06.2014; 2♀♀, 4♂♂, 15-25.06.2014; 1♀, 15-25.07.2014; 1♂, 4-14.09.2014; H3: 1♂, 15-25.05.2014.

*A. viaticus* (Linnaeus, 1758)

Material: H2: 1♀, 12-23.04.2014.

### Genus *Arachnospila* Kincaid, 1900\*

*Arachnospila alpivaga* (Kohl, 1888)\*

Material: H2: 1♂, 25.05.-4.06.2014; 1♂, 4-14.06.2014; 1♂, 15-25.07.2014.

*A. fuscomarginata* (Thomson, 1870)\*

Material: H1: 1♂, 5-15.05.2014; 1♂, 25.05.-4.06.2014; 1♀, 4-14.06.2014; 1♀, 6♂♂, 15-25.06.2014; 1♀, 25.06-5.07.2014; 1♀, 25.07-5.08.2014; H2: 1♂, 25.05-4.06.2014; 10♂♂, 4-14.06.2014; 11♂♂, 15-25.06.2014; H3: 1♀, 4-14.06.2014; 1♀, 15-25.06.2014; 1♀, 4♂♂, 25.06-5.07.2014; 1♀, 6♂♂, 5-15.07.2014; H5: 1♂, 15-27.09.2014.

*A. sogdianoides* (Wolf, 1964)\*

Material: H1: 2♂♂, 4-14.06.2014.

### Genus *Auplopus* Spinola, 1841

*Auplopus carbonarius* (Scopoli, 1763)

Material: H1: 1♀, 23.04-4.05.2014; 2♀♀, 5-15.05.2014; 3♂♂, 15-24.05.2014; 1♀, 11♂♂, 24.05.-4.06.2014; 1♀, 8♂, 4-14.06.2014; 1♀, 2♂, 15-25.06.2014; 2♀♀, 25.06-5.07.2014; 2♂♂, 5-15.07.2014; 1♂, 25.07-5.08.2014; 1♀, 15-27.09.2014; H2: 5♀♀, 5-15.05.2014; 1♂, 15-25.05.2014; 3♀♀, 5♂♂, 25.05.-4.06.2014; 4♀♀, 2♂♂, 4-14.06.2014; 1♀, 5-15.07.2014; 3♀♀, 15-25.08.2014; 1♀, 25.08.-4.09.2014; 1♀, 15-27.09.2014; H3: 1♂, 23.05.-4.06.2014, 3♀♀, 25.07-5.08.2014; 1♀, 5-15.08.2014; 4♀♀, 25.08-4.09.2014; H4: 1♀, 5-15.07.2014.

### Genus *Caliadurgus* Pate, 1946\*

*Caliadurgus fasciatellus* (Spinola, 1807)\*

Material: H1: 1♀, 4-14.06.2014; H2: 1♀, 5-15.05.2014; 1♀, 1♂, 15-25.05.2014; 1♀, 1♂, 25.05.-4.06.2014; 1♀, 4-14.06.2014; 1♀, 15-25.06.2014; 1♀, 15-25.07.2014; 1♂, 15-25.08.2014; 2♀♀, 4-14.09.2014; 1♀, 15-27.09.2014.

### Genus *Ceropales* Latreille, 1796

*Ceropales pygmaea* Kohl, 1880\*

Material: H1: 1♀, 15-25.07.2014; 1♀, 25.07.-5.08.2014.

### Genus *Cryptocheilus* Panzer, 1806

*Cryptocheilus freydessneri* (Kohl, 1883)\*

Material: H1: 1♀, 25.07-5.08.2014; H2: 2♂♂, 15-25.06.2014; 4♀♀, 15-25.07.2014; 1♀, 15-25.08.2014; H4: 1♀, 5-15.08.2014.

*C. notatus* (Rossi, 1792)

Material: H1: 1♀, 4-14.06.2014; H2: 1♀, 15-25.06.2014; 2♀♀, 5-15.07.2014; 2♀♀, 15-25.07.2014.

### Genus *Deuteragenia* Sustera, 1912\*

*Deuteragenia austriaca* Wolf, 1964\*

Material: H3: 1♀, 15-25.06.2014; 1♀, 25.06-5.07.2014.

*D. bifasciata* (Geoffroy, 1785)\*

Material: H1: 1♀, 5-15.05.2014; 1♂, 23.05.-4.06.2014; H2: 2♀♀, 15-25.05.2014; 1♀, 25.05.-

4.06.2014; 1♂, 5-15.09.2014; H3: 1♂, 5-15.05.2014; 1♂, 5-15.07.2014; 1♀, 1♂, 5-15.08.2014; 1♀, 25.08-4.09.2014.

*D. subintermedia* (Magretti, 1886)\*

Material: H1: 2♂♂, 23.05.-4.06.2014; 1♂, 4-14.06.2014; 1♂, 25.06.-5.07.2014; H2: 1♀, 1♂, 15-25.05.2014; 2♂♂, 23.05.-4.06. 2014; 1♂, 15-25.06. 2014; 1♀, 2♂♂, 5-15.07.2014; H3: 2♀♀, 3♂♂, 23.05.- 4.06.2014; 2♀♀, 1♂, 15-25.06.2014; 1♂, 5-15.07.2014; 1♂, 25.07.-5.08.2014; 1♀, 7♂♂, 5-15.08.2014; 5♂♂, 25.08.-4.09.2014; 1♂, 5-15.08.2014.

*D. variegata* (Linnaeus, 1758)\*

Material: H2: 1♀, 5-15.05.2014; 1♂, 25.05-4.06.2014; 1♂, 4-14.06.2014; 1♀, 15-27.09.2014; H3: 1♂, 15-25.06.2014; 2♂♂, 25.06.-5.07.2014; 1♂, 5-15.08.2014.

**Genus *Dicyrtomellus* Gussakovskij, 1935**

*Dicyrtomellus kizilkumi* (Radoszkowski, 1877)\*

Material: H1: 8♀♀, 23.04.-4.05.2014; 2♀♀, 5-15.05.2014; H2: 2♂♂, 15-25.07.2014; 12♂♂, 25.08-4.09.2014; 2♂♂, 5-15.09.2014; 1♀, 5-15.05.2014; H3: 1♂, 5-15.07.2014; 1♀, 5-15.08.2014; H4: 4♂♂, 5-15.08.2014; H7 1♀, 15-25.08.2014.

**Genus *Episyron* Schiødte, 1837**

*Episyron arrogans* (F. Smith, 1873)\*

Material: H2: 2♀♀, 25.05.-4.06.2014; 1♂, 1♀, 15-25.06.2014; 1♂, 5-15.07.2014; 1♂, 15-25.07.2014.

**Genus *Poecilagenia* Haupt, 1927\***

*Poecilagenia rubricans* (Lepelletier de Saint-Fargeau, 1845)\*

Material: H1: 3♂, 4-14.06.2014; 1♂, 23.06.-5.07.2014; H2: 3♂♂, 15-25.08.2014; 1♀, 5-14.09.2014.

**Genus *Priocnemis* Schiødte, 1837**

*Priocnemis coriacea* Dahlbom, 1843\*

Material: H1: 4♂♂, 2-12.04.2014; 1♀, 5♂♂, 12-23.04.2014; 1♀, 1♂, 23.04.-4.05.2014; H2: 3♂♂, 4-14.09.2014.40.

*P. exaltata* (Fabricius, 1775)\*

Material: H1: 2♂♂, 24.05.-4.06.2014; 2♂♂, 4-14.06.2014; 2♂♂, 15-25.06.2014; 4♀♀, 22♂♂, 25.06.-5.07.2014; 3♂♂, 5-15.07.2014; 4♀♀, 4♂♂, 25.07.-5.08.2014; H2: 1♂, 15-25.06.2014.

*P. fastigiata* Haupt, 1934\*

Material: H3: 1♀, 15-25.06.2014.

*P. parvula* Dahlbom, 1845\*

Material: H1: 4♂♂, 25.06.-5.07.2014.

*P. perturbator* (Harris, 1780)

Material: H1: 1♀, 37♂♂, 2-12.04.2014;

12♀♀, 13♂♂, 12-23.04.2014; 2♀♀, 1♂, 23.04-4.05.2014; 1♀, 5-15.05.2014. H2 1♀, 7♂♂, 12-23.04.2014. H3: 3♂♂, 12-23.04.2014; 1♀, 4♂♂, 23.04.-4.05.2014.

**Species previously recorded from Georgia:**

*Aporinellus sexmaculatus* (Spinola, 1806), recorded from Tbilisi [29,30]; *Anoplius viaticus* (Linnaeus, 1758), recorded from river Iori valley [31]; *Aporinellus moestus* (Klug, 1834), recorded from Lagodekhi, Tbilisi [29]; *Arachnospila anceps* (Wesmael, 1851), recorded from Batumi [30]; *Auplopus carbonarius* (Scopoli, 1763), recorded from Tbilisi [30]; *Auplopus rectus* (Haupt, 1926), recorded from Tbilisi [30]; *Ceropales cribrata* A. Costa, 1881, recorded from Tbilisi [32]; *Cryptocheilus egregius* (Lepelletier de Saint-Fargeau, 1845), recorded from Borjomi [20], Tbilisi [30] (Wolf *et al.* 2009); *C. notatus* (Rossi, 1792), recorded from Tbilisi [30]; *C. richardsi* Móczár, 1953, recorded from Tbilisi [30]; *C. rubellus* (Eversmann, 1846), recorded from Tbilisi [30]; *C. versicolor* (Scopoli, 1763), recorded from Tbilisi [30]; *Dicyrtomellus argenteus* Wahis, 1982, recorded from Tbilisi [30]; *Eoferreola manicata* (Pallas, 1771), recorded from Tbilisi [30]; *Episyron rufipes* (Linnaeus, 1758), recorded from Tsagveri [31]; *Evagetes crassicornis* (Shuckard, 1837), recorded from Adigeni [31]; *Pompilus cinereus* (Fabricius, 1787), recorded from Pitsunda, Tbilisi, Akhali Atoni [30]; *Priocnemis fallax* Verhoeff, 1922, recorded from Mtskheta, Tbilisi [30]; *P. perturbator* (Harris, 1780), recorded from Lagodekhi [33]; *P. pusilla* Schiødte, 1837, recorded from Tbilisi [30]; *P. sulci* Balthasar, 1943, recorded from Tbilisi [30].

**Family Ampulicidae**

Ampulicidae (cockroach wasps) are a small (about 210 described species), principally a tropical group of wasps perying Blattodea. The female ampulicid wasp stings the prey to induce paralysis, and then stores it into a preexisting cavity where laid its egg. Up to now only one cockroach wasp species has been reported from Georgia. One species are established in LPA.

**Genus *Dolichurus* Latreille, 1809**

*Dolichurus corniculatus* (Spinola, 1807)\*

Material: H1: 1♂, 15-25.05.2014; 1♂, 15-25.06.2014. H2: 1♀, 25.05-4.06.2014; 1♂, 15-25.06.2014; 1♀, 7♂♂, 5-15.07.2014; 1♀, 1♂, 15-25.07.2014; 3♂♂, 15-25.08.2014; 2♂♂, 25.08-4.09.2014; H3: 1♂, 25.08-4.09.2014.

Species previously recorded from Georgia:

*Dolichurus haemorrhous* A. Costa, 1886, recorded from Gudauta district, near Akhali Atoni [34].

### Family Bembicidae

The Bembicidae are predatory ground-nesting wasps, inhabiting mostly in sandy areas (except for the cleptoparasitic genera and for genus *Argogorytes*, latter nesting in rotten wood); they are solitary nest provisioners with prey of different insect orders (mainly Hemiptera and Diptera). The family comprises about 1 680 species; their greatest diversity occurs in the desert and semiarid regions of the world; the latter circumstance explains their small number for Georgia (both for Lagodekhi and elsewhere in the country). Previous studies of the sand wasps provide up to now 18 species to occur in Georgia. Four species are established in LPA.

#### Genus *Argogorytes* Ashmead, 1899

*Argogorytes mystaceus* (Linnaeus, 1761)\*

Material: H5: 1♀, 25.06.-5.07.2014.

#### Genus *Bembecinus* A. Costa, 1859

*Bembecinus tridens* (Fabricius, 1781)

Material: H1: 1♂, 15-25.08. 2014.

#### Genus *Gorytes* Latreille, 1804

*Gorytes laticinctus* (Lepelletier de Saint-Fargeau, 1832)\*

Material: H1 1♀, 15-25.07.2014.

#### Genus *Nysson* Latreille, 1802

*Nysson trimaculatus* (Rossi, 1790)

Material: H1 1♀, 25.06.-5.07 .2014; 1♀, 5-15.07.2014.

#### Species previously recorded from Georgia:

*Alysson spinosus* (Panzer, 1801), recorded from Kakheti, Lagodekhi [35]; *Ammatomus rogenhofferi* (Handlirsch, 1888), *Argogorytes fargeii* (Shuckard, 1837), recorded from Vashlovani reserve [36]; *Bembecinus hungaricus* (Fridvaldszky, 1876), recorded from Gagra district, near Gantiadi [34]; *B. tridens* (Fabricius, 1781), recorded from Mtskheta [37], Gagra district, near Gantiadi, Gudauta district near Akhali Atoni [34]; *Bembix bidentata* Vander Linden, 1829, recorded from Tbilisi [38], Gagra [39] (Nemkov 2016); *B. oculata* Panzer, 1801, recorded from Tbilisi [38], Gagra district near Gantiadi [34]; *Brachystegus scalaris* (Illiger, 1807), recorded from Tbilisi [40]; *Didineis lunicornis* (Fabricius, 1798), recorded from Gagra [34]; *Gorytes pleuripunctatus*

(A. Costa, 1859), recorded from Vashlovani reserve [41]; *G. schlettereri* Handlirsch, 1893, recorded from Mestia [42], Bakuriani [41]; *Hoplisoides punctuosus* (Eversmann, 1849), recorded from David Gareji monastery [43]; *Nysson fulvipes* A. Costa, 1859, recorded from Gagra [44], Gudauta district near Akhali Atoni [34]; *N. maculosus* (Gmelin, 1790), recorded from Tbilisi botanical garden [44]; *N. trimaculatus* (Rossi, 1790), recorded from Gagra; environs of Pitsunda [34]; *Stizoides cyanopterus* (Gussakovskij, 1928), recorded from Tbilisi [45]; *Stizus euchromus* Handlirsch, 1892, recorded from Tbilisi [46]; *Stizus rufiventris* Radoszkowski, 1877, recorded from Tbilisi [46].

### Family Crabronidae

The Crabronidae are predatory wasps, nesting in in preexisting cavities, in the ground or in twigs; the prey consists of a wide range of insects and spiders. The group is distributed around the world and embraces about 3 250 described recent species. The previous studies and records of Crabronidae for Georgia are surprisingly scarce. Considerable number of species was mentioned for Abkhazeti (Western Georgia) by Mokrousov & Popov [34]; the group includes up to now 43 established species in Georgia. Twenty-four species are established in LPA.

#### Genus *Crossocerus* Le Peletier-de-Saint-Fargeau & Brullé, 1835

*Crossocerus annulipes* (Le Peletier-de-Saint-Fargeau & Brullé, 1835)

Material: H1: 1♀, 25.07.-5.08. 2014; 1♀, 5-15.08.2014. H2: 1♂, 25.08.-4.09.2014. H3: 2♀, 15-25.06. 2014.

*C. assimilis* (F. Smith, 1856)

Material: H3: 1♀, 15-25.06.2014; 1♀, 25.06.-5.07.2014; H4: 1♂, 25.06.-5.07.2014; H6: 1♀, 25.06.-5.07.2014; H7: 1♀, 5-15.07.2014.

*C. barbipes* (Dahlbom, 1845)\*

Material: H4: 1♀, 5-15.07.2014.

*C. cetratus* (Shuckard, 1837)

Material: H1: 1♀, 25.07.-5.08.2014; H3: 1♀, 5-15.07. 2014.

*C. congener* (Dahlbom, 1844)\*

Material: H1: 3♀♀, 5.-15.05.2014; 1♀, 15.-25.05.2014; 1♀, 1♂, 15.-25.06.2014; 1♀, 15-25.07.2014, 2♀♀, 25.07.-5.08.2014; 1♀, 5-14.09.2014; H3: 2♀♀, 5-15.05.2014; 3♀♀, 15-25.05.2014; H4: 1♂, 15.-25.06.2014; 1♂, 15-25.07.2014; H6: 1♀, 15.-25.06.2014; 1♀, 15-

25.07.2014; H7: 4♀♀, 5-15.07.2014.

*C. megacephalus* (Rossi, 1790)\*

Material: H3: 1♂, 4-14.06.2014; H6: 1♀, 25.07.-5.08.2014.

*C. podagricus* (Vander Linden, 1829)\*

Material: H1: 3♀, 5-15.05.2014; 1♀, 15-25.05.2014; H3: 1♀, 5-15.07.2014.56.

*C. podagricus* (Vander Linden, 1829)\*

Material: H3: 1♀, 4-14.06.2014; 1♂, 25.06.-5.07.2014.

*C. varus* Le Peletier-de-Saint-Fargeau & Brullé, 1835\*

Material: H1: 1♀, 25.05.-4.06.2014; 1♀, 15.-25.06.2014; 1♀, 25.06.-5.07.2014; H3: 1♀, 25.05.-4.06.2014; 1♀, 4.-14.06.2014; 1♀, 15-25.06.2014; 2♀♀, 25.06.-5.07.2014; 1♀, 5-15.07.2014; H4: 1♀, 5-15.07.2014.

### Genus *Ectemnius* Dahlbom, 1845

*Ectemnius cavifrons* (Thomson, 1870)\*

Material: H2: 1♀, 15-25.05.2014; 1♀, 25.05.-4.06.2014; H4: 2♀, 25.06.-5.07.2014; 1♂, 5-15.07.2014; 2♀♀, 15-25.07.2014; 1♂, 25.08.-4.09.2014; H6: 1♀, 5-15.05.2014; 1♀, 15-25.06.2014.

*E. cephalotes* (Olivier, 1792)\*

Material: H2: 1♀, 4-14.06.2014; H3: 2♀♀, 25.05.-4.06.2014; H6: 1♀, 4-14.06.2014.

*E. lituratus* (Panzer, 1803)\*

Material: H1: 1♀, 25.05.-4.06.2014; H3: 1♀, 4-14.06.2014; 1♂, 25.07.-5.08.2014; H4: 1♀, 15.-25.06.2014; H6: 3♀♀, 15-25.07.2014.

### Genus *Entomognathus* Dahlbom, 1844\*

*Entomognathus brevis* (Vander Linden, 1829)\*

Material: H1: 1♂, 15-25.05.2014; 1♂, 4-14.06.2014; 2♀♀, 4♂♂, 25.05.-4.06.2014; 15♀♀, 9♂♂, 15-25.06.2014; 3♀♀, 1♂, 25.06.-5.07.2014; 1♂, 5-15.07.2014; H2: 2♀♀, 2♂♂, 25.05.-4.06.2014; 1♀, 15-25.06.2014; H3: 1♀, 1♂, 15-25.06.2014; 4♀♀, 7♂♂, 25.06.-5.07.2014; 1♀, 3♂♂, 5-15.07.2014.

### Genus *Miscophus* Jurine, 1807

*Miscophus bicolor* Jurine, 1807

Material: H2: 1♀, 15-25.08.2014.

### Genus *Nitela* Latreille, 1809\*

*Nitela borealis* Valkeila, 1974\*

Material: H2: 1♂, 25.08.-4.09.2014; H3: 1♀, 25.07.-5.08.2014; 1♀, 5-15.08.2014; 4♀♀, 1♂, 25.08.-4.09.2014; H4: 2♀♀, 1♂, 15-25.07.2014; H6: 1♀, 5-14.09.2014.

### Genus *Rhopalum* Stephens, 1829\*

*Rhopalum clavipes* (Linnaeus, 1758)\*

Material: H1: 1♀, 15-25.05.2014; H3:

2♀, 5-15.05.2014; 1♀, 25.05.-4.06.2014; 1♀, 25.06.-5.07.2014; 1♀, 25.07.-5.08.2014; 2♀♀, 25.08.-4.09.2014; 2♀♀, 26.08.-5.09.2014; H4: 1♀, 4-14.06.2014; 2♀♀, 2♂♂, 15-25.06.2014; 1♀, 1♂, 5-15.07.2014; 1♂, 15-25.07.2014; H6: 4♀♀, 5-15.05.2014; 1♀, 1♂, 4-14.06.2014; 1♀, 15.-25.07.2014; 4♀♀, 5.-15.08.2014; 2♀♀, 25.08.-4.09.2014; 2♀♀, 5-14.09.2014; H7: 1♀, 4-14.06.2014; 1♂, 15-25.06.2014; 1♀, 5-15.07.2014.

*R. coarctatum* (Scopoli, 1763)\*

Material: H1: 1♀, 15-25.05.2014.

### Genus *Trypoxylon* Latreille, 1796

*Trypoxylon attenuatum* F. Smith, 1851\*

Material: H1: 1♀, 15.-25.06.2014.

*T. beaumonti* Antropov, 1991\*

Material: H1: 4♀♀, 5-15.05.2014; 2♀♀, 25.05.-4.06.2014; 1♂, 15-25.06.2014; 1♀, 25.07.-5.08.2014; H2: 1♀, 15.-25.06.2014; 1♀, 15.-25.08.2014; H6: 1♀, 5.-15.05.2014.

*T. clavicum* Lepeletier de Saint-Fargeau & Audinet-Serville, 1828

Material: H3: 1♀, 25.05.-4.06.2014.

*T. fronticorne* Gussakovskij, 1936

Material: H2: 1♀, 15-25.06.2014.

*T. kolazyi* Kohl, 1893

Material: H1: 1♀, 4-14.06.2014; H2: 1♀, 25.08.-4.09.2014.

*T. minus* de Beaumont, 1945

Material: H1: 3♀♀, 23.04.-4.05.2014; 6♀♀, 1♂, 5-15.05.2014; 9♀♀, 2♂♂, 15.-25.05.2014; 1♀, 25.05.-4.06.2014; 2♀♀, 25.06.-5.07.2014; 3♀♀, 25.07.-5.08.2014; 1♀, 25.08.-4.09.2014; H2: 2♀♀, 23.04.-4.05.2014; 4♀, 1♂, 5-15.05.2014; 1♀, 15-25.05.2014; 1♀, 25.05.-4.06.2014; 1♀, 4-14.06.2014; 5♀♀, 3♂♂, 15-25.06.2014; 9♀♀, 5.-15.07.2014; 6♀♀, 15.-25.08.2014; 11♀♀, 25.08.-4.09.2014; H3: 2♂♂, 23.04.-4.05.2014; 2♀♀, 15-25.05; 2♀♀, 2♂♂, 25.05.-4.06.2014; 2♀♀, 4-14.06.2014; 2♀♀, 15-25.06.2014; 1♀, 1♂, 25.06.-5.07.2014; 1♀, 5-15.07.2014; 1♀, 25.07.-5.08.2014; 5♀♀, 2♂♂, 5-15.08; 1♂, 15-25.08.2014; 5♀♀, 4♂♂, 25.08.-4.09.2014; H4: 1♂, 25.06.-5.07.2014; 2♀♀, 5-15.08.2014; H6: 1♀, 1♂, 4-14.06.2014; 1♀, 15-25.07.2014; 1♀, 5-15.08.2014; 1♀, 5-14.09.2014; H7: 2♀♀, 5.-15.07.2014; 2♀♀, 5-15.07.2014.

*T. rubiginosum* Gussakovskij, 1936

Material: H1: 2♀♀, 5♂♂, 15-25.05.2014; 3♂♂, 25.05.-4.06.2014; 1♀, 1♂, 4-14.06.2014; 2♀♀, 1♂, 15-25.06.2014; 1♀, 25.06.-5.07.2014; 1♀, 15-25.07.2014; 3♀♀, 25.07.-5.08.2014; 1♀, 26.08.-5.09.2014; H2: 1♀, 5-15.05.2014; 1♀, 15.-

25.05.2014; H3: 1♀, 15-25.06.2014; 1♀, 25.06.-5.07.2014; 1♀, 25.07.-5.08.2014; 1♀, 5-15.08.2014; 1♀, 25.08.-4.09.2014; H4: 2♀♀, 4-14.06.2014; 2♀♀, 5-15.07.2014; 1♀, 15-25.07.2014; H6: 1♀, 5-15.05.2014; 1♀, 15-25.07.2014; 1♀, 5-14.09.2014.

#### **Species previously recorded from Georgia:**

*Crabro alpinus* Imhoff, 1863, recorded from Gagra district Mt Mamdzhishkha [34]; *Crossocerus annulipes* (Le-Peletier-de-Saint-Fargeau & Brullé, 1835), recorded from Gantiadi (Tsandripsh), Akhali Atoni [34]; *C. assimilis* (F. Smith, 1856), *C. capitatus* (Shuckard, 1837), recorded from Mestia [47]; *C. cetratus* (Shuckard, 1837), recorded from Avadhara [34]; *C. elongatulus* (Vander Linden, 1829), recorded from Gantiadi (Tsandripsh) [34]; *Ectemnius cavifrons* (Thomson, 1870), recorded from Mtirala National Park [28], Otkhara, Akhali Atoni [34]; *E. lapidarius* (Panzer, 1803), recorded from Mtirala National Park [28]; *E. nigratarsus* (Herrich-Schaeffer, 1841), recorded from Gagra [48]; *E. rubicola* (Dufour & Perris, 1840), recorded from Akhali Atoni [34]; *E. sexcinctus* (Fabricius, 1775), recorded from Borjomi [20]; *Lestica clypeata* (Schreber, 1759), recorded from Gantiadi (Tsandripsh), Akhali Atoni [34]; *Lindenius pygmaeus* (Rossi, 1794), recorded from Gantiadi (Tsandripsh) [34]; *Liris festinans* (F. Smith, 1858), recorded from Gagra, Akhali Atoni, Sokhumi district Sokhumi, Kelasuri [34]; *L. niger* (Fabricius, 1775), recorded from Alhali Atoni [34]; *Miscophus bicolor* Jurine, 1807, recorded from Gagra, Akhali Atoni [34]; *Oxybelus caucasicus* Radoszkowski, 1893, recorded from Lagodekhi [18]; *O. uniglumis* (Linnaeus, 1758), recorded from Gantiadi (Tsandripsh) [34]; *Palarus variegatus* (Fabricius, 1781), recorded from Vashlovani reserve [49], Gantiadi (Tsandripsh) [31,49, 50]; *Parapiagetia genicularis* (F. Morawitz, 1890), recorded from Lagodekhi [52]; *Pison atrum* (Spinola, 1808), recorded from Tbilisi, Sagarejo [53]; *Rhopalum austriacum* (Kohl, 1899), recorded from Akhali Atoni [31]; *Tachysphex brullii* (F. Smith, 1856), recorded from Lagodekhi [54]; *T. consocius* Kohl, 1892, recorded from Akhali Atoni [34]; *T. ferrugineus* Pulawski, 1971, recorded from Sokhumi [54], Pitsunda, Akhali Atoni [35], Sairme [55] (Straka 2016); *T. fugax* (Radoszkowski, 1877), recorded from Tbilisi 52,55], Akhali Atoni [34]; *T. helveticus* Kohl, 1885, recorded from Akhali Atoni [34]; *T. incertus* (Radoszkowski, 1877), recorded from Tbilisi [54]; *T. mediterraneus*

Kohl, 1883, recorded from Gantiadi (Tsandripsh), Gagra [34]; *T. obscuripennis* (Schenck, 1857), recorded from Gudauta district Lake Ritsa [34]; *T. pompiliformis* (Panzer, 1803), recorded from Lagodekhi [54], Akhali Atoni [31]; *T. psammobius* (Kohl, 1880), recorded from Lagodekhi [54], Gantiadi (Tsandripsh), Gagra, Mt Mamdzhishkha; Akhali Atoni [34]; *T. stachi* de Beaumont, 1936, recorded from Lagodekhi [54]; *T. unicolor* (Panzer, 1809), recorded from Tbilisi [54]; *Tachytes ambidens* Kohl, 1884, recorded from Lagodekhi [57]; *Trypoxylon clavicerum* Lepeletier de Saint-Fargeau and Audinet-Serville, 1828, recorded from Tbilisi [58], Batumi, Sokhumi [59]; *T. deceptorium* Antropov, 1991, Tamishi, Batumi, Tbilisi, Adigeni, Mtskheta [60]; *T. fronticorne* Gussakovskij, 1936, recorded from Gagra, Akhali Atoni [34]; *T. kolazyi* Kohl, 1893, recorded from Tbilisi [58]; *T. minus* de Beaumont, 1945, recorded from Akhali Atoni [34]; *T. rubiginosum* Gussakovskij, 1936, recorded from Batumi [60,61]; Primorskoe (Apkhazeti), Tbilisi, Gagra [62]; *T. scutatatum* Chevrier, 1867, recorded from Tbilisi [58].

#### **Family Pemphredonidae**

Family Pemphredonidae includes about 460 recent species of predatory wasps; they nest in soil or in twigs and prey mainly upon immature Hemiptera. Eight species (mostly from genus *Pemphredon*) have been previously recorded for Georgia. Six species of that group are established in LPA.

#### **Genus *Passaloecus* Shuckard, 1837**

*Passaloecus gracilis* (Curtis, 1834)

Material: H1: 1♀, 25.05.-4.06.2014; H2: 1♀, 15-25.06.2014; 3♀♀, 25.08.-4.09.2014; H3: 1♀, 15-25.06.2014; 1♀, 25.06.-5.07.2014; H6: 1♀, 25.06.-5.07.2014.

#### **Genus *Pemphredon* Latreille, 1796**

*Pemphredon inornata* Say, 1824\*

Material: H1: 1♀, 5-15.05.2014; 1♀, 25.05.-4.06.2014; 2♀♀, 15-25.06.2014; 1♀, 5-15.07.2014; 1♀, 15-25.08.2014; H2: 1♀, 5-15.05.2014; 1♀, 15-25.05.2014; 1♀, 25.05.-4.06.2014; H3: 1♀, 25.05.-4.06.2014; 1♀, 4-14.06.2014; 2♀♀, 5-15.07.2014; H4: 1♀, 15-25.07.2014.

*P. lugubris* (Fabricius, 1793)\*

Material: H1: 1♀, 12-23.04.2014; 2♀♀, 23.04.-4.05.2014; 1♀, 5-15.05.2014; H2: 1♀, 12-23.04.2014; H3: 1♀, 23.04.-4.05.2014; 1♀,

5-15.05.2014; 2♀♀, 15-25.05.2014; 4♀♀, 25.05.-4.06.2014; 3♀♀, 4-14.06.2014; 3♀♀, 15.-25.06.2014; 1♀, 5-15.07.2014; 1♀, 25.07.-5.08.2014; H6: 6♀♀, 4-14.06.2014; 1♀, 15-25.06.2014; 2♀♀, 25.06.-5.07.2014; 3♀♀, 15-25.07.2014; H7: 2♀♀, 2♂♂, 5-15.07.2014; 2♀♀, 1♂, 5-15.07.2014.

*P. morio* Vander Linden, 1829\*

Material: H1: 1♀, 15-25.05.2014; H3: 1♀, 5-15.08.2014.

### Genus *Spilomena* Shuckard, 1838

*Spilomena beata* Blüthgen, 1953\*

Material: H3: 3♀♀, 5-15.07.2014; 2♀♀, 25.08.-4.09.2014; H5: 1♀, 25.07.-5.08.2014; 1♀, 5-14.09.2014.

### Genus *Stigmus* Panzer, 1804\*

*Stigmus pendulus* Panzer, 1804\*

Material: H1: 1♀, 25.05.-4.06.2014; H3: 1♀, 15-25.05.2014; 1♀, 25.06.-5.07.2014.

### Species previously recorded from Georgia:

*Diodontus brevilabris* de Beaumont, 1967, recorded from Gagra district environs of Pitsunda [34]; *Passaloecus gracilis* (Curtis, 1834), recorded from Akhali Atoni [34] (Mokrousov & Popov 2016); *P. insignis* (Vander Linden, 1829), recorded from Akhali Atoni; Mt. Avadkhara [34]; *Pemphredon austriaca* (Kohl, 1888), recorded from Gantiadi (Tsandriash); environs of Pitsunda; Akhali Atoni; Sokhumi, Kelasuri Station [34]; *P. lethifer* (Shuckard, 1837), recorded from Akhali Atoni [34]; *P. lugens* Dahlbom, 1842, recorded from Mestia [63]; *P. rugifer* (Dahlbom, 1844), recorded from Akhali Atoni [35]; *Spilomena troglodytes* (Vander Linden, 1829), recorded from Akhali Atoni [34].

## Family Psenidae

Psenidae is a comparatively small family of about 580 species with worldwide distribution. These wasps are solitary predators and nest explicitly in soil; the prey includes various Hemiptera. Only three species of that group are established in LPA.

### Genus *Psenulus* Kohl, 1897

*Psenulus fuscipennis* (Dahlbom, 1843)

Material: H1: 1♀, 25.05.-4.06.2014; 1♀, 25.07.-5.08.2014; 1♀, 5-15.08.2014; H2: 2♀♀, 15-25.05.2014; H3: 1♂, 25.05.-4.06.2014; H4: 1♀, 5-15.08.2014.

*P. meridionalis* de Beaumont, 1937\*

Material: H2 1♂, 15.-25.05.2014.

### *P. pallipes* (Panzer, 1798)

Material: H1: 2♀♀, 2♂♂, 5-15.05.2014; 2♂♂, 15-25.05.2014; 4♂♂, 25.05.-4.06.2014; 1♀, 15-25.07.2014; 1♀, 25.07.-5.08.2014; 2♂♂, 5-15.08.2014; H2: 1♀, 23.04.-4.05.2014; H3: 1♀, 23.04.-4.05.2014; 4♀♀, 1♂ 5-15.05.2014; 9♀♀, 15-25.05.2014; 4♀♀, 3♂♂, 25.05.-4.06.2014; 6♀♀, 1♂, 15-25.06.2014; 5♀♀, 25.06.-5.07.2014; 3♀♀, 1♂, 5-15.07.2014; 2♀♀, 15-25.07.2014; 11♀♀, 5-15.08.2014; 1♀, 15-25.08.2014; 13♀♀, 25.08.-4.09.2014; H4: 4♀♀, 15.-25.07.2014; 1♀, 5-15.08.2014; H6: 1♀, 4♂♂, 4-14.06.2014; 2♀♀, 25.06.-5.07.2014; 1♀, 15.-25.07.2014; 1♀, 5-14.09.2014; 1♀, 5.-15.07.2014; 1♀, 5.-15.07.2014.

Species previously recorded from Georgia:

*Entomosericus concinnus* Dahlbom, 1845, recorded from 2 km SW Atskuri [64]; *Mimesa caucasica* Maidl, 1914, recorded from Lagodekhi [53]; *Mimesa crassipes* A. Costa, 1871, recorded from Tbilisi [53]; *Pseneo exaratus* (Eversmann, 1849), recorded from Gantiadi (Tsandriash); 20 km west from gagra, Leselidze, Tskaltubo, Lagodekhi reserve [65]; *Psenulus fuscipennis* (Dahlbom, 1843), recorded from Mestia [66]; *P. pallipes* (Panzer, 1798), recorded from Gantiadi (Tsandriash) [34]; *P. pan* de Beaumont, 1967, recorded from Tbilisi botanical garden [65]; *P. schencki* (Tournier, 1889), recorded from Mestia [66].

## Family Sphecidae

Sphecidae is a cosmopolitan family of about 700 species; members of the family are solitary predators/parasitoids; the prey includes arachnids or insects (Blattoptera, Orthoptera and larvae of Lepidoptera). The greatest portion of Sphecida diversity exists in the Old World, particularly in the xeric regions. In LPA Sphecidae is presented by one species so far.

### Genus *Sceliphron* Klug, 1801

*Sceliphron curvatum* (F. Smith, 1870)

Material: H4: 1♂, 25.06.-5.07.2014.

### Species previously recorded from Georgia:

*Ammophila campestris* Latreille, 1809, recorded from Georgia, not exact location is given [50]; *A. sabulosa* (Linnaeus, 1758), recorded from Mestia [67], Akhali Atoni [34]; *Palmodes melanarius* (Mocsáry, 1883), recorded from Tbilisi [67,68]; *Podalonia affinis* (W. Kirby, 1798), recorded from Tbilisi [70]; *P. caucasica* (Mocsáry, 1883),

recorded from Tbilisi [68]; *P. fera* (Lepeletier de Saint-Fargeau, 1845), Gantiadi (Tsandriphsh) [34]; *Podalonia fera* (Lepeletier de Saint-Fargeau, 1845), recorded from near Jandari [71]; *Prionyx kirbii* (Vander Linden, 1827), recorded from Gantiadi (Tsandriphsh), Akhali Atoni [34]; *P. subfuscatus* (Dahlbom, 1845), recorded from Gantiadi (Tsandriphsh) [34]; *P. viduatus* (Christ, 1791), recorded from Gantiadi (Tsandriphsh) [34]; *Sceliphron curvatum* (F. Smith, 1870), recorded from Borjomi [71], Pitsunda, Akhali Atoni [33,71]; *S. destillatorium* (Illiger, 1807), recorded from Tbilisi [66,67]; Gagra, lori river, Sagarejo [74]; *S. madraspatanum* (Fabricius, 1781), recorded from Lagodekhi [73]; *S. spirifex* (Linnaeus, 1758), recorded from Gantiadi (Tsandriphsh), Akhali Atoni [34]; *Sphex flavipennis* Fabricius, 1793, recorded from Gantiadi (Tsandriphsh), Akhali Atoni [34]; *S. funerarius* Gussakovskij, 1934, recorded from Gantiadi (Tsandriphsh) [34].

## Family Vespidae

The family Vespidae is a diverse group of hymenopterous insects exhibiting solitary, primitively social and eusocial way of life; it includes of about 4 200 described species. The members of the family construct nests, usurp nests or occupy pre-existing cavities for nesting. Most vespids are predators on other insects, however, a small group (subfamily Masarinae) supply their nests entirely with pollen and/or nectar for feeding the offspring. Fifty species of Vespidae have been previously recorded for Georgia. In LPA Vespidae are known with twelve species.

### Genus *Allodynerus* Bluethgen, 1938

*Allodynerus rossii* (Lepeletier de Saint-Fargeau, 1845)

Material: H2: 1♀, 15-25.07.2014.

### Genus *Ancistrocerus* Wesmael, 1836

*Ancistrocerus nigricornis* (Curtis, 1826)

Material: H2: 2♀, 4-14.06.2014; H3: 1♀, 15-25.05.2014; H4: 1♀, 5-15.07.2014; H5: 1♀, 15.25.06.2014; 2♀, 25.08.-4.09.2014; H6: 1♀, 25.06.-5.07.2014.

*A. trifasciatus* (O. F. Müller, 1776)

Material: H7: 1♀, 25.06-5.07.2014.

### Genus *Dolichovespula* Rohwer, 1916

*Dolichovespula media* (Retzius, 1783)

Material: H3: 1♀, 5-15.07.2014.

*D. saxonica* (Fabricius, 1793)

Material: H1: 2♀♀, 5-15.05.2014; 1♀, 15-25.06.2014; H3: 1♀, 4-14.06.2014; H4: 1♀, 5-15.05.2014; 1♀, 25.06-5.07.2014.

*D. sylvestris* (Scopoli, 1763)

Material: H1: 1♀, 23.04-4.05.2014; 1♀, 15-25.06.2014; H4: 1♀, 5-15.05.2014, 1♀, 4-14.06.2014; H6: 1♀, 5-15.07.2014.

### Genus *Odynerus* Latreille, 1802

Material: H3: 1♀, 15-25.06.2014.

### Genus *Polistes* Latreille, 1802

*Polistes dominula* (Christ, 1791)\*

Material: H1: 1♀, 15-25.06.2014; H6: 1♀, 25.07-5.08.2014; 1♂, 25.07-5.08.2014; H7: 4♀♀, 5-15.07.2014; 2♀♀, 15-25.07.2014; 1♂, 15-25.07.2014; 1♂, 25.07-5.08.2014.

*P. gallicus* (Linnaeus, 1767)

Material: H6: 1♀, 25.07-5.08.2014; 1♀, 15-25.08.2014; H7: 1♀, 15-25.07.2014; 1♀, 5-15.08.2014; 1♀, 15-25.08.2014.

### Genus *Vespa* Linnaeus, 1758

*Vespa crabro* Linnaeus, 1758

Material: H1: 1♀, 5-15.07.2014; 2♀♀, 15-25.07.2014; 2♀♀, 25.07-5.08.2014; 1♀, 5-15.08.2014; H2: 1♀, 25.05-4.06.2014.

### Genus *Vespula* Thomson, 1869

*Vespula rufa* (Linnaeus, 1758)

Material: H1: 1♀, 15-25.06.2014; 1♀, 15-25.07.2014; H4: 3♀♀, 5-15.07.2014; 1♀, 25.07-5.08.2014; H6: 4♀♀, 15-25.07.2014.

*V. vulgaris* (Linnaeus, 1758)

Material: H1: 3♀♀, 12-23.04.2014; 6♀♀, 23.04-4.05.2014; 11♀♀, 5-15.05.2014; 1♀, 15-25.05.2014; 3♀♀, 24.05-4.06.2014; 9♀♀, 4-14.06.2014; 2♀♀, 15-25.06.2014; 6♀♀, 25.06-5.07.2014; H2: 2♀♀, 23.04-4.05.2014; 2♀♀, 4-14.06.2014; 5♀♀, 15-25.06.2014; 1♀, 4-14.09.2014; H3: 1♀, 23.04-4.05.2014; 1♀, 15-25.06.2014; 1♀, 25.07-5.08.2014; 2♀♀, 5-15.08.2014; H4: 1♀, 5-15.05.2014; 2♀♀, 15-25.05.2014; 3♀♀, 4-14.06.2014; 2♀♀, 25.06-5.07.2014; 2♀♀, 5-15.07.2014; 5♀♀, 15-25.07.2014; 3♀♀, 25.08-4.09.2014; H5: 1♀, 15-27.09.2014.

### Species previously recorded from Georgia:

*Allodynerus rossii* (Lepeletier de Saint-Fargeau, 1841), recorded from Borjomi [75]; *Ancistrocerus antilope* (Panzer, 1798), recorded from Omalo, Lebarde [76]; *A. kazbekianus* (Kostylev, 1940), recorded from Stepantsminda (Kazbegi) [77]; *A. nigricornis* (Curtis, 1826), recorded from Avadkhara, Tbilisi Botanical Garden [76]; *A. oviventris* (Wesmael, 1836), recorded from Gudauri

[75], Omalo [76]; *A. terekensis* (Kostylev 1940), recorded from Stepantsminda (Kazbegi) [77]; *A. trifasciatus* (O. F. Müller, 1776), recorded from Pasanauri [75]; *Antepipona cribrata* (F. Morawitz, 1885), recorded from Lagodekhi [76]; *Antepipona deflenda* (S. Saunders, 1853), recorded from Akhaltsikhe [75]; *Celonites tauricus* Kostylev, 1935 recorded from Stepantsminda (Kazbegi) [20]; *Delta unguiculatum* (de Villers, 1789), recorded from Borjomi [20]; *Delta unguiculatum* (de Villers, 1789), recorded from Nasakirali mineral waters, Tbilisi Surroundings, Kulbaki, Sokhhumi [76]; *Dolichovespula adulterina* (du Buysson, 1905), recorded from Nikorts minda, Tlugi, Chrebalo [76]; *D. media* (Retzius, 1783); recorded from Ushguli, Tskhneti [76]; *D. norwegica* (Fabricius, 1781), recorded from Ushguli, Tsana [76]; *D. omissa* (Bischoff, 1931), recorded from Stepantsminda (Kazbegi), Lebarde [76]; *D. saxonica* (Fabricius, 1793), recorded from Kojori [77], Avadkhara, Omalo, Gurshevi, Nikorts minda, Tlugi, Tsana, Tsageri, Chrebalo [76]; *D. sylvestris* (Scopoli, 1763), recorded from Gudauri, Pasanauri, Batumi, Manglisi, Kavtiskhevi, Bakuriani, Nedzvi, Tori, Borjomi, Abastumani [78], Abano, Omalo, Gurshevi, Ketrisi, Koruldashi, Lagilda, Lagodekhi reserve, Lebarde, Kochalos mta, Manglisi, Mestia, Tetrtsklebi, Ushguli, Khashuri, Itria, Tsageri, Tsana, Tskneti, Chrebalo [76]; *Eumenes coarctatus* (Linnaeus, 1758), recorded from Klukhori and Apkhazeti [76]; *E. papillarius* (Christ, 1791), recorded from Apkhazeti [76]; *E. pedunculatus* (Panzer, 1799), recorded from Apkhazeti [76]; *Euodynerus dantici* (Rossi, 1790), recorded from Tbilisi [75]; *E. posticus* (Herrich-Schäffer, 1841), recorded from Lagodekhi [75], Nasakirali mineral waters [76]; *Hemipterochilus bembeciformis* (F. Morawitz, 1867), recorded from Eldari [76]; *Katamenes arbustorum* (Panzer, 1799), recorded from Kojori [76]; *K. flavigularis* (Blüthgen, 1951), recorded from Akhaltsikhe [79]; *Leptochilus mimulus* Gusenleitner, 1970, recorded from Akhaltsikhe [75]; *Microdynerus microdynerus* (Dalla Torre, 1889), recorded from Borjomi [74,80]; *Odynerus melanocephalus* (Gmelin, 1790), recorded from Orbeli [76]; *O. nigrospinosus* (F. Morawitz, 1895), recorded from Apkhazeti [76]; *O. serricrus* (Blüthgen, 1963), recorded from Omalo [76]; *O. spinipes* (Linnaeus, 1758), recorded from Eldari [76]; *Polistes atrimandibularis* S. Zimmermann, 1930, recorded from Lagodekhi reserve, Khashuri, Itria [76]; *P. biglumis* (Linnaeus, 1758), recorded

from Omalo, Lebarde, Tkemlovani, Ushguli, Tsageri [76]; *P. foederatus* Kohl, 1898, recorded from Gardabani, Gurshevi, Lagodekhi reserve, Lebarde, Ksani, Sokhumi, Ushguli, Khashuri, Itria, Tsana, Tskneti [76]; *P. gallicus* Linnaeus, 1761, recorded from Borjomi [20], Akhmeta, Batumi, Gentsvishi, Kvareli, Kojori, Lagodekhi reserve, Nasakirali mineral waters, Near lake Ritsa, Saguramo, Tbilisi, Rustavi, Tskadisi, Utsera (Skodora), Iori valley, Khashuri, Khulo, Tsageri, Dedoplistskaro, Tskneti, Shiraki, Shulaveri [76]; *P. nimpha* (Christ, 1791), recorded from Omalo, Lekistskali, Mestia, Nasakirali mineral waters, Tbilisi, Tskadisi, Khulo [76]; *P. semenowi* F. Morawitz, 1889, recorded from Lagodekhi reserve [76]; *Pseudepipona herrichii* (de Saussure, 1856), recorded from Borjomi, Lagodekhi [76]; Akhaltsikhe [79]; *Pterocheilus phaleratus* (Panzer, 1797), recorded from Kojori [75]; *Stenodynerus bluethgeni* van der Vecht, 1971, recorded from Borshom [75]; *S. fastidiosissimus* (de Saussure, 1855), recorded from Tbilisi [75]; *S. punctifrons* (Thomson, 1874), recorded from Omalo [76]; *S. xanthomelas* (Herrich-Schäffer, 1839), recorded from Borjomi [75]; *Symmorphus bifasciatus* (Linnaeus, 1761), recorded from Eldari [76]; *S. gracilis* (Brullé, 1833), recorded from Borjomi [75], Omalo [76]; *Vespa crabro* Linnaeus, 1758, recorded from Baatumi botanical garden, Kojori, Kutaisi, Sagoria Oak forest, Saguramo, Oni, Poti, Chala (Ozurgeti) [76]; *Vespula germanica* (Fabricius, 1793), recorded from Akhmeta, Borjomi, Ialguja, Kojori, Ozurgeti, Nasakirali mineral waters, Ateni, Bakhvi, Isriti, Kurzu, Menji, Sokhumi, Tbilisi, Khaishi, Khashuri, Iorta, Tskneti [76]; *V. rufa* (Linnaeus, 1758), recorded from Avadkhara, Omalo, Lagodekhi reserve, Tetrtsklebi, Khulo, Tsana [76]; *V. vulgaris* (Linnaeus, 1758), Avadkhara, Akhmeta, Kojori, Lagodekhi reserve, Lebarde, Nasakirali mineral waters, Ritsa, Tbilisi, Klich, Bzvani, Bakhvi, Tetrtsklebi, Khashuri, Itria, Khulo, Iupshara [76].

## Results and Discussion

### *Species inventory, Patters of Diversity and Distribution*

In total we found 54 woody and 244 herbaceous plant species [9]. Our results show, that woody plant species richness decreased with altitude (Tab. 1), while herbs reached highest richness above 2200 m (Tab. 1). Species richness of plants and Hymenoptera were negatively correlated. This pattern was

opposed to previous results on Encyrtidae [9], but similar to that of Chrysomelidae [81].

Following families: Gasteruptiidae, Heloridae and Bethyridae are new to Georgia, therefore all genera and species recorded from these families are new to Georgia too. In total 14 new genera and 57 species are new for Georgia. Prior our study 39 species have been recorded from Lagodekhi reserve, we recorded only 4 species previously recorded from this area and 87 species are new for Lagodekhi protected areas, thus the number of species increased to 126 species.

Our study increased the number of families, and species reported from Georgia. Hymenopteran species richness was not significantly related with plant richness, either with total richness (Tab. 1,  $r = -0.68$ ,  $P > 0.05$ ), nor with herb richness (Tab. 1,  $r = -0.65$ ,  $P > 0.10$ ) or woody plant richness (Tab. 1,  $r = 0.19$ ,  $P > 0.10$ ). These negative results indicate that the hosts of many of the parasitoid and predatory Hymenoptera do not directly depend on plant species richness. In turn, Hymenopteran richness (Tab. 1,  $r = -0.91$ ,  $P < 0.01$ ) and catches (Tab. 1  $r = -0.89$ ,  $P < 0.01$ ), as well as the respective catches/richness relationship (Tab. 2,  $r = -0.88$ ,  $P = 0.01$ ) decreased with increasing altitude contrary to the finding of Mumladze et al. [7](2017) how reported an inverse altitudinal gradient in the hymenopteran family Encyrtidae.

The proportion of Hymenoptera species to that of plants decreased with altitude (Tab. 2,  $r = -0.86$ ,  $P = 0.01$ ). This was mainly due to the respective relation for herbivorous species (Tab. 2,  $r = -0.84$ ,  $P = 0.02$ ).

### Acknowledgment:

We thank to Rustaveli National Science foundation for funding the project through ref: FR/221/7-110/13. Josef Gusenleitner (Austria) has provided a great deal of assistance in identifying members of family Vespidae. Dr Werner Ulrich (Ecology and Biogeography, Nicolaus Copernicus University, Toruń, Poland) made valuable comments to earlier versions of this publication. We also thank Borislav Naumov for preparing the map of the location and the borders of Lagodekhi Protected Areas.

### References

[1] Costello MJ., Wilson S., Houlding B. More Taxonomists Describing Significantly Fewer Species per Unit Effort May Indicate That Most

Species Have Been Discovered. *Systematic Biology* 62(4) (2013) 616–624.

- [2] Dunn R. Modern insect extinctions, the neglected majority. *Conservation Biology* 19 (2005) 1030–1036.
- [3] Samways M. Insect Extinctions and Insect Survival. *Conservation Biology*, 20(1) (2006) 245–246.
- [4] Andriamampianina L., Kremen C., Vane-Wright D., Razafimahatratra V. Taxic Richness Patterns and Conservation Evaluation of Madagascan Tiger Beetles (Coleoptera: Cicindelidae). *Journal of Insect Conservation* 4 (2000) 109–128.
- [5] Lobo J., Martín-Piera F. Searching for a predictive model for species richness of Iberian dung beetle based on spatial and environmental variables. *Conservation Biology* 16 (2002) 158-173.
- [6] Gutiérrez D., Menéndez R., Méndez M. Habitatbased conservation priorities for carabid beetles within the Picos de Europa National Park, northern Spain. *Biological Conservation* 115 (2004) 379-393.
- [7] Chikovani T., Gigauri G., Didmanidze E., Syroechkovskij E., Shavliashvili I. Lagodekhi Reserve. In: Sokolov, V. & Syroechkovskij, E. (editors in chief). Natural reserves in the USSR. Natural reserves in Caucasus. – “Mysl”. Moscow (1990) 210-225 (1-368) (in Russian).
- [8] Kottek M., Grieser J., Beck C., Rudolf B., Rubel F. World Map of the Köppen-Geiger climate classification updated. *Meteorol. Z.*, 15 (2006) 259-263.
- [9] Mumladze L., Ulrich W., Asanidze Z., Japoshvili G. An inverse elevational species richness gradient of Caucasian vascular plants and Encyrtidae (Hymenoptera, Chalcidoidea). *Écoscience*, 24(1-2) (2017) 75–79.
- [10] Zhang R., van Achterberg K., Tian X., He C., Tan J. Sexual variation in two species of Helorus Latreille (Hymenoptera, Heloridae) from NW China, with description of female of Helorus caii He & Xu. *Zootaxa*, 4821 (2020) 570-584.
- [11] Belokobylskij S. Ensign wasps (Hymenoptera: Evanioidea, Evaniidae) in the fauna of Russia, with description of a new Brachygaster Leach species from Tajikistan and Uzbekistan. *Zootaxa*, 4629 (2019) 379-388.
- [12] Azevedo C., Alencar I., Ramos M., Barbosa D., Colombo W., Vargas J., Lim J. Global guide

- of the flat wasps (Hymenoptera, Bethyridae). *Zootaxa*, 4489 (2018) 1–294.
- [13] Radoszkowsky O. Les Chrysidés et Sphégydes du Caucase. *Horae Societatis Entomologicae Rossicae*, 15 (1879) 140-156.
- [14] Semenov-Tian-Shanskij A. New species of gold wasps (Hymenoptera, Chrysididae). *Travaux de l'Institut Zoologique de l'Académie des Sciences de l'URSS*, 43 (1967) 118–183 (in Russian).
- [15] Rosa P., Lelej A., Proshchalykin M., Loktionov V., Mokrousov M. New records of Chrysididae (Hymenoptera) from Russia with description of five new species. *Far Eastern Entomologist*, 345 (2017) 1-33.
- [16] Rosa P., Lelej A., Belokobylskij S., Vinokurov N., Zaytseva L. Illustrated and annotated checklist of the Russian cuckoo wasps (Hymenoptera, Chrysididae). *Entomofauna, Supplementum* 23 (2019) 1-360.
- [17] Radoszkowski O. Révision des armures copulatrices des mâles de la tribu des Chrysidés. *Horae Societatis Entomologicae Rossicae*, 23 (1889) 3-40 & plates I-VI.
- [18] Radoszkowski O. Descriptions d'Hyménoptères nouveaux. *Revue d'Entomologie publiée par la Société Française d'Entomologie. Caen*, 12 (1893) 241-245.
- [19] Mocsáry A. Monographia Chrysididarum orbis terrarum universi. *Typis Societatis Franklinianae. Budapestini* (1889) 1-643 & plates I-II.
- [20] Radde G. *Die Sammlungen des Kaukasischen Museums. Band I. Zoologie. Typographie der Kanzelei des Landescheffs*. Tiflis: 521pp. (1899) (in Russian and German).
- [21] Semenov-Tian-Shanskij A. Chrysididarum species novae 05el parum cognitae (Hymenoptera). *Revue Russe d'Entomologie*, 12 (1912) 177-201.
- [22] Semenov-Tian-Shanskij A. Revisio synoptica Cleptidarum faunae rossicae (Hymenoptera Proctorypoidea). *Bulletin de l'Académie des Sciences de Russie*, 14 (1920) 303-328.
- [23] Radoszkowsky O. Enumération des espèces de Chrysidés de Russie. *Horae Societatis Entomologicae Rossicae*, 3 (1866) 295-310 & plates II-VI.
- [24] Móczár L. Revision of the Cleptes (Holcocleptes) species of the world (Hymenoptera, Chrysididae). *Acta Zoologica Academiae Scientiarum Hungaricae*, 43 (1998) 323-343.
- [25] Móczár L. Revision of the Cleptes nitidulus group of the world (Hymenoptera, Chrysididae, Cleptinae). *Entomofauna*, 18 (1997) 25-44.
- [26] Kimsey L. Designation of chrysidid lectotypes. *Pan-Pacific Entomologist*, 62 (1986) 105-110.
- [27] Mocsáry A. Additamentum primum ad Monographiam Chrysididarum orbis terrarum universi. *Természetrájszi Füzetek*, 13 (1890) 45-66.
- [28] Japoshvili G. New records of Hymenopterans for Georgia from Mtirala National Park. *Journal of the Entomological Research Society*. 17 (2015) 89-92.
- [29] Gussakovskij V. Palaarktische Pompiloides-Arten (Hymenoptera, Psammocharidae). *Konowia*, 14 (1935) 135-150.
- [30] Wolf H., Sorg M., Stenmans W., Schwan H. Wegwespen (Hymenoptera, Pompilidae) der westlichen Paläarktis bearbeitet von Heinrich Wolf 1948 - 2008. – *Mitteilungen aus dem Entomologischen Verein Krefeld*, 3 (2009) 1-395.
- [31] Loktionov VM., Lelej AS. Spider wasps (Hymenoptera: Pompilidae) of the Russian Far East. – Vladivostok: Dalnauka, 2014. – 472 p. (2014) (in Russian).
- [32] Gussakovskij V. Psammocharidarum species novae et parum cognitae (Hymenoptera). *Revue Russe d'Entomologie*, 20 (1926) 250-259.
- [33] Gussakovskij V. Review of Palaearctic species of the genus Priocnemis Schodte (Hymenoptera, Psammocharidae). *Annuaire du Musée Zoologique de l'Académie des Sciences de l'Union des Républiques Socialistes*, 31 (1930) 227–290.
- [34] Mokrousov M., Popov I. Digger Wasps (Hymenoptera, Apoidea: Ampulicidae, Sphecidae, Crabronidae) of the Black Sea Coast of Krasnodar Territory, Abkhazia, and Adjacent Areas. *Entomological Review*, 96 (2016) 559-599.
- [35] Nemkov P. The digger wasps of the genus Alysson Panzer (Hymenoptera: Crabronidae: Bembicinae) of Russia and adjacent territories, with a key to species and new synonymies. *Zootaxa*, 3838 (2014) 276-286.
- [36] Nemkov P. Sphecoid wasps of the tribe Gorytini (Hymenoptera, Sphecidae) of the fauna of the CIS. The genera Argogorytes

- Ashmead, Hoplisoides Gribodo, Psammaecius Lepeletier. *Proceedings of the Zoological Institute. St. Petersburg*, 258 (1995) 128-137 (in Russian).
- [37] Schmid-Egger C. Revision of Bembecinus (Hymenoptera: Crabronidae) of Palaearctic region. *Notes faunistiques de Gembloux*, 54 (2004) 3-69.
- [38] Handlirsch A. Monographie der mit Nysson und Bembex Verwandten Grabwespen. VII (Schluss). *Sitzungsberichte der kaiserlich Akademie der Wissenschaften in Wien. Mathematisch-naturwissenschaft Classe. Abtheilung 1*, 102 (1893) 657-942 & plates I-VII.
- [39] Nemkov P. Digger wasps of the genus Bembex Fabricius, 1775 (Hymenoptera: Crabronidae, Bembicinae) of Russia and adjacent territories. *Far Eastern Entomologist*, 313 (2016) 1-34.
- [40] Nemkov P. Review of the digger wasps of the genus Brachystegus A. Costa (Hymenoptera, Crabronidae, Bembicinae) of Russia and neighbouring countries. *Far Eastern entomologist*, 131 (2003) 1-5.
- [41] Nemkov P. Digger wasps of the tribe Gorytini (Hymenoptera, Sphecidae) of the fauna of the USSR. Genera Gorytes Latreille, Pseudoplisus Ashmead, Kohlia Handlirsch. *Revue d'Entomologie de l'URSS*, 69 (1990) 675-690 (in Russian).
- [42] Schmid-Egger C. Key and records for the western Palaearctic species of Gorytes Latreille 1804 with descriptions of a new species (Hymenoptera, Sphecidae, Bembicinae). *Linzer biologische Beiträge*, 34 (2002) 167-190.
- [43] Mokrousov M., Proshchalykin M., Maharramov M. Digger wasps of the genus Hoplisoides Gribodo (Hymenoptera, Crabronidae, Bembicinae) from the Palaearctic Region, with description of two new species. *Journal of Hymenoptera Research*, 79 (2020) 213-233.
- [44] Nemkov P. Digger wasps of the genus Nysson Latreille (Hymenoptera, Crabronidae) of the Russian fauna. *Euroasian Entomological Journal*, 7 (2008) 217-221 (in Russian).
- [45] Ohl M. A Revision of Stizoides Guérin-Méneville, 1844: Taxonomy, Phylogenetic Relationships, Biogeography, and Evolution (Hymenoptera: Apoidea, "Sphecidae"). *Mitteilungen aus dem Museum für Naturkunde in Berlin*, 75 (1999) 63-169.
- [46] Handlirsch A. Monographie der mit Nysson und Bembex Verwandten Grabwespen. VI. *Sitzungsberichte der kaiserlich Akademie der Wissenschaften in Wien. Mathematisch-naturwissenschaft Classe. Abtheilung 1*, 101 (1892) 25-205 & plates I-III.
- [47] Dollfuss H. The Crabroninae Wasps of "Biologiezentrum Linz"-Collection in Linz, Austria (Hymenoptera, Apoidea, Crabronidae), Part 2. *Linzer Biologische Beiträge*, 38 (2006) 505-532.
- [48] Dollfuss H. The Crabroninae Wasps of "Biologiezentrum Linz" Collection in Linz, Austria (Hymenoptera, Apoidea, Crabronidae). *Linzer Biologische Beiträge*, 36 (2004) 761-784.
- [49] Nemkov P. Review of the digger wasp genus Palarus Latreille in Russia and neighbouring countries (Hymenoptera, Crabronidae, Larrinae). *Entomofauna*, 26 (2005) 241-252.
- [50] Nemkov P. *Annotated catalogue of digger wasps (Hymenoptera; Sphecidae, Crabronidae) of Asian part of Russia*. Dalnauka, Vladivostok, 193pp. (2009) (in Russian).
- [51] Pulawski W., Prentice M. A Revision of the Wasp Tribe Palarini Schrottky, 1909 (Hymenoptera: Apoidea: Crabronidae). *Proceedings of the California Academy of Sciences. Fourth Series*, 59 (2008) 307-479.
- [52] Puławski W. A revision of the Old World Parapiagetia Kohl (Hymenoptera, Sphecidae). *Polskie Pismo Entomologiczne*, 47 (1977) 601-669.
- [53] Gussakovskij V. Espèces paléarctiques des genres Didineis Wesm., Pison Latr. et Psen Latr. (Hymenoptera Sphecidae). *Travaux de l'Institut Zoologique de l'Academie des Sciences de l'URSS*, 4 (1937) 599-698 & one plate (in Russian & Latin).
- [54] Puławski W. *Les Tachysphex (Hym., Sphecidae) de la région paléarctique occidentale et centrale*. Panstwowe Wydawnictwo Naukowe. Wrocław, 464pp. (1971)
- [55] Straka J. Tachysphex austriacus Kohl, 1892 and T. pompiliformis (Panzer, 1804) (Hymenoptera, Crabronidae) are a complex of fourteen species in Europe and Turkey. *ZooKeys*, 577 (2016) 63-123.
- [56] Pulawski W. The Wasp Genus Tachysphex Kohl, 1883, of Sahara, Sub-Saharan Africa, the Arabian Peninsula, and Madagascar (Hymenoptera: Apoidea: Crabronidae).

- Proceedings of the California Academy of Sciences. Fourth Series, 58, Supplement 1* (2007) 1-698.
- [57] Radoszkowski O. Faune Hyménoptérologique Transcaspienne. *Horae Societatis Entomologicae Rossicae*, 20 (1886) 3-56 & plate I-XI.
- [58] Gussakovskij V. Les espèces paléarctiques du genre Trypoxylon Latr. (Hymenoptera, Sphecidae). *Travaux de l'Institut Zoologique de l'Académie des Sciences de l'URSS*, 3 (1936) 639-667 (in Russian & Latin).
- [59] Antropov A. New species of digger wasps of the genus Trypoxylon (Hymenoptera, Sphecidae) from Transcaucasia. *Zoologicheskij Zhurnal*, 64 (1985) 630-633 (in Russian).
- [60] Antropov A. On taxonomic rank of Trypoxylon attenuatum Smith (Hymenoptera, Sphecidae). *Entomologicheskoe Obozrenie*, 70 (1991) 672-685 (in Russian).
- [61] Antropov A. A new species of digger wasps of the genus Trypoxylon (Hymenoptera, Sphecidae) from Adzharia. – *Zoologicheskij Zhurnal*, 63 (1984) 626-628 (in Russian).
- [62] Antropov A. To the knowledge of the digger wasps of the tribe Trypoxylini (Hymenoptera, Sphecidae, Larrinae) of the Palearctic fauna. – *Byulleten Moskovskogo Obshestva Isspytateley Prirody. Biological section*, 94 (1989) 55-58 (in Russian).
- [63] Dollfuss H. The sphecid wasps from the genus Pemphredon Latreille 1796 of "Biologiezentrum Linz" collection in Linz, Austria (Hymenoptera, Sphecidae). *Linzer Biologische Beiträge*, 33 (2001) 269-273.
- [64] Mokrousov M. To the knowledge of digger wasps of subfamily Pemphredoninae (Hymenoptera: Crabronidae) of Russia. *Far Eastern Entomologist*, 337 (2017) 1-16
- [65] Budrys E. New and little-known species of digger wasps of tribe Psenini (Hymenoptera, Sphecidae). *Trudy vsesoyuznogo Entomologicheskogo Obshchestva*, 70 (1988) 101-116 (in Russian).
- [66] Dollfuss H. The Pemphredoninae Wasps of "Biologiezentrum Linz" Collection in Linz, Austria (Hymenoptera, Apoidea, Crabronidae). *Linzer Biologische Beiträge*, 36 (2004) 105-129.
- [67] Dollfuss H. The Ammophilini Wasps of the "Biologiezentrum Linz"-collection in Linz, Austria (part 2) including the Genera Ammophila Kirby and Podalonia Fernald (Hymenoptera, Apoidea, Sphecidae), and description of the hitherto unknown male of Podalonia erythropus (F. Smith 1856). *Linzer Biologische Beiträge*, 45 (2013) 565-591.
- [68] Mocsáry A. Hymenoptera nova europaea et exotica. *Természettudományok Köréből*, 13, (11) (1883) 1-72.
- [69] Kohl F. Die Hymenopterengruppe der Sphecinen. I. Monographie der natürlichen Gattung Spheg Linné (sens. lat.). (II. Abteilung). *Annalen des kaiserlich-königlichen naturhistorischen Hofmuseums. Wien*, 5 (1890) 317-462 & plates VIII-XII.
- [70] Kohl F. Die Hymenopterengruppe der Sphecinen. III. Monographie der Gattung Ammophila W. Kirby (sens. lat. = Ammophilinae Ashmead). Abteilung A. Die Ammophilinen der paläarktischen Region. *Annalen des kaiserlich-königlichen naturhistorischen Hofmuseums. Wien*, 21 (1906) 228-382 & plates VII-XIII.
- [71] Danilov Y., Mokrousov M. New data on the distribution and taxonomy of some Palearctic species of Sphecidae (Hymenoptera: Apoidea). *Euroasian Entomological Journal*, 16 (2017) 107-113.
- [72] Četković A., Mokrousov M., Plečaš M., Bogusch P., Anti D., Đorović-Jovanović L., Krpo-Četković J., Karaman M. Status of the potentially invasive Asian species Sceliphron deforme in Europe, and an update on the distribution of S. curvatum (Hymenoptera: Sphecidae). *Acta entomologica serbica*, 16 (2011) 91-114.
- [73] Kohl F. Die Hautflüglergruppe "Sphecinae". IV. Teil. Die natürliche Gattung Sceliphron Klug (= Pelopoeus Latr.). *Annalen des kaiserlich-königlichen naturhistorischen Hofmuseums in Wien*, 32 (1918) 1-171.
- [74] Dollfuss H. The Chloriontinae, Sceliphrinae and Sphecinae Wasps of the "Biologiezentrum Linz" – Collection in Linz, Austria including the Genera Chalybion Dahlbom, Chlorion Latreille, Dynatus Lepelletier de Saint-Fargeau, Penepodium Menke, Podium Fabricius, Sceliphron Klug, Stangeella Menke and Trigonopsis Perty (Hymenoptera, Apoidea, Sphecidae) and description of the new species Chalybion ohli from Madagascar. *Linzer biologische Beiträge*, 48 (2016) 1149-1185.
- [75] Morawitz F. Materialien zu einer vespidenfauna des Russischen Reiches. *Horae Societatis*

- Entomologicae Rossicae*, 29 (1896) 407-493.
- [76] Kobakhidze D. Vespidofofauna (Vespidae, Hymenoptera) of Georgia. – *Materials of fauna of Georgia* (1966) 46-58 (in Russian).
- [77] Kostylev G. Espèces nouvelles et peu connues de Vespides, d'Eumenides et Masarides paléarctiques (Hymenoptera). *Bulletin de la Société des Naturalistes de Moscou. Section Biologique*, 49, (5–6) (1940) 24–42.
- [78] Birula A. Über die russischen Wespen und ihre geographische verbreitung (Dritter Beitrag). *Annuaire du Musée Zoologique de l'Académie des Sciences de l'URSS*, 31 (1931) 291-339.
- [79] Blüthgen P. Neue oder bemerkenswerte paläarktische Falten-wespen aus der Zoologischen Staatssammlung in München (Hym. Vespidae, Eumenidinae.) *Mitteilungen der Münchnener Entomologischen Gesellschaft*, 41 (1951) 166-201.
- [80] Morawitz F. Eumenidarum species novae. *Horae Societatis Entomologicae Rossicae*, 19 (1885) 135-182.
- [81] Gul Aslan E., Mumladze L., Japoshvili G. List of leaf beetles (Coleoptera: Chrysomelidae) from Lagodekhi reserve with new records for Transcaucasia and Georgia. *Zootaxa*, 4277(1) (2017) 86-98.
- [82] Semenow, A. 1892. Revisio Hymenopterorum Musei Zoologici Academiae Caesareae Scientiarum Petropolitanae. III Familia Evaniidae. – *Bulletin de l'Académie Impériale des Sciences de St.-Petersbourg. Nouvelle Série* 3, (35): 9-30.

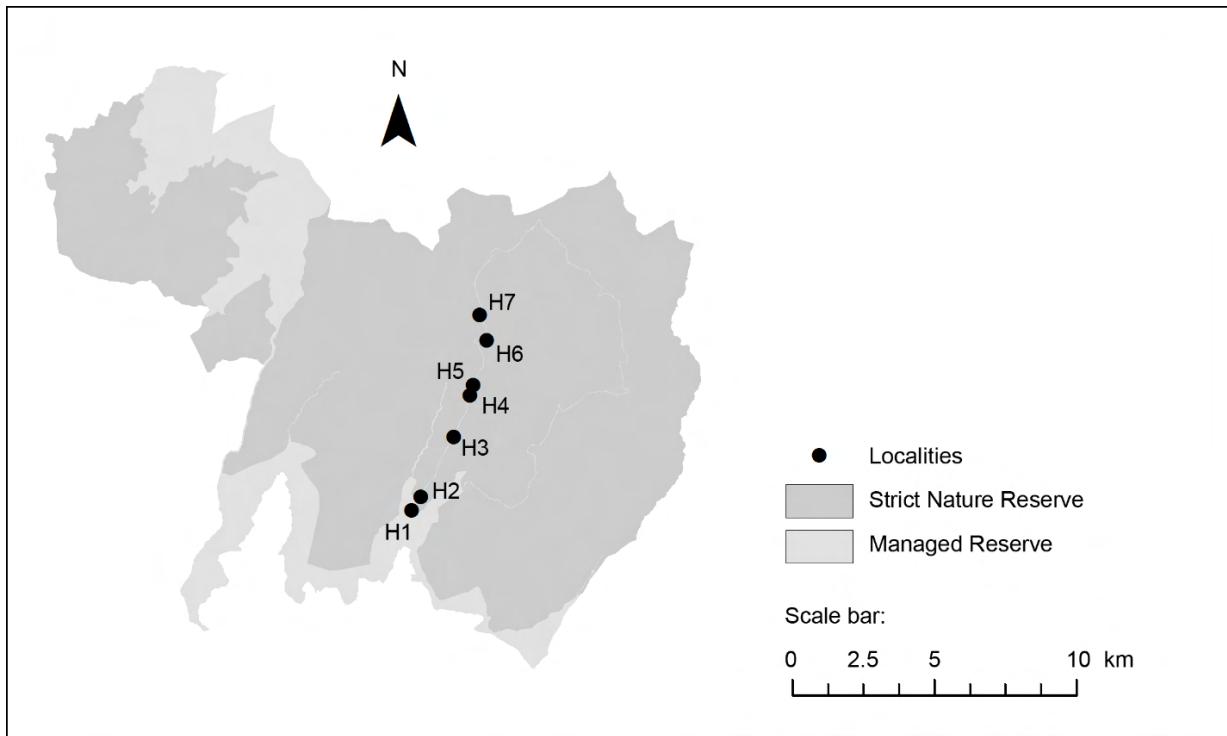
**Figure 1.** Geographic location and the limits of Lagodekhi Protected Areas. Points on the map show the sampling sites from the lowest (H1) to the highest (H7).

**Table 1.** Basic data on sample sites, elevation, hymenopteran catches, and hymenopteran and plant richness. Estimated richness is based on the second order jackknife.

Sam- pling loca- lities	Eleva- tion	Total hymenop- teran catches	Hyme- nopteran species richness	Expected hymenop- teran richness	Unique species at this level	Species caught only once	Species caught twice	Total plant richness	Woody plant richness	Herb rich- ness
H1	665	571	58	76	11	19	10	51	12	39
H2	841	448	46	62	9	13	5	50	14	35
H3	1350	590	53	65	10	17	12	39	10	28
H4	1840	83	26	44	1	12	4	34	10	22
H5	1920	13	6	10	2	4	0	90	17	69
H6	2230	68	21	29	0	9	5	139	7	127
H7	2559	28	10	18	1	4	1	119	3	112

**Table 2.** Basic relationships between plant and hymenopteran richness based on the data in Table 1.

Sampling localities	Hymenoptera catches / richness	Hymenoptera richness / plant richness	Hymenoptera richness / woody plant richness	Hymenoptera richness / herb richness
H1	9.84	1.14	4.83	1.49
H2	9.74	0.92	3.29	1.31
H3	11.13	1.36	5.30	1.89
H4	3.19	0.76	2.60	1.18
H5	2.17	0.07	0.35	0.09
H6	3.24	0.15	3.00	0.17
H7	2.80	0.08	3.33	0.09



**Fig.** *Areas of study in Lagodekhi Protected Areas*

Points on the map show the sampling sites from lowest (H1) to the highest (H7).

H1 – mountain low zone; H2, H3 – mountain middle zone; H4, H5 – mountain upper zone;  
H6 – subalpine zone; H7 – alpine zone.



## Role of the subalpine forest ecosystems (on example of the southern slope of Great Caucasus)

L. Dolidze<sup>a,\*</sup>, M. Bachilava<sup>b\*</sup>

<sup>a</sup>Georgian Academy of Agricultural Science  
51, I. Javakhishvili Str., Tbilisi, 0102, Georgia

<sup>b</sup>Ministry of Environmental Protection and Agricultural of Georgia,  
LEPL Scientific-Research Center of Agriculture, 6, Marshal Gelovani Str., Georgia, 0159, Georgia

Received: 10 December 2019; Accepted: 23 April 2020

### ABSTRACT

The current situation of subalpine forest ecosystem is discussed in this article. Investigations were carried out in the typical Duruji river basin, where because of anthropogenic influence occurred degradation of forest and grass cover, followed by deterioration of physical characteristics of soil, increasing of surface runoff and soil erosion causing significant decreasing of the upper boundary of forest from 2500-2600 m to 2100-2200 m above sea level.

The main physical characteristics of soil: such as dispersivity, hydrofiltration, indexes of erosion was studied for the Duruji River muflow basin. Soils of subalpine forests ecosystem characterized by good water regulation functions and they are natural mechanisms for runoff regulation. Afforestation-melioration activities should be carried out for the deforested areas resulted from anthropogenic influence which are under serious threat of erosion. Recommendations are developed according to the selected species, soil conditions and slope declinations.

**Keywords:** Subalpine, Ecosystem, Soil erosion, Restoration, Forest, Measure.

\*Corresponding author: Maia Bachilava; E-mail address: [m.bachilava@agrni.edu.ge](mailto:m.bachilava@agrni.edu.ge)

### Introduction

Sub-alpine forests are significantly important among the Georgian mountain forests. According to V.Gulisashvili [1], subalpine zone in Georgia located at different altitudes, on steep slopes of the Greater and Lesser Caucasus. In the sea climate conditions it occurs at the altitude from 1900-2000 m. to 2100-2150 m., in the continental climate conditions at the altitude 2150-2300 m.

Subalpine forest ecosystems according to L.Makhatadze [2] are divided into three categories: colhic, boreal and transcaucasian.

Colhic subalpine forest ecosystems occurs in the west part of Georgia and creates kroomm-

holzed beech, birch and Pontus Oak stands. Subalpine stands are presented by acer, birch, spruce, in understory there are following species: *Rhododendron luteum*, *Prunus laurocerasus* and *Vaccinium arctostaphylos*.

Boreal forest ecosystems spread on the north slopes of the Caucasus – in Kazbegi region, Mta-tusheti, near to Enguri, Kodori, Bzipi river basin. Above mentioned forest ecosystems are presented by *Betula* spp. and *Pine* spp. subalpine low density stands.

Transcaucasus forest ecosystems are presented mainly by subalpine low density stands, sometimes beech kroommholzed stands. These type of forests occurs in Meskheta and Trialeti ranges. In Kartli and Kakheti there are stands of the following

species, such as *Betula* spp, *Acer trautvetteri medw.*, *Quercus macranthera*, *Fagus orientalis L* and *Pinus* spp. [3].

Beech and birch kroommholzed stands are not more then 3-4 meter and mainly located in the upper part of natural range, near to alpine zone [4].

Climate of subalpine zone is moderately cold [5]. It is characterized by short and warm summer, severe and long winter, with variations in temperature, reduced vegetation period, early autumn and late summer frosts, resulted in development of woody plants in wervtical direction, above which forest doesn't occur and replaced by meadow vegetation.

Vegetation of subalpine zone formed by forest, shrubs and meadow type of species. Subalpine forest charachterized by low growth and low productivity. As a result of anthropogenic influence (unreasonable selective cuttings, unsustaible grazing induced degradation of forest and grass cover, furtthemore often ocured deterioration of physical characteristics of soil, encreasing of surface runoff and soil erosian processes, negatively affecting on the development of plants.

As a result of above mentioned processes the upper boundary of forest range decreased from 2500-2600 m above sea level, causing significant erosive processes and formation of dramatic mudflows in mountain river basins [6-11].

Protection of existed vegetation and planned reforestation activities is a major chalange in the above mentioned ecosystems.

**Reseach objectives**

Studies were conducted in typical Duruji River mudflow basin. There are three zones in this basin: 1. The lower zone (from 450-1000 m. above sea

level); 2. The middle zone (from 1000-1800 m. above sea level); 3. The upper zone (1800-2200 or 2300 m).

Researches were carried out for this purpose in typical Duruji River mudflow basin, where the forest zone strating from 450 m and reache to 2200-2300 m. There are three basins: 1. The lower zone (from 450-1000 m. above sea level); 2. The middle zone (from 1000-1800 m. above sea level); 3. The upper zone (1800-2200 or sometimes 2300 m).

The first and the second zone are charachterized by high density (0.6 and 0.7) forest ecosystems, which distinguished by their protective-ecological and erosian resistant functions.

The third zone, sublapine zone presented by the following forest types:

1. Fagus forest with bracken;
2. High mountain Quercus;
3. Acer forest with high grass cover;
4. Rare Betula.

According to the data of G.Kharaishvili [12]; T.Urushadze, T.Kvrivishvili [13]; T.Urushadze [14] subalpine zone mainly are presented by mountainous-forest-meadow type soils.

Woody and grassy vegetation support formation of stable soil structure, which improving its characteristics and prevent development of erosion processes. After destruction of vegetative cover soil loses stability and by the influence of water easily fall into the pieces. This enhance blocking of capillary pores of the soils and reduce water permeability. As a result, liquid runoff will be developed and depletion of soil will be occurred.

Studies of the physical characteristics of soil show that soil have good physical properties in Duruji River mudflow basin and in subalpine zone low density stands. (Table 1).

**Table 1.** Physical properties of the soils of Duruji Basin

Altitude, m	Exposition	Vegetation	Depth, cm	Porosity (%)			
				Total	Capillary	Non capillary	Conduct of the 10 cm water pillar (min)
1930	N-W	Fagus forest+A	0-10	57.6	45.8	11.2	0.7
2050	NN-W	Acer forest + F,B	0-10	60.4	47.9	12.5	0.7
2100	W	Betula forest with grass	0-10	55.5	41.4	14.1	0.8

2180	N-E.	Betula forest with Rhododendron	0-10	47.3	29.2	18.1	–
1900	E	Quercus + Acer + Ulmus	0-10	47.7	36.2	11.5	–

In beech, birch and acer forest ecosystem non-capillarity of soil is varied from 11.8-14.1 %, water permeability – 0.7-0.8 min. Birch forest with its *Rhododendron caucasicum* understory is also very important, where non-capillarity of soil is about 18%.

The soil erosion indicators (Table 2) studied by us show the following results: upper soils of headwaters of Duruji River, where dispersion index is -  $D = 0.68$ , belongs to the group of mean dispersion indicator [15]), and the lower layers belongs to the high dispersion indicator ( $D = 0.96$ ).

**Table 2.** Erosion proof of river Duruji Basin

Altitude	Vegetation	Depth, cm	Dispersion			Hidrof.				Unit		
			<0,05 sm without chemical farm (%)	0,05 cm with chemical farm (%)	Dispersion	Max hygros. (%)	Content of colloids (%)	Equivalent humidity (%)	Indicator of hydrof. (C)	>0,25 mm unit (%)	Indicator of aggregates (a)	(E)
1930	Fagus forest with Acer and Sorbus	3-18	63.81	92.22	0.68	10.16	38.86	33.68	0.99	91.48	0.63	1.06
2050	Acer forest with Fagus, Betula and Sorbus	2-15	70.05	86.61	0.80	14.05	46.83	44.33	0.94	92.88	0.43	1.77
		20-45	78.87	94.39	0.83	9.95	33.16	42.17	1.17	88.80	0.52	2.03
2300	Subal pine meadow without pastures	0-10	61.13	75.37	0.81	12.79	42.95	59.39	1.38	81.20	0.33	3.38
		15-40	64.80	76.14	0.95	10.00	33.33	47.98	1.43	78.40	0.39	5.27
2400	Subal pine meadow without pastures	0-10	65.92	80.89	0.81	13.89	46.30	88.99	1.92	77.08	0.30	5.18
		15-25	70.63	84.12	0.84	8.60	28.66	70.47	2.45	76.92	0.39	5.27

The upper layers of beech forest soil are much less susceptible to the erosion processes than the lower ones, index of dispersion ranges from 0.81-0.85. These soils belong to the high desperation rate.

According to the hydrophilicity parameters the upper soil layers in Duruji river headwaters and subalpine zone belongs to the low range ( $H < 1.0$ ), but lower layers belong to the average ( $H = 1.0-1.25$ ). Soils in the alpine are more susceptible to the erosion than in subalpine zone. Furthermore, plots where occurred unsustainable grazing tend to be more susceptible than the areas without grazing.

According to the aggregate index investigated soils (a) can be grouped in the following maner: the soils of beech forest where  $a > 0.60$  belong to the group of very high aggregate index, the soils of subalpine low density stands and alpine meadows, where  $a = 0.30-0.60$  belong to the group of high aggregate index.

So, the highest aggregate index have forest soils ( $a = 0.60-0.64$ ), followed by forest soils of subalpine zone ( $a = 0.43-0.52$ ) and the lowest index have the soils of alpine zone where occurred unsustainable grazing.

Indicator of soil erosion (E) for investigated soils varies between (1.06 -5.27). This indicator is different for the soils in forest areas and for non-forest areas. For example, indicator of soil erosion in the upper layers in beech and acer forest ecosystems is about  $E = 1.06 - 5.27$  and in the upper layers of alpine zone is -  $E = 3.88 - 5.18$ .

Investigations showed that the soils of subalpine forest ecosystem have good water regulative functions and act as a natural protective mechanism to regulate both: liquid and solid runoff. Namely, stands of evergreen *Rhododendron caucasicum* and low density birch stands.

Other forest stands according to water regulation functions are grouped in the following order: birch stands, birch, pine and oak low density stands.

Soils of stands of *Rhododendron caucasicum*, *Betula spp* and *Ulmus spp*. of subalpine zone of the Greater Caucasus mountain range are characterized by the best water regulation functions.

Other formations of subalpine forest ecosystems according to the water regulations functions are grouped in the following order: birch stands, kroomholzed birch stands, birch low density stands and stands of *Pinus sosnowskyi*.

The above mentioned beneficial functions of forest ecosystems, such as water regulative, soil protective and other ecological functions should

become basis for formation of targeted forest stands against soil erosion, which as a result anthropogenic influence are deforested and now there is serious threat of development of erosive processes.

Therefore one of the main activities should be considered banning of cattle grazing, implementation of forest-melioration activities, considering increasing of the upper boundary up to its natural range (2500-2600 m.above sea level).

Forest ecosystems against soil erosion in Subalpine zone should be compound with *Shade-tolerant species* in the second floor. For the good water regulation purposes are recommended to use the following species:

1. Tree -shrub type, with oak dominant species
  1. Tree -shrub type, with oak dominant species
  2. Tree -shrub type, with pine dominant species;
  3. Tree -shrub type, with beech dominant species;

Shrub type for the following types the following species will be selected:

East Georgia:

- 1 – type: trees – birch (*Betula spp*), *Acer pseudoplatanus*, *Sorbus aucupa*;  
Shrubs – nut, *Viburnum lantana*, *Caucasian Honeysuckle*
- 2 – type: trees - *Quercus macranthera*, *Acer trautvetteri*, birch Shrubs: nuts, *Viburnum lantana*, *Caucasian Honeysuckle*
- 3 – type: trees - *Pinus sosnowskyi*, birch, *Acer trautvetteri*;  
shrubs: nuts, *Ribes nigrum*, *Viburnum lantana*.
- 4 –type: trees - *Fagus orientalis L.*, *Sorbus aucupa*, *Acer trautvetteri*;  
shrubs: *Caucasian Honeysuckle*, *Viburnum lantana*, *Ribes nigrum*.
- 5 – type: trees – nuts, *Ribes nigrum*, *Rhododendron caucasicum*, *Azalea pontica L.*,  
*Salix caprea* and other local shrub species.

On the slopes with inclination  $< 6^\circ$  m, soil should be completely cultivated with belts varies between  $6^\circ$   $12^\circ$  and terraces from  $12^\circ$   $20^\circ$ . For the slope with inclination  $20^\circ$  and more should be made pits with chess layout.

On the slopes with inclination ( $20^\circ <$ ) it is reasonable planting of forest plantations in the total area of watershed (at least 10,000 hectares per 1 ha).

Selection of woody species should be made according to the inclination and erosion rate: for the steep inclination ( $20^\circ$  and  $>$ ) – 50% of total number of selected plants, for the medium inclination ( $< 20^\circ$ ) – 40 %.

In terms of guarantee the best water regulation

functions it is recommended used equal ratio (50-50%) of coniferous and broadleaf species.

Natural regeneration activities should be carried out in the low density subalpine forest ecosystems where are not *Rhododendron caucasicum* and *Rhododendron luteum*. It is recommended planting in pits with chess layout.

During carrying out melioration activities special attention should be paid to the to preserving of existing forest ecosystems and restoring of degraded. For this purpose new established forest plantations in the future should give us at least medium (0.5-0.6) dense stands [16-18].

Tending of forest plantations depends on local natural-climatic conditions and is recommended to be continued for 4-5 years. Namely, in the first year number of activities should be 5, in the second year 4, in the third year 3, in the fourth – 2, in the fifth – 1.

Tending of forest plantations usually involves soil timely cultivation, weeding. However, on the slopes where forest plantations are established, grazing should be banned until fully formation of stands.

## Conclusion

1. Subalpine forest ecosystems of River Duruji with higher density (0,8) are natural mechanism of regulating of liquid and solid runoff.
2. Grove of evergreen azaleas and low density stands of subalpine birch croomholz with *Sorbus aucuparia* and with undersory (*Rhododendron caucasicum*) characterized by the best water regulation functions.
3. Uncontrolled grazing negatively impacted on the development of Subalpine forest ecosystems.

## References

- [1] V. Gulisashvili, Nature and nature zones of Georgia, „Sabchota Sakartvelo“, Tbilisi, 244 pp (in Georgian).
- [2] L. Makhatadze, Types of forests of Trialeti Range and they use in forestry, Works of Tbilisi Institute of Forestry, Lesnaia Promishlenost , Tbilisi, 1962 (in Russian).
- [3] G. Gigauri, Biodiversity of Forest of Georgia, Rariteti, Tbilisi, 2000, 160 pp. (in Georgian).
- [4] V. Gulisashvili, Nature zones and natural and historic areas of Caucasus, Nauka, Moscow, 1964, 327 pp, (in Russian).
- [5] M. Kordzakhia, Climate of Georgia, Sabchota Sakartvelo, Tbilisi, 1961, 246 pp. (in Georgian).
- [6] G. Kharashvili, O. Dvalishvili, R. Chagelishvili, L. Dolidze, Water Regulation and Soil Protected Functions, Measures of its Conservation,, „Momavlidan“, Tbilisi, 2007, 238-247, (in Georgian).
- [7] G. Kharashvili, Ravine erosion and Fight with them by Forest-Melioration, Measures, Poligrafist“, Tbilisi, 2009. 84 pp, (in Georgian).
- [8] G. Gigauri, Forests of Georgia, Forestry in changing societies in Europe, Silva, Network, 1999.
- [9] R. Chagelishvili, G. Kharashvili, Measures of Protecting of Mountain Forest from Erosion Processes, J. Agricultural of Georgia, 12 (1970), 6-11 (in Georgian).
- [10] L. Dolidze, G. Kharashvili, O. Dvalishvili, Role of Water Regulation and Fights Against Erosion of the mountain forests of West Georgia, Scientific Works of the Institute of Mountain Forestry, Metsniereba, 2003, 94-107, (in Georgian).
- [11] L. Dolidze, Scientific Bases for Forest Ecosystems optimization and Stabilization of East Georgia, Tbilisi, 2019, 250 pp, (in Georgian).
- [12] G. Tarasashvili, Mountain-forest and Mountain Meadow Soils of East Georgia, Metsniereba, Tbilisi, 1956, 220 pp, (in Russian).
- [13] T. Urushadze, T. Kvrivishvili, Guide of Georgian Soils, Mtsignobari, Tbilisi, 2014, 135 pp (in Georgian).
- [14] T. Urushadze, Forest Soils of Georgia, Metsniereba, Tbilisi, 1972, 244 pp, (in Georgia).
- [15] A. Vosnesenski, Laboratory Method to determine of anti-erosion capacity of soils, “Nauka”, Tbilisi”, 1940 (in Russian).
- [16] Sh. Khidasheli, Ecological-technological basis of transformation of degradation forest plant in forest-garden, Metsniereba, Tbilisi, 2005 (in Russian).
- [17] G. Kharashvili, Protection of the Subalpine forests and principles of its Creation in Georgia, Works of the Forest Mountainous Institute, Metsniereba, Tbilisi, 1985, pp. 16-37 (in Russian). L. Dolidze, Cutting and Renewal of Forest, J. Agrarian Science, 1, (2006) 18-19 (in Russian).



## Annals of Agrarian Science

Journal homepage: <http://journals.org.ge/index.php>



# Survey results of sanitary-hygienic conditions and landscaping of urban forest parks in the Republic of Armenia (RA)

M.H. Galstyan<sup>a</sup>, H.Ya.Sayadyan<sup>b,\*</sup>, K.Sh. Sargsyan<sup>a</sup>, Z.H. Hoveyan<sup>a</sup>,  
I.L. Hakobjanyan<sup>a</sup>

<sup>a</sup>Armenian National Agrarian University; 74, Teryan Sr., Yerevan, 0009, Republic of Armenia

<sup>b</sup>Yerevan State University; 1, Alex Manukyan Str., Yerevan, 0002, Republic of Armenia

Received: 26 September 2021; Accepted: 24 October 2021

## ABSTRACT

The survey results conducted on the evaluation of sanitary and hygienic conditions, services, and landscaping in urban forest parks of the RA are introduced in the current article.

According to the study and survey data the blue and green areas (parks, lakes, alleys, etc.) of the republic are mainly used as intended by the population. Anyhow, there are some obstacles for the enhancement of management efficiency in the mentioned areas, particularly in terms of increasing and expanding the people's attendance, which are especially related to the pollution issues, poor conditions of individual parks, lack of signboards, improper organization of public transport and catering, as well as their availability, hence calling attention of the relevant structures and organizations.

**Keywords:** Urban forest park, landscaping, survey, evaluation, pollution, sanitary hygiene, availability, preferences.

\*Corresponding author: Hovik Sayadyan; E-mail address: [hovik.sayadyan71@gmail.com](mailto:hovik.sayadyan71@gmail.com)

## Introduction

Since time immemorial the humanity has been conscious of the role and significance of the settlement gardening and artificial afforestation, particularly in woodless areas.

In the southern located countries mitigation of the solar radiation intensity is of utmost importance. Upon multiple investigations it has been substantiated that inside the green plantations the solar radiation is about ten times weaker, than in open areas. Providing shade in forest parks and green areas is the most important issue, since it ensures the organization of people's proper rest in southern conditions. In forest parks and green zones, the heat is reduced more intensively as compared to other types of green plantations, since in the green

masses the sun rays first fall on the surface of the plants foliage and are mostly absorbed by them. In green areas the air temperature is at least 3-4 0C lower than that of recorded in the surrounding areas. In the inner layers of the tree and shrub foliage grown in recreation areas the temperature is considerably lower due to the circumstance, that via the evaporation process the leaves saturate the environment with humidity [1-3].

The studies have shown that in conditions of Yerevan city the temperature inside the foliage of the trees and shrubs grown in the intensely landscaped parks is 5-8 0C lower than that of in open, non-landscaped areas [1- 4]. It has been also found out that the effect of the green areas on the air temperature is figured out not only inside the plantations but also in their surroundings, sometimes

in the distance of up to 0.5 km. The mentioned effect is mainly related to the size of green areas: the larger the area is, the higher its favorable effect on high temperature mitigation is.

Landscaping is one of the key problems for the current urban development; it has a multifaceted significance. Trees and shrubs absorb 72.0 % of the dust suspended in the air, 60.0 % of the sulfur gas and other harmful substances [2,3,4]. Regulating the temperature and relative air humidity saturating air with oxygen and simultaneously imbibing carbon dioxide from the atmosphere, the green areas, particularly coniferous species, emit phytoncides, which have bactericidal properties, in addition, they also kill pathogens dangerous for human health. According to the data introduced by numerous authors [4 - 8], the green areas in the cities absorb 26.0 % of the overall sound energy fallen on those territories. The researchers have justified that in the high-rise buildings located at the roadside the borders of which are lacking any green plantations, trees and shrubs, the noise is 5 times higher than in the buildings near the walkways with trees and greens. Urban parks should be looked upon as ecosystems, which can be effective for a long time in case of some human intervention (support). This is not about the periodic spraying of the parks with chemical pesticides, which contaminate (poison) the whole environment, but it is about the reasonable regulation of the animal and plant amount rather. It is necessary to sow wild grasses, where insects dwell, which birds feed on, to hang birds' nest in the parks and feed them in winter, etc.

Thus, by so much emphasizing the role of parks, green areas and urban forest parks in the recovery of human health and labor capacity, it is also of extreme significance to have an insight about their state, so as to develop the needed measures in the given city or settlement to improve such an important branch as proper which would be aimed at the human health and labor capacity recovery and proper organization of environmental work.

## Materials and methods

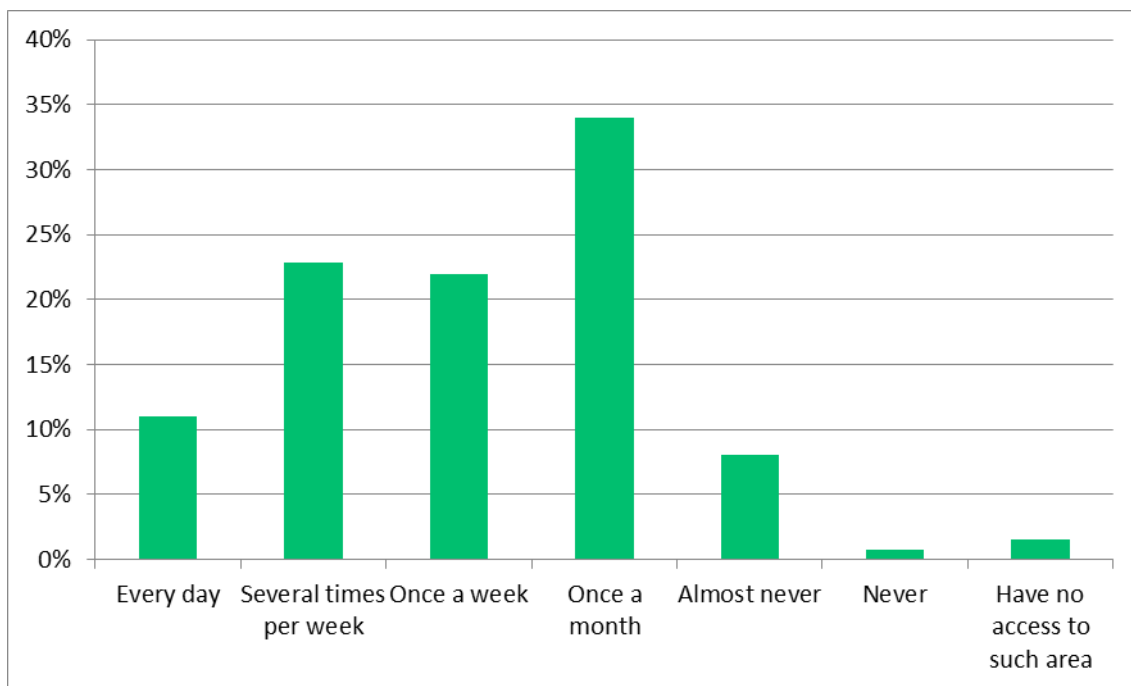
The Armenian National Agrarian University (ANAU) participated in "Urban Blue-Green Infrastructures in Eastern and Western Europe" education-research grant program supported by the Swedish Institute (SI). This is multi-national (Sweden, Ukraine, Georgia and Armenia) project with the aim to identify and evaluate the sanitary and

hygienic state of urban forest parks, health resorts and recreation sites within that specific country, the frequency and ability of the people to visit those areas, as well as to implement activities concerning the internationalization of the collaborating universities. To accomplish the mentioned goals, upon the multiple discussions held with the researchers engaged in the grant programs implemented at the partner universities of the mentioned countries, questionnaires were compiled, based on the answers (online and face-to-face) of which the state of the mentioned areas in the preferable cities of the certain country was evaluated. Then after some analyses and summary evaluation of the received results a system of required measures was developed in order to expand the blue and green areas in the settlements and to promote the population's healthy lifestyle, as well as to ensure life expectancy extension via their effective functionality.

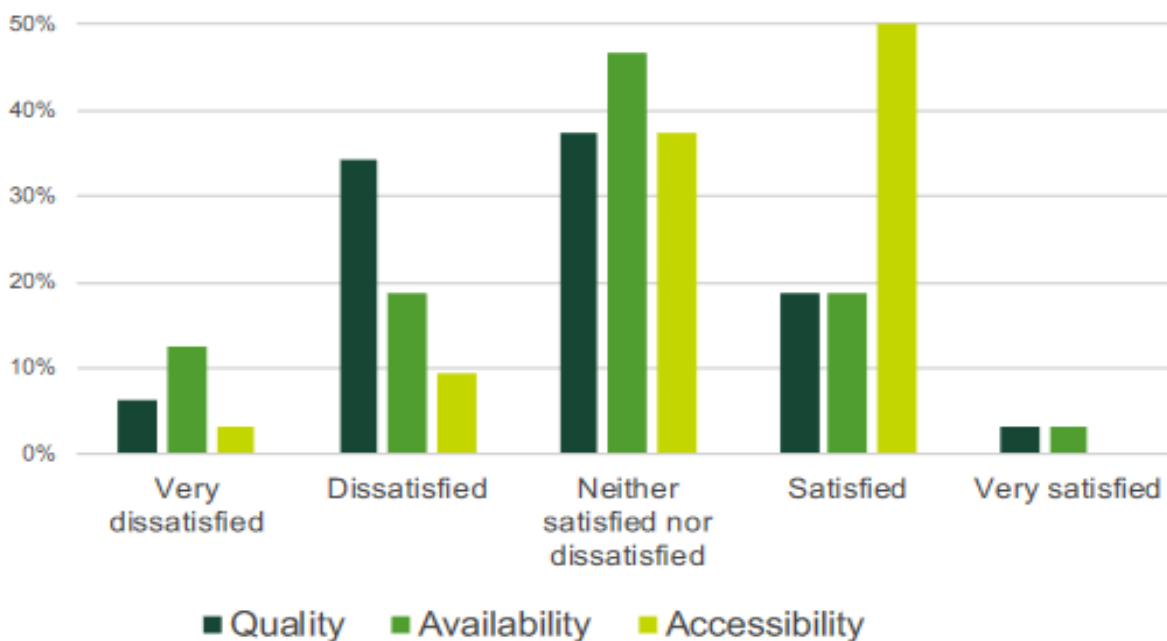
## Research results

The results of the investigations (surveys) carried out in the Republic of Armenia (RA) have indicated that out of about 1200 respondents more than 81.5 %, highly prioritize the role of green and blue areas, visit those sites with varying frequency and take rest there. Besides, 10 % of the attenders or 120 people visit parks every day, 24.5 % (294 people) – several times a week, 17.0 % (194 people) – once a week and 27.5 % or 330 people visit parks once a month (Fig. 1), while only 18.5 % of the respondents hardly ever attend such places, which is accounted for the fact, that 35.0 % of the visitors are dissatisfied with green and blue spaces availability, service quality, and what is more important, they are short of time and have health problems (Figure 2).

As the survey results conducted for the park availability, affordability and service quality have shown, only 17 % of the respondents (204 people) are satisfied or very satisfied with the parks' availability and service quality and 50 % - with its affordability (Fig. 2). From the rest of the respondents about 5 % or 60 people are very dissatisfied, 35 % are dissatisfied with the mentioned criteria, while 37.0 % expressed neutral opinion towards the criteria of availability, affordability and service quality, which, once more, comes to prove the fact that there are some people in the society, who are indifferent to the environmental protection, particularly towards the parks, their conditions and to the rendered service quality.



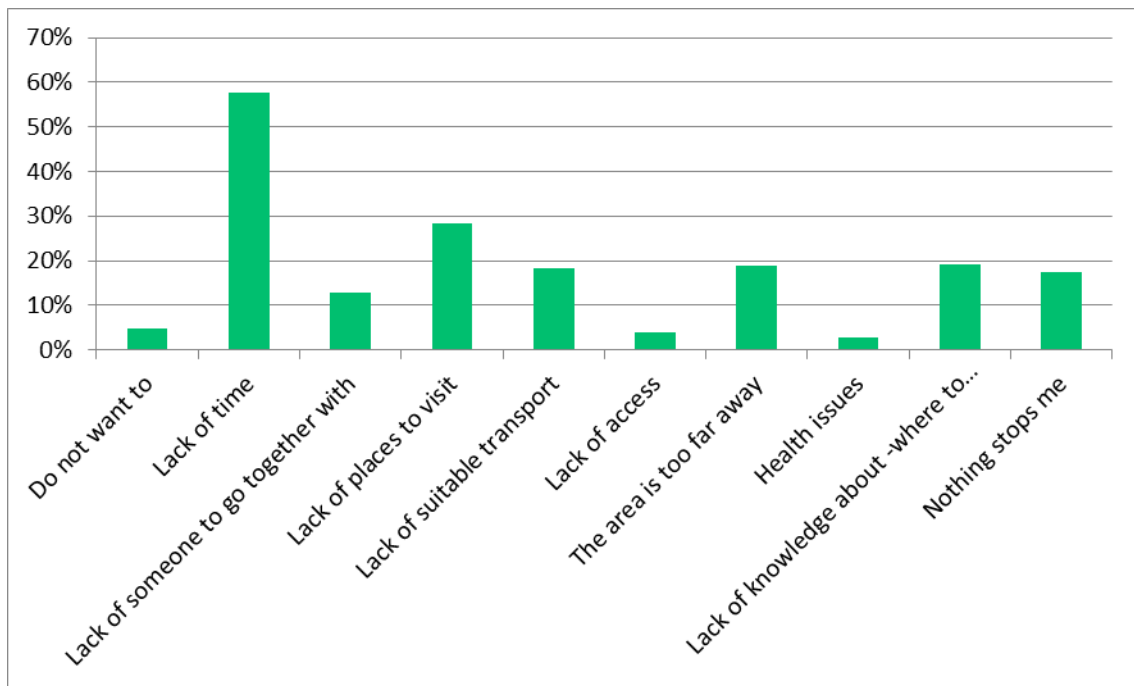
**Fig. 1.** Frequency of urban green areas' use



**Fig.2.** Perceived quality, availability and accessibility of urban green areas

The 74.5 % of the overall respondents or 894 people expressed their great concern about the pollution of the parks and 25.0 % mentioned that they didn't see any problems in the parks which they had visited. Meanwhile there is a certain number of people among the respondents, with

negligible percentage, who were in the opinion that the frequency of the visits can be affected by the problem related to lack of signboards in the parks, existence of overgrown or toxic plants, the hazard of getting hurt due to the relief characteristics and the noise of children (Fig. 3).

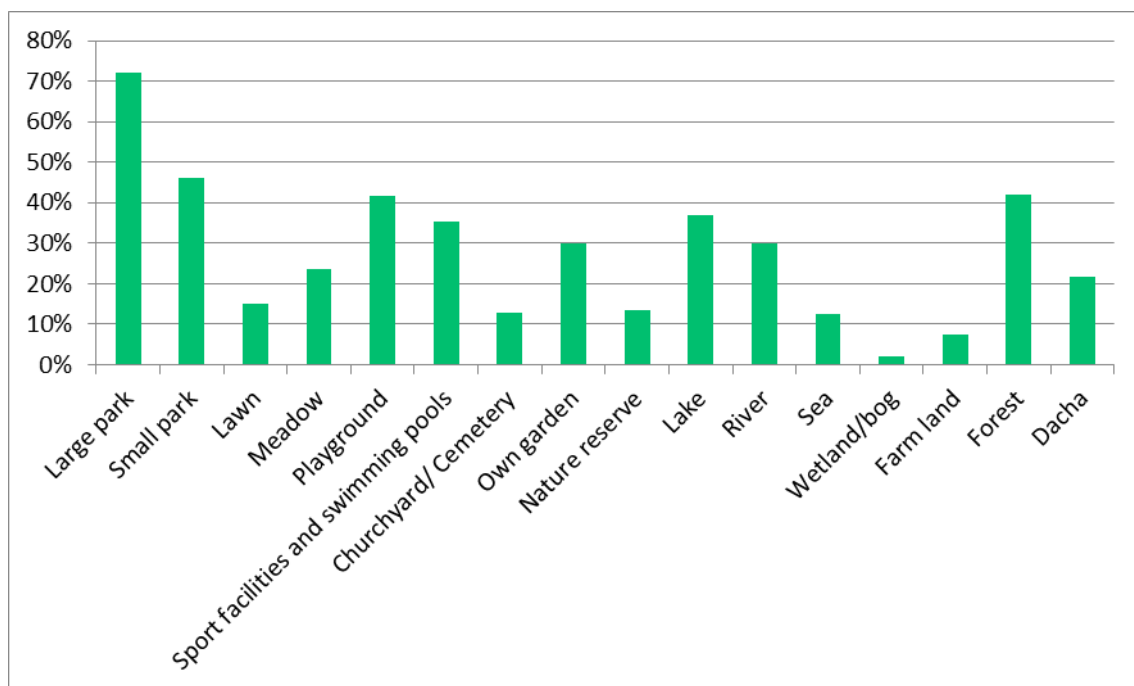


**Fig.3.** Perceived problems

The results of the surveys conducted about the preferable blue and green areas by the respondents are introduced in Fig. 4. According to those data, 72.0 % of the respondents or 826 people prefer visiting large parks, 46 % (529 people) – small parks and 42.0 % or 483 people visit forests.

All respondents indicated that parks should have

playgrounds (35.0 %) and it is desirable that they had swimming pools (37.0 %), where apart from swimming it would be also possible to hold sports events. The interviews also show data retrieved from the respondents who had alternatives to the abovementioned rest areas, such as own garden, lake, river, sea, farmland, etc.



**Fig.4.** Selected urban blue-green areas

### Conclusions and recommendations

- [1] Thus, according to the survey results, the targeted use of blue and green areas (parks, lakes, alleys, etc.) of the Republic of Armenia, expansion of those areas and increase of people's attendance to the mentioned sites, as well as the enhancement of their efficiency are particularly affected and interfered with such circumstances as pollution, poor conditions of individual walkways, lack of signboards, improper organization of public transport and catering services and their affordability, which are claimed to be overcome with the support of relevant state bodies.
- [2] In order to handle the current situation related to the blue and green areas and to establish efficient management system, as a vital component in the ecological and economic complex policy of the cities, it is required to implement reforms in the management policy of green plantings and recreation sites.
- [3] In the contemporary conditions the promotion of urban landscaping and the proper management of green plantings and parks can be possible only in case when the management of the mentioned areas is implemented via the consistent combination of the following three components: service, development and control.
- [4] Special accent should be put on the vertical landscaping of the buildings, as a result of which the microclimatic and sanitary-hygienic conditions of the cities, particularly those of located in the southern belts, such as Yerevan, will improve, and at the same time the buildings and constructions will look more spectacular.

### References

- [1] Galstyan M.H., Mkrtchyan A.L., RA Natural Resources. Teaching Guide for Higher Education Institution, Yerevan, ASAU, Yerevan, 2013 (in Armenian).
- [2] Harutyunyan L.V., Armenian Nature, the Ways of its Conservation and Enrichment. Nahapet, Yerevan, 2005, (in Armenian).
- [3] Harutyunyan V.S., Sargsyan K.Sh., Teaching Guide for the Laboratory and Practical Classes in the Course of Environmental Monitoring, ASAU, Yerevan, 2012 (in Armenian).
- [4] Melkumyan L.S., Galstyan M.H., Basics of Nature Protection, Teaching Guide, "Zangak –

97", Yerevan, 2010 (in Armenian).

- [5] Sayadyan H.Ya., Vulnerability of Forest Ecosystems in Armenia and the Development of Greenhouse Absorbents "Climate Change Issues of Armenia" (Collection of Articles), 1999, pp. 77-82 (in Armenian).
- [6] Harutyunyan L.V., Landscaping of Yerevan Related to Some Peculiarities of the Microclimate, Bulletin of the Agricultural Sciences of the Armenian SSR, T X111, No 5 (1960) 65-72 (in Armenian).
- [7] Vardanyan Zh.A., Grigoryan A.A., Actual Problems of Landscaping in Yerevan, Mat. Int. Scientific. Conf. "Problems of Modern Dendrology", Moscow, 2009, pp. 431-433 (in Russian).
- [8] Sargsyan K.Sh., Green Ring of Yerevan. History of Establishment and Ways of Restoration, Asoghik, Yerevan, 2007 (in Armenian).



## Determining the likelihood of emerging illegal dumpsite on the example of Georgia

N. Dzebisashvili<sup>a\*</sup>, N. Buachidze<sup>a</sup>, G. Berechikidze<sup>b</sup>

<sup>a</sup>Georgian Technical University, Institute of Hydrometeorology;  
150g, David Agmashenebeli Ave., Tbilisi, 0112, Georgia

<sup>b</sup>National Forestry Agency of Georgia; 6, G. Gulua Str., Tbilisi, 0114, Georgia

Received: 22 June 2021; Accepted: 03 July 2021

### ABSTRACT

On the example of the Mtskheta-Tianeti highland rural region of Georgia, the probability of illegal dumpsites/ landfills is studied using the mathematical equation developed within the research.

Statistical and experimental data were collected and processed to calculate the likelihood of illegal landfills as a result of surveys and visual inspections of municipal services and residents. As a result of airbrushing and visual field work, the results of determining the probability of illegal landfills in the study area obtained by theoretical calculations were verified.

It is established that the theoretically obtained results, which depend on the reliability of the data available in the municipality, are in good agreement with the real picture. It is also estimated that, on average, the probability of illegal landfills in the study highland rural areas is 52%. At the same time, the number of illegal landfills in the highland rural areas of five municipalities in the Mtskheta-Mtianeti region varies from 0.1 to 1.6 units per village.

**Keywords:** Illegal Dumpsite, Likelihood of Emerging, Identification, Equation, Statistical data, Highland rural settlements, Mtskheta-Mtianeti region.

\*Corresponding author: Dr. N. Dzebisashvili; E-mail address: [n.dvalishvili@gtu.ge](mailto:n.dvalishvili@gtu.ge),  
[emc.buachidze@yahoo.com](mailto:emc.buachidze@yahoo.com)

### Introduction

The uncontrolled occurrence of illegal dumpsites (landfills) in populated areas is a global problem. It is especially relevant for Georgia as local governments are unable to provide appropriate services in the regions. This problem becomes even more urgent for the high mountainous rural regions.

Georgia's highmountainous regions are characterized by significant structural weaknesses compared to the lowlands and urban areas - weak economic diversification, population migration, extreme poverty, weak infrastructure, lack of health

care facilities, limited access to public services, and more. 66% of the territory of Georgia consists of mountainous areas, where 6.5% of the country's population lives permanently [1,2]. The Mtskheta-Mtianeti region is particularly interesting in this regard, as 75% of the villages in the region ( $\approx 450$  units) belong to high mountainous villages, and the population living in these villages is 22% of the total population of the region [3].

Mtskheta-Mtianeti region is located in eastern Georgia and includes the following historical-geographical provinces: Khevi, Ertso-Tianeti, Pshavi, Khevsureti, Mtiuleti, Gudamakari and

partly Ksniskhevi. It is bordered on the north by the republics of the Russian Federation: North Ossetia, Ingushetia and Chechnya, on the east by the Kakheti region, on the south by Kvemo Kartli, and on the west by Shida Kartli. Mtskheta-Mtianeti region is a heterogeneous and, therefore, quite specific terrain, of which more than 4/5 belongs to the high mountainous category. The administrative center of the region is Mtskheta, and the region consists of the following administrative units: Akhlagori Municipality, Dusheti Municipality, Tianeti Municipality, Mtskheta Municipality, Kazbegi Municipality. The total area of the Mtskheta-Mtianeti region is 5.8 thousand sq. km. (8.3% of the total territory of Georgia), and the population in recent years is 94 3701 people (2.53% of the population of Georgia); Population density - 1 sq. Km. 16.3 men per km [4].

Today, 40% of the region's population lives in the territory of Mtskheta Municipality, and the lowest, 5% - in Kazbegi Municipality (Table 1) [5]. As mentioned, the share of rural population in the total population of the region is 75%. The region is characterized by an abundance of small villages. There is only one village in the whole region with 5,000 inhabitants (Mukhrani village of Mtskheta municipality, which does not belong to the category of high mountainous villages) [3].

## Objectives and methods

The aim of the study was to develop and use an integrated methodology for determining the probability of illegal landfills in the border, highland rural regions of Georgia.

The research methodology is an innovative approach that involves determining the likelihood of illegal landfills in a geographically complex area of the country (particularly highland villages) based on statistics provided by municipalities. The results obtained using the developed theoretical methodology were verified by visualization (using a remote-controlled unmanned aerial vehicle-drone and with the help of expedition work) and a population survey. Based on the collection of statistical data on the study areas, relevant municipalities and population, the probability of illegal landfills was determined using a mathematical formula developed by us(1). This formula allows to determine the approximate amount of waste left without service in the study area and, consequently, to determine the likelihood of the existence/

occurrence of an illegal landfill/landfills. In order to collect data, questionnaires were compiled and used for the representation of both the population and the municipality representatives, which were filled out on the basis of the on-site (expedition) survey. In particular, the questionnaire was filled with the following data - population, availability of specific service/frequency/efficiency, number of containers (by size), amount of waste per capita, presence of waste recycling, etc. (Table 2).

$$D = ((AC - (Ln(L) + Sn(S))f(q/h)) / AC) \cdot 100\% \quad (1)$$

where,

D- Percentage generating possible illegal dumpsite;

A- amount of waste per capita;

C- population;

L- Large container capacity, kg;

n (L) - number of large containers, pieces;

S- small container capacity, kg;

n (S) - number of small containers, pieces;

f- collection frequency (frequency of waste truck service per week), coefficient from 0 to 1;

q- collection efficiency (ratio between the result achieved and the resources used, which implies the actual state of waste collection), coefficient from 0 to 1;

h - the relative rate of container overload, a ratio of 1 to 10, which shows how full the container is at the time of its emptying, and which depends on the population and the number of containers).

Parameter A: From 2015 to 2017, using the gravimetric method, we examined the amount of municipal solid waste (MSW) per capita and its morphological composition, which was supported by the Shota Rustaveli National Science Foundation (SRNSFG) [6] project "Development of a methodology for determination of amounts and morphological composition of municipal solid waste and creation of a data base" #FR/88/9-220/14 (Table 1) [7,8,9,10]. Accordingly, the average quantity of municipal solid waste (MSW) per capita living in cities in Georgia is 250 kg/person/year, and 75-80 kg/person /year per capita living in the region. This sharp difference, as already mentioned above, is due to the difference in living conditions and peculiar standards.

**Table 1.** *Morphological Composition of Municipal Solid Waste in Georgia*

#	Regions	Glass	Paper	Metal	Plastic	Nappies	Rubber / Leather/ Textile	Wood/ green waste	Fine Fraction	Hazar- dous	Food	Total
1	Tbilisi	3.5	13.2	1.7	15.2	8.0	2.2	0.7	3.7	0.8	51.1	100.0
2	Ajara	2.8	15.5	2.5	15.4	7.2	1.7	0.2	9.8	0.8	44.1	100.0
3	Guria	2.2	11.6	3.5	15.5	5.7	0.4	0.5	17.7	0.8	42.2	100.0
4	Samegrelo-Zemo Svaneti	2.6	9.9	1.9	12.1	7.0	1.2	1.5	19.5	0.8	43.8	100.0
5	Racha-Lechkhumi	2.4	13.5	1.5	13.2	9.0	1.3	0.2	16.5	0.4	42.0	100.0
6	Imereti	0.5	9.2	0.3	1.9	9.1	1.0	0.1	29.2	0.6	48.2	100.0
7	Mcxeta-Mtianeti	1.5	9.9	3.8	15.5	5.9	1.8	0.7	16.0	1.0	43.9	100.0
8	Shida Kartli	3.1	11.3	2.8	15.6	5.2	0.5	0.7	16.7	0.3	43.7	100.0
9	Kvemo Kartli	0.7	11.3	3.0	13.8	7.9	1.6	0.4	19.2	0.7	41.4	100.0
10	Samtskhe-Javakheti	2.8	12.0	3.2	13.8	6.8	0.8	0.8	10.8	0.4	48.7	100.0
11	Kakheti	2.8	11.2	2.9	11.5	5.0	1.0	0.3	22.6	0.5	42.3	100.0
12	Average	2.4	11.9	2.7	14.2	6.8	1.3	0.6	15.3	0.8	44.1	100.0

The parameters C, L, S, n(L), n(S) provided and subsequently determined by the specialists of the municipal service and as a result of a population survey.

Table 2 presents an example of a questionnaire compiled for municipalities to determine the above parameters.

**Table 2.** *Questionnaire for Municipal Service*

#	1	2
1	Municipality	Name
2	Population	Person
3	Population which have not served of waste disposal service	Person/household
4	Number of villages	Units
5	Number of high mountain villages	Units
6	The least populated high mountain villages	Units and Name
7	High mountain villages with the densest settlements	Units and Name
8	High mountain villages where there is no road infrastructure	Units and Name
9	Total number and type of waste containers (volume)	Units and Name
10	High mountain villages where there are not waste containers	Units and Name
11	High mountain villages where there is no waste truck	Units and Name
12	Total number of waste trucks	Units and Name
13	High mountain villages where illegal dumpsites are registered	Units and Name

Table 3 shows the gradations of the parameters f, q and h in Formula 1, which are selected during the calculations by visual evaluation at the study site.

**Table 3.** Ratios Classification of Waste Collection Frequency and Waste Collection Efficiency

f	Waste Collection Frequency	q	Waste Collection Efficiency	h	Relative rate of container overload
0	Not	0	Not	0	There is no container
0.2	Once a week	0.2	Very bad	1	Too overloaded
0.4	Twice a week	0.4	Bad	2	Overloaded
0.6	Every second day	0.6	Satisfactorily	4	Full
0.8	Every day	0.8	Good	6	Incompletely filled
1	Twice a day	1	Very good	8-10	Less than half / insignificant

Additional information about the survey region was obtained with the help of the online resource of the National Statistics Office of Georgia [11], (Table 4).

Currently, about 1/6 of Mtskheta Mtianeti - the entire territory of Akhagori Municipality is occupied by the Russian Federation and information about it is not officially found (Map 1). A total of 601 villages are registered in the regions controlled by Georgia, of which 448 are mountainous villages, the total population of the municipality is 102,044 people (in the case of Akhagori municipality according to the Geostat data of 2008), territorial area: 6 785 km<sup>2</sup>. The share of the population using the municipal cleaning service is 65%, which is

served by three official dumpsites.

The average result of the calculation by our proposed formula for the individual regions studied is given in Table 4, which shows that the average likelihood of illegal dumpsites in the high mountainous rural areas for the study region is 52%. In addition, significant differences were identified between municipalities in terms of waste management (Table 4). Table 4 shows that the situation is more difficult in the mountainous villages of Dusheti and Mtskheta municipalities, where the result is more than 50%, while the situation is better in Kazbegi municipality, which is mainly explained by the influence of tourism policy and responsible local government work.

**Results and analysis**

**Table 4.** Calculation of Likelihood of Emerging Illegal Dumpsites in High Mountainous Rural Areas of Some Border Region of Georgia

Parameters	Measurement	Symbol	Index of Regions					
			Dusheti	Tianeti	Kazbegi	Mtskheta	Akhagori	Total
Quantity of MSW	kg/Person/day	A	0.3	0.3	0.3	0.3	0.3	0.3
Quantity of village	Units	-	289	87	47	62	116	601
Quantity of high mountain village	Units	-	242	87	47	19	53	448
Population	Person	C	9100	7700	2469	1 632	1500	22 401
Residents of high mountain village who are not provided with a waste disposal service	Person	-	6777	664	0	1632	1500	10573
Value of big containers	kg	L	220	220	220	220	220	220
Quantity of big containers	Units	n(L)	90	60	40	0	0	190
Value of small containers	kg	S	20	20	20	20	20	20
Quantity of small containers	Units	n(S)	0	0	0	0	0	0

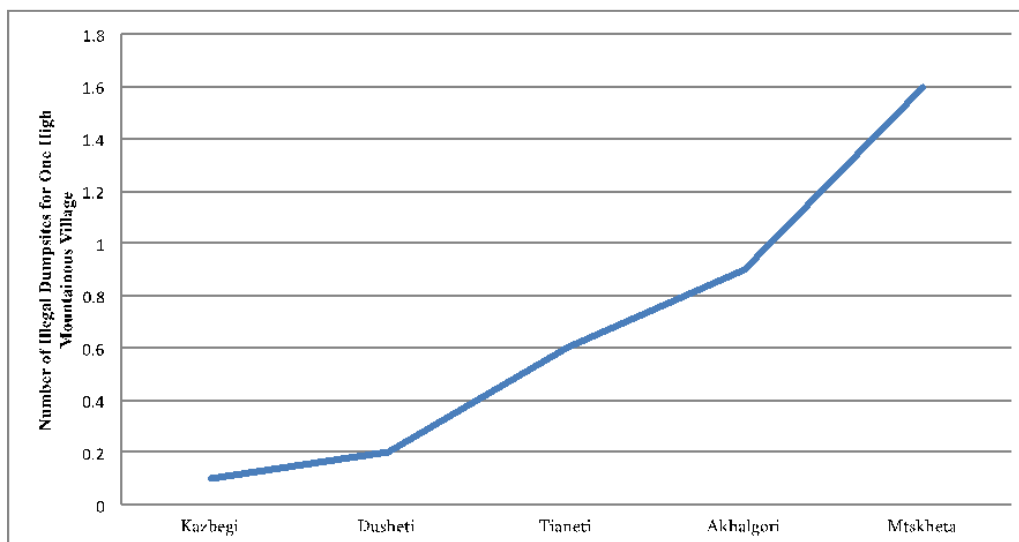
Waste Collection Frequency	0-1	f		0.2	0.2	0.6	0	0.2	0.4
Waste Collection Efficiency	0-1	q		0.3	0.5	0.8	0	0.2	0.4
Relative indicator of container load	1-10	h		1	1	6	0	1	1.8
Total quantity of emerging waste	kg/day	B	AC	2730	2310	740.7	489.6	450	6720.3
Total quantity of disposal waste	kg/day	E	$L*n(L)+S*n(S)*f*(q/h)$	1188	1320	704	0	0	3212
Total quantity of remaining waste	kg/day	H	B-E	1542	990	36.7	489.6	450	3508.3
Likelihood of Emerging Illegal Dumpsites	%	D	H/B*100	56.48	42.85	4.95	100	100	52.20
Parameters	Measurement	Symbol	Index of Regions						
			Dusheti	Tianeti	Kazbegi	Mtskheta	Akhalgori	Total	
Quantity of MSW	kg/Person/day	A		0.3	0.3	0.3	0.3	0.3	0.3
Quantity of village	Units	-		289	87	47	62	116	601
Quantity of high mountain village	Units	-		242	87	47	19	53	448
Population	Person	C		9100	7700	2469	1 632	1500	22 401
Residents of high mountain village who are not provided with a waste disposal service	Person	-		6777	664	0	1632	1500	10573
Value of big containers	kg	L		220	220	220	220	220	220
Quantity of big containers	Units	n(L)		90	60	40	0	0	190
Value of small containers	kg	S		20	20	20	20	20	20
Quantity of small containers	Units	n(S)		0	0	0	0	0	0
Waste Collection Frequency	0-1	f		0.2	0.2	0.6	0	0.2	0.4
Waste Collection Efficiency	0-1	q		0.3	0.5	0.8	0	0.2	0.4
Relative indicator of container load	1-10	h		1	1	6	0	1	1.8
Total quantity of emerging waste	kg/day	B	AC	2730	2310	740.7	489.6	450	6720.3
Total quantity of disposal waste	kg/day	E	$L*n(L)+S*n(S)*f*(q/h)$	1188	1320	704	0	0	3212
Total quantity of remaining waste	kg/day	H	B-E	1542	990	36.7	489.6	450	3508.3
Likelihood of Emerging Illegal Dumpsites	%	D	H/B*100	56.48	42.85	4.95	100	100	52.20

By entering some of the values taken from Table 4 into Equation 1, we obtain the corresponding results for the likelihood of emerging an illegal dumpsite, according to the districts we have selected. The results show which of them is more unsatisfactory or better, and also where and what type of work needs to be done to improve the current situation.

The results can be presented as follows - for example: in the example of Kazbegi in 47 high mountainous villages (MMS) we got the likelihood

of only 5 illegal dumpsites, if we recalculate these values for one village (or how many dumpsites can be emerging in one village), we get that in one village Generate only 0.1 dumpsites; According to the relevant data - the likelihood of creating a dumpsite in one of the high mountainous villages of Dusheti district is 0.2 dumpsites; 0.6 dumpsites in one village of Tianeti, 0.9 dumpsites in Akhlagori, 1.6 dumpsites in Mtskheta.

The obtained results are well presented in Fig.



**Fig.** Likelihood of Quantitative Emerging of Illegal Dumpsites for One High Mountainous Village (Example of Mtskheta-Mtianeti Region).

## Conclusion

1. It is established that the results obtained using Equation-1 (with their values) are descriptive and fairly close to the real picture observed by the fieldwork team in the field of the project.
2. The number of illegal landfills that may arise in each municipality has been reduced to one village. Accordingly, it was determined how many illegal landfills are likely to occur in a single village in a particular district. The results show that this figure ranges from 0.1 to 1.6 units.
3. Based on the data obtained from the study, the most satisfactory situation is in Kazbegi region, while the situation is relatively poor in the villages of Akhlagori and Mtskheta regions, where the local government pays less attention to the population living in these mountainous villages. In particular, they are not provided with all the necessary services needed to solve these existing problems

## Acknowledgement

We would like to thank the Shota Rustaveli National Science Foundation for its financial support of the SRNSFG [Grant №FR-18-718] research.

## References

- [1] Internet resource: <https://1tv.ge/news/maghalmtiani-dasakhlebis-statusi-1715-dasakhlebas-maghalmtian-dasakhlebashimudmivad-mckhovrebi-piris-statusi-ki-237-469-moqalaqes-mienicha> (in Georgian);
- [2] Buachidze N. , Dvalishvili N. Determining a Probability of the uncontrolled landfills emergence in Georgia's Rural Areas using Integrated Methodology, 16 th International Conference on Environmental Science and Technology Rhodes, Greece, 2019, [https://cest2019.gnest.org/sites/default/files/presentation\\_file\\_list/cest2019\\_00882\\_](https://cest2019.gnest.org/sites/default/files/presentation_file_list/cest2019_00882_)

- [posterf\\_paper.pdf](#) ;
- [3] Report of Mtskheta-Mtianeti Regional Administration, <https://mtskheta-mtianeti.gov.ge/>;
- [4] Mtskheta-Mtianeti, <https://en.wikipedia.org/wiki/Mtskheta-Mtianeti>;
- [5] Population by cities and boroughs, as of 1 January 2021, <https://www.geostat.ge/en/modules/categories/41/population>;
- [6] Shota Rustaveli National Science Foundation (SRNSFG), <http://rustaveli.org.ge>;
- [7] N. Dvalishvili, Report of the SRNSFG project FR/ 88/9-220/14: “Development of a methodology for determination of amounts and morphological composition of municipal solid waste and creation of a data base”, <http://ecohydmnet.ge/projects.html> (in Georgian);
- [8] N.L. Dvalishvili, Assisment of OF Eco-Efficiency of Separation of some Fractions of MSW: a Case Stady of Georgia, South Caucasus, Waste Management and the Environment IX, WIT Transactions on Ecology and the Environment, 2019, <https://www.witpress.com/elibrary/wit-transactions-on-ecology-and-the-environment/231/37093>
- [9] N.L. Dvalishvili, Establishment of energy potential of Norio landfill of municipal solid waste of Tbilisi, Procedia Environmental Sciences, Volume 35, 2016, Pages 377-380, <https://www.sciencedirect.com/science/article/pii/S1878029616301062>;
- [10] N.L. Dvalishvili, M.S. Tabatadze, The influence of municipal solid waste of Georgia on climate changes, Waste Management and Resource, 2019 – Springer, [https://link.springer.com/chapter/10.1007/978-981-10-7290-1\\_16](https://link.springer.com/chapter/10.1007/978-981-10-7290-1_16);
- [11] National Statistics Office of Georgia, <http://geostat.ge/en>.



## Annals of Agrarian Science

Journal homepage: <http://journals.org.ge/index.php>



# Farming according to the Grand Defter of Gurjistan Vilayet of 1595

**D. Nikolaishvili<sup>a\*</sup>, D. Sartania<sup>b,c</sup>, A. Ujmajuridze<sup>c</sup>**

<sup>a</sup>Ivane Javakhishvili Tbilisi State University, Faculty of Exact and Natural Sciences, Department of Geography; 3, Ilia Chavchavadze Ave; Tbilisi, 0128, Georgia

<sup>b</sup>Ivane Javakhishvili Tbilisi State University, Museum of TSU; 3, Ilia Chavchavadze Ave; Tbilisi, 0128, Georgia

<sup>c</sup>Ivane Javakhishvili Center; 33, Tsinamdzgvrishvili Str; Tbilisi, 0112, Georgia

Received: 02 June 2021; Accepted: 16 July 2021

## ABSTRACT

The article describes the economic situation, in particular, the tradition of Georgian farming culture, in the south-western part of Georgia concurred by the Ottoman Empire in the XVI century and called Gurjistan Vilayet by the Ottomans. Gurjistan Vilayet covered a great area of south-western Georgia, with only small part of it being a part of Georgia and the remained territory being a part of the Republic of Turkey, the legal successor of the Ottoman Empire. The study is mainly based on the Grand Defter of Gurjistan Vilayet. In the course of the study, the geographical objects (villages, sites of ancient villages, sowing areas, etc.) given by the Defter were identified, systematized and classified and the GIS database and relevant maps was developed. The objects plotted on the map were identified by comparing with other sources that was used to identify the types and amounts of taxes set by the Ottoman for the population, group them thematically and draw the picture of the economic potential of the region. It was found that the Georgian economic tradition was solid and sustainably profitable, but the Ottoman ideological press had certain effect on it.

**Keywords:** Gurjistan Vilayet, Liva, Nahiyah, Ottoman Defters, XVI century, Farming.

\*Corresponding author: Dali Nikolaishvili; E-mail address: [dali.nikolaishvili@tsu.ge](mailto:dali.nikolaishvili@tsu.ge)

## Introduction

Following the conquest of the southwestern part of Georgia by the Ottomans in the 16<sup>th</sup> century, a new administrative-territorial unit - "Gurjistan Vilayet" was formed, where Muslimization and gradual eradication of the remnants of Georgian culture started, though this process was slow and gradual. The first change made by the Ottomans on the occupied territories was the introduction of the Ottoman tax system, which was based on religious discrimination, in particular, the Christians paid higher taxes than the Muslims. This supported the substitution of the Christian faith with the Islam what was dangerous for the Georgian traditional culture in general. As to when the forced transformation of the Georgian traditional culture

started in the given area, how it developed and which areas it covered, is an important issue of a scientific study. It is virtually impossible to study this issue through scarce, old Georgian sources. Consequently, the old Ottoman documents are an irreplaceable source. The most important of them are the population census books.

"The Grand Defter of Gurjistan Vilayet" dated by 1595 is the first document translated and published in Georgian, which describes the political, socio-economic and cultural situation of this newly conquered territory of Georgia. The Grand Defter was a subject of interest of many scholars [1-6]. However, due to its diversity and information value, many interesting novelties are still found in the document.

## Methods and initial data

The study is based on the Grand Defter of Gurjistan Vilayet of 1595 prepared by the Ottoman Empire and translated from Turkish to Georgian and published in 1941 by S. Jikia. The Turkish original of the Grand Defter of Gurjistan (hereinafter “The Defter”) was written on 490 quite large pages and is preserved at the Korneli Kekelidze Georgian National Center of Manuscripts [Fund # 478]. The document gives extremely rich and diversified data about the social-economic situation of then-time Georgia, regarding which S. Jikia, the translator and researcher of the Defter wrote: “*The feudal Ottoman Empire, owing to unequal forces, succeeded in isolating it from Georgia, which had the economic riches and traditions of high culture of the many-century-long history of Georgia*” [1].

“The map of Gurjistan Vilayet” [7], different-scale (1:100,000; 1:50,000) Soviet topographic maps, maps in the old verst system (scales: 1 inch: 5 versts and 1 inch : 10 versts), 3-dimensional Google Earth geo-images were also used as primary sources.

The given paper relies on the agricultural data given in the “Defter”, in particular, the information related to farming, which tells us about the kinds of crops the people of the given region grew and the geography and intensity of the given crops. The data the Defter are compared with the later data, including the ethnographic studies of the 20th century. The study used comparative, semantic, quantitative and retrospective mapping methods.

## Object of the study

As per the Defter of 1595, Gurjistan Vilayet was divided into 8 Livas and 28 Nahiyah. These Livas are: Akhaltsikhe, Khertvisi, Akhalkalaki, Chrdili, Potskhovi, Petre, Didi-Artaani and Panak. According to the map compiled by Al. Aslanikashvili [7], there were 9 Livas and 37 Nahiyahs in Gurjistan Vilayet, although the Defter says that Oltisi Liva inscription was made sometime later [1], i.e. it does not belong to the Defter of 1595.

The area of Gurjistan Vilayet was 11 791 km<sup>2</sup> (excluding Oltisi Liva) [8]. The Livas of Gurjistan Vilayet occupied completely different areas as a result of their different natural conditions. After all, the borders between the Livas were fixed with mountain systems and river valleys. The biggest (Akhalsikhe) Liva was almost 4 times bigger than the smallest (Potskhovi) Liva. There were total 5 big Livas in the Vilayet: Akhalsikhe, Akhalkalaki,

Didi Artaani, Chrdili and Panak, while the area of 3 other Livas (Petre, Khertvisi and Potskhovi) was less than 950 km<sup>2</sup>.

## Main results

**General features of agriculture.** Regarding “The Grand Defter of Gurjistan Vilayet” of 1595, Sh. Lomsadze wrote: “*It shows us enslaved people deprived of all rights and grouped by villages and cities; the heads of the families who are referred to by their names and patronymics (as it was common in Turkey) ... the sites of ancient villages and cities, desolate and “empty of Rayah (peasant)”*” [8]. And truly, the Defter gives such a picture: almost 1/3 of the villages are deserted.

As per our calculations, there were 1070 objects named in the Vilayet (excluding Oltisi Liva), but only 726 of them had permanent residents, while the rest of the locations were deserted, i.e. were sites of ancient villages. The agricultural lands in these villages were used by the people from the surrounding villages who therefore paid relevant agricultural taxes. It is of these taxes; these villages were included in the Defter. Presumably, the Vilayet had much more sites of ancient villages that were completely abandoned not only by their permanent inhabitants, but also by the farmers from the neighboring villages, who would cultivate the abandoned land to gain harvest. Such state of affairs surely had an impact on the economy of the Vilayet as it is clearly seen by the statistics of the Defter.

The residents of Gurjistan Vilayet paid 64 different types of taxes, including 55 agricultural and 9 non-agricultural taxes. The highest taxes were paid by Akhaltsikhe and Didi Artaani Livas, and the least taxes were paid by Petre Liva (Fig. 1).

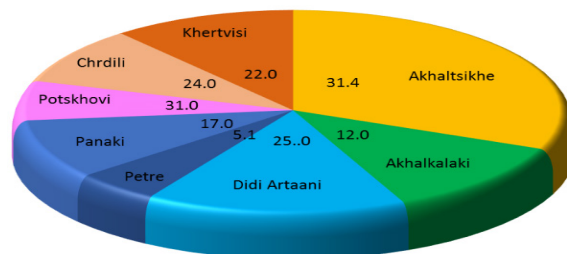


Fig. 1. Total taxes according to Livas (%)

The Vilayet paid a total of 9 391 732 akçe (the chief monetary unit of the Ottoman Empire and neighboring countries in XIV-XIX cc). Clearly, the highest taxes were paid by the biggest Livas, namely, the first three biggest Livas were: Akhalsikhe

(paying 2 942 868 akçe), Didi Artaani (paying 1 458 612 akçe) and Akhalkalaki (paying 1 120 480 akçe) Livas. The lowest tax (475,305 akçe) was paid by Petre Liva.

The situation will be completely opposite if calculating the total taxes by homesteads. On average, the population of Vilayet paid 593 akçe per homestead. Petre Liva was charged with a maximum tax (1657 akçe) and Didi Artaani Liva

paid the minimum tax (340 akçe), the former paying a 5 times higher tax than the latter meaning that virtually, the inhabitants of Didi Artaani Nahiyah must have had higher incomes. This is logical, since by then, Petre Nahiyah was already quite depopulated and most sites of ancient villages of all Vilayet were found on its territory (Fig. 2), while the situation in Didi Artaani Liva was “calmer” and this figure was close to the average value of the Vilayet.

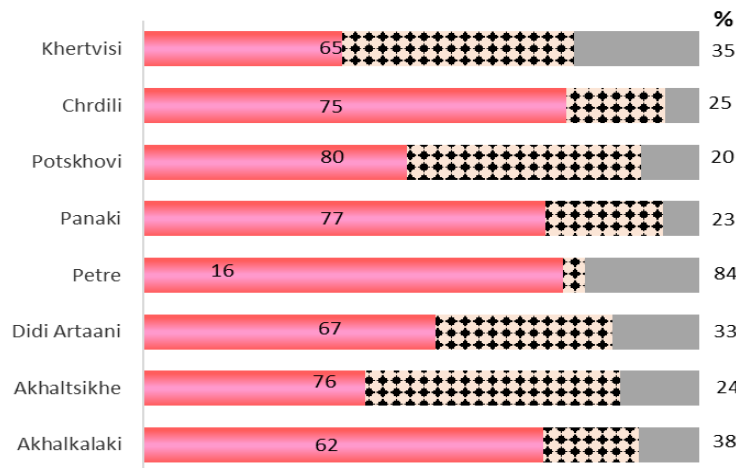


Fig. 2. Share of villages and ancient villages in Gurjistan Vilayet (%)

There was no big difference between the amounts and types of agricultural and non-agricultural taxes in different Livas (Fig. 3). An average difference for non-agricultural tax was 8.8%. There is a bigger difference in different

Nahiyahs and villages. All over the Vilayet, the maximum (56.0%) non-agricultural taxes were paid by Panaskerti Nahiyah of Panak Liva, while the minimum (2.9%) taxes were paid by Petre Nahiyah of Petre Liva.

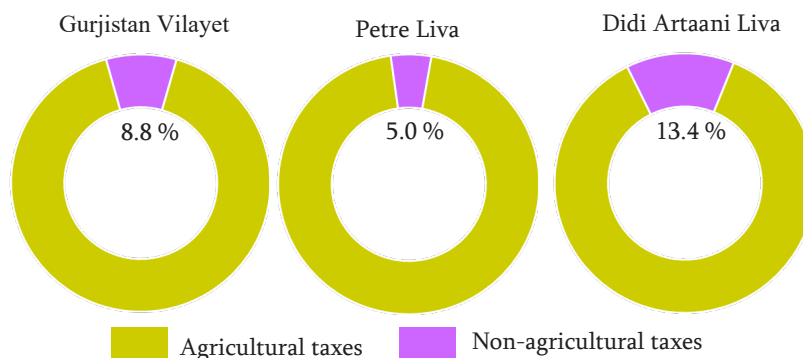


Fig. 3. Agricultural and non-agricultural taxes

There is an even greater disproportion between the villages. Across the Vilayet, the maximum (46.2%) non-agricultural taxes were paid by village Gura of Mzvare Nahiyah of Didi Artaani Liva. This was due to two circumstances: 1) the village had many (133) households with their majority being Christians who were consequently charged with

higher non-agricultural taxes (hispenje, murahasia), i.e. non-agricultural taxes. They account for the largest share (39%) of total taxes; 2) low tax on grain (wheat, barley), just 3600 akçe. This is a very low tax for the village for so many homesteads. For comparison, take village Zanazgomi with only 13 households paying exactly the same tax (3600 akçe)

on grain as big village Gura with many homesteads used to pay. At the same time, we would note that such a big difference in taxes between these two villages evidences a big difference in the incomes of the two. Village Zanazgomi had a much higher income, i.e. the natural conditions in this village were most favorable to grow grain.

Several agricultural branches were developed in Gurjistan Vilayet. The taxes in farming were much higher than in cattle-breeding (Fig. 4), with 92% and

8% specific weight ratios. This ratio varied more or less across the Livas, Nahiyahs and villages, with a particularly great difference between the villages. For example, in one of the estates of Mzvare Nahiyah of Didi Artaani Liva [“Defter”, p. 405, #3], the share of cattle-breeding was almost 27%. The situation is similar in village Patara Alabala of the same Nahiyah where the share of cattle-breeding was much higher than the average value of the Vilayet.

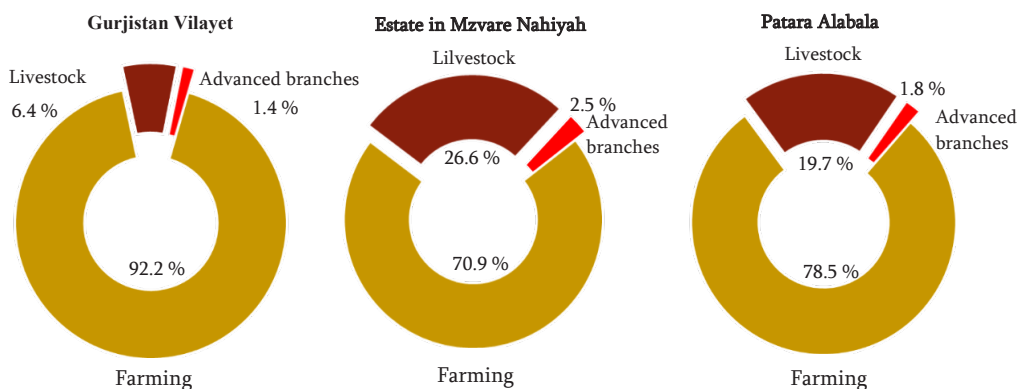


Fig. 4. Branch structure Gurjistan Vilayet

Grain-growing was the most developed branch all over Gurjistan Vilayet. The total of 5,954,870 akçe that is almost 64% of the total tax and 86% of the agricultural tax, was paid by the Vilayet. The highest tax (2,025,492 akçe) was paid by Akhaltsikhe Liva, which accounted for more than 1/3 of the relevant Vilayet tax. Didi Artaani Liva ranked the second (with 923,986 akçe). Both of these Livas paid almost 1/2 of the entire tax. Petre Liva was charged with the least tax (50,750 akçe).

However, a homestead-based calculation gave a completely different picture. The highest tax on cereals per household was paid by Petre Liva (1450

akçe), with all other Livas felling much behind it. This tax was the least (341 akçe) in Didi Artaani Liva. Such a large imbalance with the major agricultural crop is the sign of different economic standing of the population. In this respect, Petre Liva was in the most unfavorable situation.

As it is clear from the taxes, the people grew 4 cereal varieties: wheat, barley, rye and millet. The most important cereals were apparently wheat and barley. The total wheat tax was 2,994,912 akçe and the total barley tax was 2,635,172 akçe. These crops were grown in all Livas and Nahiyahs (Fig. 5).

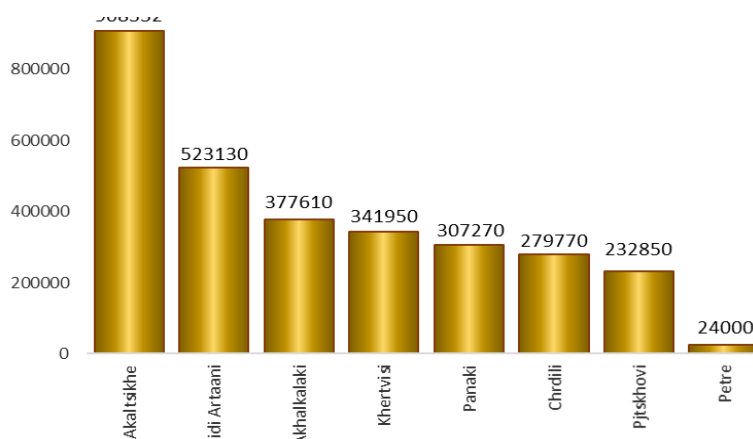


Fig. 5. Taxes on wheat according to Livas (Akçe)

As already mentioned, the highest was the wheat tax amounting to 2,994,912 akçe, i.e. ½ of the cereal tax. The cereal tax was particularly high (908,332 akçe) in Akhaltsikhe Liva, accounting for almost 1/3 of the Vilayet wheat tax. This tax was similarly high in Didi Artaani (523,130 akçe) and Akhalkalaki (377,610 akçe) Livas. The aggregate data of all three Livas are over 60% of all Vilayet taxes. Thus, the given Livas were the main wheat “producers”. The least tax (24,000 akçe) was paid by Petre Liva. The forested Nahiyah of Didi Artaani Liva paid the highest tax (227,000 akçe). It was followed by Akşehir Nahiyah of Akhalkalaki Liva (221,110 akçe). With this tax too, none of Nahiyahs of Akhaltsikhe Liva is ahead of others what is easy to explain: Akhaltsikhe Liva occupied a large area and was administratively divided into many (8) units so that the total value was distributed among those units.

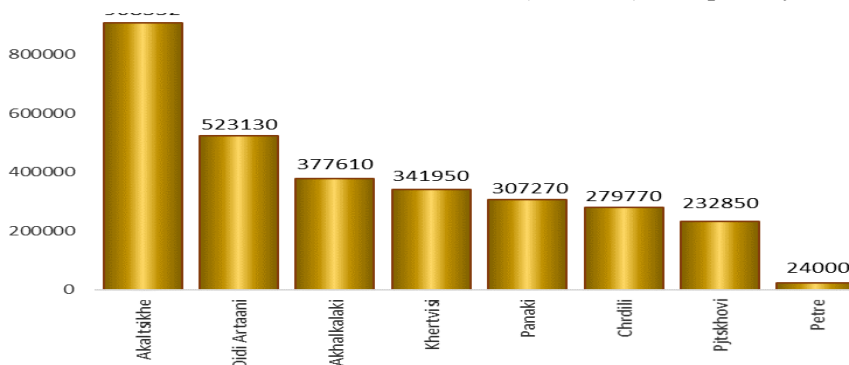


Fig. 6. Taxes on barley according to Livas (Akçe)

Rye and millet must have been much less important, as evidenced by tax amounts. This is evidenced by the fact that the sum of taxes on two cereal varieties paid by the Liva was less than the taxes paid by individual Nahiyahs (Akhaltsikhe, Akhalkalaki, Didi Artaani and Khertvisi) on wheat. A total of 230,936 akçe was paid as a rye tax by whole Vilayet. A particularly high tax (116,600 akçe) was paid by Akhaltsikhe Liva, which was more than ½ of the total rye tax of the Vilayet. This was an exceptionally high value, in proof whereof it may be said that this tax was almost 3 times higher than that of Akhalkalaki Liva (41,320 akçe) ranking the second. Akhalkalaki (39.8 akçe) and Khertvisi (39.3 akçe) Livas paid the highest tax per household and the least tax (0.9 akçe) was paid by Artaani Liva. The least common cereal was millet and a total of 93,850 akçe was levied on it making only 1.5% of all cereal taxes. As millet was the least common crop, Didi Artaani and Chrdili Livas did not pay any tax for it. This tax was very small for Petre and Akhalkalaki Livas and not all Nahiyahs of

The second leading crop was barley. A total of 2,635,172 akçe, i.e. more than 44% of cereal tax was levied on it. At the same time, alongside with barley, wheat accounted for the largest share of the grain tax (almost 95%) in Gurjistan Vilayet. Like wheat, barley was grown in all of Livas and Nahiyahs (Fig. 6). The highest barley tax was paid by Akhaltsikhe Liva (more than 1/3 of the barley tax of the Liva). With this tax too, Didi Artaani Liva ranked the second (397,776 akçe), Khertvisi Liva ranked the third (305,120 akçe) and Petre Liva paid the least tax (26,000 akçe). However, with this value, the latter exceeded the corresponding wheat tax. Of Gurjistan Vilayet, Petre Liva was the only one where the barley tax was higher the wheat tax. The situation was completely different with total barley tax per homestead, where the maximum tax per household (743 akçe) was paid by Petre Liva and the minimum tax (111 akçe) was paid by Didi Artaani Liva.

other Livas paid it. Akhaltsikhe Liva paid the highest tax (67,620 akçe) making more than 72% of total rye tax. All Nahiyahs in the said Liva paid this tax, with Ude Nahiyah paying the highest tax. The tax was also high (16,400 akçe) in Panak Liva.

Several studies were used to establish that Georgia is located in the geographical area where wheat crops were cultivated as evidenced by 6 endemic wheat species and their varieties found in Georgia [10; 11; 12]. A special role in this respect was played by historical Meskheta or the region where Gurjistan Vilayet was located for some time. Such cereals as wheat and barley were widely used by the people until the mid-XX century [13]. Alongside with the endemic wheat varieties, more than 50 botanical forms of barley are found in Georgia [14]. The fact of wheat and barley species and their varieties having survived to the industrialization epoch proves their great importance for Georgian farmers, and “The Grand Deftar of Gurjistan Vilayet” clearly proves its importance.

They also grew **legumes** in Gurjistan Vilayet.

The legumes, alongside with the cereals, played an important role in the agricultural life of the Georgians. Legumes are known to be rich in proteins and to be very important for living organisms. In Georgia, “in the past, people traditionally did not eat meat every day, and fasting prohibitions of the Christian religion played quite an important role in it. Therefore, the deficit of meat proteins in human food was compensated by the products rich in legume proteins and nitrogenous substances” [15]. Thus, the legumes have been known in Georgia since the ancient times. A total tax of 26 755 akçe was levied on the legumes in Gurjistan Valayet. This is a very low figure compared to cereal tax. At the same time, only 3 Livas were charged with legume tax: Akhaltsikhe (24,965 akçe), Panak (120 akçe) and Khertvisi (1690 akçe).

The Defter shows that 3 legume varieties were grown to Gurjistan Vilayet: peas, lentils and broad beans. Peas (accounting for 43% of total tax on legumes) and lentils (35%) must have been widely spread. Only the residents of Akhaltsikhe Liva paid taxes on broad beans. The highest tax on all three legume varieties was paid by Ude Nahiyah of Akhaltsikhe Liva accounting for almost 1/3 of the tax on legumes in Akhaltsikhe Liva. Particularly high taxes were paid by villages: Uraveli and Tskordza, paying 2.5-3 times higher legume taxes than other villages.

**Flax** must have been another important agricultural crop. With the exception of Petre and Panak Livas, all of its Livas were taxed. The total tax amounted to 23,294 akçe. Particularly high tax on flax (11,550 akçe) was paid by Akhaltsikhe Liva making almost ½ of its total taxes. At the same time, flax must have been a widely spread crop in Liva as its tax was paid by all Nahiyahs. Of villages, the maximum tax (250 akçe) was paid by village Kvemo Pamaji of Chrdili Nahie of the same Liva. Flax tax was similarly high in Akhalkalaki Liva (4408 akçe).

**Vegetable-growing** was one of the most advanced branches in Gurjistan Vilayet as evidenced by the size of its total tax and the vast majority of villages paying it (Fig. 7). The population of the Vilayet paid a total of 84,731 akçe on vegetables accounting for more than 1/5 of the total taxes on farming (except cereals) what was quite a big value. In fact, the vegetable patch tax must have been even higher. In particular, 2 more taxes should be added to this value: 1) the tax levied on sowing areas (57,330 akçe), which obviously refers to arable lands, i.e. vegetable patches and orchards, and 2) tax levied on fruits and vegetables (4811 akçe), which makes it impossible to determine the exact share of vegetables, but we can probably assume tax of ½-½ per each. This means that the share of vegetable-growing and gardening was taxed by a total of 142 261 akçe, i.e. making more than 1/3 of the total taxes on farming (excluding cereals).

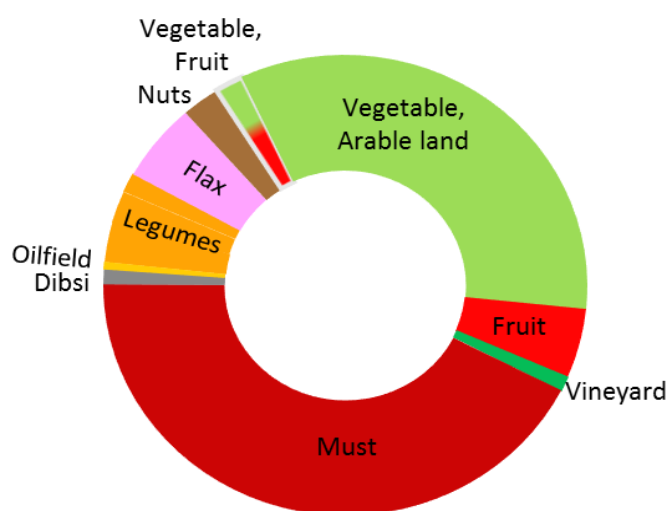


Fig. 7. Some land taxes (excluding wheat)

As M. Svanidze wrote, based on the XIX-century data, alongside with vegetables and watermelons, they grow pumpkins, dill, cabbage, melons, celery, garlic, mint, carrots, coriander, savory, beets,

onions, etc. all over Principality of Samtskhe [4]. It is not excluded that the listed products were the traditional agricultural crops of this region and they were grown even in the XVI century.

The highest tax on vegetables and watermelons was paid by Akhaltsikhe Liva (28,127 akçe), accounting for almost 1/4 of total taxes on vegetables in the Vilayet. It was followed by Didi Artaani Liva (18,221 akçe). Elsewhere, the maximum vegetable tax alone was 9000 akçe. Of the Nahiyahs, the highest tax (8024 akçe) was paid by the forested Nahiyah of Didi Artaani Liva. All over the Vilayet, the vegetable tax averaged 6.7 akçe per household. The highest (14.3 akçe) tax was paid by Petre Liva, and the least tax (3.8 akçe) was paid by Panaki Liva.

The vegetable tax was levied in fact on every village. The only exceptions are a very small village with 2 or 3 households, or estates, where not all types of taxes were enlisted. Consequently, it may be said that vegetable-growing was common everywhere, although it seems that it was not equally developed everywhere, and hardly survived at the devastated locations, where the Ottoman officials had not even mentioned it. The highest taxes were paid by some villages, with the first three villages of Klde of of Mzvare Nahiyah of Akhaltsikhe Liva (790 akçe), village Okami of Akshehir Nahiyah of Akhalkalaki Liva (600 akçe) and village Panakis Rabati of Panak Nahiyah of Panak Liva (534 akçe). It is noteworthy that these villages are not large, with the number of households of 20-38. Therefore, per household tax was high there (15-30 akçe). An opposite picture is seen in some other villages with more households where the vegetable tax is much lower, e.g.: the villages of Panak Nahiyah of Panak Liva: Bariq (with 42 households paying vegetable tax of 150 akçe), villages Zemo Arsenak and Shua Arsenak (with 138 households paying 242 akçe) and village Kotik (with 40 households paying 150 akçe).

**Viticulture and fruit-growing** must have been one of the most advanced branches in Gurjistan Vilayet as evidenced by several circumstances, primarily the taxes on fruit, vineyards and must. The highest land tax (almost 1/2 of all taxes) excluding cereals was must. The fruit and vineyard tax proportions were much lower.

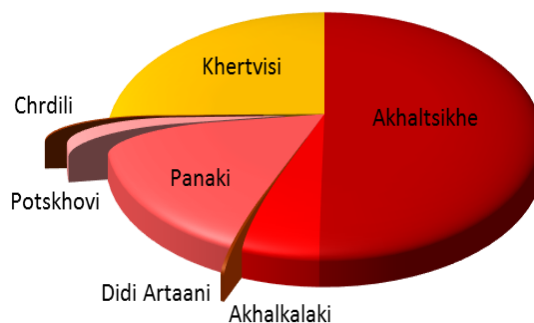
Vineyard tax was levied in 3 Livas and made total 1951 akçe. The maximum tax was paid by Akhaltsikhe Liva (871akçe) and Panak Liva (550 akçe). Khertvisi Liva ranked the third (410 akçe). All three of these Livas accounted for 95% of the vineyard tax. It is also interesting to note that the vineyard grew in the Livas located farther south, i.e. where today the local people do not grow vines. Such Livas are Akhalkalaki and Potskhovi. In proof, we cite the relevant data from “Meskhetian

Chronicles of the Psalter”, the same as “Meskhetian Chronicles” (XVI c.). There is also a reference where the village of Oloda, located in the south-western part of modern Akhalkalaki Plateau, is mentioned. It was located at an altitude of 1900-1940 m above sea level. The document states that in addition to fruit-growing and cattle-breeding, viticulture was also developed in the area [16]. This is confirmed by later sources as well: “According to Ballas, in 1895, there were only 10 vineyards of 3 dessiatines in Akhaltsikhe Province” [17]. Another interesting fact is that Didi Artaani Liva located even further south was charged with vineyard tax. These issues need further in-depth study.

A higher (20 395 akçe) than the vineyard tax was levied on fruit. Akhaltsikhe and Khertvisi Livas had to pay the highest taxes (16 046 and 2075 akçe, respectively), although Akhaltsikhe Liva was still leading, paying 8-9 and even several tens of times higher tax than other Livas. Particularly high taxes were paid by Atskuri (3930 akçe), Chacharaki (3350 akçe) and Mzvare (2430 akçe) Nahiyahs.

It is interesting to note that must (grape of fruit juice) was similarly high in Akhaltsikhe and Khertvisi Livas. Two Livas: Didi Artaani and Panak did not pay fruit tax. The latter is of a particular interest, since the Liva paid vineyard tax and must, but not fruit tax. This evidences the intensive development of viticulture in the region.

Gurjistan Vilayet paid 179,540 akçe as must. This tax varied a lot across the area (Fig. 8). The Livas in the northern and southern parts of the Vilayet paid the taxes, while the Livas in the central part were not taxed. Only 2 Livas (Didi Artaani and Oltisi) paid this tax. 3 Livas (Akhaltsikhe, Khertvisi, Panak) were charged with the highest tax, with Akhaltsikhe Liva ranking the first paying 90 440 akçe. This tax for Akhaltsikhe Liva was twice as much as in Khertvisi Liva, which ranked the second. It was these 3 Livas paying the major proportion of must (92%).



**Fig. 8.** Taxes on must according to Livas

## Conclusion

The analysis of the statistical data given in the Defter evidences that farming was a leading branch in Gurjistan Vilayet, where grain-growing was particularly important. Grain-growing was more or less common in all Livas and Nahiyahs. There were two other advanced agricultural branches in the area: fruit-growing and vegetable-growing. Vegetable-growing seemed to be developed all over the region, particularly in Akhaltsikhe and Didi Artaani Livas. An exception was Petre Liva, who was depopulated that diminished many agricultural sectors. Fruit-growing and viticulture were developed in all Livas, but showed more imbalance. Fruit-growing and viticulture were particularly well developed in Akhaltsikhe, Akhalkalaki and Khertvisi Livas.

It is known that farming has always been the most important branch in Georgian agriculture. The data of the Defter evidence that farming in southwestern Georgia, which was conquered by the Ottomans by the end of the XVI century, was quite a well-developed field of agriculture. The Defter evidences that although the conquest of the region reduced the population and hampered the development of the region, it did not harm the Georgian agricultural traditions as evidenced by the diverse and highly productive cereals and legumes in the region. The data of the Defter also evidence quite well-developed viticulture in the given region proving that the Georgians firmly maintained this traditional branch.

## Acknowledgements

The authors cordially thank the Shota Rustaveli Science Foundation for support, under whose financial aid it became possible to accomplish the present work within the scope of project "Mapping and analyzing Georgian cultural heritage of south-west Georgia according to old Turkish Population census books", Grant #HE-18-448, 26.07.2018). The authors also cordially thank members of the project, especially Manana Kvetenadze who fulfilled calculations of taxes.

## References

- [1] S. Jikia, The Great Book of Gurjistan Vilayet, Book II, Publishing House of the Academy of Sciences of the Georgian SSR, Tbilisi, 1941, In Georgian.
- [2] S. Jikia, The Great Book of Gurjistan Vilayet, Book III, Publishing House of the Academy of Sciences of the Georgian SSR, Tbilisi, 1941, In Georgian.
- [3] M. Svanidze, From the history of the Georgian-Ottoman relations in the XVI-XVII centuries. Tbilisi, 1971, In Georgian.
- [4] M. Svanidze, Agriculture of South-West Georgia in the XVI century, Metsniereba, Tbilisi, 1984, In Georgian.
- [5] G.A. Tivadze, Ottoman Dervishism and Peasant Uprisings in the XVI Century, by discussing some additional issues, Technology and Labor, Tbilisi, 1946, In Georgian.
- [6] N. Shengelia, Ottoman taxes and liabilities according to the "Grand Defter of Gurjistan Vilayet", Proceedings of the Tbilisi State University, # 91, Series of Oriental Studies, II, Tbilisi State University Publishing House, Tbilisi (1960) 277-304, In Georgian.
- [7] Al. Asklanikashvili, Map of Gurjistan Vilayet. scale: 1:300 00, 1953, In Georgian.
- [8] Sh. Lomsadze, Samtskhe-Javakheti: From mid-XVIII century to the mid-XIX century, S. Janashia State Museum, Metsniereba, Tbilisi, 1975, In Georgian.
- [9] M. Kelberashvili, The Demographic situation in Gurjistan Vilayet at the end of the XVI century, TSU, Tbilisi, 2017, In Georgian.
- [10] N. Bregadze, Wheat, Historical-Ethnographic Atlas of Georgia, Tbilisi, 1980, In Georgian.
- [11] V. Menabde, Georgian Wheats and Their Role in General Wheat Evolution, Proceedings of the Institute of Botany of Georgian Academy of Sciences, v. 21, 1961, In Georgian.
- [12] P. M. Zhukovsky, Cultural Plants and Their Varieties, Lenigrad, 1970, In Russian.
- [13] L. Beriashvili, Farming in Meskheta, Tbilisi, 1961, In Georgian.
- [14] N. Bregadze, Barley, Materials for Georgian Ethnography, v. 19. Tbilisi, 1978, In Georgian.
- [15] L. Beriashvili, Legumes of Georgia. Kidney beans. Materials for Georgian Ethnography, v. 19, Tbilisi, 1978, In Georgian.
- [16] D. Nikolaishvili, D. Sartania, A. Ujmajuridze, // Materials of scientific conference "Regional Development Perspectives: Samtskhe-Javakheti". Tbilisi (2016) 43-60.
- [17] G. Beridze, Wine and Cognac of Georgia, Sabchota Sakartvelo, Tbilisi, 1965.



## New probiotic produced from spore forming *Bacillus* cultivated on local agro-industrial wastes substituting antibiotic in broiler nutrition

A. Chkuaseli<sup>a</sup>, A. Chagelishvili<sup>a</sup>, M. Khutsishvili-Maisuradze<sup>a</sup>, V. Elisashvili<sup>a</sup>,  
T. Khardziani<sup>a</sup>, T. Lashkarashvili<sup>b</sup>, G. Chagelishvili<sup>a</sup>, R. Lapachi<sup>a</sup>,  
K. Samkurashvili<sup>c</sup>, V. Berikashvili

<sup>a</sup>Agricultural University of Georgia, Kakha Bendukidze University Campus; 240, David Aghmashenebeli Alley, Tbilisi, 0159, Georgia

<sup>b</sup>LTD „Nutrimax“; L. Gogoberidze 67<sup>b</sup>, Tbilisi, 0165, Georgia

<sup>c</sup>LTD „Roster“; Gardabani, Teleti, 1300, Georgia

Received: 02 July 2021; Accepted: 25 July 2021

### ABSTRACT

In Georgia, poultry farms continue to use antibiotics as a food supplement to prevent poultry diseases. Given these circumstances and the benefits of using probiotics, local market requirements, availability of resources and the lack of probiotics production in the South Caucasus, the Agricultural University of Georgia team, with the financial support of the “Shota Rustaveli National Science Foundation of Georgia”, has conducted research which enabled us to study the physiological mechanisms that regulate and improve the use of *Bacillus amyloliquefaciens* spore forming probiotics in broiler nutrition. The growing demand to increase and improve the quality of poultry production has put on the agenda the search for alternative methods of removing the antibiotic load in the bird's body. To achieve this, new strains of *Bacillus amyloliquefaciens* were updated in the collection, which were tested in broiler feed. A new probiotic from spore forming *Bacillus amyloliquefaciens* cultivated on local agro-industrial raw materials was mixed with 0.05%, 0.04% and 0.03% of the feed in the broiler plant as a food supplement in the form of a feed additive in 3 test groups. In control group was applied feed with antibiotic. Both broiler and control group broilers were fed a complete combined feed in appropriate phases, which met the broiler's demand for nutrients, minerals, and biologically active substances. Based on the experiment, it was found that the optimal dose of the new probiotic *Bacillus amyloliquefaciens*, as a food additive in the first period of growth (start, grower) is 0.04-0.03%, and in the concluding period (finish) – 0.05%.

**Keywords:** Broiler, Antibiotic, Probiotic, Efficiency, Experiment, Probiotic optimal dose.

\*Corresponding author: Amrosi Chkuaseli; E-Mail address: [a.chkuaseli@agruni.edu.ge](mailto:a.chkuaseli@agruni.edu.ge)

### Urgency of the issue

Due to the growing demand for broiler meat, both the number of poultry farms and their capacity have increased significantly. The use of antibiotics in the production of broiler meat is accepted as a prophylactic as well as the use of growth-promoting [1] antibiotics. The use of antibiotics led not only to the emergence of pathogens resistant to multibiotic drugs but also to a reduction in the microflora

and immune function [2] required in the broiler, resulting in production losses and a significant increase [3] in overall costs. Potential transfers of antibiotic resistance from animals to humans [4] have been introduced. Due to this problem, the use of antibiotics as growth stimulants has been banned in Europe and other developed countries [5]. It has therefore become mandatory to replace antibiotics with other effective means. The use of probiotics as micro-organisms that create a natural

protective barrier between the broiler organism and pathogens has become the most realistic natural alternative to gastrointestinal antibiotic therapy [6], which promotes broiler productivity and product safety [7].

The *Bacillus subtilis* and *Bacillus amyloliquefaciens* application in broiler feed are characterized by high adaptation to environmental conditions and high growth rate on plant raw materials. Thermoresistant spores are stable to long-term storage without refrigeration, moreover, the spores have the ability to survive in the gastrointestinal tract even at low pH levels [8-10]. Thus, the full dose of spore bacteria invariably reaches the small intestine, which is not the case for all species of *LactoBacillus* [11,12]. In addition, the secretion of antimicrobial compounds (coagulin, amikoumacin, and subtilizine) provides a probiotic effect by inhibiting the growth of both competing bacteria and enteric pathogens. Also, the vegetative forms of *Bacillus* and *Bacillus amyloliquefaciens* produce extracellular enzymes (protease, cellulase, xylanase, pectanase and lipase) that promote the absorption of nutrients [13]. The use of probiotics in broiler production has made it possible to improve: broiler maintenance, live weight, feed conversion [14] economic efficiency [15-17].

### Aim and objective of research

Probiotics produced on agro-industrial waste raised the issue of studying the effectiveness of the use of new probiotics from *Bacillus subtilis* and *Bacillus amyloliquefaciens* as a dietary supplement of antibiotic in broiler feeding, which formed the basis of the research work.

The aim of the study was to determine the optimal dose of *Bacillus subtilis* and *Bacillus amyloliquefaciens* in broiler feeding.

Testing of probiotic drugs, confirmation of its best effect was carried out in the poultry enterprise "Roster" Ltd. The biological efficacy, feed conversion, broiler growth and productivity of the new probiotic drug *Bacillus subtilis* and *Bacillus amyloliquefaciens* were evaluated. The study was conducted on several experimental and control groups.

### Testing material and methodology

In order to study the effectiveness of new spore forming *Bacillus subtilis* and *Bacillus*

*amyloliquefaciens* probiotics, new strong strains with superior antimicrobial and probiotic action were selected in the laboratory of the Georgian Agrarian University [18]. New nutrient cultivation areas with unique composition have been developed, laboratory technology for the production of one of the highest yield spores in the world, using solid-state fermentation (SSF), which has been tested using a variety of plant raw materials. [19-21]. The obtained probiotics were tested and their influence was confirmed by the livestock and veterinary specialists of the Agrarian University in the production environment, in the poultry enterprise "Roster" Ltd. The broiler was fed phased whole food, the zootechnical analysis were carried out in the accredited laboratory "Chirina" Ltd. For the experimental groups broiler, probiotics of *Bacillus Subtilis* and *Bacillus Amyloliquefaciens* were mixed separately with feed at the required concentration using a rotary mixer.

### Research results on industrial level

The experiments were conducted at a broiler plant where the broiler feeding was implemented in phases: start 1-10 days, grower 11-28 days and finish 29-35 days. Combined feed was prepared for the control and experimental group birds in "Roster" Ltd. In order to apply new spore forming *Bacillus subtilis* and *Bacillus amyloliquefaciens* and to determine the optimal dose for experiments in "Roster" Ltd. In each experiment participated 400-400 oneday birds, 100 birds in group.

Each experiment lasted 35 days. During the experiment we studied: broiler live weight dynamics, individual weighing of poultry was undertaken at 1, 14, 28 and 35 days, absolute and daily increments, maintenance, feed intake during rearing per 1 bird and feed conversion per 1 kg weight, meat output, meat category, morphological and biochemical parameters of the blood, a broiler growth efficiency index.

In both experiments, chemical analysis of the feed showed that the starting protein content in the starter food was 23.6%, energy 305 kcal, raw protein content in the Grower period 20.5%, energy 309 kcal, and in the finish period 18.5% and 316 kcal, respectively. The content of other nutrients was also within the norm and fully met the requirements of the broiler "Ross-308" for nutrients in all ages.

*Bacillus subtilis* application scheme is as follows.

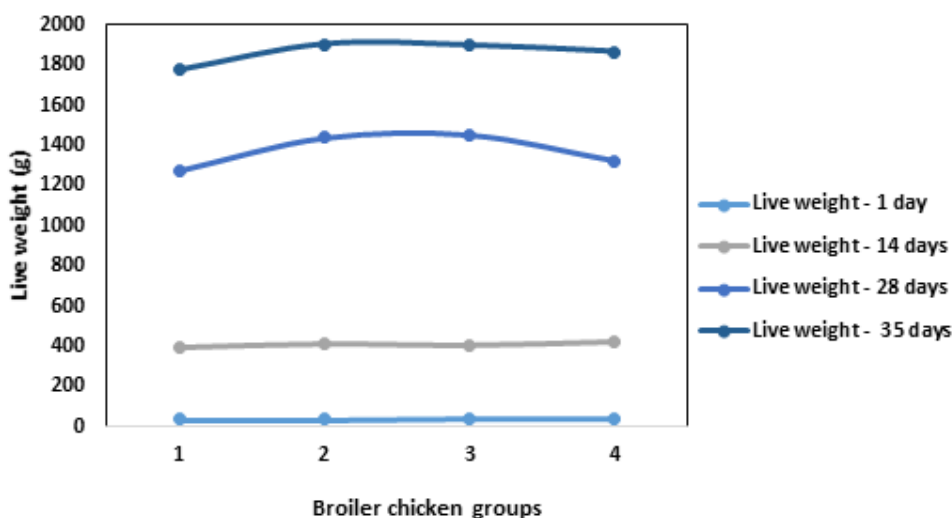
**Table 1.** *Bacillus subtilis* test scheme

Group	Feed	Probiotic/Antibiotics	Quantity
Stage I			
I Group (control)	Combined feed with probiotic	Enrafloxacin	100
II Group	Combined feed with probiotic	<i>B. subtilis</i> 10 <sup>8</sup> spore/gr 0.05%	100
III Group	Combined feed with probiotic	<i>B. subtilis</i> 10 <sup>7</sup> spore/gr 0.04%	100
IV Group	Combined feed with probiotic	<i>B. subtilis</i> 10 <sup>6</sup> spore/gr 0.03%	100

According to the experimental scheme, the first group was the control group, during the whole period of growth (1-35 days) they received a complete combined feed with no probiotics and in the first days of growth (1-3 days) they took the antibiotic Enrafloxacin with water. The second, third, and

fourth groups were experimental and consumed the probiotic *Bacillus subtilis* in the amount of 0.05%, 0.04%, and 0.03%, respectively.

The growth dynamics of the broiler during the experiment.

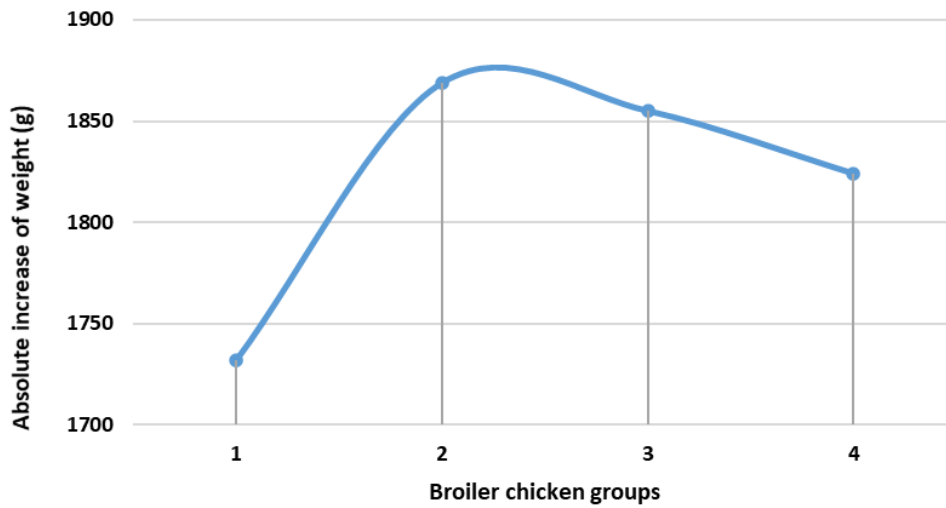


**Fig. 1.** Live weight growth dynamic

The live weight of a one-day trial broiler is the same in all four groups of 40.7-40.8 g, which indicates a high uniformity of the test chickens. The live weight of the control broiler at age of 14 days was 395 g, and the broiler weight of the experimental groups with application of new probiotic *Bacillus subtilis* from day one was 408.4-427.2 g, which was 3.4-8.1% higher compared to the control group ( $P \geq 0.01-0,001$ ). At age of 28 days, the live weight of the experimental group broiler wighted 1322-1452 g, which is 4.0-14.3% higher than the control group ( $P \geq 0.01-0,001$ ). The broiler of the 3rd experimental group had the highest live weight during this period - 1452 g, which is 14.3% higher ( $P \geq 0,001$ ) than the broiler live weight of the control

group and 9.83% higher ( $P \geq 0.01$ ) higher than the live weight of 4th experimental group. At the age of 35 days, at the end of growth period, the highest live weight was observed in the 2nd experimental broiler group - 1903 g, which is 7.3% ( $P \geq 0,001$ ) higher than the control and 2.0% higher than in 4th experimental group. The live weight of broilers in the 3rd and 4th experimental groups at the age of 35 days is 5.2-6.9% higher than in control group ( $P \geq 0.01$ ). The absolute increase (Diagram 2) over the course of 35 days in the 2nd and 3rd test groups is almost the same - 1869-1855g and 123-137g by 7.1-7.9% ( $P \geq 0.01$ ) but greater than in control group.

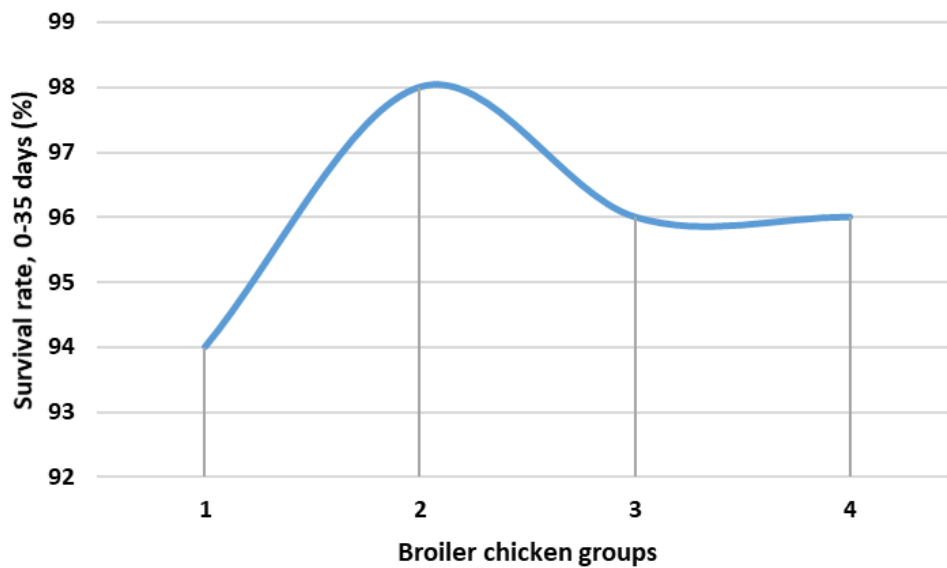
During the experiment we also studied the daily weight gain of the broiler.



**Fig. 2.** Broiler daily weight gain

In the same groups, the highest daily gain was 53.0-53.2 g, which is 3.5-3.7 g higher than in control group.

During the experiment was studied an absolute increase of weight in groups.

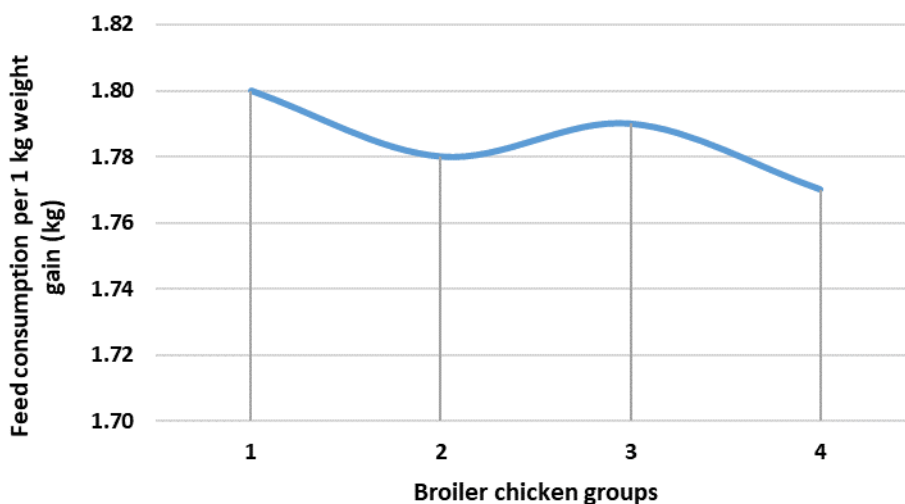


**Fig. 3.** Absolute increase of weight of broilers

Absolute increase of broiler weight in 0-35 days. The absolute increase in the experimental groups was the highest in the 2nd experimental group and amounted to 1869 g, while in the 3rd and 4th experimental groups this figure was 1824-

1855 g. During the growth period, the data of all experimental groups were higher than the control group.

Broiler survival rate during the age of 35 days period was different in groups.

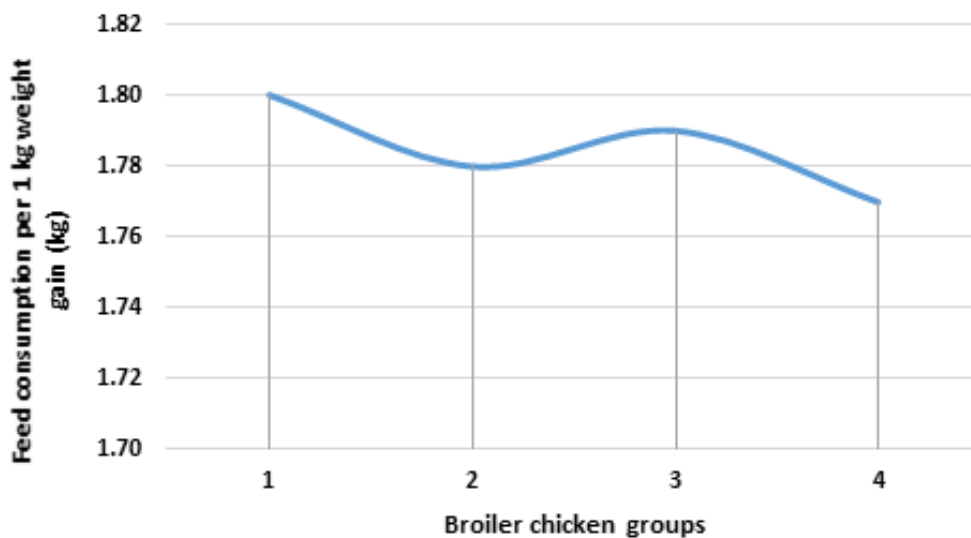


**Fig. 4.** Broiler survival rate

Broiler survival rate in control group was 94%, which is 2-4% lower than in the test groups (Diagram 4). The reason for the bird loss in the control group, even though they were given an antibiotic during the first period of growth, was a gastrointestinal disorder. In the first period of growth in the test

groups, there was no bird loss due to gastrointestinal disorders. The main reason for the decline in these groups in late period of growth was myocardia.

During the broiler rearing period was studied the feed consumption per 1 kg of body weight.



**Fig. 5.** Feed consumption per 1 kg of body weight

Feed consumption per 1 kg body weight in the four groups is practically the same in the range of 1.77-1.80 kg, although the control group has a

tendency to increase feed consumption.

At the end of the experiment we calculated the European (productivity) index of the broiler.

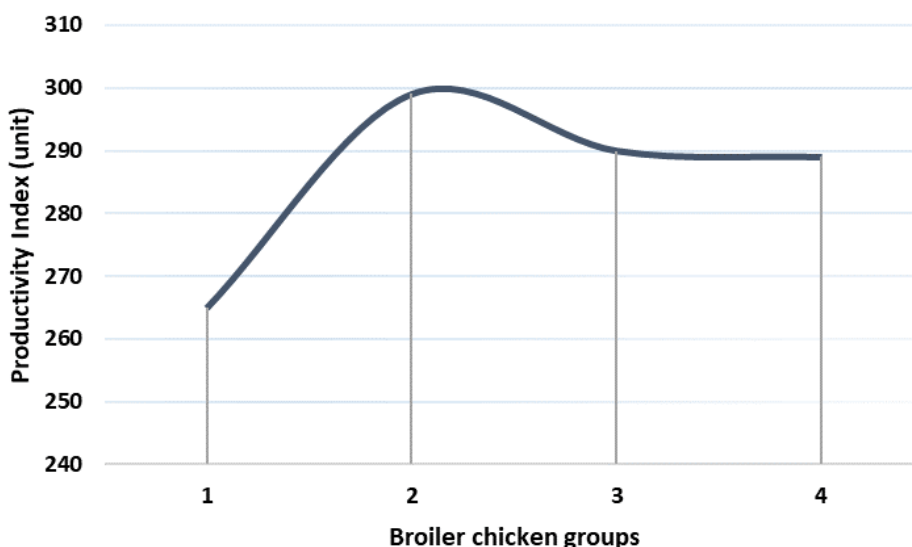


Fig. 6. European (productivity) index

The productivity index in the experimental groups is almost the same and is quite high 289-299 units, which is 24-34 units higher than in control group.

Thus, the optimal dose of the new probiotic as a feed additive in the broiler feed is 0.04% of the new is as follows:

probiotic *Bacillus Subtilis*, produced on local agro-industrial raw materials.

In addition to *Bacillus Subtilis*, our goal was to use the new spore-producing *Bacillus Amyloliquefaciens* and determine its optimal dose.

*Bacillus amyloliquefaciens* application scheme

Table 2. *Bacillus amyloliquefaciens* test scheme

Group	Feed	Probiotic/Antibiotics	Quantity
Stage II			
I Group (control)	Combined feed with probiotic	Ernafloracillin	100
II Group	Combined feed with probiotic	<i>B. amyloliquefaciens</i> 10 <sup>8</sup> spore/gr 0.05%	100
III Group	Combined feed with probiotic	<i>B. amyloliquefaciens</i> 10 <sup>7</sup> spore/gr 0.04%	100
IV Group	Combined feed with probiotic	<i>B. amyloliquefaciens</i> 10 <sup>6</sup> spore/gr 0.03%	100

According to the experimental scheme, the first group was the control group, during the whole period of growth (1-35 days) they received a complete combined feed with no probiotics and in the first days of growth (1-3 days) they took the antibiotic Ernafloracillin with water. The second, third, and

fourth groups were experimental and consumed the probiotic *Bacillus amyloliquefaciens* in the amount of 0.05%, 0.04%, and 0.03%, respectively.

The growth dynamics of the broiler during the experiment.

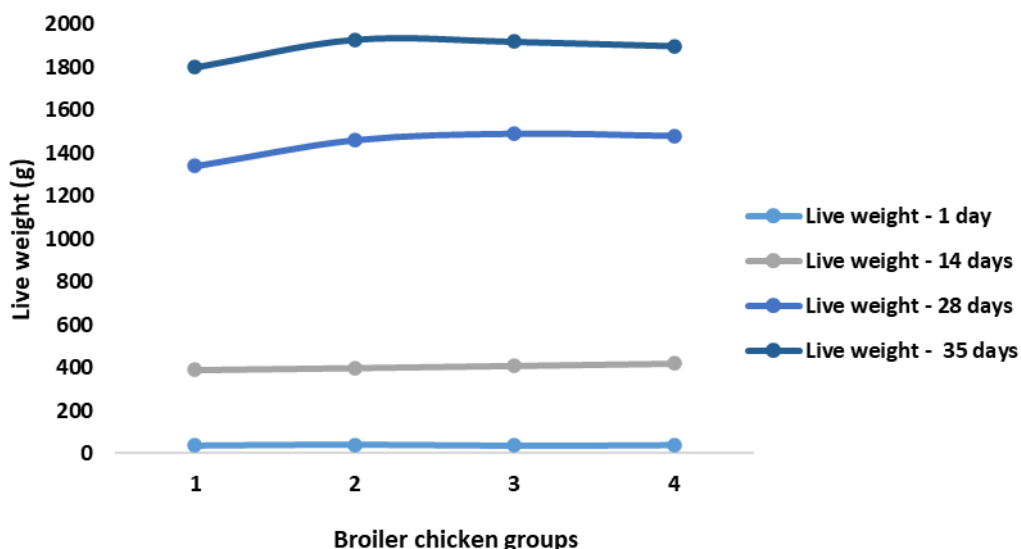


Fig. 7. Broiler live weight growth dynamic

The live weight of a one-day broiler is the same in all four groups of 39,7-40,2 g, which indicates a high uniformity of the test chickens. The live weight of the control broiler in forth group at age of 14 days was 420 g, Which is 7.7% higher compared to the control group ( $P \geq 0,001$ ), as for the 2nd and 3rd experimental groups, they also exceeded the data of the control group by 2.4-5,0% in live weight.

The difference between the 2nd and 3rd experimental groups was 10-20 g compared to the 4th group. At 28 days of age, the live weight in the broiler of the experimental group was 1460-1490 g and exceeded the live weight of the broiler in the

control group by 8.9-11.2% ( $P \geq 0,001$ ). However at this age the relatively high live weight of the 2nd group broiler was 1490 g. And the difference in live weight between the experimental groups at this age is 0.0-1%, which is negligible. At the age of 35 days or at the end of the experiment, the highest live weight of group 2 broilers was 1930 g, which is 7.3% higher than the control ( $P \geq 0,001$ ). As for the 3rd and 4th experimental groups, they were slightly 0.5-2.0% behind the 2nd group and exceeded the index of the control group by 5.5-6.7% ( $P \geq 0.01$ ).

During the broiler rearing period (0-35 days) we studied the daily weight gain of the broiler.

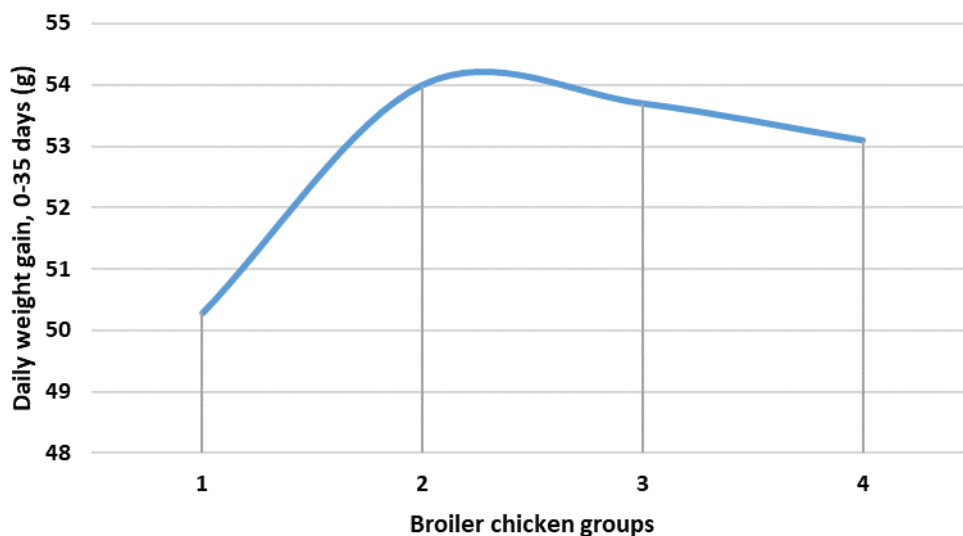


Fig. 8. Daily weight gain of the broiler

Calculation according to the daily weight gain in groups showed that the broiler of all three experimental groups had the highest daily increments of 35.1-54.0 g during the 35-day period,

while the lowest control group had 50.29 g. It should be noted, however, that the highest daily weight gain during the growing period was in the 2nd experimental group broiler at 54.0 g.

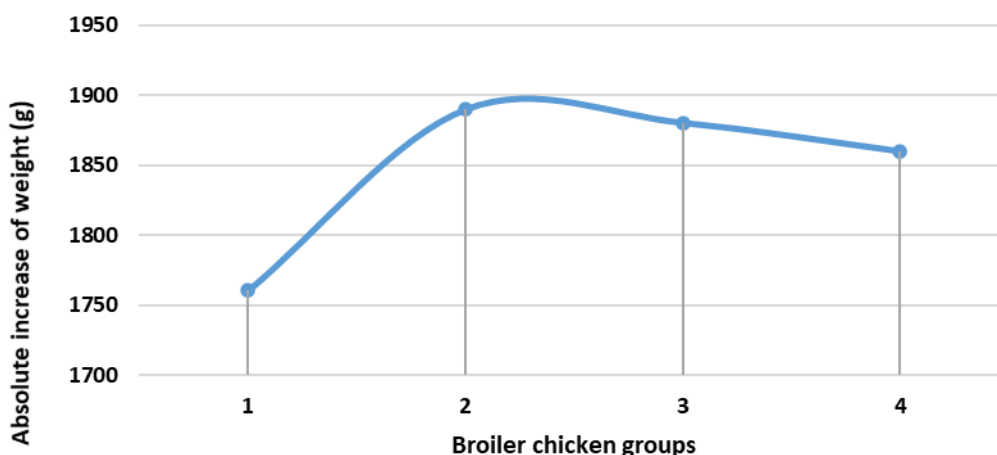


Fig. 9. Absolute increase of weight

Absolute increase of broiler 0-35 days. The absolute increase in the experimental groups was the highest in the 2nd experimental group and amounted to 1890 g, while in the 3rd and 4th experimental groups this figure was 1880-1860 g. During the

growth period, the data of all experimental groups are higher than those of the control group.

As for the survival rate of the broiler during the growing period, the data in the experimental and control groups are shown in the diagram.

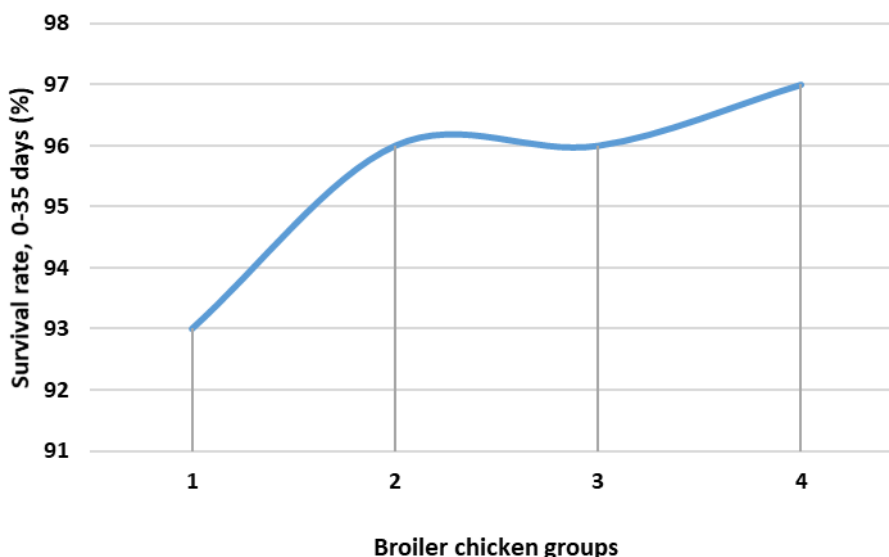


Fig. 10. Broiler survival rate

Broilers survival rate by groups (0-35 days). Broiler survival is different in the experimental and control groups. The highest maintenance was observed in the 4th trial group-97%. The maintenance of the broiler of the 2nd and 3rd experimental groups was

the same and amounted to 96%. The maintenance of the control group broiler was -93%, 3-4% less than in the experimental groups.

Feed consumption during the broiler rearing period is shown on the diagram.

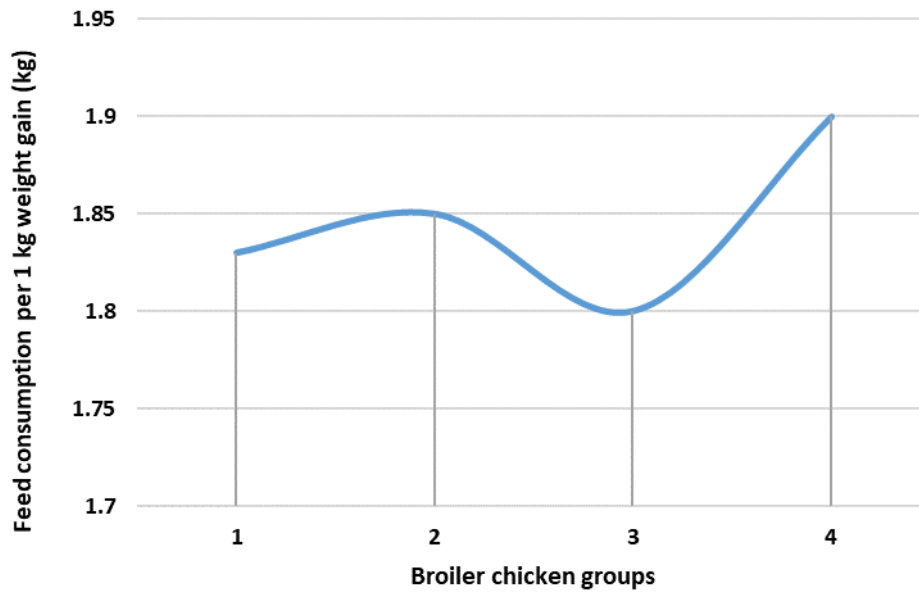


Fig. 11. Feed consumption per 1 kg of body weight

Feed consumption per 1 kg of body ranged from 1.85 to 1.90 kg in the experimental groups and in

the control group it was 1.83 kg.

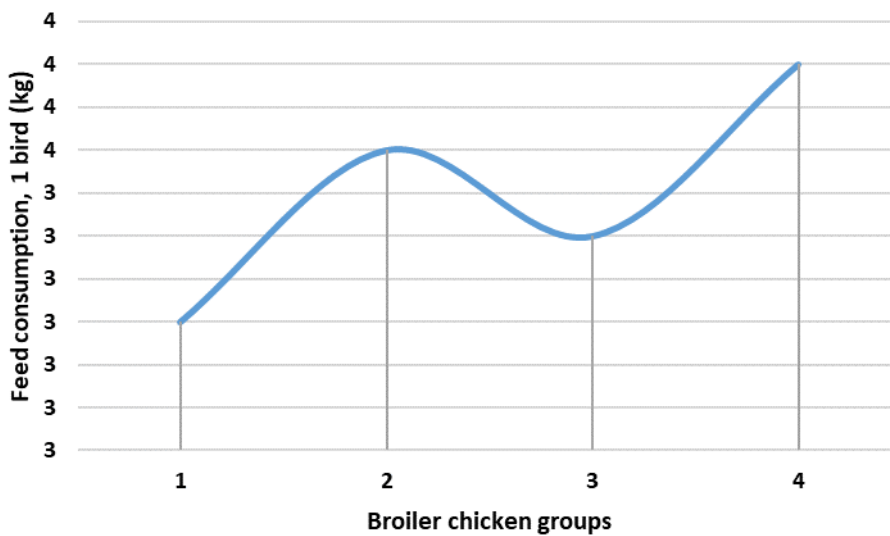
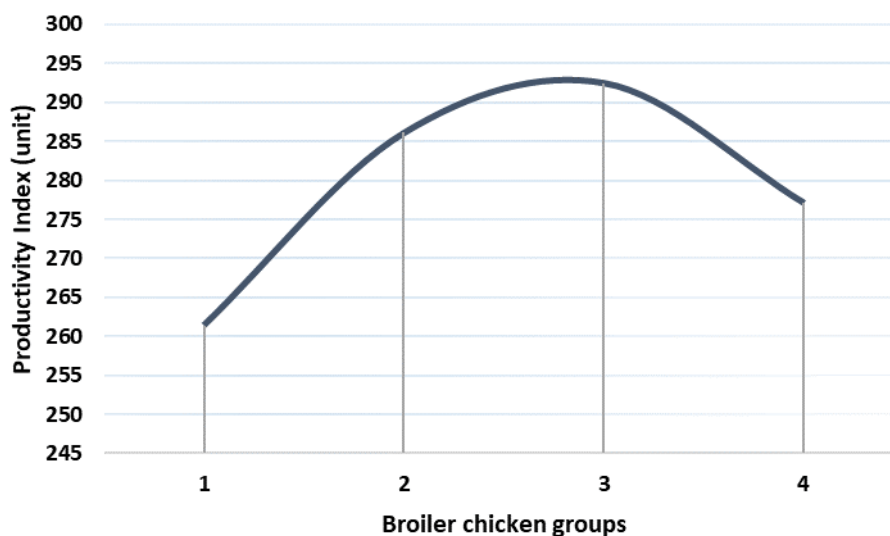


Fig. 12. Feed consumption, 1 bird

Feed consumption per 1 bird ranged 3.5-3.6 kg in the experimental groups and in the control group it was 3.3 kg.

At the end of the experiment we calculated the European (productivity) index of the broiler.



**Fig.13.** Broiler productivity index

The calculation of the productivity index showed that this indicator was the highest in the 3rd group - 292.5 units, which is 31.1 units higher than the control group. According to the mentioned parameter, the control group was 16-25 units behind the data of the second and fourth test groups.

The experiment showed that the optimal dose of new spore-producing *Bacillus amyloliquefaciens* probiotics in the first period (start, grower) of broiler rearing is 0.04-0.03%, and in the last period (finish) -0.05%

The application of new probiotics at a dose of 0.04% *Bacillus subtilis* and 0.05% dose of *Bacillus amyloliquefaciens* had a positive effect on broiler productivity.

### Acknowledgement

The research was conducted with the financial assistance of “Shota Rustaveli National Science Foundation of Georgia” (Grant Project CARYS-19-2268).

### References

- [1] World Health Organization. Joint FAO/OIE/WHO Expert Workshop on Non-Human Antimicrobial Usage and Antimicrobial Resistance: Scientific Assessment; World Health Organization: Geneva, Switzerland (2003); No. WHO/CDS/CPE/ZFK/2004.7.
- [2] Roth, N.; Käsbohrer, A.; Mayrhofer, S.; Zitz, U.; Hofacre, C.; Domig, K.J., The application of antibiotics in broiler production and the resulting antibiotic resistance in *Escherichia coli*: A global overview, *J. Poult. Sci.*98 (2019) 1791–1804.
- [3] A. Sundsfjord, M. Sunde. *Tidsskr Nor Laegeforen*, Antimicrobial resistance after antibiotic use in animals- impact on human health, 98 (2008) 2457-61.
- [4] M.T. Madigan, J.M. Martinko, K.S. Bender, F.H. Buckley, D.A. Stahl, *Brock Biology of Microorganisms*. 14th ed. Illinois, J. Pearson International (2014) 1006.
- [5] U. Gadde, W.H. Kim, S.T. Oh, H.S. Lillehoj, Alternatives to antibiotics for maximizing growth performance and feed efficiency in

- poultry: a review, *J. Animal Health Res Rev* (2017)26-45.
- [6] A.Grant, G.Gay, H.S. Lillehoj, [Bacillus spp. as](#) direct-fed microbial antibiotic alternatives to enhance growth, immunity, and gut health in poultry, *J. Avian Pathol.* 47(4)(2018) 339-351.
- [7] ERL. Pelicano, P.A. de Souza, HBA. de Souza, Oba A, EA. Norkus, LM. Kodawara, T.M.A. de Lima, Effect of different probiotics on broiler carcass and meat quality, *J. Poult. Sci.*5 (2003) 207–214.
- [8] Hong, H.A., Duc, L.H. and Cutting, The use of bacterial spore formers as probiotics, *J. FEMS Microbiol Rev.*29 (2005) 813–835.
- [9] M.Brashears , A.Amezquita, D.Jaroni, Lactic Acid Bacteria and Their Uses in Animal Feeding to Improve Food Safety, *J. Advances in Food and Nutrition Research* (2005).
- [10] T.M. Barbosa, C.R. Serra, R.M. La Ragione, M.J. Woodward, A.O. Henriques, **Screening for Bacillus isolates in the broiler gastrointestinal tract**, *J. Appl. Environ. Microbiol.*71 (2005) 968-978.
- [11] J.I. Cañanón, History of the use of antibiotic growth promoters in European poultry feeds, *J. Poultry Science.*86 (2007) 2466-2471.
- [12] [K.M. Tuohy](#), [M. Pinart-Gilberga](#), [M. Jones](#), [L. Hoyles](#), [A. L. McCartney](#), [G. R. Gibson](#), Survivability of a probiotic *LactoBacillus casei* in the gastrointestinal tract of healthy human volunteers and its impact on the faecal microflora, *J. Appl Microbiol* (2007) 1026-1032.
- [13] Guo, J.J., Liu, K.F., Cheng, S.H., Chang, C.I., Lay, J.J., Hsu, Y.O., Yang, J.Y. and Chen, T.I., Selection of probiotic bacteria for use in shrimp larviculture, *J. Aquacult Res* 40 (2009) 609–618.
- [14] Chaucheyras-Durand, F. and Durand, H., Probiotics in animal nutrition and health, *J. Benef Microbes* (2010) 1, 3–9.
- [15] [V. Kurtoğlu](#) , [F. Kurtoğlu](#), [B. Coşkun](#), Effect of probiotic supplementation on laying hen diets on yield performance and serum and egg yolk cholesterol, *J. Food Additives and Contaminants* (2004) 817-823.
- [16] Duc, L.H., Hong, H.A., Barbosa, T.M., Henriques, A.O. and Cutting, Characterization of *Bacillus* probiotics available for human use, *J. Appl Environ Microbiol.*70 (2004) 2161–2171.
- [17] [Arun Panda](#), [Sv Rama Rao](#), [M V L N Raju](#), Effect of probiotic (*LactoBacillus sporogenes*) feeding on egg production and quality, yolk cholesterol and humoral immune response of White Leghorn layer breeders, *J. Science of Food and Agriculture* (2008) 43–47.
- [18] [Algburi](#), [C. Cugini](#), [E.Walsh](#), [A. Volski](#), Safety Properties and Probiotic Potential of *Bacillus subtilis* KATMIRA1933 and *Bacillus amyloliquefaciens* B-1895, *J. Advances in Microbiology* (2016) 432-452.
- [19] T. Khardziani, E. Kachlishvili, K. Sokhadze, V. Elisashvili, R. Weeks, M.L. Chikindas, V. Chistyakov, Elucidation of *Bacillus subtilis* KATMIRA 1933 potential for spore production in submerged fermentation of plant raw materials, *J. Probiotics and Antimicrobial Proteins.*9 (2017) 435-443.
- [20] T. Khardziani, K. Sokhadze, E. Kachlishvili, V. Chistyakov, V. Elisashvili, Optimization of enhanced probiotic spores production in submerged cultivation of *Bacillus amyloliquefaciens* B-1895, *J. Microbiol Biotechnol Food Sci.* 7 (2017) 132-136.
- [21] V. Elisashvili, M.khutsishvili, A.Chagelishvili et al., -Poultry-beneficial solid-state *Bacillus amyloliquefaciens* B-1895 fermented soybean formulation, *J. Bioscience of Microbiota, Food and Health.*34 (2015) 25-28.



## Annals of Agrarian Science

Journal homepage: <http://journals.org.ge/index.php>



# Development and laboratory and field testing of new combined insecticides against the resistant and non-resistant populations of the Brown Marmorated Stink Bug (*Halyomorpha Halys*)

A. Chirakadze <sup>a</sup>, N. Mitagvaria <sup>b</sup>, N. Lomidze <sup>a</sup>, M. Ambokadze <sup>a</sup>, A. Gigineishvili <sup>a</sup>, Z. Buachidze <sup>a</sup>, T. Chichua <sup>a</sup>, A. Jishiashvili <sup>a</sup>, K. Chigogidze <sup>a</sup>, Z. Chekurashvili <sup>c</sup>, Z. Lipartia <sup>c</sup>, N. Meskhi <sup>c</sup>, L. Zazadze <sup>a</sup>

<sup>a</sup>Department of Engineering Physics, Georgian Technical University, 77 Kostava Str., Tbilisi, 0160, Georgia

<sup>b</sup>Department of Brain Blood Circulation and Metabolism, Ivane Britashvili Center of Experimental Biomedicine, 14 Gotua Str., Tbilisi, 0160, Georgia

<sup>c</sup>National Food Agency of Georgia, Ministry of Environment Protection and Agriculture of Georgia, 6 Marshal Gelovani Av., Tbilisi, 0159, Georgia.

Received: 10 September 2021; Accepted: 19 October 2021

## ABSTRACT

The Brown Marmorated Stink Bug (BMSB) is among the most serious pests for agriculture. It can cause a significant social nuisance. Development and laboratory testing of new synergistic insecticidal mixtures highly effective against this invasive pest and less toxic to the living environment (especially humans, mammals, birds, pollinators and other useful insects, aquatic organisms) is an extremely urgent challenge for the modern applied entomology. Synergy of pyrethroidal (bifenthrin, gamma-cyhalothrin) and organophosphorous (malathion, dimethoate) active substances was investigated and the contribution of additional components (mineral dusts, essential oils, emulsifiers, dispersers and hydroxyethylcellulose) to the total joint biological effectiveness of the mixtures was assessed. It was found that the both studied mixtures (with bifenthrin + malathion and gamma cyhalothrin + dimethoate) showed a clearly expressed synergy and increase of biological effectiveness in comparison to the separate components. At the same time, the observed synergistic effect of malathion- and bifenthrin- based mixture against the insect populations was obviously lower in Abasha and Senaki municipalities, than in Kobuleti and Khelvachauri municipalities of Georgia. Difference in biological effectiveness of gamma-cyhalothrin- and dimethoate-based mixtures for the insect groups was significantly lower. The most reliable explanation of this shift could be the resistance developed by Abasha and Senaki populations due to the five-year long enhanced treatment with bifenthrin based formulations. The acute toxicity of the newly developed combinations to mammals and bird embryos was found to be 3-5 times lower, than of the widely applied insecticidal modalities. The data of laboratory testing of the biological effectiveness of insecticidal combinations were supported by the results of field trials of the five insecticidal formulations in a corn field and a hazelnut plantation held with the technical support of the National Food Agency of Georgia of the Ministry of Environment of Georgia.

**Keywords:** Brown Marmorated Stink Bug, Synergy, Biological effectiveness, Combined insecticides, Resistance, Acute toxicity, Nano-insecticides.

\*Corresponding author: Archil Chirakadze; E-mail address: [achikochirakadze@gmail.com](mailto:achikochirakadze@gmail.com)

## Introduction

**Importance and necessity of BMSB management in Georgia.** Development and study of a wide range of new highly effective pesticides

having low toxicity against the living environment (primarily - humans, mammals, bees and other beneficial insects, aquatic organisms, reptiles, etc.) is of a paramount importance for agriculture

and environmental safety. One of the most acute current task in Georgia and globally is to combat the polifague invasive pest called the Brown Marmorated Stink Bug (BMSB, *Halyomorpha halys*) [1-14]. Management of BMSB in Georgia is provided by the Ministry of Environment and Agriculture of Georgia with an active support of the Food and Agriculture Organization of the United Nations (UNFAO), the United Nations Development Program (UNDP) and the United States Agency for International Development (USAID) within the framework of the programs implemented by the National Food Agency of Georgia [15, 16].

#### **Relevance and the main aim of the study.**

In recent years, special attention has been paid to the development and testing of synergistic combinations of active substances of different chemical class and types of action, which should be highly toxic against the nymphs, adult and mature instars and, at the same time, provide effective inhibition of the development of pest resistance to insecticides and acaricides [3, 5-8]. This approach gives us the possibility of making a significant reduction of the applied doses of the active substances and minimizes their negative impact on human, pollinators and other useful insects, farming animals, cattle, poultry and wildlife.

For taking into account the possible low effectiveness of the developed combinations against drug resistant pest populations and achieving the high environmental and economic effect, it is necessary to conduct laboratory and field testing of the combined drugs on the both resistant and non-resistant population [9-14]. One of the most important characteristics of insecticides is their acute toxicity to warm-blooded animals. At the same time, the requirements for the standards of humane treatment of test animals (which should exclude killing of animals, causing them severe pain, significant deterioration of health, prolonged immobilization and anesthesia, etc.) are becoming increasingly stringent [5-7, 17-20].

One of the most important aims of this study is to determine the biological effectiveness and validity terms of the developed insecticides against BMSB populations, spread in the Abasha-Senaki and Kobuleti-Khelvachauri municipalities, and their acute toxicity to mammals (white rats) using new methods developed and tested at the Georgian Technical University and I. Beritashvili Center for Experimental Biomedicine and adhering to all modern 4 R principles

of humane treatment of test animals.

An especially important part of the study is the field testing of the developed combinations with the aim of demonstrating applicability of the developed and used methods for the accurate and reliable determination of the main characteristics of the pesticides.

Evaluation of the acute toxicity involved the long-term continuous monitoring of cognitive, behavioral, physiological and rheology characteristics of the exposed groups of test animals, with the aim of excluding any kind of testing contradicting to the 4R principles of humane treatment of laboratory animals. Preliminary testing of nano-based combinations containing nanostructured alumina was carried out to examine the prospective of nanoparticles and nanobased complexes as active components of insecticidal combinations with increased biological effectiveness and safety.

## **Materials and methods**

### **Test insects**

The main subjects of the three-year laboratory study were as follows: 3900 adult pests of both sexes (Group A), provided by the Georgian National Food Agency and collected from the farmers in Abasha and Senaki municipalities; 3900 adult pests of both sexes collected in the Autonomous Republic of Adjara from the farmers in Kobuleti and Khelvachauri municipalities (Group B); 900 adult pests of both sexes collected from the farmers in Abasha and Senaki municipalities (control group C); 900 adult pests of both sexes collected from the farmers in Kobuleti and Khelvachauri municipalities (control group D). The purpose of dividing into groups was to determine whether there was a noticeable prevalence in resistance to the widely used insecticide bifenthrin in the populations of Abasha and Senaki municipalities compared to populations of Kobuleti and Khelvachauri municipalities. The reported study showed that the biological effectiveness of combinations and synergy of components depends significantly on the ratio of synthetic components of the combination.

### **Components of the developed insecticidal combinations**

Synthetic and natural components for the insecticidal combinations used in this study

were selected according to the following criteria: registration in the US, Australia, EU or Georgia; widespread and free availability to farmers; the low prescribed concentrations of the active substances and low recommended rates of application, high lethality to mature BMSB adults. The choice between essential oils with the approximately equal effectiveness [21–23] was made in favor of the less harmful rosemary oil. Among the pyrethroid insecticides, bifenthrin (widely used and well studied in Georgia [24, 25]) and gamma-cyhalothrin (which has a sufficiently high biological effectiveness against the target pest [26, 27]) have been selected. Dichlorvos was initially excluded from the list of potentially applicable organophosphorus insecticides, as its use is banned or severely restricted in many parts of the world [28, 29]. Among other organophosphorus insecticides, malathion [30, 31] and dimethoate [32, 33] were selected, as they showed noticeably higher synergies with bifenthrin and gamma-cyhalothrin than the other eight widely used pyrethroid insecticides probed during the pre-testing period. We also selected Lansperse-BIO868 [34] (a well-known bio-based 100 % biodegradable surfactant) from “Lankem” (UK) and Hydroxyethylcellulose (HEC) [35] (widely used in medicine and pharmacology) from “Sakshi Chem Sciences Private Limited” (India), which allowed us to provide highly stable disperse insecticidal combinations.

One of the selected mineral components DE (amorphous diatomaceous earth or diatomite) [36-40] is practically non-toxic to mammals and warm-blooded animals, and is widely used in agriculture and veterinary medicine (as a pesticide, anti-parasitic drug and animal food additive), in cosmetics, hygien products and food industry (for composition of food additives, various types of filtration, food protection, soil cultivation,

in hydroponic mixtures, etc.). The insecticidal-acaricidal action of amorphous diatomaceous earth (diatomite) is mainly due to the effect of the mineral on the cuticle and exoskeleton causing dehydration of constituent substances and absorption of fatty compounds. Micro-powder of the DE mined in Georgia (Kisatibi mine), dried at a temperature of 150 °C and ground to a size of tens of micrometers, is noticeably less toxic to bees than to target pests due to several differences in the structure of the protective covering layer and lifestyle of insects. The chemical content of the utilized DE was practically the same as the typical composition given in [40]. The second mineral component used by us was kaolin (aluminum hydrosilicate) [41, 42] with the chemical formula  $Al_2Si_2O_5(OH)_4$ . Kaolin is a widely used phyllosilicate clay compound which consists of silicate sheets ( $Si_2O_5$ ) bonded with layers of aluminum oxide/hydroxide ( $Al_2(OH)_4$ ) and is widely used for manufacture of medicines, textiles, petroleum and building materials. It has high thermal stability, porosity and a large area of the capillary surface, which (among other properties) provides its sufficiently high insecticidal properties (including the ability to dehydrate insects' exoskeleton). Due to its lower strength and toughness, damaging effect of kaolin on the outer layer of pollinators covered with body-hairs is insignificant, and it is practically harmless or less harmful for different species of bees. In all insecticidal combinations we utilized the food grade product from “Neelkanth Finechem LLP” (Boranada, Jodhpur, Rajasthan, India). The chemical content of kaolin micro powder dried at 200°C was practically the same as the typical composition given in paper [42]. Pyrethroidal and organophosphorus synthetic drugs were selected primarily considering the higher lethality index and low recommended doses of application [43-46].

**Table 1.** Basic synthetic components of insecticidal combinations

Active substance	Name of the drug	Registration in Georgia, EU, Australia, USA	Content of the active substance, mass %	Lethality index to BMSB, % [41-42]
Bifenthrin	Insakar	Georgia, 1/8/2015, 137	10	91.5
Gamma-cyhalothrin	Trojan 150 CS	EU, 11/07/2009	15	64.2
Malathion	Malafos	Georgia 12/2/2015, 144	50	83.8
Dimethoate	Dimevit	Georgia 9/25/2017, 288	40	93.3

In all insecticidal combinations (see Table 1) we used the widely applied insecticides registered in EU or Georgia: “Insacar” (“Parijat Industries PVT. Ltd”, India) with 100 ml of bifenthrin per liter; “Trojan 150 CS” (FMC, Australia) with 150 ml/l of gamma-cyhalotrin per liter; “Malafos” (“Parijat Industries PVT. Ltd”, India), with 500 ml of malathion per liter; and “Dimevit” (“Ukravit Ltd.”, Ukraine - Georgia), with 500 ml of dimethoate per liter.

Emulsifier-dispersant (surfactant) “Lansperse BIO-868” has entirely natural and 100%-biodegradable components, does not contain any volatile organic compounds (VOCs), does not cause flocculation (coagulation), provides a balanced homogenous distribution of micro-particles, does not cause skin irritation, is slightly eco-toxic (in terms of used concentrations and dosages is practically not-eco-toxic). Hydroxyethylcellulose (HEC) is widely used as lubricant, water-binder and thickening agents in many industry applications, that is, personal care products, pharmaceutical formulations, building materials, adhesives, etc., and as stabilizer for liquid soaps, provides high stability of dispersions and is practically non-toxic.

## Methodology

### Insecticidal formulation for control (relative) measurements

One of the main difficulties of the study was the fact that (due to various objective reasons) the number of test insects was limited and, consequently, the number of replications in the laboratory testing process was reduced. Therefore, in order to increase the accuracy and reliability of testing, it became necessary to make relative measurements in comparison to a combined “reference” insecticidal formulation comprehensively described in the literature, widely used in practice and characterized by a high biological effectiveness and a low persistence in processed plants. ProStore 420 EC supplied by FMC was studied and chosen as the most appropriate insecticidal formulation [47]. This combined drug contains malathion (up to 45%), bifenthrin (up to 5%), calcium dodecylbenzenesulfonate (up to 5%), 2-ethylhexane-1-ol (up to 5%), ethoxylated polyarylphenol (up to 5%), petroleum and heavy aromatic hydrocarbons (up to 50%), 1,2,4-trimethylbenzene (up to 2%). In order to compare the biological effectiveness and

toxicity of the studied insecticidal combinations of the warm-blooded mammals, the following composition was chosen: malathion 400 g/l and bifenthrin 20 g/l, which is characterized by especially high biological effectiveness against various harmful pests in cereals, flour and groats.

### Preparing of disperse insecticidal combinations

Food grade freshwater diatomite of the Kisatibi (Southern Georgia) deposit was immersed in water for 30 minutes, filtered, and after 45 minutes of heating in a microwave oven at 135 °C ground into a 10-50 µm particle micro-powder. All other components were dispersed in water using a standard homogenizer at a frequency of 15000 rpm for 8–10 min. Diatomaceous earth micro-powder was added to the dispersion under constant mixing conditions and treated in an ultrasonic homogenizer for 25–30 min to obtain a stable dispersion. Food grade kaolin supplied by Neelkanth Finechem LLP (Jodhpur, Rajasthan, India) was thermally processed for 10 minutes in a microwave oven at 155 °C. All other components were dispersed using a standard homogenizer at 12000 rpm, followed by a continuous stirring for 4-5 minutes in a microwave oven at 65-70 °C. The yielded mixture was dispersed using an ultrasonic homogenizer for 15-20 minutes to obtain a stable dispersion.

### Methodology of the biological effectiveness study

The essence of the synergistic approach is the determining of optimal ratio of the amounts of active components, solvents and emulsifier-dispersers and of the biological effectiveness and terms of action of the corresponding combinations. Optimization of the ratio of each of the active insecticidal synthetic and natural substances should ensure maximum synergistic effect, while the optimal ratio of natural materials (diatomite, kaolin), emulsifier-disperser, Hec (Hydroxyethyl –cellulose) and water content should ensure the production of the long-lasting disperse mixtures. So, the following sequence of the relevant operation stages was identified: dispersing of diatomite or kaolin, rosemary, and hydroxyethyl-cellulose to obtain the most stable dispersions in water and emulsifier; dispersing of appropriate

ratios of synthetic pyrethroid and organophosphorus insecticides in the manufactured stable dispersions; determining the biological effectiveness of the developed and tested combinations; evaluation of the total biological effect and synergy of active components (compared to the reference insecticidal formulation). Based on the obtained results, it became possible to assess the acquired resistance of pests, evaluate the total share of the combined mineral and essential oil components and assess the acute toxicity of the tested drugs to warm-blooded mammals and avian embryos.

## Experimental: laboratory research and field trials

### Composition of the tested insecticidal combinations

Formulations made from the components selected on the basis of literal data and the results of the preliminary research must meet the following major requirements relevant to the main purposes of the study: concentration of synthetic components in combinations should not exceed the values recommended by the relevant regulations; their concentration and the average total biological effectiveness must be relatively low to allow an

accurate estimation of the “strong” synergistic peaks; their average biological effectiveness must be high enough for observing and accurate estimation of the relatively “weak” peaks and “non-synergistic” (slowly varying) contributions; the active component ratio step should be small enough to detect and evaluate all noticeable peaks of synergy.

Considering these requirements, 37 combinations with the conditional name “Dibifmal” (combinations D-1 – D-37) and 37 combinations with the conditional name “Kagcidim” (combinations K-1 – K-37) were prepared. In combinations D-1 – D-37 the mass content of active synthetic components varied in a constant step as follows: “Insakar - from 0 to 10 %, “Malafos” - from 10 % to 0. The mass content of active synthetic components in K-1 – K-37 combinations varied in a constant step as follows: “Trojan 150 EC - from 0 to 10%, Dimevit - from 10% to 0. Content of the active synthetic components in the tested insecticidal combinations and their expected total relative biological effectiveness are given in Tables 2, 3 and Figures 1, 2. The expected total biological effectiveness of the tested combinations (ETBE) characterizing the summarized effectiveness of components in absence of any synergistic (super-additive or antagonistic) interaction can be easily calculated using literary data (e. g. [24-27, 30-33, 43]).

**Table 2.** Composition of the tested malathion-, bifenthrin- and diatomite-based insecticidal combinations D1- D7 (water - 73.3 %, HEC - 1.2 %, BIO-868- 3.3 %, essential oil – 0.2 %, diatomaceous earth –12 %).

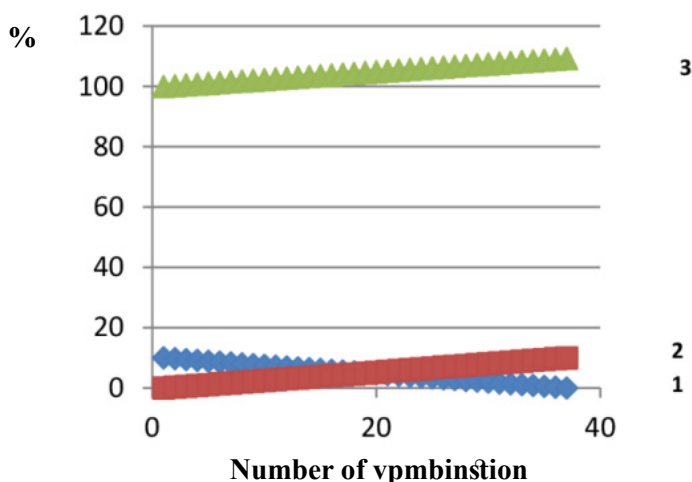
No	Malafos, mass %	Insakar, mass %	ETBE, %	No	Malafos, mass %	Insakar, mass %	ETBE, %
D1	10	0	100	D20	4.72	5.18	104.88
D2	9.7	0.28	100.25	D21	4.45	5.55	105.14
D3	9.4	0.56	100.51	D22	4.19	5.81	105.4
D4	9.16	0.84	100.77	D23	3.92	6.08	105.66
D5	8.88	1.12	101.03	D24	3.64	6.36	105.91
D6	8.6	1.4	101.29	D25	3.36	6.64	106.17
D7	8.32	1.68	101.55	D26	3.08	6.92	106.43
D8	8.04	1.96	101.8	D27	2.8	7.2	106.69
D9	7.78	2.22	102.05	D28	2.52	7.48	106.94
D10	7.51	2.49	102.30	D29	2.24	7.76	107.2
D11	7.24	2.76	102.56	D30	1.96	8.04	107.46
D12	6.96	3.02	102.81	D31	1.68	8.32	107.71
D13	6.7	3.30	103.06	D32	1.4	8.6	107.97
D14	6.42	3.58	103.32	D33	1.12	8.88	108.23
D15	6.16	3.84	103.58	D34	0.84	9.16	108.48
D16	5.9	4.10	103.84	D35	0.56	9.44	108.74
D17	5.63	4.37	104.11	D36	0.28	9.72	109
D18	5.36	4.64	104.36	D37	0	10	109.24
D19	5.09	4.91	104.62				

**Table 3.** Composition of the tested dimethoate-, gamma-cyhalothrin- and kaolin-based insecticidal combinations K1-K37 (water - 73.3 %, HEC - 1.2 %, BIO-868 - 3.3 %, essential oil – 0.2 %, diatomaceous earth –12 %).

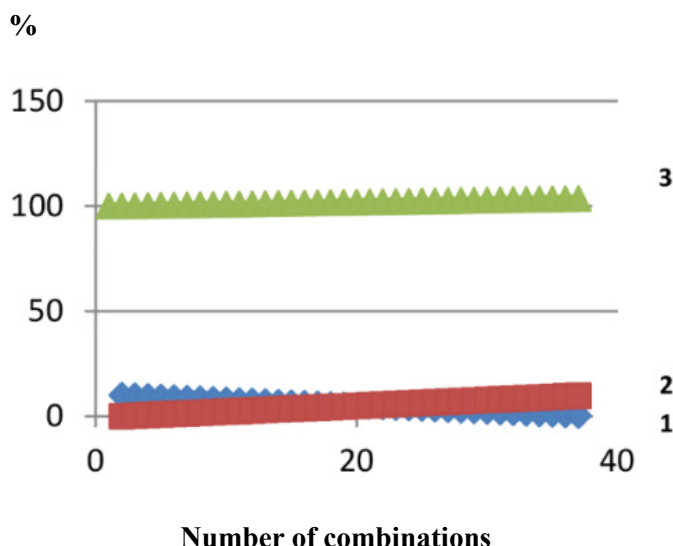
No	Dimevit, Mass%	Trojan 150 CS, mas %	ETBE, %	No	Dimevit, mass%	Trojan 150 CS, mas %	ETBE, %
K1	10	0	100	K20	4.72	5.18	101.9
K2	9.72	0.28	100.1	K21	4.45	5.55	102.
K3	9.44	0.56	100.2	K22	4.19	5.81	102.1
K4	9.16	0.84	100.3	K23	3.92	6.08	102.2
K5	8.88	1.12	100.4	K24	3.64	6.36	102.3
K6	8.6	1.4	100.5	K25	3.36	6.64	102.4
K7	8.32	1.68	100.6	K26	3.08	6.92	102.5
K8	8.04	1.96	100.7	K27	2.8	7.2	102.6
K9	7.78	2.22	100.8	K28	2.52	7.48	102.7
K10	7.51	2.49	100.9	K29	2.24	7.76	102.8
K11	7.24	2.76	101	K30	1.96	8.04	102.9
K12	6.96	3.02	101.1	K31	1.68	8.32	103
K13	6.7	3.30	101.2	K32	1.4	8.6	103.1
K14	6.42	3.58	101.3	K33	1.12	8.88	103.2
K15	6.16	3.84	101.4	K34	0.84	9.16	103.3
K16	5.9	4.10	101.5	K35	0.56	9.44	103.4
K17	5.63	4.37	101.6	K36	0.28	9.72	103.5
K18	5.36	4.64	101.7	K37	0	10	103.6.
K19	5.09	4.91	101.8				

To check the liability of the calculated data on the expected biological effectiveness, we prepared 6 water solutions corresponding to formulations D-1, D-18, D-37, K-1, K-18, K-37 containing only the active synthetic insecticidal components (without diatomite, kaolin, hydroxyethyl- cellulose, rosemary essential oil and emulsifier-disperser Lansperse-BIO8) and determined their biological effectiveness against the test insects of groups E

and F. The comparison with the formulations D-1, D-18, D-37, K-1, K-18, K-37 allowed us to estimate the joint contribution of the mineral components, hydroxyethyl-cellulose, rosemary essential oil and emulsifier-disperser in the integral biological effectiveness of the mixtures. In principle, in such a way it is possible to evaluate the contribution of each component to the integral (total) biological effectiveness.



**Figure 1.** Content of “Malafos”(1, mass %) and “” “Insakar” (2, mass %) in insecticidal combinations D1-D37, and the dependence of expected relative biological effectiveness% (3, %) on the number of combination



**Figure 2.** Content of “Dimevit” (1, mass %) and “Trojan” (2, mass %) in insecticidal combinations D1- D37, and the dependence of expected relative biological effectiveness% (3, %) on the number of combination

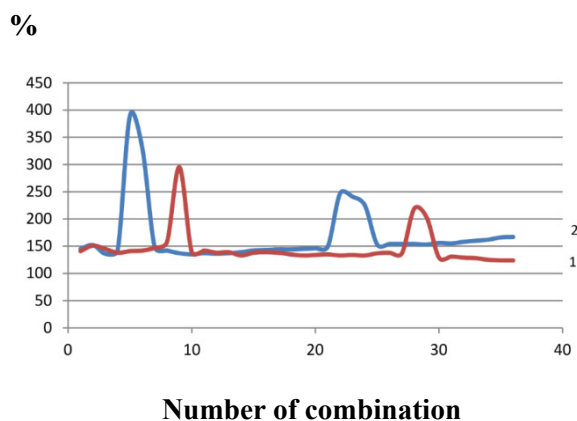
### Determination of the relative biological effectiveness of developed insecticidal combinations

The biological effectiveness of all the above insecticidal combinations was compared to the biological effectiveness of D-1 and K-1 combinations respectively to determine their relative biological effectiveness utilizing the experimental method and design given in [48]. The only difference was that the test insects were disposed in 5 l volume transparent containers (25 insects in each) with sprouted wheat. The provided and controlled testing conditions were as follows: air temperature  $T = 25 \pm 2$  °C, relative humidity RH= 55-65 %, bright time - 16 hours, dark time - 8 hours. An important advantage of the developed experimental design was the possibility of the direct evaluation of the change of residual biological effectiveness over the testing time. All containers were sprayed just before the first group of insects was placed in the containers. During the week all insects in the test and control groups were observed and the dead insects were daily removed and counted. On the last day of the week all the dead and survived insects were removed and counted. The food layovers were daily carefully removed. The described procedure was repeated for two times. Biological effectiveness was determined daily and weekly, summarized and averaged during the whole period of testing,

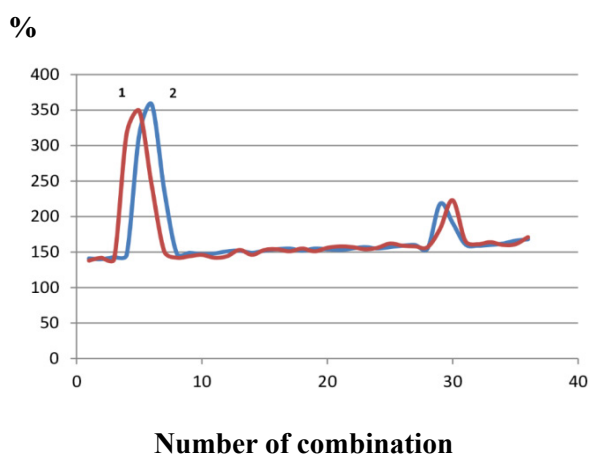
providing the data on daily, weekly and total (averaged) biological effectiveness of the tested combinations and its dynamics. According to [4] and [43] we expected to have moribund insects when applying the bifenthrin containing combinations, but no-one moribund case was fixed during our 3-week study. Probably, the insecticidal impact of diatomite, kaolin and rosemary oil did not allow the moribund insects to recover. All insects were exposed to the equal dose of the tested combinations (50 ml per container) which was approximately 5 times lower than the recommended application dose of malathion formulations [43]. The results of testing are given in Figures 4-7. The biological effectiveness (BE) of the combinations was determined using the well-known Abbott formula (“modified” by us to account for the insect mortality in the control group):

$$BE (\%) = [1 - (n_1 + k \times n_2) / n_3] \times 100 \quad (1)$$

where, BE is the biological effectiveness of the insecticide,  $n_1$  is the number of insecticide-treated surviving insects in the test group (in our case, groups A and B),  $n_2$  is the number of insects killed in the control group, and  $n_3$  is the number of surviving insects in the control group,  $k$  is the empirical coefficient close to 1 (we used the value  $k = 1$ ).



**Figure 3.** Dependence of the relative biological effectiveness of malathion-, bifenthrin- and diatomite-based insecticidal combinations against group A (1, %) and B (2, %) insects on the number of combination.



**Figure 4.** Dependence of the relative biological effectiveness (RBE) of gamma-cyhalothrin-, dimethoate- and kaolin-based insecticidal combinations against the group A (1,%) and B (2, %) insectson the number of combination.

It should be noted that the modified formula can be easily applied both to the laboratory and field trial data. The determined total (averaged) relative biological effectiveness of the malathion–bifenthrin-diatomite based and the dimethoate-gamma-cyhalothrin-kaolin based insecticidal combinations against group A and B insects is given in Figures 3 and 4.

**Determination of the synergy (co-toxicity) factor.** The relative synergy (co-toxicity) coefficient  $C_o$  is usually applied in laboratory research [21, 43-46] to characterize synergy of components (see formula 2):

$$C_o (\%) = 100 \times [(Observed\ Mortality - Expected\ Mortality) / Expected\ Mortality] \quad (2)$$

We introduced and used a modified version (formula 2') which is more precise and simple in use and can be used for an arbitrary number of components of mixture and takes into account the natural mortality (mortality of insects in the control group):

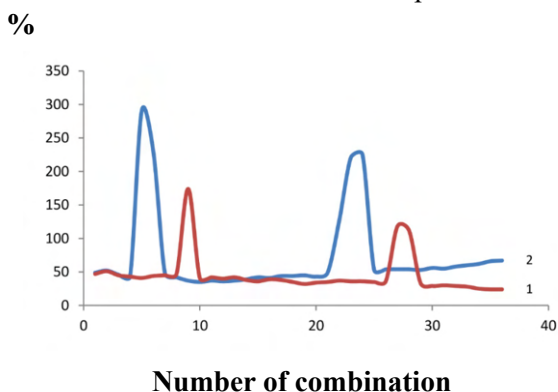
$$CT (BE, \%) = 100 \times (BEM - BEE) / BEE \quad (2')$$

where, BEM and BEE respectively are the experimentally measured and predicted (expected) values of the relative biological effectiveness.

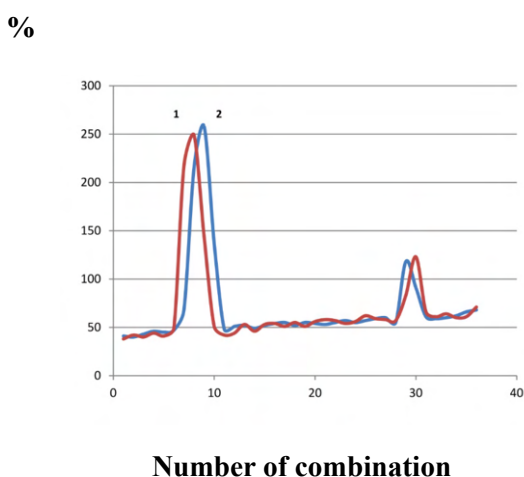
The above formula does not require the data on lethal doses and can be easily used both in the laboratory and field trials. In this case, the measured relative biological effectiveness (BEMR) is determined using the data of field trials in the test sites after applying the tested insecticidal combinations, while the expected relative BE (BETR) will be a sum of BEMRs of all components determined using the data of field trials conducted in the control sites.

Taking into account that almost all data on the lethality and biological effectiveness of insecticides are determined by testing of non-resistant populations of insects (e. g. [43-45]) and that the non-synergistic part of the biological effectiveness of bifenthrin-based combinations against insects collected in Abasha-Senaki sites decreased with the increase of content of bifenthrin, indicating a significant resistance to bifenthrin, we decided to apply the formula (2'), where the expected biological effectiveness(BEE) is supposed to be equal to the linearized non-synergistic (lowly varying) part of the measured biological effectiveness. This approach allows several times to reduce the amount of time and labor expended to quantify the synergy of the components and assess the possible practical benefit from the use of insecticidal “cocktails” against both resistant and non-resistant pest populations. The accuracy of the results obtained using this approach can easily be verified by measuring the biological effectiveness of several specific formulations of the studied combinations and determining the average correction factor. The obtained data on the relative synergy (co-toxicity) factor values are given in Figures 5 and 6. It is especially important that both combinations show a pronounced synergy of components, but

the synergy peaks of the malathion-bifenthrin-diatomite based combinations in case of group A insects are noticeably “weaker” than in case of group B insects, while the synergy peaks of the dimethoate-gamma-cichlothrin-kaolinbased combination are practically the same for the both groups of insects. Another non-trivial result is the observed shift of the synergy peaks towards the higher content of bifenthrin in case of A group insects. In our opinion, this shift can be explained by the increased amount of bifenthrin required to neutralize the additional mechanism(s) of protection against bifenthrin, which is (are) the main cause of resistance development.



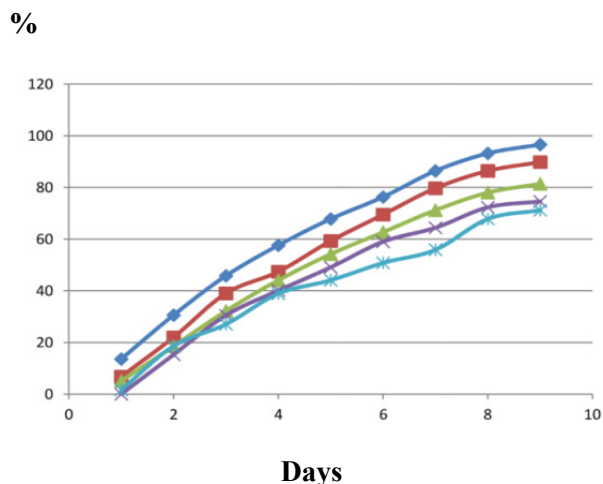
**Figure 5.** Dependence of the synergy (co-toxicity) factor CT of malathion-, bifenthrin- and diatomite-based insecticidal combinations against group A (1, %) and group B (2, %) insects on the number of combination.



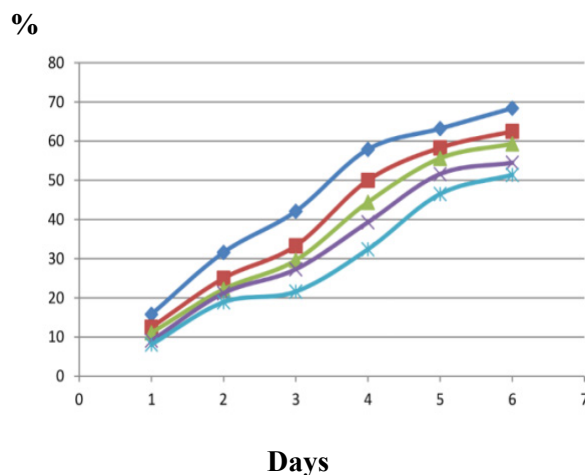
**Figure 6.** Dependence of the synergy (co-toxicity) factor CT of dimethoate-, gamma-cyhalothrin- and kaolin-based insecticidal combinations against group A (1, %) and group B (2, %) insects on the number of combination.

### Field trials in the cornfield located in Senaki municipality

The field testing of the developed insecticidal combinations was held in the cornfield in village Ettseri of Senaki municipality located in the subtropical clay podzole zone which is typical to many districts of the Western Georgia (mainly in Imereti, Guria, Samrgrelo and Ajara regions) which is strongly infested by BMSB. The trials were carried out by the researchers of the Institute for Problems of Engineering Physics of the Georgian Technical University (GTU) and agro-technical group of the National Food Agency (NFA) of Georgia, with the help and under supervision of NFA. The corn field was divided horizontally and vertically into six “columns” and five conditionally “rows”, each with 18 observation plants located at equal distances. The distance between first five “columns” was 20 m. The sixth (control) “column” was separated from all other “columns” by a 50 m wide fruit garden with trapping host plants, which were assumed to prevent migration of insects to the test site. The number of BMSB imago insects on all selected trees was counted and averaged. In all cases their number ranged from 44 to 71. Finally, the average number of insects per plant was estimated as  $59 \pm 2$ . On the first day, from 8 to 9 o’clock p.m., six “columns” were sprayed with the most effective developed combinations (K5, D9, K24 and D28 respectively) and the insecticide Prostore 420 EC using backpack-type “Solo” engine. Thereafter, during the next 9 days from 5 to 6 o’clock p.m., the survived insects in all six “columns” were daily counted by the local volunteers. On the 10-th day the BMSB two-part pheromone lures supplied by the „Evergreen Growers Supply“ (Clackamas, Oregon, US) were fixed to the observation trees to attract insects, and on the 11-th day field trials were continued. The data on the biological effectiveness of the four tested and one control insecticidal formulation are given in Figures 7 (days 1 to 9) and 8 (days 12-17). Systemized data of 18 days of the field trial are given in table 4.



**Figure 7.** Biological effectiveness of the field tested insecticides (from up to down) K5 (%), D9 (%), K30 (%), D28 (%) and control insecticide ProStore 420 EC (%), days 1-9).



**Figure 8.** Biological effectiveness of the field tested insecticides (from up to down) K5 (%), D9 (%), K30 (3, %), D28 (%) and control insecticide ProStore 420 (%), days (12-18)

The average number of insects  $N_1$  counted on the test plants of the corresponding five “columns” was taken as the number of insects  $N_1$  survived in the test groups. The averaged reduce of the insects on the plants of the sixths (control) “column” was taken as the number of insects  $N_2$  killed in the control group. The averaged number of insects  $N_3$  counted in “column” 6 was taken as the number of insects survived in the control group. Calculation of the biological effectiveness was made using formula (3):

$$BE (\%) = [1 - (N_1 + k \times N_2) / N_3] \times 100 \quad (3)$$

where, BE is the biological effectiveness of the insecticide,  $N_1$  is the number of surviving insecticide-treated insects on the plants (“columns” 1-5),  $N_2$  is the decrease of the average number of insects in the control group (“column 6”), and  $N_3$  is the average number of surviving insects in the control group (“column” 6),  $k$  is the empirical coefficient close to 1 (here we used the value  $k = 1$ ).

**Table 4.** Data on the biological effectiveness of insecticidal formulations during the field trial

Day of the trial	The average number of insects on the plants in the test and control “columns” 1 to 6						Decrease of the average number of insects on the plants in the test and control “columns” 1 to 6						Biological effectiveness of insecticidal combinations in the test “columns” 1 to 5, %				
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5
0	59	59	59	59	59	59	-	-	-	-	-	-	-	-	-	-	-
1	48	52	53	54	55	56	11	7	6	5	4	3	14	7	5	3	1.7
2	38	43	45	47	48	56	21	16	14	12	11	13	31	22	19	15	12
3	32	36	40	41	43	59	27	23	19	18	16	0	46	39	32	31	27
4	27	33	35	37	38	61	32	26	24	22	21	-2	58	47	44	40	39
5	18	23	26	30	32	58	41	36	33	29	27	1	68	59	54	49	44
6	14	19	22	24	29	59	45	41	37	35	30	0	76	70	62	59	51
7	10	14	19	23	28	61	49	45	40	36	31	-2	86	79	71	65	56
8	7	11	16	19	22	62	52	48	43	40	37	-3	93	86	78	72	68
9	4	8	13	17	19	61	55	53	48	42	42	-2	97	90	81	75	71
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

12	19	24	27	33	37	59	-	-	-	-	-	-	-	-	-	-	-
13	18	23	26	32	36	61	1	1	1	1	1	-2	16	13	11	9	8
14	12	19	22	27	31	60	7	5	5	6	6	-1	32	25	22	21	19
15	10	15	18	23	28	58	9	9	9	10	9	1	42	33	29	27	22
16	9	13	16	21	26	60	10	11	11	12	11	-1	58	50	44	39	32
17	8	11	13	17	21	60	11	13	24	14	16	-1	63	58.3	57	51.6	47
18	6	9	11	15	18	60	13	16	16	18	19	0	68	62	59	54.5	51

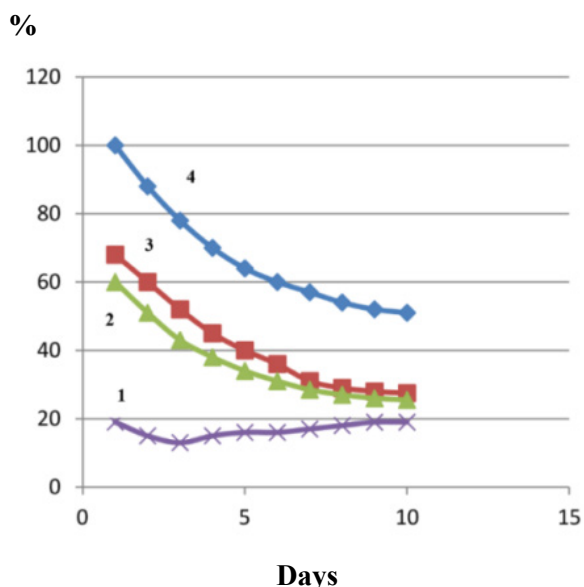
### Testing of the acute toxicity of the developed compositions to mammals

One of the most important characteristics of insecticides is their acute toxicity to the warm-blooded mammals. On the other hand, modern requirements for the standards of humane treatment of test animals (which should exclude the death of animals, causing them severe pain, significant deterioration of health, prolonged forced immobility, whole body anesthesia, etc.) are becoming increasingly stringent, and the modern methods of animal testing must be fitted to all modern 4 R (replace, refine, reduce, responsibility) principles of humane treatment of laboratory animals. The acute toxicity of developed drugs was tested using an original methodology developed and tested at the Georgian Technical University and Ivane Beritashvili Center for Experimental Biomedicine [49-51]. Methodology is based on the observation of the behavioral and physiological parameters of white rats in a standard branched training maze consisting of lighted and darkened sections.

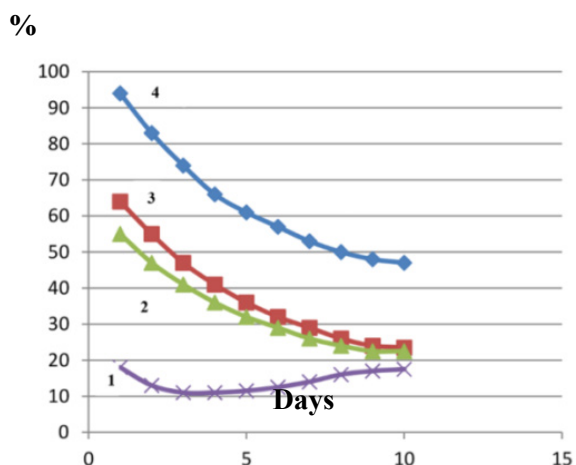
In addition to behavioral and mental characteristics, the four indicators, measured by means of the veterinary system of non-invasive blood pressure measurement of rodents "Systola", the non-contact infrared thermometer for animal research BIO-IRB153, the Free Radical Analytical System FRAS5 for measuring oxidation stress caused by reactive species (RS) and reactive oxygen species (ROS), and the pulse oximeter transfectance sensor NONIN 2000T were used for characterization of the general acute toxicity of insecticidal formulations to the exposed animals. The acute toxicity induced by injection of 30 mg/kg of ProStore 420 EC was taken as a 100% value. The acute toxicity was characterized by the Combined Toxicity Index (CTI) calculated according to formula (3):

$$CTI = [(N_1/N) \cdot (T_c + T_d) / T] \cdot (\Delta B_p \cdot \Delta B_t \cdot \Delta ROS / S)^2 \quad (4)$$

where CTI (dimensionless quantity) is the combined toxicity index,  $N_1$  is the number of errors made during passage through the maze,  $N$  is the total number of decisions made during passing through the maze,  $T$  is the total time of passing through the branched maze,  $T_1$  is the time spent in the lighted sections of the maze,  $T_d$  is the time spent in the darkened sections of the maze,  $\Delta B_p$  is a relative change of blood systolic pressure,  $\Delta B_t$  is a relative change of body temperature,  $\Delta ROS$  is a relative change of reactive oxygen species in blood, whereas  $S$  is a relative change in blood oxygen saturation as a result of passing through the maze [18–20]. During the first 3 days of observation, 96 white rats divided into 8 sub-groups were injected intramuscularly with equal doses 30 (10 + 10 + 10) mg / kg of solution of tested formulations (D18 or K18), one reference insecticidal formulation (ProStore 420 EC) and standard saline solution total dose. 3-day exposure was followed by standard maze tests for 10 days, under monitoring of all parameters given in formula (3) using the above listed devices. With aim to estimate the possible influence of other abiotic and biotic stresses (high temperature, dehydration, low humidity, long-term immobilization, restriction of mobility, lack of oxygen, etc., another group of 96 white rats was 3-times exposed to the Whole Body Hyperthermia (WBH) in a special camera at  $42 \pm 1$  °C for 10 minutes per day and then tested during 10 days according to the above scheme. The averaged results of the carried out research of hyper-thermally untreated and hyper-thermally treated animals are given in Figures 9 and 10.



**Figure 9.** Acute Toxicity (CTI) of the standard physiological solution (1, %), formulation D18 (2, %), formulation K18 (3, %), and insecticide ProStore 420 EC (4, %) against the group A insect.



**Figure 10.** Acute toxicity (CTI) of the standard physiological solution (1, %), formulation D-18 (2, %), formulation K-18 (3, %), and insecticide ProStore 420 EC (4, %) against group A insects previously treated in WHB camera.

However, the WBH exposure has noticeably improved all registered parameters and reduced the CTI for all tested combinations, which can be the result of a strong immune response of the organism to the hyperthermia stress providing (due to the Hormesis phenomenon) a higher resistance to the inhibitory impact of toxicants on the endocrinal metabolic activity of the affected organism [49].

### Testing of the acute toxicity of the developed compositions to bird embryos

In order to support or refute the reliability of the results of above experimental studies, we decided to prove their reliability using an alternative method of the nondestructive diagnostics of bird embryos. Among the appropriate modern methods of the visible, infrared and ultrasonic ovoscopy [52-55] widely applied in the medical, veterinary and agricultural research, we have chosen the most easily available, simple and low cost method of the visible light ovoscopy. The results of our study based on the visible range ovoscopy of eggs of chicken, duck and goose eggs exposed to various kinds of potential toxicants (combined nanofluids, radiomimetics, anticancer drugs, radiation, short-term mild hyperthermia, etc.) will be published in the nearest future.

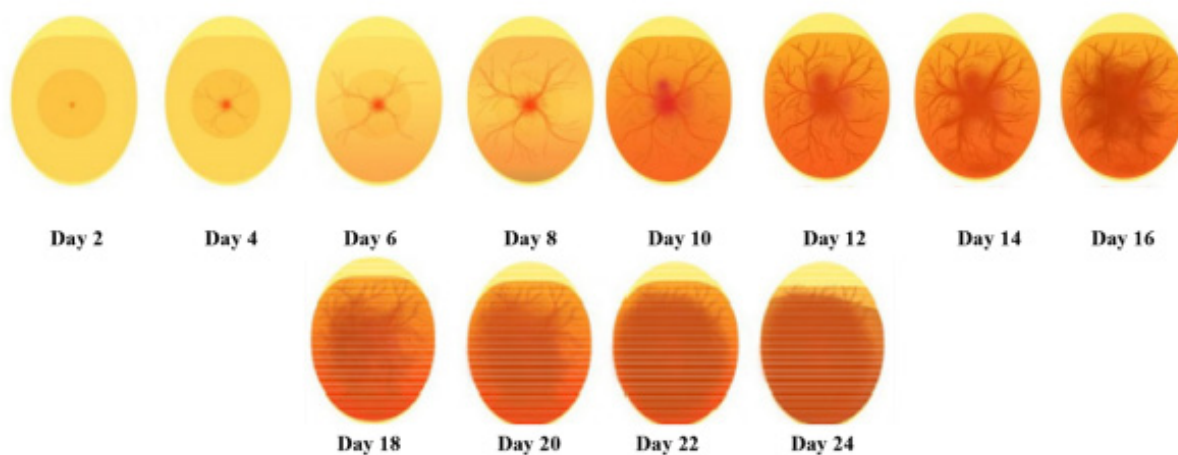
The main criteria for the evaluation of toxicity was the rate of mortality of duck and chicken embryos, mortality of hatched ducklings chicks and disruption of the normal development of embryos during their development within 21 and 24 days, depending on the bird species. The useful additional criteria are as follows.

The visible delamination of the shell membrane is manifested by the displacement of the air chamber in the lateral direction or towards the sharp side of the egg; an abnormally large air chamber indicates that the egg is old and stale, which means it is not suitable for hatching; if the translucent egg looks completely orange or orange-red, it means that the yolk has burst and mixed with the protein; if the grains are torn off, then the yolk will dangle freely throughout the intranasal space; If the yolk sticks to one wall of the shell, it also means that the egg is either old or stale. The appearance of blood rings foreshadows the death of embryos in the early stages of incubation, when the yolk is overgrown with blastoderm. A dark embryo adhering to one side of the shell indicates the absence or insufficient development of the allantois blood vessels (in later stages, the embryo is immobile and the circulatory system is underdeveloped); with insufficient heating of the eggs, the growth of embryos slows down, the development of embryos is delayed in time and the number of suffocations increases; overheating causes uneven development, resulting in ducklings and chickens hatching with an unstretched yolk sac or unused protein; with a lack of moisture, eggs lose much in weight, heat exchange increases,

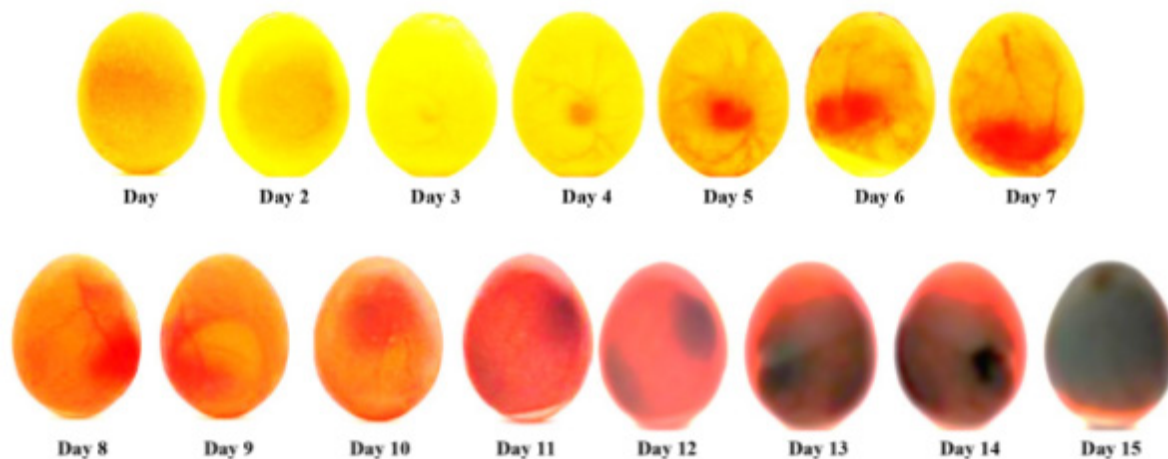
which leads to an increase in temperature inside the incubation material and causes various developmental disorders, ducklings and chickens hatch early, the shell becomes dense, shrinks and it is much more difficult to pierce it; with an excess of moisture, an increase in amniotic fluid occurs, which is detrimental to the chicks (when they try

to break through the shell, they swallow this liquid and suffocate).

Typical images derived using the visible light ovoscopy of the duck and chicken eggs with the normally developing embryos are given in Figures 11 and 12.



**Figure 11.** Typical images derived using the visible light ovoscopy of the duck eggs with normally developing embryos.



**Figure 12.** Typical images derived using the visible light ovoscopy of the chick eggs with the normally developing embryos.

The test and control groups of 128 eggs in each were incubated and monitored in the 80 Wt and 120 V AC / 12 V DC computer controlled mini-incubators with red light emission diodes (LEDs) and self-assembled detection systems.

The incubation temperature was  $(38 \pm 0.5) \text{ C}^0$  and the relative humidity (RH) was  $(68 \pm 1) \%$ . About 1900 images were made, transferred to a computer program and evaluated.

## Results and discussions

### Biological effectiveness and observed synergy of the components of developed insecticidal combinations

The results of the reported research gave us the possibility of experimental evaluation of the following significant characteristics of the developed insecticidal combinations, such as: a) relative biological effectiveness against the BMSB and its dependence on the ratio of active synthetic components; b) relative synergy against the BMSB and its dependence on the ratio of active synthetic components; c) acute toxicity of tested and control drugs to the warm-blooded mammals and bird embryos. It should be noted that the step of changing the ratio of the active synthetic components appeared to be small enough to make it possible to fix and evaluate the synergistic effect. At the same time, the use of smaller steps and the sequential removal of the active components can enable a more accurate estimation of the contribution of each component to biological effectiveness and synergy of the whole combination.

Biological effectiveness of the developed and tested bifenthrin-based insecticidal combinations against the insects collected in Abasha and Senaki municipalities was found to be significantly lower than against the insects collected in Kobuleti and Khelvachauri municipalities. At the same time, the indicated difference for gamma-cyhalothrin based formulations was many times smaller.

In contradiction to the results of calculations based on the data from publications [43-46], the non-synergistic part of the biological effectiveness of bifenthrin-based combinations against insects collected in Abasha-Senaki sites decreased with increase of content of bifenthrin. Meanwhile, the non-synergistic part of the biological efficacy of the combination against test insects supplied from the Kobuleti and Khelvachauri regions increased with increasing of the bifenthrin content. It is also noteworthy, that the height of synergy peaks of bifenthrin based formulations in case of insects supplied from Kobuleti and Khelvachauri municipalities was significantly more than the height of synergy peaks in case of the insects supplied from Abasha and Senaki municipalities.

The results of our study clearly show that the non-synergistic relative biological effectiveness of the insecticidal combinations based on dimethoate, gamma-cyhalothrin and kaolin increases with

increasing of gamma-cyhalothrin content in both studies, while the peaks of synergy have a practically equal height.

In our opinion, all these facts can be explained by the fact that significantly more intensive spraying with bifenthrin was carried out in the Abasha-Senaki region than in the Kobuleti and Khelvachauri municipalities, which led to the development of high resistance to bifenthrin in BMSB population being widespread there.

Importantly, in addition to strong synergy peaks, all of the investigated combinations have significant non-synergistic (or less synergistic) components due to mineral (diatomite, kaolin) and essential oils (rosemary essential oil), accounting for about one-third of the total non-synergistic part. This component can become important in case of strong resistance to the synthetic components of the pesticides. In addition, it is highly probable that the synergy of synthetic insecticides, diatomite, kaolin and essential oils against the Brown Marmorated Stink Bug and other agricultural pests can be used for long-term storage and transportation of cereals and other food products, which is becoming increasingly relevant because of tightening of international regulations for the synthetic pesticides (e. g. [56]).

### The acute toxicity of the developed compositions to mammals

The results of testing in white rats showed that: a) the negative effect caused by the effect of the tested combinations on the first day of observation is almost twice less than the negative effect of the control drug; b) the tested animals within 10 days after exposure practically completely recovered their behavioral and physiological functions (activity, memory and learning skills, blood oxygen saturation, systolic pressure and body temperature stability, oxidative stress level, etc.). Further prolonged (30 days) observation showed that their general state of health is practically unchanged).

The above results in more than 93 % of cases were supported by the data of the computer assisted visible light ovoscopy of the duck and chick embryos, which can be regarded as a highly prospective tool for replacing of a considerable part of the living animal testing. Ultrasound sonography can be an important step to a more informative non-invasive study of the acute toxicity of the developed formulations to bird embryos.

## **Prospective of the farther development of insecticidal combinations with increased biological effectiveness and implementation of the findings of the reported research**

### **Testing of the acute toxicity of the developed compositions to pollinators and aquatic organisms**

The studies carried out are a part of the comprehensive research of the developed insecticidal formulations necessary for their registration and introduction in Georgia and other countries. This process will be continued with the help and guidance of the National Food Agency of Georgia. An important part of the further research will be the study of acute toxicity in relation to pollinators (honey bees) and aquatic organisms (freshwater fish *Cyprinus Carpyo* and other freshwater fish species) which should be performed in the Institute for Problems of Engineering Physics of the Georgian Technical University, in the Science Research Center of the Ministry of Environment Protection and Agriculture of Georgia, in the laboratory of the technological and special measures for the prevention of bee diseases of the National Research Center of P. Prokopovych Institute of Beekeeping of the National Academy of Agrarian Sciences of Ukraine, in the company Ukrainian Bee LLC, and in the Department of pond aquaculture and hydrobiont ecology of the Institute of Fisheries of National Academy of Agrarian Sciences of Ukraine.

Honey bee is among the five most important pollinators over the world and the main pollinator of the agricultural plants. Synthetic insecticides and their residues (together with *Varroa destructor* mite), due to their high lethality to living organisms and extremely wide spread in the environment, form the main hazard threatening this insect, which is vitally necessary for humans and all living nature. The widely used lethal doses of the acute toxicity ( $LD_{10}$ ,  $LD_{25}$ ,  $LD_{50}$ ,  $LD_{75}$ , etc.), cannot directly reflect the short- and long-term impact of insecticides on the vital characteristics of honey bees like the moving and foraging activities. At the same time, in our opinion, applying of the lethality doses approach to the honey bees and other important pollinators is in a direct conflict with the modern principles of scientific ethics and ecological thinking. Moreover, even the use

of sub-lethal doses close to lethal, in our opinion, is meaningless, since in the overwhelming majority of cases it does not provide the necessary information about the real magnitude of the impact during the massive and long-term use of insecticides in food and ornamental plant growing, forestry, vector deinfestation, etc. Therefore, in the last two decades, in laboratory and semi-field studies more and more often are used the doses at least an order of magnitude less than the lowest lethal dose ( $LD_0$ ) measured at that time for the tested kind of insects. So, foraging behavior and colony conditions become the main indicators for assessing the toxicity of pesticides and their combinations. The typical identified cases of the impact of insecticides on honey bees are the following (e. g. [57]) : precocious foraging; no hypopharangeal gland development; inhibited hypopharangeal gland development; regression of hypopharangeal glands; significantly suppressed hypopharangeal gland development; early degeneration of hypopharangeal gland; decreased acquisition and persistence in conditioned response test; decreased house-cleaning; decreased learning ability, abnormal behavior (antennae leaning, rubbing together of hind legs, decreased flight activity and olfactory discrimination performance; increase in number of trials to abolish or induce habituation; slower learning of odor-mediated response; incorrect direction (angle of dance) on vertical surface; incorrect distance on horizontal surface; failure to return to colony; increased self-cleaning; abdomen tucking; rotating and cleaning of abdomen plus rubbing hind legs together; decreased walking; body insertion in cells; decreased brood, pollen patty consumption and foraging; hypothermia; differences in queen egg laying cycle, and numbers of larvae and pupae; less eggs laid; reduction in surviving brood; lower numbers of larvae ejected and increase brood production; elimination of brood production; decrease of sucrose intake; arrested egg development and larval mortality; queens ceased laying; increased queen supersedure; stimulated growth and maturation, failure to spin cocoon, hypersensitivity in simulation; adults emerge with wing malformation, stunted bodies, crippled legs and crippled or shortened wings; transient inhibition of activity; reduced honeybee visitation. Taking into account the modern principles of scientific ethics and ecological thinking we, like a number of researchers [e.g., 63] have chosen the

following two Behavioral Sublethal Effects bioassays for determination of significantly sublethal toxicity of the developed and control insecticidal formulations against the species of the Ukrainian honey bee (*Apis mellifera scoticorum* Engel): the Forager Free Choice test and the Walking Activity Bioassay of Adult Workers.

Toxicity to aquatic organisms is also one of the main characteristics of insecticidal preparations (e. g. [58]). According to the generally accepted classification, impacts on aquatic organisms should be distinguished by their duration (long-term and short-term) and by the degree of threat to the life of living organisms (lethal and sub-lethal). Long-term exposures are considered to last more than 96 hours, and lethal ones lead to irreversible consequences and, during or shortly after exposure, bring to the death of the tested organism. For the above ethical reasons, we give up the use of lethal doses and concentrations from our studies and give preference to sub-lethal exposures excluding death as the endpoint of testing. In accordance with a preliminary agreement with the Department of pond aquaculture and hydrobiont ecology of Institute of Fisheries of the National Academy of Agrarian Sciences of Ukraine, the long-term monitoring of social behavior (locomotion, anxiety, and startle responses), other behavioral and respiratory disfunctions and alterations in respiratory rate and food consumption in various several fish species will be carried out during the sub-lethal testing.

### **Development and testing of the nano based insecticidal combinations**

Along with the rapid increase of the world population the need for a substantially more productive agriculture is also growing. Weathering, erosion and desertification of fertile soils put on the order of the day the real danger of hunger not only in underdeveloped, but also in developing and developed countries on all continents of the globe. Irreparable climate change, and weathering, erosion and desertification of fertile soils put on the order of the day the real danger of hunger not only in underdeveloped, but also developing and developed countries on all continents of the globe. Even before the COVID-19 pandemic, around 690 million people worldwide suffered from hunger, which is 8.9 percent of the world's population,

according to a UN report. Moreover, over the past year, this number has increased by 10 million, and over five years - by almost 60 million. The number of those experiencing food shortages is also growing. Last year, their number was about 750 million - this is almost every tenth inhabitant of the planet. Today, almost two thirds of severely food insecure people live in just 8 countries: Afghanistan, Democratic Republic of Congo, Ethiopia, Nigeria, South Sudan, Sudan, Syria and Yemen.

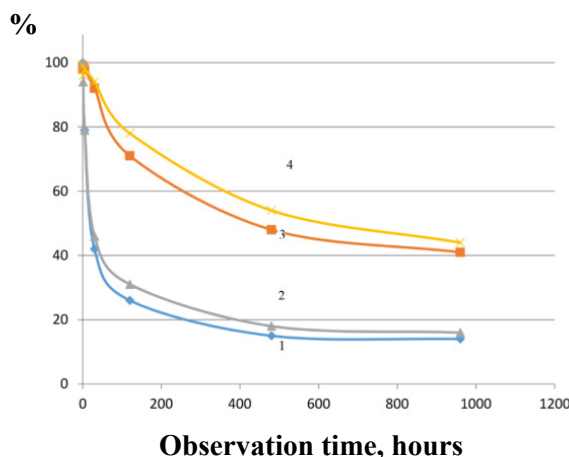
Last year, 21.3 percent of children under 5 years of age were stunted due to malnutrition, and 6.9 percent suffered from wasting; another 5.6 percent of children in this age group were overweight, which, as experts have repeatedly noted, is also associated with inadequate and irregular nutrition. The Global Food Crisis Network, which includes 15 major international partners, including UNICEF and the World Food Program (WFP), estimates that the number of people facing severe food insecurity will grow extra-linearly and could have disastrous consequences. 20 countries around the world are in urgent need of food aid, otherwise they face full-scale famine. This warning was issued by two UN food agencies. Residents of Yemen, South Sudan and Northern Nigeria are already on the verge of starvation, warn the report prepared by the World Food Program (WFP) and the Food and Agriculture Organization of the United Nations (FAO). While the vast majority of countries facing hunger are located in Africa, the problem also applies to other regions. Afghanistan in Asia, Syria and Lebanon in the Middle East, Haiti in Latin America - these and other states will not do without the help of the international community, since 34 million people are experiencing "the fourth degree of acute food shortage", that is, they will be one step away from starvation.

Despite the huge efforts of the most important international organizations (UN, UNICEF, WFP, FAO, etc.) the problem cannot be solved without increasing of the fertility of soils and saving more of the harvested food. The existing and widely used fertilizers and pesticides are unlikely to be able to provide the necessary increase in the yield and safety of food, since a further increase in their use will certainly lead to an irreversible impact on the soil, surface and underground waters, wildlife and human health. It seems that synergistic combinations, including the use of nano-scale particles (e. g. [59]) will help humanity to significantly mitigate the

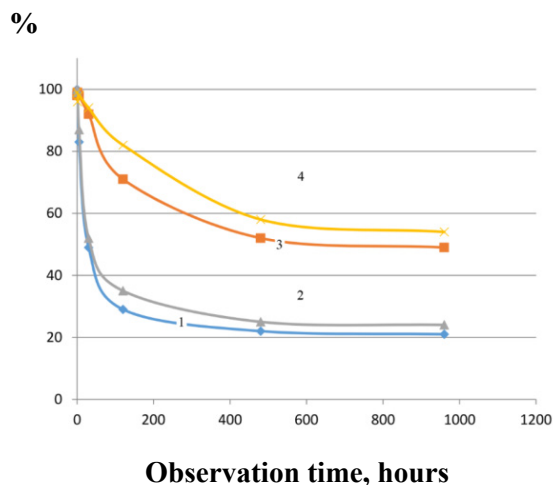
terrible threat of mass starvation. Every year, about a third of all food produced in the world is either lost or wasted. In developing countries, 40 percent of food is lost during the harvest or processing phase - this is called food waste.

To increase the yields of crops it becomes necessary to increase continuously the frequency and volume of fertilizer and pesticide applications, while the development of resistance in pest populations makes the problem even more acute. Pollution with various agricultural chemicals causes a growing severe environmental impacts on soil quality, purity of water resources, safety of useful soil microorganisms, non-targeted insects including pollinators, reptiles, birds (including poultry), mammals (including cattle and other home animals) and human health and life. Moreover, the uncontrolled use of pesticides can, in principle, bring to a dramatic decrease of populations of pollinators and other useful insects and, therefore, cause even lower yields. The attempt to replace the hazardous synthetic compounds with the organic natural substances has a considerable potential, but seems to be useless against the most hazardous pests like BMSB and locusts.

Use of nano-materials (especially of natural mineral origin) as synergistic components of the fertilizers and pesticides can likely provide a novel approach to come closer to a more sustainable balance between the sharp increase in agricultural production and conservation of nature. However, due to the peculiarity of the nano-scale objects (which exhibit many properties that are completely different from those of bulk materials) toxicity of nanoparticles and their suspensions should be studied with special care. From general considerations, the use of nanofertilizers and nanopesticides should be allowed only in cases where the ecological effect of reducing the applied doses is several (at least 2-3) times higher than the negative effect of their measured or estimated toxicity. At the same time the so called nano-insecticides can significantly slow down the degradation of "usual" pesticides, enhance their persistence and increase their acute and chronic toxicity to the living nature (e. g. [60]). That is why, beginning from 2015 the Organization of Economic Cooperation and Development (OECD) established, developed and published the risk-assessment guidelines and various regulatory documents regarding manufactured nano-materials (e. g. [61]).

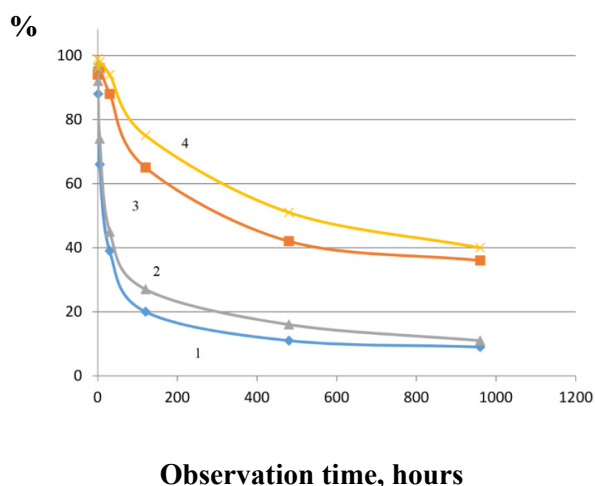


**Figure 13.** Typical dependence of the sedimentation rate of 50 nm alumina at room temperature in combinations: D18A before sonification (1, %), K18A before sonification (2, %), K18 A after sonification(3, %) and K18A (4, %) after sonification.



**Figure 14.** Typical dependence of sedimentation process of 40 nm alumina at room temperature in combinations: D18A before sonification (1, %), K18A before sonification (2, %), K18 A after sonification (3, %) and K18A (4, %) after sonification.

Taking into account the above given considerations, nano based insecticidal combinations D1A - D37A and K1A - K37A (similar to combinations D1 - D37 and K1 - K37) were developed, with the only difference that instead of diatomite and kaolin powders the nano structured alumina with average particle sizes 40 nm, 50 nm and 100 nm supplied by MK Nano and SS Nano was added in the same proportions and processed according to paper [62] using mechanical mixing and ultrasound agitation. Preliminary study of the stability and agglomeration of the prepared dispersions were studied also according to [62].



**Figure 15.** Typical sedimentation process of 100 nm alumina at room temperature in combinations: D18A before sonification (1, %), K18A before sonification (2, %), K18 A after sonification (3, %) and K18A (4, %) after sonification.

Sedimentation rates in alumina containing insecticidal combinations were measured and compared to sedimentation rates in alumina-water-ethanol mixtures. The sedimentation measured rates of 50 nm nano alumina in the insecticidal combinations were about 40 - 60-times lower than in ethanol-water (25 wt%) mixture. Ultrasonic agitation increased the stability of dispersions about 1.8 – 2.8 times. Stability of dispersion, determined considering the height of the white colored columns in liquids, significantly depended on the concentration and the averaged size of alumina nanoparticles (see figures 13 – 15). In the nearest future we are going to carry out a comprehensive laboratory and field study of the toxicity of developed nano based combinations against BMSB, Italian locust, honey bee, fish and mammals (white rat) using all the methods given above. Special attention will be paid to pesticide exposure safety issues and risk assessment indicators (e. g. [63]), prospective of use of nanoparticles for environmental clean-up (e. g. [64]) and to market analysis of the nanoparticle based insecticidal combinations (e. g. [65]).

## Conclusions

The reported study showed that it is necessary to perform a large number of laboratory experiments and field tests clarifying the further details on the basis of systematization and reliable statistical analysis of the accumulated data about the optimal composition and concentration of the newly developed combined formulations, and taking into

account the specific field conditions and climatic factors. At the same time, even today it is possible to conclude with a high probability that in certain rural areas invaded by resistant populations of insects it will be preferable to use insecticidal combinations based on dimethoate and gamma-cyhalothrin, while in other areas the combinations based on malathion and bifenthrin will still be more effective.

The results of the field trials are surely affected by a number of natural factors (weather and humidity, migration activity of insects, counting errors, etc.). Many of the above factors can attenuate the difference between the measured effectiveness of the tested insecticidal combinations. Anyway, the results of the field trials are in good coincidence with the data of laboratory testing, although more studies are required to increase the accuracy and reliability of both the laboratory and field trial data. Analyzing the data given in Figures 7 and 8 we concluded that the biological effectiveness of the developed and control combinations keep sufficiently high during all the period of the field trial, although the effectiveness during the last six days was for 15-20 % lower than during the first six days of the field trials. In our opinion, the relatively low biological effectiveness during the first day can be explained as the result of the prolonged release of insecticidal solution “trapped” in the diatomaceous earth and kaolin.

Considering that the relative biological effectiveness of the developed combinations in the area of maximum synergy is about 1.4-1.5 times higher, while the relative acute toxicity to warm-blooded animals (rats) is about twice less than that of the control insecticide (“ProStore 420 EC), we assume, that it is about 2 times safer for humans and the living environment than all widely used analogues. The results of the non-invasive testing of acute toxicity to white rats and bird embryos are in a good coincidence and show that insecticide “cocktails” can be used successfully against the agricultural pests, as a highly effective insecticide-acaricide agent, less harmful for the living environment. Grounding on the research conducted, we can also conclude that the algorithm and method developed and used by us for the study of synergy and acute toxicity can become a basis for standardized methods of the objective quantitative testing for the biological efficacy and safety of insecticides. To enhance the accuracy and reliability of the main results and findings of the reported experimental study, improve the efficiency and

safety of the insecticidal combinations and provide the successful implementation of the findings of reported study a long list of experimental works should be executed in the nearest future. A special interest should be paid to the recent publications on the acute and chronic toxicity of nano insecticides to soil microorganisms, plants, invertebrates, non-targeted and useful insects (especially –pollinators), reptiles, aquatic organisms and fish, birds, mammals and human biological effectiveness against a broad spectrum of pests.

Numerous authors have published articles with the aim of predicting the toxicity and assess the possible negative impact using analysis of various direct and indirect indicators for the reliable estimation of environmental and human health risks. A big variety of physical and biological methods has been used to provide enough information for the predictive models based on the qualitative and semi quantities data on the morphology (size, specific area, hydrophobic and mechanical properties, solubility, stability of dispersions, tribo-charging, ability to damage DNA and discompose protein expression, capacity of dehydration of tissues and generate reactive oxygen species, cause cell membrane depolarization and cell death. Several authors also made attempts to analyze more complex mechanisms of affecting living organisms through provoking proteolysis and metabolic disorders, pro-inflammatory factors, carcinogenicity and immune toxicity, etc. of the nanostructured nano-alumina and other nanoparticles.

## Acknowledgement

The Authors thank Professor Greg Kravczyk from the Pensilvania University, Professor Sabu Thomas from the Mahatma Gandhi University, Professor Giorgi Pichkhaia from the Alexandre Natishvili Institute of Morphology of Ivane Javakhishvili Tbilisi State University, Professor Giorgi Kvartskhava from the Georgian Technical University, Professor Nelly Sapojnikova from the Ivane Javakhishvili Tbilisi State University for their kind help in experimental design and interpretation of the obtained results. We also would like to thank the students of the Faculty of Agrarian Sciences and Bio systems Engineering of the Georgian Technical University for their help in laboratory testing and field trials.

## References

- [1] University of Missouri. IntegratedPestManagement, 2017(accessed 02.09.2021).
- [2] United States Department of Agriculture (USDA):Research, Education and Economics Information System. Pennsylvania State University. Facilitating Registrationof Pest Management Technology for Spacialty Crops and Specialty Uses. [https://www.nimss.org/projects/\\_/view/mrp/outline/17316](https://www.nimss.org/projects/_/view/mrp/outline/17316),2018 (accessed 02.09.2021).
- [3] M. Sparks, R. Bansal, J. Benoit, et al. Brown marmorated stink bug, *Halyomorpha halys* (Stål), genome: putative underpinnings of polyphagy, insecticide resistance potential and biology of a top worldwide pest. *BMC Genomics*. 21, (2021) 227-238.
- [4] T. Leskey, D-H. Lee, B. Short, et al. Biology, Ecology, and Management of Brown Marmorated Stink Bug (Hemiptera:Pentatomidae). *J. Integ. Pest Mngmt.* 5, 3(2014) 188-198.
- [5] M. Amobokadze, N. Lomidze, A. Chirakadze, et al.. Selection, application and experimental testing using quantitative methods for determination of synergy rate and biological effectiveness of synergistic insecticidal composition against the Brown marmorated stink bug (*Halyomorpha Halys*). *Proceedings of Archil Eliashvili Institute Of Control Systems Of The Georgian Technical University*. 24 (2020) 39-43,.
- [6] M. Amobokadze, N. Lomidze, A. Chirakadze., et al. Quantitative in vivo determination ofthe biological effectiveness of a new combined insecticidal composition against the *Calliptamus italicus*, *Leptinotarsa decemlineata*, *Zabrus tenebrioides elongatus*, *Anisoplia alazanica* and *Mayetiola destructor* and proof of the reliability of the obtained experimental data using variation criteria. *Proceedings of Archil Eliashvili Institute of Control Systems of the Georgian Technical University*. 24(2020) 39-43.
- [7] A. Chirakadze, M. Ambokadze,Z. Buachidze., et al. Preliminary testing of the natural, combined and synthesized insecticidal formulations against BMSB (*Halyomorpha Halys*) and a simplified methodology for use in the environmental school programs. *Georgia Chemical Journal*. 1(2018)167-171.

- (in Georgian).
- [8] A. Chirakadze, N. Kavlashvili, Z. Buachidze, N. Meskhi, A. Gigineishvili, A. Jishiashvili, A. Laperishvili, L. Zazadze, N. Khuskivadze, I. Khomeriki, M. Taktakishvili. Study of synergy of insecticidal formulations against BMSB using the mathematical isobole method. Proceedings of Archil Eliashvili Institute of Control Systems of The Georgian Technical University. 23(2019) 42-47.
- [9] K. Dang, J. Doggett, G. Veera Singham, et al., Insecticide resistance and resistance mechanisms in bed bugs, *Cimex* spp. (Hemiptera: Cimicidae). Parasites and Vectors. 10 (2017) 318 -326.
- [10] A. A. Enayati, H. Ranson, J. Hemingway. Insect glutathione transferases and insecticide resistance. *Insect Mol Biol.* 14 (2005) 3–8.
- [11] H. Ranson, G. Claudianos, F. Orтели, et al. Evolution of supergene families associated with insecticide resistance. *Science.* 298 (2002) 179–181.
- [12] J. Oakeshott, A. Devonshire, C. Claudianos, et al. Comparing the organophosphorus and carbamate insecticide resistance mutations in cholin- and carboxyl-esterases. *Chem Biol Interact.* 157, 158 (2005) 269–75.
- [13] L. Field, A. Devonshire. Evidence that the E4 and FE4 esterase genes responsible for insecticide resistance in the aphid *Myzus persicae* (Sulzer) are part of a gene family. *Biochem J.* 330, 1 (1998) 169–73.
- [14] N. Liu, M. Li, Y. Gong, F. Liu, et al. Cytochrome P450s--Their expression, regulation, and role in insecticide resistance. *Pestic. Biochem. Physiol.* 120(2015) 77–81.
- [15] Ministry of environment protection and agriculture of Georgia. Execution of the state program of managing BMSB. 2021 (accessed 03.09.2021).
- [16] The annual measures in the frame of the BMSB management program are successfully completed. <http://nfa.gov.ge/Ge/News/Details/214/>, 2021 (accessed 03.09.2021).
- [17] Lee K., Lee D., Kang, B. The ‘R’ principles in laboratory animal experiments. *Lab Anim Res.* 36, 45 (2020), 825-831.
- [18] A. Chirakadze, D. Jishiashvili, N. Mitagvaria, et al. Studies of the comparatively low-temperature synthesis and preliminary toxic characteristics of silver doped lanthanum manganite nanoparticles using conventional and microwave heating. Proceedings of MTP: Modern Trends in Physics, Baku, 01-03 May. 1(2019) 47-51.
- [19] N. Mitagvaria, I. Lazrshvili, M. Devdariani, et al. Hormesis - a basis for homeostasis in the presence of stressors. An example of hyperthermic stress. *Journal of Biological Physics and Chemistry.* 15 (2015) 187-193.
- [20] A. Chirakadze, N. Mitagvaria, D. Jishiashvili, et al. A new alternative for reducing and refining of the animal use in research and testing utilizing continuous observation of behavioral, blood pressure, oxygen saturation and body temperature indexes in white rats. Deposited the Georgia Center of Intellectual Property (“Sakpatenti”), Certificate No 0188, 23/10/2020.
- [21] E. J. Norris, A. D. Gross, L. C. Bartholomay, et al. Plant essential oils synergize various pyrethroid insecticides and antagonize malathion in *Aedes aegypti*. *Med Vet Entomol.* 33, 4 (2019) 453–466.
- [22] Z. Abdelatti, M. Hartbauer. Plant oil mixtures as a novel botanical pesticide to control gregarious locusts. *Journal of Pest Science.* 93 (2020) 341–353.
- [23] I.M. Bosman, J. Wilson, R. Bradbury. Insecticidal Activities of Commercial Rosemary Oils (*Rosmarinus officinalis*.) Against Larvae of *Pseudaletia unipuncta*. and *Trichoplusia ni*. in Relation to Their Chemical Compositions. *Pharmaceutical Biology.* 46, 1-2 (2008) 82-87.
- [24] Chemical book: Bifenthrin. [https://www.chemicalbook.com/ChemicalProductProperty\\_EN\\_CB1714667.htm/](https://www.chemicalbook.com/ChemicalProductProperty_EN_CB1714667.htm/), 2021 (last access 14/2/2021).
- [25] Pesticide Properties Database (PPDB): Bifenthrin (Ref: FMC 54800). <http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/78.htm/>, 2021 (accessed 03.09.2021).
- [26] Chemical book: Gamma-cyhalothrin. [https://www.chemicalbook.com/ChemicalProductProperty\\_EN\\_CB8778657.htm/](https://www.chemicalbook.com/ChemicalProductProperty_EN_CB8778657.htm/), 2021 (accessed 03.09.2021).
- [27] Pesticide Properties Database (PPDB): Gamma-cyhalothrin (Ref: DE 225). <http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/369.htm/>, 2021 (accessed 03.09.2021).
- [28] List of Banned Pesticides and Pesticides Watchlist, Version 1.0. List of Banned Pesticides (by active ingredient). <https://utz.org/>

- [org/wp-content/uploads/2015/12/EN\\_UTZ\\_List-of-Banned-Pesticides-Watchlist\\_v1.0\\_2015.pdf/](http://org/wp-content/uploads/2015/12/EN_UTZ_List-of-Banned-Pesticides-Watchlist_v1.0_2015.pdf/) , 2021 (accessed 03/09/2021).
- [29] PesticideProperties Database(PPDB): Dichlorvos (Ref: OMS 14).<http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/220.htm/> , 2021 (accessed 03.09.2021).
- [30] Chemical book: Malathion, [https://www.chemicalbook.com/ChemicalProductProperty\\_EN\\_CB7958834.htm/](https://www.chemicalbook.com/ChemicalProductProperty_EN_CB7958834.htm/), 2021 (accessed 03.09.2021).
- [31] PesticidePropertiesDatabase (PPDB): Malathion (Ref: OMS 1). <http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/421.htm/>, 2021 (accessed 03.09.2021).
- [32] Chemical book: Dimethoate. [https://www.chemicalbook.com/ChemicalProductProperty\\_EN\\_CB\\_0459837.htm/](https://www.chemicalbook.com/ChemicalProductProperty_EN_CB_0459837.htm/), 2021 (accessed 03.09.2021).
- [33] Pesticide Properties Database (PPDB): Dimethoate (Ref: OMS 94). <http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/421.htm/>, 2021 (accessed 03.09.2021).
- [34] Lankem: Lansperse BIO868, BioLoop Dispersing Agent for Aqueous Systems. <https://www.lankem.com/lansperse-bio868/>, 2021 (accessed 03.09.2021).
- [35] Chemical book: HEC (Hydroxyethyl Cellulose).[https://www.chemicalbook.com/ChemicalProductProperty\\_EN\\_CB0459837.htm/](https://www.chemicalbook.com/ChemicalProductProperty_EN_CB0459837.htm/),2021 (accessed 03.09.2021).
- [36] Z. Korunic, V. Rozman, A synergistic mixture of diatomaceous earth and deltamethrin to control stored grain insects.Julius-Kühn-Archiv, 425, 2010.10<sup>th</sup> International Working Conference on Stored Product Protection. Proceedings, 1 (2010) 894-898.
- [37] B. Subramanyam, R. Roesli, Inert dusts, in: B. Subramanyam, D.W.Hagstrum, (Eds), Alternatives to Pesticides in Stored-Product IPM. Kluwer Academic Publishers, Dordrecht, the Netherlands, 2000, pp. 321-380.
- [38] T. Ramasubramanian, A. Regupathy, Joint action of insecticides against *Helicoverpa armigera* Hubner. Resistance Pest Management Newsletter. 13 (2003) 36-38.
- [39] Chemical book: Diatomaceous earth. [https://www.chemicalbook.com/ChemicalProductProperty\\_EN\\_CB8199393.htm/](https://www.chemicalbook.com/ChemicalProductProperty_EN_CB8199393.htm/), 2021 (accessed 03.09.2021).
- [40] T. Benkacem, B. Hamdi, A. Chamayou, et al. Physicochemical characterization of a diatomaceous upon an acid treatment: a focus on surface properties by inverse gas chromatography. Powder Technology. 294 (2016) 498-507.
- [41] Chemical book: Kaolin.[https://www.chemicalbook.com/ChemicalProductProperty\\_EN\\_CB6300504.htm/](https://www.chemicalbook.com/ChemicalProductProperty_EN_CB6300504.htm/), 2021 (accessed 03.09.2021).
- [42] H. Wang, C. Li, Z. Peng. S. Zhang. Characterization and thermal behavior of kaolin. J Therm Anal Calorim. 105 (2011)157–160.
- [43] T. Leskey, D.-H Lee., B. D. Short, et al. Impact of Insecticides on the Invasive Halyomorpha halys (Hemiptera: Pentatomidae): Analysis of Insecticide Lethality. Journal of Economic Entomology. 105, 5 (2012) 1726-1735.
- [44] N. Cedergreen. Quantifying Synergy: A Systematic Review of Mixture Toxicity Studies within Environmental Toxicology. ,2021 (accessed 03.09.2021).
- [45] N. Cedergreen, A. Christensen, A. Kamper, et al. A review of independent action as a reference model for binary mixtures of compounds with different molecular target sites. Environ Toxicol Chem. 27 (2008) 1621–1632.
- [46] K. Rice, C. Bergh, E Bergmann, et al. Biology, Ecology, and Management of Brown Marmorated Stink Bug (Hemiptera: Pentatomidae). J. Integ. Pest Mngmt. 5, 3 (2014), 1-13.
- [47] FMC: Prostore 420 EC. MSDS, 6241-NPF. [https://admin.fmc.com.ua/media/fmc/productpdf/442bd51d-8041-4a0b-95a6-34f55b3773ebEE\\_PROSTORE\\_420\\_EC\\_2.pdf/](https://admin.fmc.com.ua/media/fmc/productpdf/442bd51d-8041-4a0b-95a6-34f55b3773ebEE_PROSTORE_420_EC_2.pdf/), 2019 (accessed 03/09/2021).
- [48] A. Chirakadze, G. Meskhi, Z. Buachidze, et al. Study of the biological effectiveness of new highly efficient broad spectrum combined synergistic compositions and development of a new method of their validity time testing. Deposited to the National Center of the Intellectual Property of Georgia (“Sakpatenti”), Certificate No 0193, 23/10/2020.
- [49] N. Mitagvaria, A. Chirakadze, M. Devdariani, et al. Whole Body Hyperthermia Induced Phenomenon of Hormesis (Experimental Study). Bulletin of the Georgian National Academy of Sciences. 14, 4 (2020) 68-74.

- [50] N. Mitagvaria, A. Chirakadze, M. Devdariani, et al. Development and Testing of Nanoparticles for Treatment of Cancer Cells by Curie Temperature Controlled Magnetic Hyperthermia. *Bulletin of the Georgian National Academy of Sciences*. 15, 1 (2021) 91-98.
- [51] A. Chirakadze, N. Mitagvaria, D. Jishiashvili, et al. Microwave synthesis, characterization and testing of acute toxicity of boron nitride nanoparticles by monitoring of behavioral and physiological parameters. *Bulletin of the Georgian National Academy of Sciences*, 15, 2 (2021) 120-126.
- [52] E. Onler, I. Celen, T. Gulhan, et al. A study regarding the fertility discrimination of eggs by using ultrasound. *Indian Journal of Animal Research*. 51, (2017) 322-326.
- [53] M. Boga, K. Cevik, H. Kocer, et al. Computer-assisted automatic egg fertility control. *Kafkas Univ. Vet. Fak. Derg.* 25, 4 (2019) 567-574.
- [54] A. Adegbenjo, L. Liu., M. Ngadi. Non-Destructive Assessment of Chicken Egg Fertility. *Sensors*. 20 (2020) 5546-5568.
- [55] J. Vilches-Moure. Embryonic Chicken (*Gallus gallus domesticus*) as a Model of Cardiac Biology and Development. *Comp Med*. 69, 3 (3), 2019, 184-203.
- [56] List of Banned-Pesticides Watchlist\_v1.015.pdf. <https://utz.org/wp-content/uploads/2015/12/EN/>, 2018 (accessed 03.09.2021).
- [57] M. H. Pham-Delegue, A. Decortye, L. Kaiser, et al. Behavioural methods to assess the effects of pesticides on honey bees *Apidologie*. 33, 5 (2002) 425-432.
- [58] D. Hemalatha, A. Amala, B. Rangasamy, et al. Sublethal toxicity of quinalphos on oxidative stress and antioxidant responses in a freshwater fish *Cyprinus carpio*. *Environ Toxicol*. 31, 11 (2015) 1399-1406.
- [59] M. Kah, R. S. Kookana, A. Gogos, et al. A critical evaluation of nanopesticides and nanofertilizers against their conventional analogues. *Nat. Nanotechnol.* 13 (2018) 677-684.
- [60] Publications in the Series on the Safety of Manufactured Nanomaterials. <https://www.oecd.org/env/ehs/nanosafety/publications-series-safety-manufactured-nanomaterials/>, 2019 (accessed 12.09.2021).
- [61] T. Stadler, M. Buteler, S. R. Valdez, et al. Nanoinsecticides: A New Concept in Insect Pest Management. <http://dx.doi.org/10.5772/intechopen.72448/>, 2019 (accessed 12.09.2021).
- [62] S. U. Ilyas, R. Pendyala, N. Marneni. Stability and Agglomeration of Alumina Nanoparticles in Ethanol/Water Mixtures. *Procedia Engineering* 148 (2016) 290 – 297.
- [63] Damalas CA, Eleftherohormos IG. Pesticide exposure, safety issues and risk assessment indicators. *Int. J Environ Res Public Health*. (2011) 8, 1402–19.
- [64] S. S. Patil, Y. Y. Shedbalkar, B. A. Truskewycz, et al., Nanoparticles for environmental clean up: a review of potential risks and emerging solutions. *Environ Technol Innov.* 5 (2016) 10–21.
- [65] M. N. Rittner. Market analysis of nano structured materials. *Am. Ceram. Soc. Bull.* 81 (2002) 33-36.



## Annals of Agrarian Science

Journal homepage: <http://journals.org.ge/index.php>



### Arsenic contamination in Racha and Lower Svaneti district of Georgia, consequences and remediation strategies

E. Bakradze<sup>a\*</sup>, T. Butkhuzi<sup>b</sup>, S. Khmiadashvili<sup>c</sup>, E. Bunin<sup>b</sup>, K. Didebulidze<sup>b</sup>, A. Gujabidze<sup>b</sup>, N. Dumbadze<sup>b</sup>, T. Glurjidge<sup>b</sup>, A. Giorgadze<sup>b</sup>

<sup>a</sup>LEPL Environmental Pollution Monitoring Department, The National Environmental Agency, David Aghmashenebeli Ave. 150, Tbilisi, 0112, Georgia

<sup>b</sup>Agricultural University of Georgia; 240, David Aghmashenebeli Alley, Tbilisi, 0159, Georgia

<sup>c</sup>LEPL National Environmental Agency, Ambient Air, Water and Soil Laboratory, Marshal Gelovani Ave. 6, Tbilisi, 0159, Georgia

Received: 20 March 2021; Accepted: 20 May 2021

#### ABSTRACT

In Georgia, arsenic mining, processing and storage has been undertaken for up to 52 years, arsenic sulfide ore mining, processing and production of arsenic containing drugs was carried out for decades in Racha-Lechkhumi and lower Svaneti. In the Article is presented studies of eco-chemical condition for soils near contaminated areas by arsenic in the villages Uravi - Ambrolauri Municipality and village Tsana. Research offers solutions - innovative remedial technology based on the ability of plants and microorganisms to absorb a wide range of chemical pollutants from the environment.

**Keywords:** Environment, Pollution, Arsenic, Remediation

\*Corresponding Author: Elina Bakradze; E-mail address: [h.bakradze@gmail.com](mailto:h.bakradze@gmail.com)

#### Introduction

Arsenic is a natural component of the earth's crust and is widely distributed throughout the environment in the air, water and land. It is highly toxic in its inorganic form. People are exposed to elevated levels of inorganic arsenic through drinking contaminated water, using contaminated water in food preparation and irrigation of food crops, industrial processes, eating contaminated food.

Therefore, it is important to prevent the pollution of the natural resources (water and soil) with arsenic and also to prevent its occurrence in the drinking water and food products. For this purpose, the legislation was developed in Georgia, Slovakia and in the European Union level as well. These legal documents were assessed and

compared to propose the changes (measures) in the legislation.

Toxicity of arsenic and its compounds is well known. In the seventies of the last century, their carcinogenic properties were established. Still, arsenic compounds are widely used in technology, agriculture, medicine, etc., Arsenic is a natural component of the earth's crust and is spread in any environment, air, water and soil. In a number of countries arsenic is naturally of high contents in the ground waters. This element is present in nature in both organic and inorganic forms, and the latter is very toxic. Long-term exposure to arsenic from consumed food can cause cancer and skin lesions [1,2]. Hence, control of arsenic content in natural waters, soils and food products and other objects and studying the regularity of redistributions

(pathways and mobility) and behaviour is one of the most important and topical issues in the world.

Arsenic is included in the list of 10 toxic elements (Hg, Pb, Cd, As, Ni, etc.) that create important problems for human health (WHO) [3].

Arsenic and arsenic compounds belong to the confirmed carcinogens, the Type I Hazard (IARC) for humans [4].

Beside the fact that skin and the respiratory tract can be the paths of the arsenic penetration into organism still mostly arsenic occurs in human body from the food and drinking water. Organic arsenic species are most often met in seafood and in units of terrestrial products. This terrestrial product is basically 3-5 valence arsenic forms. Therefore, arsenic enters the food chain mainly from contaminated soil and water [5,6].

Soil is a complex and multifactor system changeable according to the climatic and landscape characteristics of an area. Plants can maximally accumulate toxic substances from soil with their further migration to the human organism through a natural migratory circle – soil-plant-man; soil-water-man. In this connection, of importance is to study the principal contaminants of the environment (soil, water, etc.) in the region.

Arsenic is the most toxic of the elements that people use in their various areas of activity. Arsenic and its compounds are included in the composition of various pesticides, widely used in the manufacture of various types of glass, anticorrosive alloys, coatings, ammunition, accumulators. High purity arsenic is a necessary component in solar batteries, light-emitting diodes, lasers, integrated circuits, semiconductors, etc. Sh. Until the 70s of the last century, inorganic arsenic compounds were used in medicine as well; in particular, this element contained leukemia, psoriasis and asthma.

Arsenic tops the list of the 20 most dangerous compounds compiled jointly by The Agency for Toxic Substances and Disease Registry (ASTDR) and the US Environmental Protection Agency (EPA). Arsenic and its compounds are also considered by the World Health Organization (WHO) to be the most problematic substances for human health [7], and the International Agency for Research on Cancer (IACR) ranks them as the first category of carcinogenic hazards [8]. The Basel Convention of 22 March 1989, concerning the transboundary movement of hazardous waste and its disposal, classified arsenic as controlled waste. All of the above indicates that Arsenic is included in the list of

standardized substances [9].

Arsenic containing compounds can be entered from food and drinking water, also penetrate through skin and respiratory system. In food chain arsenic is entered from polluted soil and water. Arsenic has the different oxidation state -3, 0, +3, +5. The most abundant are arsenic (III) oxide  $As_2O_3$  and arsenic (V) oxide. According to toxicity, important compounds are arsenic (III) chloride ( $AsCl_3$ ), hydrogen compound – arsine ( $AsH_3$ ) and salts, such as lead (II) arsenate, copper (II) acetarsenate and etc. For fresh water the main sources of contamination are arsenopyrite ( $FeAsS$ ), auripigment (orpiment) ( $As_2S_3$ ) and other compounds. Contamination is mostly performed from former manufacturing factories, that results in the accumulation of arsenic containing compounds in water, soil and plants at nearby territory. This causes the transferring of arsenic in animal tissue and milk, and finally in human body. Eventually arsenic is accumulated in skin, nails, hair, as well as bones and muscles.

The As in water sources and soils consist mainly of natural source and anthropogenic source. Industrial and agricultural activities raise arsenic concentration in the natural environment, such as mining, smelting, burning of fossil fuels, using of pesticides and fertilizers. In the past it was found that pilot region areas have faced to the problems with the arsenic pollution.

Arsenic contamination is a pressing issue in Georgia, where there are many natural or anthropogenic sources. Arsenic ore extraction, processing and production of arsenic-containing preparations have been carried out on the territory of Georgia, particularly in Racha and Kvemo Svaneti for decades [10].

The processing of the ore, which is located in Ambrolauri region started in 1937. Main products of production were metallic arsenic of high purity,  $As_2O_5$ ,  $As_2S_5$  and tin arsenate. In the former Soviet Union countries these products were used for military purposes as well as civil purposes (manufacturing of leather, chemicals, electronics). It should be mentioned that technological cycle in factory was very simple and includes thermal treatment of ore. Amount of waste is about 60 tons, which contains up to 1%  $As_2O_5$ . Waste was not used and it was stored at Kajiani territory in special hydrotechnical building, called tailings. The mining of ore was carried out at Lukhuni ore, processing was performed in the factory nearby village Uravi. Lukhuni ore deposit is special, because arsenic which is produced from

this place does not contain “forbidden” elements for microelectronics, and gives possibility to get metallic arsenic of appropriate purity with low cost. The factory has stopped functioning since 1991. Nowadays Uravi factory, with all additional buildings and full infrastructure is destroyed. The waste dump of the Uravi plant, located 14 km from the plant, is not canted. The roof tiles in some places are submerged, precipitated water meets toxic waste, then leaks out of the tomb and into the environment. The tomb is also in Uravi, in the factory area, which is canted but not hermetically sealed - its roof damaged. There is a risk that if the Lukhuni River bursts, the storage wall will be washed away and a large amount of arsenic can enter the river. Also, the preparations for the production of metallic arsenic and preparations for the production of white arsenic roasting at “Jvari” were destroyed.

Chemical mining factory in village Tsana, Lentekhi region started working is 1938. The main products were metallic arsenic and refined “white arsenic” ( $As_2O_3$ ) (I grade – 99.9%, II grade – 99.5%). This substance is poorly soluble in water and permanently can have great negative influence on environment. Processing of ore was carried out from the mining near the territory the village Kuruldashi, as well as Racha ores. The factory in Tsana, administrative buildings and warehouse farming are fully destroyed and collapsed. There is no fence around the territory. Arsenic kilns and containers are taken from the ground. Local population activities cause the distribution of arsenic and its ores all over the territory. There are scattered opened containers with arsenic ore, with capsules of white arsenic. Approximately 800 to 1000 opened containers of arsenic ore are located above the territory of factory. The territory of factory and nearby territory is extremely polluted by production waste.

Thus the problem is arsenic containing waste and soils. Until today the great amount of toxic waste of arsenic production is stored in villages Uravi and Tsana [7,10], near the territory of the factories (more than 120 000 tons waste, containing 4-9 % of white arsenic), which was not located safely and there was a high risk of ecological disaster in rivers, and soils, especially risks of natural disasters (floods, rockslide, erosion and etc.). Uravi’s concrete burial ground, which covers up to 60,000 tons of arsenic, was crushed by the side of Lukhuni River and washed directly in the river, further exacerbating the expected dangers [11].

The arsenic can occur in compounds in four oxidation levels (+ V, + III, 0, -III), its highest representation is in oxidation levels (+ V) and (+ III). In nature, it occurs especially in minerals and ores (arsenopyrit – grey arsenic,  $FeAsS$ ; arsenolit – white arsenic,  $As_2O_3$ ; realgar – red arsenic,  $As_4S_4$ ; auripigment – yellow arsenic,  $As_2S_3$ ).

In water environment, the arsenic naturally occurs usually in a soluble form as As (II) - As(III) and As(V). The occurrence of these forms in natural water depends especially on oxidation-reduction potential and pH of water.

The arsenic toxicity and carcinogenicity depend on forms of its occurrence [12]. In general, it is presented that the form As (III) is more toxic than the form As(V). The toxicity of the form As (III) is almost 70-times higher than the organic form As and 10-times higher than the form As (V). Many studies [13] indicated that drinking of water with higher arsenic content may cause the cancer of kidneys, skin, bladder, liver, lungs. It has also non-cancer effects including cardiovascular, lung, immunologic, neurological, reproductive and endocrine problems.

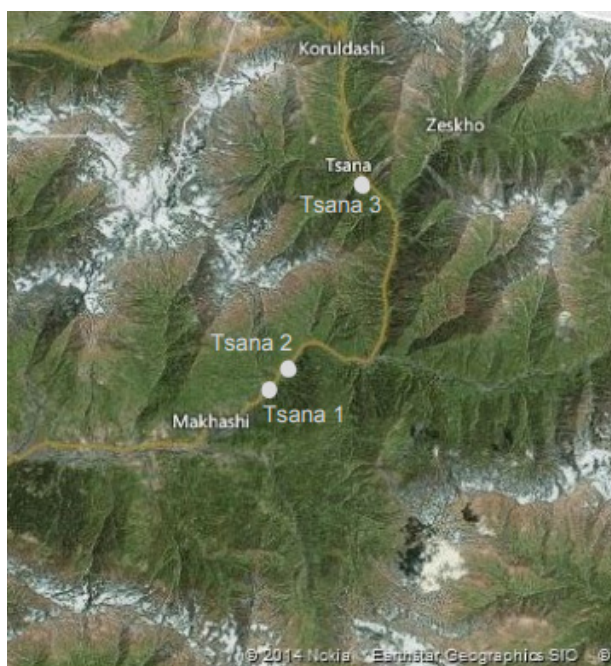
## Objectives and methods

The aim of the study was to establish the basis for arsenic-contaminated soils and cleaning technology in Georgia, which will use diagnostic and remediation technologies [14]. In order to achieve the aim the following objectives are set: 1. Determination of arsenic content in contaminated soil and water; 2. Creation of DNA-chip and study of microbiota in polluted environment by using of chip; 3. Isolation and characterization of active strains of microorganisms from contaminated soil; 4. Study the possibility of cleaning water polluted with arsenic by using of alga *Spirulina*; 5. Selection of plants that have ability to uptake arsenic; 6. Test of selected microorganisms and plants for phytoremediation of soil polluted with arsenic.

The expedition was conducted for sampling activities of soils and waters. Racha-Kvemo Svaneti, Time and date - 10/08/2020 - 16/08/2020. The samples were taken from the source of pollution 100, 300, 500 and 1000 m, the upper (0-10 cm) of the soil and lower (20-25 cm) layers ISO 10381-1, 10381-2 and 5667-4: 1987 According to the methodology. Locations were fixed by GPS. Total 61 samples have been taken from 6 locations, namely:

Gadamshi (control);  
 Uravi - 1;  
 Uravi - 3;  
 Tskhenistskali (control);  
 Tsana 2;  
 Koruldashi Factory.

**Map.1** *sampling points*



Preparation for preliminary processing and chemical analyzes of soil samples (drying, plotting, exchanging, excretion, etc.) were conducted according to methods - ISO-11464; EPA 3050; EPA- TCLP-1111. Determining the common forms of arsenic in the soil samples (A + 3, As + 5) - ISO 11885: 2007; According to EPA 200.2, which is based on Evins's method, which implies as a caliper, as well as direct iodometry (ST.15642052-002-98). This method is a very strong acid digestion that dissolves almost all elements that could become "environmentally available."

All samples were mixed thoroughly to achieve homogeneity and sieve, if appropriate and necessary, using a USS #10 sieve. All equipment used for homogenization were cleaned according to the guidance to minimize the potential of cross-contamination. For each digestion procedure, soil was weighed to the nearest 0.01 g and transferred to a 1 g sample (dry weight) to a digestion vessel.

For the digestion of samples for analysis were added 2.5 mL conc. HNO<sub>3</sub> and 10 mL conc. HCl

to a 1-2 g sample (dry weight) and covered with a watch glass or vapor recovery device. Samples were digested by the digester. After digestion procedure samples were cooled. Filtered where it was necessary through Whatman No. 41 filter paper (or equivalent) and filtrate were collected in a 100-mL volumetric flask. filter paper was washed, while still in the funnel, with no more than 5 mL of hot (~95EC) HCl, then with 20 mL of hot (~95EC) reagent water. washings were collected in the same 100-mL volumetric flask.

For each batch of samples that were processed, a method blank was used throughout the entire sample preparation and analytical process according to the frequency described in method Chapter One. These blanks are useful in determining if samples are being contaminated. After the QA-QC Procedures samples were analyzed by the spectrometry.

ICP-OES (Inductively coupled plasma - optical emission spectrometry) is a technique in which the composition of elements in (mostly water-dissolved) samples can be determined using plasma and a spectrometer. The solution to analyze is conducted by a peristaltic pump through a nebulizer into a spray chamber. The produced aerosol is lead into an argon plasma. Plasma is the fourth state of matter, next to the solid, liquid and gaseous state. In the ICP-OES the plasma is generated at the end of a quartz torch by a cooled induction coil through which a high frequency alternating current flows. As a consequence, an alternate magnetic field is induced which accelerated electrons into a circular trajectory. Due to collision between the argon atom and the electrons ionization occurs, giving rise to a stable plasma. The plasma is extremely hot, 6000-7000 K. In the induction zone it can even reach 10000 K. In the torch desolvation, atomization and ionizations of the sample takes place. Due to the thermic energy taken up by the electrons, they reach a higher "excited" state. When the electrons drop back to ground level energy is liberated as light (photons). Each element has an own characteristic emission spectrum that is measured with a spectrometer. The light intensity on the wavelength is measured and with the calibration calculated into a concentration.

## Results

**Table 1.** *Resultes of the analyses of the samples taken In Racha and Lower Svaneti district - Georgia*

Sample #	Sampling place	Depth, cm	Coordinates	Sampling date	Arsenic concentration, ppm	
1	Gadamshi-1100	0-10	N-42.62343	10.08.2020	67	
2	Gadamshi-1100	20-25	E-43.34136		140	
3	Uravi-3 1100-1	0-10	N-42.66743	11.08.2020	130	
4	Uravi-3 1100-1	20-25	E-43.30085		160	
5	Uravi-3 1100-2	0-10	N-42.66760		600	
6	Uravi-3 1100-2	20-25	E-43.30048		6300	
7	Uravi-3 1100-3	0-10	N-42.66768		140	
8	Uravi-3 1100-3	20-25	E-43.30021		110	
9	Uravi-3 1250-1	0-10	N-42.66495		290	
10	Uravi-3 1250-2	0-25	E-43.29653		330	
11	Uravi-3 1050-1	0-10	N-42.66854		150	
12	Uravi-3 1050-1	20-25	E-43.30276		410	
13	Uravi-3 1050-2	0-10	N-42.66816		820	
14	Uravi-3 1050-2	20-25	E-43.30333		140	
15	Uravi-3 1050-3	0-10	N-42.66615		440	
16	Uravi-3 1050-3	20-25	E-43.30277		290	
17	Uravi-1 850-1	0-10	N-42.63792		12.08.2020	330
18	Uravi-1 850-1	20-25	E-43.28826			74
19	Uravi-1 850-2	0-10	N-42.63776	82		
20	Uravi-1 850-2	20-25	E-43.28778	180		
21	Uravi-1 850-3	0-10	N-42.63724	2900		
22	Uravi-1 850-3	20-25	E-43.28884	93		
23	Uravi-1 850-4	0-10	N-42.63766	110		
24	Uravi-1 850-4	20-25	E-43.28700	74		
25	Tsana-2 1350-1a	0-10	N-42.81442	13.08.2020	260	
26	Tsana-2 1350-1a	20-25	E-43.11556		290	
27	Tsana-2 1350-2a	0-10	N-42.81425		100	
28	Tsana-2 1350-2a	20-25	E-43.11586		7400	
29	Tsana-2 1350-3a	0-10	N-42.81422		1100	
30	Tsana-2 1350-3a	20-25	E-43.11592		560	
31	Tsana-2 1350-4a	0-10	N-42.81411		220	
32	Tsana-2 1350-4a	20-25	E-43.11601		290	
33	Tsana-2 1350-5a	0-10	N-42.81394		100	
34	Tsana-2 1350-5a	20-25	E-43.11608		74	
35	Tsana-2 1350-1b	0-10	N-42.81418		11200	
36	Tsana-2 1350-1b	20-25	E-43.11539		13000	
37	Tsana-2 1350-2b	0-10	N-42.81403		11900	
38	Tsana-2 1350-2b	20-25	E-43.11567		10400	
39	Tsana-2 1350-3b	0-10	N-42.81396		9700	
40	Tsana-2 1350-3b	20-25	E-43.11580		1600	

41	Tsana-2 1350-4b	0-10	N-42.81382	13.08.2020	93
42	Tsana-2 1350-4b	20-25	E-43.11588		370
43	Tsana-2 1350-5b	0-10	N-42.81381		280
44	Tsana-2 1350-5b	20-25	E-43.11595		93
45	Tsana-2 1350-1c	0-10	N-42.81392		930
46	Tsana-2 1350-1c	20-25	E-43.11548		197
47	Tsana-2 1350-2c	0-10	N-42.81416		280
48	Tsana-2 1350-2c	20-25	E-43.11572		187
49	Tsana-2 1350-3c	0-10	N-42.81452		187
50	Tsana-2 1350-3c	20-25	E-43.11597		47
51	Tsana-2 1800	0-10	N-42.82259	14.08.2020	93
52	Tsana-2 1800	20-25	E-43.11097		740
53	Tsana-2 1600	0-10	N-42.813906		370
54	Tsana-2 1600	20-25	E-43.193175		187

Of the numerous minerals containing arsenic, the following sulfide ores are the most important:  $As_2S_3$  – Arsenic sulfide,  $As_4S_4$  – Pararealgar,  $FeAsS$  – arsenopyrite,  $FeAs_2$  –loellingite. Rarely is arsenic found as an element. The oxygen containing compound of arsenic present in the waste in the form of arsenic trioxide-  $As_2O_3$ , is formed in nature as a result of depletion of arsenic ores in nature. Also arsenic (III) oxide as a residue is formed during the combustion of arsenic containing ores. In our opinion, from eco-chemical point of view, according to the contact duration with soil and water and physico-chemical conditions, conversion of arsenic (III) oxide ( $As_2O_3$ ) and sulfides can be carried out based on their main characteristic chemical reactions. Dissolution of arsenic (III) oxide in water is very slow process, but it is enhanced in the presence of hydrogen ions and hydroxide ions. Unlike oxides sulfides are not dangerous for environment due to their low solubility. However, sulfide ores and fumes can be converted into mobile forms in oxidizing conditions.

Based on conducted investigations it is established that contamination of soils is especially important with industrial waste. Soot that is accumulated in the warehouses of chemical mining factories in Racha and Lower Svaneti, represent loose, porous material.

Regardless of several attempts to dispose of arsenic industrial waste, industrial recycling process was not performed, so arsenic industrial waste remains a powerful anthropogenic source of environmental pollution.

For evaluation an intervention value for soil (20

cm) of 200 mg/kg for agricultural and residential land uses was applied.

54 sample were analyzed and in 30 samples were exceeding the norm.

Maximum was in Tsana-2 1350-1b; 20-25 cm - 65 times the norm;

Minimum was in Gadamishi-1100; 0-10 cm (67 mg/kg).

The best solution is to make a phytoremediation, which will be based on the local microflora, and the soil samples will be used to be analyzed, taking into consideration arsenic content.

## Conclusions

This study highlighted once again the dangerous ecological situation created in Ambrolauri and Lentekhi regions of Georgia and environment related security risks that may arise from this old soviet legacy. After the plants producing arsenic concentrate were closed in the 90s., plant premises were demolished and drums with arsenic waste material remain scattered around openly on the sites, polluting the environment and causing risks to human health. About 100,000 tons of wastes containing arsenic are accumulated in the villages of Tsana and Uravi. The sites are located in the basin of the Tskhenistskhali and Rioni rivers and there is an existing high risk of arsenic leakage. In the past the Ministry of Environment took several efforts as well as initiated discussions on the issue with different line ministries, scientific institutions, NGOs, other experts as well as local population to develop and implement effective measures. The importance of the matter is underlined in the

National Environmental Action Programme for 2012-2016 and the issue is identified as particularly dangerous. In accordance with the agreement reached in 2012 with the government of the Kingdom of the Netherlands a project for arsenic containing mine waste in Georgia is conducted by “Witteveen+Bos”, Dutch consultancy 22nd OSCE Economic and Environmental Forum “Responding to environmental challenges with a view to promoting cooperation and security in the OSCE area” FIRST PREPARATORY MEETING Vienna, 27-28 January 2014 Session IV cont. Georgia, EEF. DEL/6/14/Add.1 28 January 2014 ENGLISH only and engineering firm. A study of arsenic-containing ashes and sludge in villages of Tsana and Uravi has been conducted. Nevertheless, implementation of the urgent measures, sarcophagus construction and safe disposal of arsenic containing waste materials requires additional funding for insuring the population and environmental safety in Georgia.

Arsenic (As) is a top human carcinogen widely distributed in the environment. As-contaminated soil exists worldwide and poses a threat on human health through water/food consumption, inhalation, or skin contact. More than 200 million people are exposed to excessive As concentration through direct or indirect exposure to contaminated soil. Therefore, affordable and efficient technologies that control risks caused by excess As in soil must be developed. The presently available methods can be classified as chemical, physical, and biological. Combined utilization of multiple technologies is also common to improve remediation efficiency. This research article presents the research progress on different remediation technologies for As-contaminated soil. For chemical methods, common soil washing or immobilization agents were summarized. Physical technologies were mainly discussed from the field scale. Phytoextraction, the most widely used technology for As-contaminated soil, was the main focus for bioremediation. Further research directions are proposed based on literature analysis.

It is important not only to evaluate the extent of arsenic removal from water and soil, but also to assess its biological impacts on ecosystems using ecotoxicological biotests. A number of aquatic toxicity testing methods have been published to assess the effects of substances on aquatic ecosystems. Based on the obtained ecotoxicological data, it is possible to assess ecological risks and propose possible new

approaches to the regulation and control of toxic chemicals in various ecosystems.

### Acknowledgments

This work was supported by grant # CARYS-19-179 from Shota Rustaveli National Science Foundation (SRNSF).

### References

- [1] L. Shavliashvili, E. Bakradze, M. Arabidze, G. Kuchava, Arsenic pollution study of the rivers and soils in some of the regions of Georgia, international Journal of Current Research. 9, 2 (2017) 47002 – 47008.
- [2] M.V. Gagoshidze, M.O. Antelava, A.G. Zedgenidze. Influence of the arsenic contaminated environment on the genetic apparatus of children and adolescents Georgian Medical news, 117 (2004) 59-62. (In Russian).
- [3] 10 chemicals of public health concern, <https://www.who.int/news-room/photo-story/photo-story-detail/10-chemicals-of-public-health-concern>; 2020 (accessed 14.10.2021).
- [4] Arsenic and Arsenic Compounds IARC Monographs – 100C, (IARC, 1980, 1987, 2004) <http://monographs.iarc.fr/ENG/Monographs/vol100C/mono100C-6.pdf> (accessed 14.10.2021).
- [5] M.V. Gagoshidze, M.O. Antelava, A.G. Zedgenidze., N.Sh. Manjavidze, Influence of the ecological situation on the morbidity of children with acute respiratory diseases. Georgian Medical news. 1, 118 (2005) 49-52. (In Russian).
- [6] M.G. Skalnaya, A.V. Skalny, V.A. Demidov, Dependence of the increased oncologic morbidity rate from the excessive contents of arsenic and other toxic chemical elements in environment. (2001) 32-35.
- [7] A. Chirakadze, Z. Buachidze, I.O. Khomeriki, V. Gvakharia, M. Stamateli, N. Chakvetadze, M. Chokheli, W.A. Toscano, K. Chigogidze, L. Gvertsiteli, N. Bagrationi, Arsenic pollution of soils and morbidity prevalence in Racha-lower Svaneti district of Georgia International Journal of Global Warming (2016) <https://experts.umn.edu/en/publications/arsenic-pollution-of-soils-and-morbidity-prevalence-in-racha-lowe-2>.
- [8] IACR monographs on the identification of carcinogenic hazards to human. Last update:

- 27 September, 2021. <https://monographs.iarc.who.int/agents-classified-by-the-iarc/>
- [9] The Basel Convention on the "Control of Transboundary Movements of Hazardous Wastes and their Disposal" 1989, 22 March, Basel (Switzerland) Georgia is a party of the convention from (1999).
- [10] N. Bagrationi, V. Gvakharia, A. Chirakadze, L. Gverdtsiteli. General analysis of ecochemical study results of soils of contaminated territory of Racha and lower Svaneti. Georgian Chemical Journal, 15, 2 (2015) 190-198.
- [11] I. Gurguliani, Arsenic contamination in Georgia Environment related security risks from old soviet legacy; 22nd OSCE Economic and Environmental Forum (2014). [https://www.osce.org/files/f/documents/a/a/110702.pdf?fbclid=IwAR3kxzaNbiAOxqTprXPefadxizIkaQQNx\\_HBeGjr6MhC\\_oLLWJFDWYHvvGc](https://www.osce.org/files/f/documents/a/a/110702.pdf?fbclid=IwAR3kxzaNbiAOxqTprXPefadxizIkaQQNx_HBeGjr6MhC_oLLWJFDWYHvvGc) (accessed 14.10.2021).
- [12] E. Bakradze, Y. Vodyanitskii, T. Urushadze, Z. Chankseliani, M. Arabidze. About rationing of the heavy metals in soils of Georgia. Annals of Agrarian Science. 16 (2018), 1-6.
- [13] K. Laperashvili, Food safety. Georgian strategic researches and development center Bulletin N111 (2008).
- [14] G. Khatisashvili, T. Varazi, M. Kurashvili, M. Pruidze, E. Bunin, K. Didebulidze, T. Butkhuzi, E. Bakradze, N. Asatiani, T. Kartvelishvili, N. Sapojnikova, Remedial Approaches against Arsenic Pollution (July 2nd 2021). Remedial Approaches against Arsenic Pollution [Online First], IntechOpen, DOI: 10.5772/intechopen.98779. <https://www.intechopen.com/online-first/77387>.



## Bover-Ge – mycopesticides for the control Brown marmorated stink bug - *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae)

M. Burjanadze\*, N. Kharabadze, M. Arjevanidze

Agricultural University of Georgia, Vasil Gulisashvili Forest Institute; 240, David Agmashenebeli Alley, Tbilisi 0159, Georgia

Received: 16 August 2021; Accepted: 17 September 2021

### ABSTRACT

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) (BMSB) is an exotic invasive insect which has spread extensively and established in new area of Black Sea regions of Georgia. Mycopesticide, trade mark- Bover-Ge™ based on a local strain of entomopathogenic fungus *Beauveria bassiana*, with tree concentrations ( $1 \times 10^6$ ,  $1 \times 10^7$  and  $1 \times 10^8$  conidia/ml) was tested on the adults of *H. halys* in laboratory and efficiency reached 73.3-93.3%. Also, established postmigration efficacy on the migration stage was tested and mortality achieved 64.6%.

**Keywords:** Brown marmorated stink bug (BMSB), Entomopathogenic fungi (EPF), Bover-Ge, Bioassay, effectiveness.

\*Corresponding author: Medea Burjanadze; E-mail address: [m.burjanadze@agruni.edu.ge](mailto:m.burjanadze@agruni.edu.ge)

### Introduction

#### *Brown marmorated stink bug*

The brown marmorated stink bug (BMSB), *Halyomorpha halys*, is native for East Asia and distributed throughout the US, Canada, Europe and become a severe invasive agricultural pest [1,2]. BMSB is highly polyphagous, it feeds on over 170 host plants, many of them are of agricultural importance, such as fruit, vegetables, row crops, and ornamentals. It is also a structural pest, with high reproductive output, potentially enabling its spread and success in invaded regions, as large populations invade houses, trying to overwinter. BMSB is capable of long-distance flight [3,4].

Following its first detection in 2015, *H. halys* has spread extensively and established in new areas of Black Sea regions and has become a key pest in many crops in West Georgia [5]. At present BMSB is very active and characterized by the massive

increase and formation of foci in agricultural and urban landscapes of Western Georgia, where situation is quite alarming. Georgia is the third hazelnut-producing country worldwide after Turkey and Italy (FAOSTAT 2017). Nowadays, due to the lack of specific natural enemies, population density of this insect is not downregulated.

#### *Entomopathogenic fungi*

Entomopathogenic fungi (EPF) are important natural enemies of many harmful pest species, providing important ecosystem services. They possess many positive attributes including broad host range, but restriction to and thus safety for non-target organisms and the environment; ease of mass-production and application; rapid host mortality; and potential for recycling in the environment. The EPF-based bioinsecticides have significant advantages over other entomopathogenic microorganisms, because of their ability to actively penetrate directly through natural openings and the cuticle of insects;

they do not have to be eaten unlike viruses or bacteria. They infect harmful pests of 48 families from orders Hemiptera, Coleoptera, Lepidoptera, Thysanoptera, Orthoptera Entomopathogenic fungi *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae), are ubiquitous organisms that are pathogenic to various arthropod pests [1-8].

### ***Bover-Ge***

In 2019, proposed product, mycopesticide, trade mark- Bover-Ge™, was registered by National food agency (NFA) of Georgia as a biopesticides. Bioformulation are based on local strain of entomopathogenic fungus *Beauveria bassiana*, isolated from soil of high mountain of Caucasus region, gave a unique cultural number (code) - IMI # 501799, and kept in CABI Genetic Recourse Collection.

The aim of our study was to tested Bover-Ge on the *H. halys* and evaluate their potential for the its control.

## **Materials and methods**

### ***Insect collection***

Overwintering adult of *H. halys* were collected from gardens and hazelnut orchards from different site of Western Georgia (Samegrelo, Guria, Imereti) during 2020-2021. *H. halys* were transferred in the laboratory and kept a few days at room temperature 22–23°C, 60–65% RH, 16 h light (L):8 h dark (D) photoperiod before the bioassay. As a food source for the insects, fresh apples and carrots were used.

### ***Bioassay***

The adults target insects - *H. halys* performed for the bioassay and treated with suspension of Bover-Ge with tree concentration, based on *Beauveria bassiana* (Bb007 strain). Formulations contained 12.5 % fungal conidia and as active ingredients 87.5% inert material ( $2.2 \times 10^{10}$  conidia g<sup>-1</sup>). The formulation were diluted to deliver the desired test concentrations  $1 \times 10^6$ ;  $1 \times 10^7$  and  $1 \times 10^8$  conidia ml<sup>-1</sup>. For control treatments normal, tap water were used. *H. halys* were placed in a glass jar with carrot and corn and kept at room temperature ~23 °C (day)/18 ~ °C (night), RH with 14/10 light/dark regime. Dead or infected larvae with fungal symptoms were removed

and placed in moister environment for development of conidia. Mortality of larvae were recorded on 3-18 days after treatment [32].

### ***Data analysis***

The recorded mortality data were corrected for mortality in the control group using the Abbott equation (Abbot, 1925).

Data were analysed by IBM SPSS 23.0, using a probit analysis method to determine the lethal concentration (LC<sub>50</sub>) for the different treatments. The mortality data were transformed to probits, while the concentrations were transformed into Probit log<sub>10</sub> (dose). Before analysis LC<sub>50</sub> values were estimated from the probit lines.

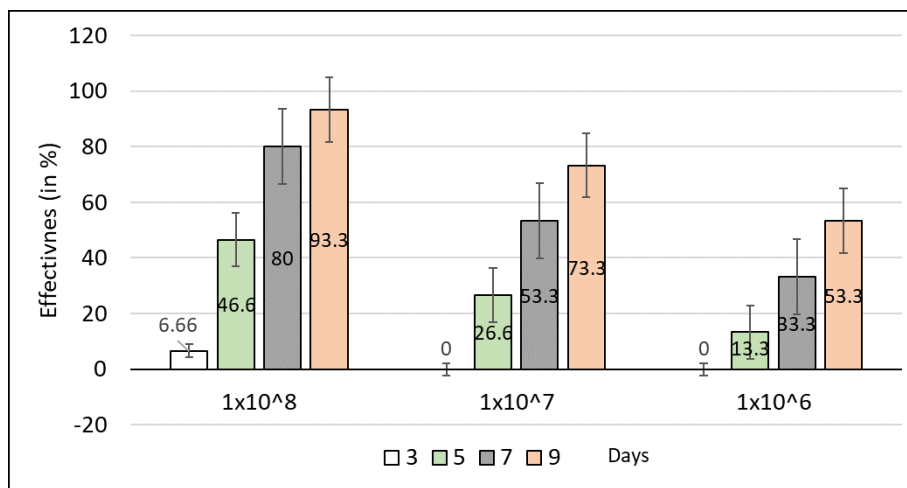
For the determination of lethal time (LT<sub>50</sub>) probit analyses, the method of Finney (1971) was used. Calculation of the lethal concentration (LCs) at their 95% confidence limits (CLs) was based on an accurate estimation of log (LC) variances (Hayes & Kruger, 2014). The Kaplan–Meier survival analysis technique was used to determine both the mean survival and the median lethal time (LT<sub>50</sub>), the number of days until 50% of insects were dead, for each treatment (SPSS 23.0). In order to calculate significant differences between doses and exposure times, a one-way analysis of variance (ANOVA) using the SPSS 23.0 software package at P < 0.01 and P < 0.05 levels was carried out.

## **Results and Discussion**

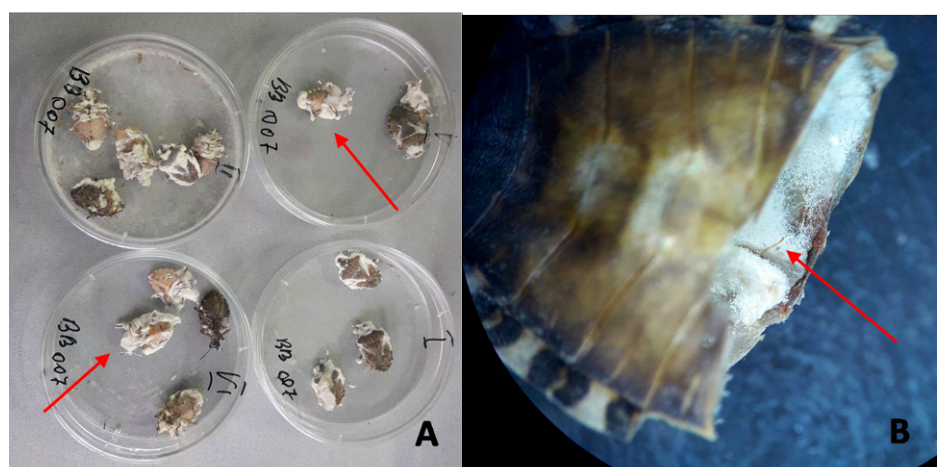
Experimental data showed that Bover-Ge in all tested concentrations were able to kill *H. halys* (Figure 1). The infected adults were counted 3,5,7,9 days after treatment. All dead specimens showed symptoms of infection with fungi developing on the surface and into adult bodies (Figure 2).

Significant differences were observed between concentration. At the end of experiment (9 days), cumulative mortality at high concentration ( $1 \times 10^8$  conidia/ml) reached 93.3% and 53.3%, whereas at concentrations of  $1 \times 10^7$  and  $1 \times 10^6$  conidia/ml the mortality ranged 80-33.3% on 7 day and 46.6-13.3%73.3 on 5 Day (Fig. 1).

The susceptibility of *H. halys* to a particular concentration is a key factor for LC<sub>50</sub> levels. The concentration required to cause 50% mortality of *H. halys* for different are presented in Table 1. All



**Fig. 1.** Efficiencies of Bover-Ge on the adult *H. halys* according to days in laboratory



**Fig. 2.** Efficiencies Bover-Ge on the adult *H. halys* in laboratory

three concentration are pathogenic with a mean mortality ranging from 33.3 to 93.3% ( $P \leq 0.05$ ). The highest percentage of mortality was observed 9 days after treatment. Bover-Ge in high concentration  $1 \times 10^8$  conidia/ml significantly more virulent than the other tested concentrations. Probit analysis used to analyse the mortality results revealed that  $1 \times 10^8$  had the lowest median lethal concentration value (Table 1).

The concentration required to cause 50% mortality of *H. halys* for the three tested concentration at high, medium and low concentrations is presented in Figure 4(A–D). One-way ANOVA analysis shows that the average mortality was significantly ( $P \leq 0.05$ ) affected by exposure of *H. halys* insects to different concentrations of Bover-Ge suspensions.

Insects exposed during the period 3–9 days post infection at the highest concentration ( $1 \times 10^7$  and  $1 \times 10^6$  conidia/ml), had significantly increased death rate. The lethal survival time ( $LD_{50}$ ) of *H. halys* (Figure 5) ranged from a minimum of 4.3

to a maximum of 6.6 days (Table 2). Survival curves for treatments with a concentration of 1000 IJs/adult, all differed based on the Kaplan–Meier method. Pearson's Chi-square statistic test (all values of  $P > 0.05$ ) indicated that the data fitted the regression models according to Breslow (Generalised Wilcoxon).

Time-Response Bioassay Survival analysis of *H. halys* adults exposed to the control and the selected concentration  $1 \times 10^7$  and  $1 \times 10^6$  conidia/ml adult indicated no significant difference between the mortality times (Tables 3 and 4). Survival curves for treatments with concentration  $1 \times 10^7$  (Figure 6) Pearson's Chi-square statistic test (all values of  $P > 0.05$ ) indicated that the data fitted the regression models, where  $x^2 = 0.292$ ,  $df = 1$ ,  $sig = 0.589$  and with the concentration of  $1 \times 10^6$  (Figure 7)  $x^2 = 0.839$ ,  $df = 1$ ,  $sig = 0.360$  according to Breslow (Generalised Wilcoxon). Survival curves for all treatments differed by the Holm–Sidak method ( $P < 0.05$ ).

**Table 1.** Median lethal concentration LC<sub>50</sub> of *H. halys* adult treated by the Bover-Ge in deferent concentration (ANOVA, P ≤ 0.05)

Strain	Slope±SE	LC%50 PPM	95% Fiducial CI		Chi-test (χ <sup>2</sup> ) Sig	df	P-value
			Lower	Upper			
1×10 <sup>8</sup> conidia/ml	0.721±0.322	879.3	205.4	3764.2	0.871	1	0.015
1×10 <sup>7</sup> conidia/ml	1.255±0.228	64.2	22.9	179.6	0.997	1	0.034
1×10 <sup>6</sup> conidia/ml	0.951±0.245	656.7	217.6	1982.0	0.882	1	0.039

**Table 2.** Mortality (%), mean survival time and LT<sub>50</sub> of *H. halys* adult on 9 days after treatment by Bover-Ge

Nematode strains	Mortality % <sup>a</sup>	Mean survival time ± SE <sup>b</sup>	LT50 (95% CI)	N <sup>c</sup>
1×10 <sup>8</sup> conidia/ml	93.3	6.6±0.417	7.0	15
1×10 <sup>7</sup> conidia/ml	73.3	4.3±0.182	4.0	15
1×10 <sup>6</sup> conidia/ml	53.3	6.6±0.417	7.0	15

<sup>a</sup> Percent of dead individuals at the end of experiment corrected for mortality in control using Abbott's formula;

<sup>b</sup> The mean survival time and its standard error were underestimated because the largest observation was censored;

<sup>c</sup> Total number of individuals in bioassay.

**Table 3.** Mortality (%), mean survival time and LT<sub>50</sub> of *H. halys* adult 7 days after treatment by Bover-Ge

Nematode strains	Mortality% <sup>a</sup>	Mean survival time ± SE <sup>b</sup>	LT50 (95%CI)	N <sup>c</sup>
1×10 <sup>8</sup> conidia/ml	80.0	6.4±0.510	6.0	15
1×10 <sup>7</sup> conidia/ml	53.3	6.6±0.510	7.0	15
1×10 <sup>6</sup> conidia/ml	33.3.	6.0±0.577	6.0	15

<sup>a, b, c</sup> For explanations see Table 2

**Table 4.** Mortality (%), mean survival time and LT<sub>50</sub> (days) of *H. halys* adult 5 days after treatment by Bover-Ge

Concentration	Mortality% <sup>a</sup>	Mean survival time ± SE <sup>b</sup>	LT50 (95% CI)	N <sup>c</sup>
1×10 <sup>8</sup> conidia/ml	46.6	6.6±0.510	7.0	15
1×10 <sup>7</sup> conidia/ml	26.6	6.8±0.510	7.0	15
1×10 <sup>6</sup> conidia/ml	13.3	6.8±0.477	7.0	15

<sup>a, b, c</sup> For explanations see Table 2.

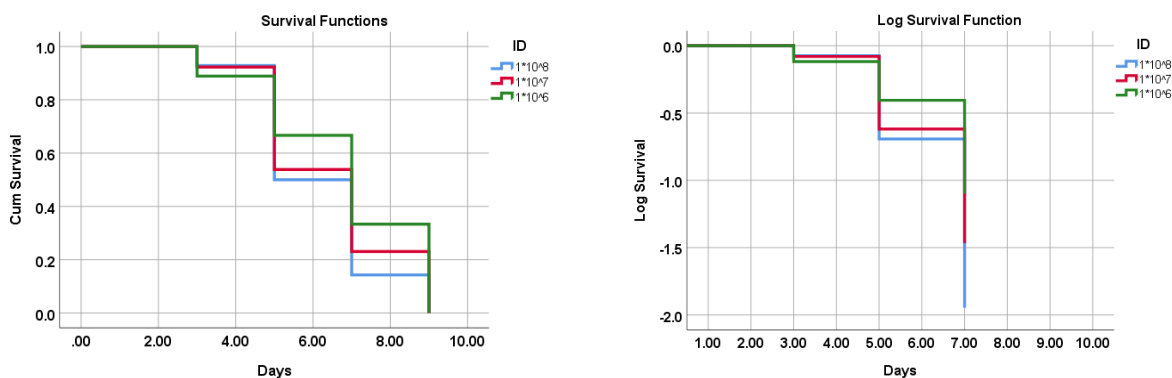


Figure 5. Survival curves (Lethal concentration  $LC_{50}$ ) of *H. halys* adults treated by Bover-Ge.

Also to establish postmigration efficacy of mycopesticides, Bover-Ge was tested on the migration stage of *H. halys* adults, and mortality reached 64,6% (Fig. 3).

Variation	Data of insects in %		C (variation coefficient)
	Alive	Dead	
Control ( no treatment)	93,2	6,8	± 8,9
Control ( With water)	62,0	38,0	± 42,51
Bover-Ge ( $1 \times 10^8$ conidia/ml )	35,4	64,6	± 11,8

Figure 3. Efficiencies Bover-Ge on the migration stage of *H. halys*

### Conclusion

These bioassay results confirm that significant mortality of *H. halys* can occur following a spray treatment of the Bb007 strain of *B. bassiana* found in the commercial fungal-based product Bover-Ge.

At 9 days after treatment with the Bb007 strain applied at a concentration of  $1 \times 10^8$  conidia/ml , 93.3% mortality was obtained for adults .

The infected adults were counted 3,5,7,9 days after treatment. All dead specimens showed symptoms of infection with fungi developing on the surface and into adult bodies (Figure 2). Significant differences were observed between concentration. At the end of experiment (9 days), cumulative mortality at high concentration ( $1 \times 10^8$  conidia/ml ) reached 93.3% and 53.3%, whereas at concentrations of  $1 \times 10^7$  and  $1 \times 10^6$  conidia/ml the mortality ranged 80-33.3% on 7 day and 46.6-13.3% 73.3% on 5 Day.

Based on these results and those from our previous research, the adult stages of *H.halys* are

impacted by treatment with the Bb007 strain. In addition, the immature stage is more sensitive to infection than the adults, therefore, growers may want to specifically target that stage to maximize reduction of the pest population. However, although these results show promise, trials are still needed to fully assess the efficacy of these fungal formulations under different environmental field conditions. In general, many comparisons of results from lab-based and field trials on the lethality of various chemical pesticides to *H. halys* have shown that mortality is considerably less in the field than in the laboratory.

### Acknowledgments

The research has been supported by NFA-SRNSFG project # 18-350 “ Develop ecofriendly tools for control Brown marmorated stink bug (BMSB) *Halyomorpha halys* in Georgia”.

## References

- [1] Leskey TC. *Bull. Entomol. Res.* 2015, 105, 566–73
- [2] Wiman NG, Walton VM, Shearer PW, Rondon SI, Lee JC., *J. Pest Sci.* 2014, 88, 37–47
- [3] Hoebeke ER, C. E. *Proc Entomol Soc Wash*, 2003, 105, 225–237
- [4] Leskey, T. C. *Outlooks Pest Man.*, 2012, 23(5), 218-226.
- [5] Lee D.-H. B. *Environ Entomol.*, 2013, 42(4), 627-641.
- [6] Gapon D.A. *Entomol Rev.*, 2016, 96, 1086–1088
- [7] Sugie H, Yoshida M, Kawasaki K, et al. *Applied Entomology and Zoology*, 1996, 31(3), 427-431.
- [8] Zhang A, Khirmian A, Aldrich JR, Leskey TC, Weber DC, 2013. U.S. *Provisional Application No. 61/724,475.*, USA.
- [9] Behle, R.W., Jackson, M.A. and Flor-Weiler, L.B. (2013) Efficacy of a Granular Formulation Containing *Metarhizium brunneum* F52 (Hypocreales: Clavicipitaceae) *Microsclerotia* against Nymphs of *Ixodes scapularis* (Acari: Ixodidae). *Journal of Economic Entomology*, 106, 57-63. <https://doi.org/10.1603/EC12226>
- [10] Bidochka, M.J., Kasperski, J.E. and Wild, G.A.M. (1998) Occurrence of the Entomopathogenic Fungi *Metarhizium anisopliae* and *Beauveria bassiana* in Soils from Temperate and Near-Northern Habitats. *Canadian Journal of Botany*, 76, 1198-1204. <https://doi.org/10.1139/b98-115>
- [11] Gouli, V., Gouli, S., Skinner, M., Hamilton, G., Kim, J.S. and Parker, B.L. (2012). Virulence of Select Entomopathogenic Fungi to the Brown Marmorated Stink Bug, *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae). *Pest Management Science*, 68, 155-157. <https://doi.org/10.1002/ps.2310>
- [12] Padulla, L.F.L. and Alves, S.B. (2009) Suscetibilidade de ninfas de *Diaphorina citri* a fungos entomopatogênicos. *Arquivos do Instituto Biológico*, 76, 297-302.
- [13] Reddy, G.V.P., Zhao, Z. and Humber, R.A. (2014) Laboratory and Field Efficacy of Entomopathogenic Fungi for the Management of the Sweet Potato Weevil, *Cylas formicarius* (Coleoptera: Brentidae). *Journal of Invertebrate Pathology*, 122, 10-15.
- [14] Roberts, D.W. and St. Leger, R.J. (2004) *Metarhizium* spp., Cosmopolitan Insect Pathogenic Fungi: Mycological Aspects. *Advances in Applied Microbiology*, 54, 1- 70.
- [15] Zimmerman, G. (2008) The Entomopathogenic Fungi *Isaria farinosa* (Formerly *Paecilomyces farinosa*) and the *Isaria fumosorosea* Species Complex (Formerly *Paecilomyces fumosoroseus*): Biology, Ecology, and Use in Biological Control. *Biocontrol Science and Technology*, 18, 865-901.
- [16] <https://doi.org/10.1080/09583150802471812>
- [17] Kevin B Rice, Robert H Bedoukian, et al. *Journal of Economic Entomology*, 2018, 111(1), 9, 495–499.
- [18] Gouli, V., Gouli S, Skinner M., Hamilton G., Kim JS, Parker B. *Pest management Sciences*, 2012, 68(2), 155-15
- [19] Parker BL., Skinner M., Gouli S., Gouli V., Kim JS. *Insects*, 2015, 6 (2), 319-324
- [20] H. Hagiwara, T. Okabe, H. Ono, V. P. Kamat, T. Hoshi, T. Suzuki, M. Ando. *J. Chem. Soc., Perkin Trans.* 2002, 1, 895–900
- [21] T. C. Leskey, A. Khirmian, D. C. Weber, J. C. Aldrich, B. D. Short, D.-H. Lee, W. R. Morrison. *J Chem Ecol* , 2015, 41, 418–429
- [22] Leskey, T. C., A. Agnello, J. C. Bergh, G. P. Dively, G. C. Hamilton, P. Jentsch, A. Khirmian, G. Krawczyk, T. P. Kuhar, D.-H. Lee, et al. *Environ. Entomol.* 2015. 44, 746–756
- [23] Leskey TC. *Bull. Entomol. Res.*, 2015, 105, 566–573.
- [24] Jaronski, S.T., Grace, J.A., Schlothauer, R. *Proceedings, 33rd Biennial Meeting, American Society of Sugar Beet Technologists.* 2005, 185-187
- [25] Jaronski, S. *Mass Production of Beneficial Organisms.* USA: Academic Press. 2013, 357-415
- [26] *The manual of biocontrol agents: a world compendium*, 2009, 851.
- [27] Douglas A., Landis Stephen D., et al, *Annual Review of Entomology*, 2000, 45, 175-201
- [28] *Manual of Techniques in insect pathology*, 1997
- [29] *Manual of Techniques in insect pathology*, by Lawrence A Lacey, 1997, 409
- [30] *Bioassays of Entomopathogenic Microbes and Nematodes: Navon*, 2000
- [31] Navon A.. *Bioassays of Entomopathogenic Microbes and Nematodes: In Bioassays of Entomopathogenic Microbes and Nematodes*, 2000, 1-25.
- [32] Leonard G. Copping. *The Biopesticide Manual*, 1998, 333
- [33] Lawrence A. Lacey, Harry K Kaya. *Field Manual of Techniques in Invertebrate Pathology: Application and Evaluation of Pathogens for Control of Insects and other Invertebrate Pests*, 2007, 587
- [34] Abbott WS.. *J Econ. Entomol.*, 1925, 18, 265-267



## Microorganisms and Plants as Tools for Phytoremediation of Soil Polluted with Different Forms of Arsenic

N. Asatiani<sup>a</sup>, E. Bakradze<sup>b</sup>, T. Butkhuzi<sup>c</sup>, K. Didebulidze<sup>c</sup>, A. Gujabidze<sup>c</sup>,  
T. Kartvelishvili<sup>a</sup>, G. Khatisashvili<sup>c</sup>, S. Khmiadashvili<sup>d</sup>, M. Kurashvili<sup>c\*</sup>,  
M. Pruidze<sup>c</sup>, D. Razmadze<sup>c</sup>, N. Sapojnikova<sup>a</sup>, T. Varazi<sup>c</sup>

<sup>a</sup> Andronikashvili Institute of Physics, I. Javakhishvili Tbilisi State University, 6 Tamarashvili Str., 0162, Tbilisi, Georgia

<sup>b</sup> LEPL Environmental Pollution Monitoring Department, The National Environmental Agency, David Aghmashenebeli Ave. 150, Tbilisi, 0112, Georgia

<sup>c</sup> Agricultural University of Georgia; 240, David Aghmashenebeli Alley, Tbilisi, 0159, Georgia

<sup>d</sup> LEPL National Environmental Agency, Ambient Air, Water and Soil Laboratory, Marshal Gelovani Ave. 6, Tbilisi, 0159, Georgia

Received: 10 June 2021; Accepted: 24 July 2021

### ABSTRACT

The presented work concern to establish the basis of cleaning technology for arsenic-contaminated soils in Georgia. To achieve this aim, the selected microorganisms and plants for phytoremediation of soil polluted with different forms of arsenic ( $As^{+3}$  - and  $As^{+5}$ ) have been tested.

Model experiments were carried out to test the suitability of plants - fern and sunflower for phytoremediation of artificially contaminated soils with arsenite or arsenate. Thus, among the plants tested in the experiments, the most effective was sunflower which can decrease arsenic content in soil at 200 ppm contamination with  $As^{+3}$  by about 35% and in case of contamination with  $As^{+5}$  - by almost 50%. It is important that the degree of purification of soil contaminated with arsenic increased by plant if the soil is enriched with bacterial strains that have the oxidizing-reducing ability of arsenic containing compounds.

**Keywords:** Phytoremediation, soil pollution, Arsenic, arsenate, arsenite.

\*Corresponding author: Maritsa Kurashvili, E-mail Address: [m.kurashvili@agruni.edu.ge](mailto:m.kurashvili@agruni.edu.ge)

### Introduction

The study deals with chemical pollution of the environment, in particular, arsenic contamination of soil and water, which is a highly pressing problem for both Georgia and the world. The solution is to develop innovative remedial technology based on the ability of plants and microorganisms to absorb a wide range of chemical pollutants from the environment [1].

Arsenic is the most dangerous pollutants because practically all of its compounds have high toxicity, and they can cause various pathologies, including cancer diseases [2, 3].

On the territory of Georgia during Soviet Union period, intensive work was carried out for the mining and production of arsenic, which today has led to a high level of pollution with dangerous wastes [4].

Preventing of natural waters and soils from

pollution in potentially contaminated regions, evaluation of pollution and health risks, to provide ecosystem enhancement and population safety, is extremely important for the reality of Georgia.

In modern ecological biotechnologies, which means cleaning the environment polluted by chemicals, the most effective is phytoremediation, from economically and ecologically point of view, which means to develop selected plants in polluted regions [1]. It is necessary to choose plant for phytoremediation according to the type of chemical contaminant, and characteristics of polluted environment. It is also necessary to study the level of pollution, chemical and physical characteristics of soil, and condition of soil microbiota. This factor is important due to the fact, that microorganisms have already established consortium, which on the one hand is adapted to exist in polluted environment and on the other hand to determine conditions for development and growth of plants. In some cases, they cause transformations of contaminants, which increases ability of plants to assimilate pollutants.

The aim of the presented work is to establish the basis of cleaning technology for arsenic-contaminated soils in Georgia. To achieve this aim, the selected microorganisms and plants for phytoremediation of soil polluted with arsenic have been tested.

## Materials and methods

The model experiments were carried out to test the phytoremediation method for clean-up of artificially contaminated soils with arsenic. For testing the following plants - fern (*Pteris vittata*) and sunflower (*Helianthus annuus*) have been used.

The soil has been artificially contaminated by the following way: carbonate-type soil was sieved into a 2 mm sieve and then dried in the air. Then arsenate or arsenite containing solutions were added to the soil. The initial contamination was 100 or 200 ppm. 100 g of each soil sample was placed in a 100 ml plastic cup in which the plant was sown.

In some variants, 10 ml of suspension of relevant bacteria was added to the soil which were extracted from the Georgian soils contaminated with arsenic, based on the results obtained using

the biochip [5]. They have the ability to participate in the oxidation-reduction of arsenic compounds, in particular, these are *Shewanella spp.* (SH04) and an unknown strain X02, which could not be identified at this time.

Each model experiment was carried out in 4 variants:

1 - control in which the contaminated soil was placed.

2 - contaminated soil in which the plant was sown.

3 - contaminated soil in which was added bacterial strains.

4 - contaminated soil with bacterial strains in which the plant was sown.

The duration of model experiment was 37 day-night, temperature 20-25°C; artificial lighting – 14L/10D.

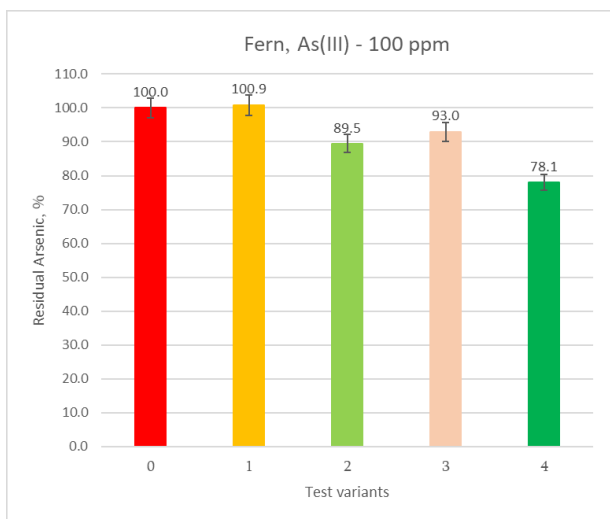
After incubation from soil samples were removed the plant samples, shredded, dried and was determined the arsenic concentration by ICP-AES method ISO 11885: 2007; EPA 200.2 [6].

## Results and discussion

Model experiments were carried out to test the suitability of plants for phytoremediation of artificially contaminated soils with different forms of arsenic ( $As^{+3}$  and  $As^{+5}$ ). In the experiments besides to plants were used bacteria of the genus *Shewanella* isolated from soils of Georgia contaminated with arsenic, which during the study showed the ability to participate in the oxidation-reduction of arsenic compounds.

Model experiments on ferns and sunflowers have shown that these plants can grow in soils contaminated with both arsenite and arsenate. It should also be noted that these plants grew better on contaminated soils in which bacteria were present. It seems that under the influence of these strains, arsenic transfer into a less toxic form in the soil, which increases plant tolerance.

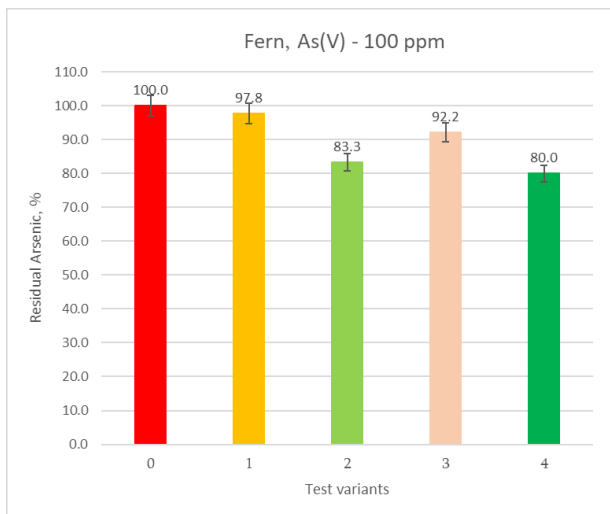
Determination of arsenic content in the soil after plant growth showed that fern insignificantly - by about 20% decreased soil contamination in the case of both  $As^{+3}$  and  $As^{+5}$  (Figs.1 and 2).



**Fig. 1.** Model experiment: clean-up of the soil artificially contaminated with arsenic by fern. Initial pollution - 100 ppm (calculated as  $As^{+3}$ ). Duration - 37 days, temperature 20-25 °C; artificial lighting - 14L/10D.

Test variants:

- Initial contamination
- Control variant (without plant and bacteria)
- Plants without bacteria
- Bacteria without plant
- Plants and Bacteria.

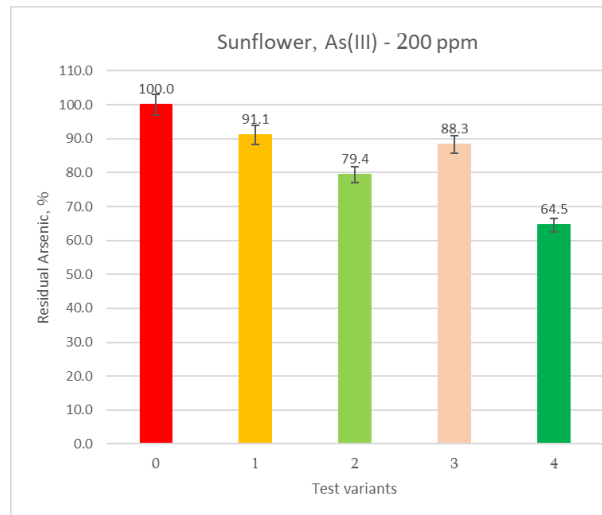


**Fig. 2.** Model experiment: clean-up of the soil artificially contaminated with arsenate by fern. Initial pollution - 100 ppm (calculated as  $As^{+5}$ ). Duration - 37 days, temperature 20-25 °C; artificial lighting - 14L/10D.

Test variants:

- Initial contamination
- Control variant (without plant and bacteria)
- Plants without bacteria
- Bacteria without plant
- Plants and Bacteria.

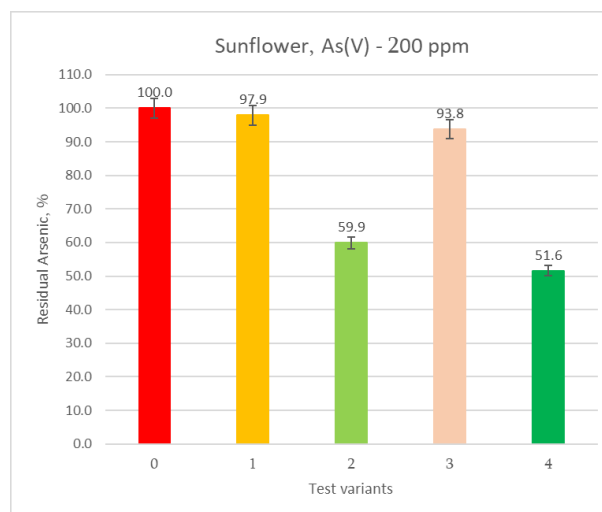
As for sunflower, in these conditions it performs the role of phytoremediator much better: it decreases arsenic content in soil treated with bacteria at 200 ppm contamination with  $As^{+3}$  by about 35% (without bacteria by 20%) (Fig. 3) and in case of contamination with  $As^{+5}$  - by almost 50% (without bacteria by 40%) (Fig. 4).



**Fig. 3.** Model experiment: clean-up of the soil artificially contaminated with arsenite by sunflower. Initial pollution - 200 ppm (calculated as  $As^{+3}$ ). Duration - 30 days, temperature 20-25 °C; artificial lighting - 14L/10D.

Test variants:

- Initial contamination
- Control variant (without plant and bacteria)
- Plants without bacteria
- Bacteria without plant
- Plants and Bacteria.



**Fig. 4.** Model experiment: clean-up of the soil artificially contaminated with arsenate by sunflower. Initial pollution - 200 ppm (calculated as  $As^{+5}$ ). Duration - 30 days, temperature 20-25 °C; artificial lighting - 14L/10D.

Test variants:

- Initial contamination
- Control variant (without plant and bacteria)
- Plants without bacteria
- Bacteria without plant
- Plants and Bacteria.

Thus, among the plants tested in the experiments, the most effective was sunflower, which can effectively uptake both  $As^{+3}$  and  $As^{+5}$  forms of arsenic from contaminated soil. Sunflower increased the degree of purification of soil contaminated with arsenic if the soil is enriched with bacterial strains that have the oxidizing-reducing ability of arsenic containing compounds.

Model experiments have shown that these microorganisms promote the uptake of arsenic by ferns and sunflowers while growing on contaminated soil, which allowing the development of phytoremediation technology.

## Acknowledgment

This work was supported by Shota Rustaveli National Science Foundation (SRNSF) - Applied Research Grants Program CARYS #19-179.

## References

- [1] G. Kvesitadze, G. Khatisashvili, T. Sadunishvili, J.J. Ramsden. Biochemical Mechanisms of Detoxification: Basis of Phytoremediation, Springer, 2006.
- [2] M.V. Gagoshidze, M.O. Antelava, A.G. Zedginidze, N.Sh. Mandzhavidze. Dependence of child health on the ecology, Georgian Medical news, 118 (2005) 49-52.
- [3] IARC monographs: Arsenic and arsenic compounds.– 100C, (IARC, 1980, 1987, 2004) <https://monographs.iarc.who.int/ENG/%20Monographs/vol100C/mono100C-6.pdf>
- [4] R.I. Gigauri, Sh. S. Japaridze, T.B. Gogiberidze, N.S. Khavtasi. The study of the toxicity level of soils contaminated with Arsenic in Racha and Svaneti, Annals of agrarian science, 12 (2014) 42-44 (in Georgian).
- [5] G. Khatisashvili, T. Varazi, M. Kurashvili, M. Pruidze, E. Bunin, K. Didebulidze, T. Butkhuzi, E. Bakradze, N. Asatiani, T. Kartvelishvili, N. Sapojnikova, Remedial Approaches against Arsenic Pollution (July 2nd 2021). Remedial Approaches against Arsenic Pollution [Online First], IntechOpen, DOI: 10.5772/intechopen.98779. <https://www.intechopen.com/online-first/77387>.
- [6] J.T. Creed, T.D. Martin, S.E. Long. Method 1640 – Determination of trace elements in waters and wastes by inductively coupled plasma – Mass Spectrometry, 1997.



## Annals of Agrarian Science

Journal homepage: <http://journals.org.ge/index.php>



# Estimation of biomass and carbon stock of components of subalpine birch forest of Georgia

G. Vachnadze\*, Z. Tiginashvili\*, G. Tsereteli\*\*, B. Aptsiauri\*

\*Vasil Gulisashvili Forestry Institute of Tbilisi State University, \*\*Micheil Sabashvili

Institute of Soil Science, Agrochemistry and Melioration Kakha Bendukidze campus, 240,

David Aghmashenebeli Alley, Tbilisi, 0159, Georgia

Received: 25 June 2021; Accepted: 05 September 2021

## ABSTRACT

In Georgia birch is spread in the mountain subalpine belt on the altitudes 1,800 - 2,250 m above sea level, mostly in the form of subalpine sparse crooked forests of Litvinov birch (*B.litwinowii* A.Dol), as well as common birch (*B.verrucosa* Ehrh). The paper considers the biomass (2,628.5 Gg) of the forest stand and the subordinate layer (young, undergrowth, a living cover of the forest) of the subalpine birch forests and carbon stock (1,298.5 Gg C), sequestered in it, their distribution by age groups and fractions of the forest. The carbon stock of dead organic mass (dead standing trees and fallen dry branches, dead forest cover). Mean annual increment of carbon in 63,781 ha of subalpine birch forests of Georgia.

**Keywords:** forest stand, age group, fraction, young, undergrowth, living cover, dead cover, soil.

\*Corresponding author: G. Vachnadze, E-mail address: [givi.vachnadze@gmail.com](mailto:givi.vachnadze@gmail.com)

## Introduction

Today the biological productivity of forests is determined as their main characteristic that taking into account the global change determines processes in forest ecosystems and is used in ecological monitoring, simulation of forest productivity [1-3], estimation of a diversity of forest structure, and forest cover, carbon sequestration of forests [4-8].

The biological productivity of forests is determined by climatic factors, but there are also ontogenetic, cenotic, edaphic, and other factors of its variability [9]. Biological productivity varies according to the horizontal as well as the vertical zoning of the Earth. There is a statistically significant increase in both above-ground biomass and net primary product in birch forests from the north (middle taiga) - 113 Mg $\cdot$ ha $^{-1}$  to the south (broadleaf and steppe sub-zone) - 225-283 Mg $\cdot$ ha $^{-1}$ ,

but further southward (Caucasus and Asia Minor regions) the total biomass of birch forests decreases to 56 Mg $\cdot$ ha $^{-1}$  [9, 10].

Climate change with the tendency of warming on Earth has caused the raising of the upper boundary of the spread of forest species in the mountains. Analysis of aerial photographs and landscape photographs, as well as a description of the upper forest line, revealed that between 1956 and 2005, during the last 50 years, the composition and structure of the northern Ural Mountains changed and the forest line has raised by 40 meters [11, 12]. Data from space in the Caucasus Reserve revealed a raising tendency of the upper limit of the distribution of subalpine deciduous forests.

The growing threat of global warming on Earth has significantly increased the role of the forest as one of the important reservoirs for the absorption and sequestration of atmospheric carbon in the gaseous

interchange process of carbon. In this regard, the determination of carbon stocks sequestered from the atmosphere by subalpine forests, including subalpine birch forests, is a very topical but little-studied issue and requires proper attention. Their study is important in terms of both vegetation dynamics of the upper forest line in the mountains and their biosphere role.

The subalpine forests of Georgia are represented by crooked, sparse groves and shrubs. Their species composition includes the following plant formations: beech, maple, birch, pine, spruce and fir (Western Georgia) and evergreen azalea groves, Pontic azalea thickets, junipers.

Birch (*Betula*) is a representative of the boreal zone of forest vegetation in the northern hemisphere. In Georgia, in conditions of moderate climate, it is spread in the mountain subalpine belt, on altitudes from 1,800 m to 2,250 m above sea level in the form of subalpine sparse groves and crooked trees (crooked trunk is a result of prolonged exposure of a large mass of snow on birch trunks and rooting of decumbent trunks on the ground). The climate is cold, with short cool summers and harsh long winters. The average annual temperature is 3.2-4.1°C. The duration of the vegetation period is three to four months. Subalpine birch forest is a forest formation that is above all other formations in the mountains. It is an important biogeographical element of the upper forest line in the mountains.

The subalpine belt of Georgia by its environmental conditions and vegetative cover is heterogeneous. Geobotanists [13-19] distinguish two sub-belts in the subalpine belt: the lower sub-belt, which extends from 1,800 m to 2,200-2,300 m above sea level, the vegetation of which is complex and is represented by beech trees (*Fagus orientalis* Lipsky), highland maples (*Acer trautvetter* Medw.), highland oak woods (*Quercus macranthera* F. et M.), rowan (*Sorbus caucasigena* Kom.) in the form of sparse groves and crooked trees of Litvinov birch

(*Betula Litvinowii* A. Doluch.). In Eastern Georgia (in conditions of semi-dry climate) Sosnovski pine (*Pinus sosnovskyi* Nakai.) is added to them, and in Western Georgia (in conditions of moderately humid climate) alpine oaks are replaced by spruce and fir groves (*Picea orientalis* L., *Abies nordmanniana* (Stev.) Spach).

The upper sub-belt of the subalpine belt, up to altitudes 2,200-2,500 m above sea level, is represented by mesophilic high-grass formations and evergreen azalea groves, in the form of bound thick undergrowth.

Five types of birch grow in Georgia. Medvedev birch (*B.medvedewii* Rgl) grows in the upper mountain belt and subalpine crooked forests in Adjara-Guria; also in the Lesser Caucasus [13, 20], Megrelian birch (*B.megrelica* D. Sosh) is the endemic species of Kolkheti, is also found in the forests of Samegrelo and Svaneti; Radde birch (*B. raddeana* Trantzy) - in the central and eastern part of the northern slopes of Caucasus. All three species of birch are spread in small areas. Litvinov birch (*B.litwinowii* A. Dol) is the most spread species, widely represented on the slopes of the Caucasus and southern highland mountains, as well as common birch (*B.verrucosa* Ehrh). Their distribution areas coincide with each other [21]. In Georgia, subalpine birch forests are often mixed with single trees or small groups of rowan (*Sorbus caucasigena* Com. et Gatch), highland oak (*Quercus macranthera* F.et M.), and on relatively moist slopes - mountain maple (*Acer trautvetteri* Medw.), in western Georgia, not infrequently, crooked beech. Litvinov birch is characterized by good seed regeneration on open slopes, covered with stones and loose rocks, where it forms sparse stands; it also well regenerates by sprouts.

During the forest inventory, the arboreal areas and timber volumes in Georgia are presented in a form of cumulative birch - Litvinov birch forests, as the most common species. The total area of birch forests is 63,781 hectares, with a timber volume of 3,105.4 thous. m<sup>3</sup> (Tab. 1).

**Table 1.** Distribution of areas and timber volume of birch forests spread in Georgia by age groups, ha/thous.m<sup>3</sup>, as of Jan. 1, 2003,\*

Area/ stock, ha/ thous. m <sup>3</sup>	Age group, ha/thous. m <sup>3</sup>					Total average increment, thous. m <sup>3</sup>	Average age, yr.
	Young, I-II cl	Average age	Ripening	Mature and older than mature			
				In total	In their number, oldest		
63,781	845	40,510	11,156	11,270	617	64.8	46
3,105.4	14.5	1,554.8	678.6	857.5	67.1		

\*Unified registration of the Georgian State Forest Fund has not been conducted since 2003. Currently, since 2017, a complete inventory of Georgian forests is being underway with the support of the German government.

## OBJECTIVES AND METHODS

### a) determination method of biomass and carbon stock of the forest stand

Phytomass and carbon stock in birch cenoses were assessed by the conversion-volume method [22, 23]. The conversion-volume coefficient is the ratio of phytomass ( $B - \text{Mg}\cdot\text{ha}^{-1}$ ) of a separate fraction of the forest (stem, branch, leaf, root) to the volume of stem wood (with bark) ( $M - \text{m}^3\cdot\text{ha}^{-1}$ ).

First, the wood biomass was determined by the equation  $B_{\text{st}} = M_{\text{st}} \times D$ , where  $B_{\text{st}}$  is the biomass of timber (with bark) in tons,  $M_{\text{st}}$  is the stem volume in  $\text{m}^3$ , and  $D$  is the basic density of birch (for birch  $D = M_o / V_{\text{wet}} = 0,56 \text{ t/m}^3$ )

The method is based on the state materials of forest inventory. The data on areas of birch forests and volume of timber (stem), for different age groups, have been found according to the available materials of forests management and the “United Indices of Inventory of Forest Fund” of the Forestry Department of Georgia [24] and also according to the Guidelines for Effective Management for the Forestry Sector [25] and the Guidelines of the National Greenhouse Gases Inventory [26].

We do not have any birch timber evaluation tables or biomass database in Georgia, therefore, in the percentage distribution of biomass of birch forest fractions, we use the “conversion ratios”, converting timber volumes to the biomass of the forest fractions of the birch forests in the southern zone of Russia [22].

The mean annual increment in biomass volume of subalpine birch forests was determined by the ratio of living biomass of the forest stand and the subordinate layer for the average age of the forest, with the formula:

$$G = B_{\text{total}} / A, \text{ where}$$

$G$  – mean annual increment of the total biomass of birch forest,  $\text{Mg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ ;

$B_{\text{total}}$  - living biomass of birch forest (without leaf and forest grass cover),  $\text{Mg}$ . According to research materials;

$A$  – average age of birch forests, year. From materials of inventory.

### b) determination method of biomass and

### carbon stock of the subordinate layer of the subalpine birch forest

Inventory materials include: species composition of young growth and undergrowth on sample areas and their number; dominant species of living soil cover, percentage of its coverage; thickness of dead soil cover and the degree of coverage, but their biomass and sequestered carbon stock that is of interest to us, are not available. The mentioned issue has been studied on the example of the Lagodekhi State Reserve, where subalpine forests are distributed in the subalpine belt of the southern slopes of the Greater Caucasus from 1,800 m to 2,200 m above sea level. The area of subalpine forests has been greatly reduced due to prolonged and strong anthropogenic impact on the forest (unsystematic felling, uncontrolled grazing of livestock in the forest). Only small groves and fragments of crooked birch groves and birch-rowan groves reach the natural upper forest line (about 2,500 m above sea level) [20]

We have used the results, obtained in the research according to the 2013 grant of Shota Rustaveli National Scientific Foundation of Georgia: “Determination of carbon ( $\text{C-CO}_2$ ) stocks sequestered in the vegetative cover of Lagodekhi State Reserve” on subalpine birch forests [27]. In the Lagodekhi Reserve, birches are represented by crooked trees of Litvinov birch (*Betula litwinowii*) and they occupy 128 ha, with a small timber volume - 23 thous.  $\text{m}^3$ . 8 sample areas are taken there, each with an area of 1000  $\text{m}^2$ . Also, 6 sample areas from the inventory materials were analyzed.

In determining the total phytomass of the forest, it is necessary to evaluate the biomass and carbon stock sequestered in all the morphological components of the forest: the forest stand, undergrowth, young growth, living soil cover, as well as dead soil cover, dead standing trees, and fallen deadwood. Species composition, number, and dendrometric indicators were determined on the sample areas. Average model trees were selected, from which average samples of stem and branch (leaf) were taken to determine biomass, taking into account their number per hectare.

Living and dead soil cover was described on the sample areas. The type, thickness, and degree of coverage of the dead soil cover were determined.

Biomass of living soil cover and carbon sequestered in it were determined by weight method of grass mass mowed on discount areas ( $1 \times 1$  m), taking into account the moisture content and the percent of coverage of grass cover. The stock of biomass of dead soil cover was determined by drying the samples (number 10) taken by metal molding blocks ( $25 \times 25 \times 20$  cm) on the sample areas to absolute dry weight; by converting their average weight per hectare and the percent of coverage of dead cover.

### c) determination method of the biomass of organic substances and volume of sequestered carbon in soils of subalpine birch forests

Morphological description of soil cuts taken from the sample areas was performed; Soil density was determined in samples taken from genetic horizons [28-31]. Granulometric and chemical analyzes have been performed; according to these characteristics, the stock of organic matter in the soil was determined by the formula:

$$C_{soil} = a \times 10\,000 \times b \times p/100, \text{ where}$$

$C_{soil}$  – humus stock, t/ha

a- thickness of soil layer, m

b- density of soil layer, g/cm<sup>3</sup>

p- humus content, %

## RESULTS AND ANALYSIS

### 1. Estimation of biomass and carbon dioxide stock in the forest stand of the subalpine birch forests

One of the important issues in the study of the regularity of development of forest biogeocenoses is the assessment of phytomass and carbon stocks of all forest components and its fractions; distribution of stocks by age groups; increase the carbon capacity of forests; maintaining climate resilience by slowing down the ongoing warming process on the planet. The results of scientists working on this issue are reflected in the publications [32-37].

The stock of biomass of the forest stand in the forest and the amount of carbon sequestered in it directly depends on the species composition of the forest, the age of the forest, the taxation frequency, the quality class - in total, on timber volume. Using cameral treatment of field materials, inventory materials, and appropriate Tables, the biomass and carbon stocks in subalpine birch forests are determined by forest age groups and regions (Table 2) and forest fractions and age groups (Table 3).

**Table 2.** Biomass and carbon stock of the birch forests of Georgia by age groups and region (B/C in Gg)

Territorial forestry service (region)	Total area/stock ha/thous. m <sup>3</sup>	Age group, B/C					In total
		Young	Average age	Ripening	Mature	Older than mature	
Eastern Georgia							
Kakheti	3 384	-	33.85	17.84	36.48	1.91	90.08
	119.4	-	16.89	8.9	18.2	0.95	44.94
Kvemo Kartli	85	-	0.79	-	0.44	-	1.23
	1.6	-	0.39	-	0.22	-	0.61
Mtskheta-Mtianeti	14 159	0.88	201.94	165.66	215.43	-	583.91
	775.5	0.43	100.72	82.67	107.47	-	291.29
Shida Kartli	8 188	0.61	188.34	56.22	56.96	34.42	336.55
	439.9	0.3	93.93	28.06	28.41	17.18	167.88
Samtskhe-Javakheti	6 428	1.22	67.95	64.89	145.55	3.89	283.5
	379.3	0.61	33.89	32.38	72.6	1.93	141.41
In total, East. Georgia	32 244	2.71	492.87	304.61	454.86	40.22	1 295.27
	1 715.7	1.34	245.82	152.01	226.9	20.06	646.13
Western Georgia							
Imereti	29	0.17	1.03	-	-	-	1.2
	1.5	0.09	0.51	-	-	-	0.6
Guria				is not spread			
Racha-Lechkhumi-Kvemo Svaneti	4 098	0.17	61.62	10.86	15.49	10.72	98.86
	128.6	0.09	30.73	5.42	7.72	5.34	49.3

Samegrelo-Zemo Svaneti	24 857	8.74	611.28	173.81	103.57	0.07	897.47
	1 160.8	4.33	304.87	86.73	51.66	0.04	447.64
Abkhazia A.R.	2 343	0.52	62.17	2.06	1.54	-	66.29
	84.1	0.26	31.01	1.02	0.77	-	33.06
Adjara A.R.	210	0.35	0.87	6.75	2.94	-	10.91
	14.7	0.17	0.43	3.37	1.47	-	5.44
In total, West. Georgia	31 537	9.95	736.97	193.48	123.54	10.79	1 074.73
	1 389.7	4.94	367.55	96.549	61.62	5.37	536.04
Georgia	63 781	12.66	1 229.84	498.09	578.4	51.01	2370
	3 105.4	6.28	613.37	248.56	288.52	25.44	1 182.17
Percentage distribution of carbon		0.5	51.9	21.0	24.4	2.2	100

The total area of the subalpine birch forest in Eastern Georgia is 32,244 hectares, with a biomass supply of 1,295.27 Gg, with the largest share coming from the Dusheti region of Mtskheta-Mtianeti Forest Service - 13,976 ha, with a biomass supply of 570.12 Gg. The total biomass supply of Mtskheta-Mtianeti birch forests was determined at 583.91 Gg, carbon stock - 291.29 Gg, which is 45.1% of the total biomass and 52.1% of the carbon stock of birch forests of Eastern Georgia. In the birch forests of Eastern Georgia, the average aged (492.97 Gg) and mature (9,454.86 Gg) stands are dominant. In the subalpine birch forests, the biomass of the older than mature and young birch forests is small, sometimes of insignificant quantity.

In Western Georgia, the birch forests are

spread on 31,537 hectares. 78.8% of them (24,857 ha) accounts for Samegrelo-Zemo Svaneti region: in particular, 15,604 ha for Upper Svaneti and 9,041 ha - Lentekhi district. The total biomass supply of birch forests of Samegrelo-Zemo-Svaneti is 897.47 Gg with 447.64 Gg of carbon sequestered in them. The birch forests of Adjara A.R. are characterized by the smallest area (210 ha) and consequently with the smallest biomass - 10.91 Gg, but are distinguished by high productivity, 52.0 t/ha per unit area when the supply of the rest of the region was 34.1 t/ha. The average age stands prevail in birch forests by biomass supply and carbon stock - 51.9%. The biomass supply is small in two age groups of birch forests: young growth (0.5%) and older than mature (2.2%).

**Table 3.** Distribution of biomass supply and carbon stock of birch forests of Georgia by fractions and age groups (B/C in Gg)

Age group, duration, year	Total area/stock ha/thous. m3	Fractions, B/C				
		Stem	Branch	Leaf	Root	In total
Young 1-20	845	7.19	1.75	1.05	2.67	12.66
	14,5	3.6	0.88	0.47	1.33	6.28
	% of biomass	57 %	14 %	8 %	21 %	100 %
Average age 21-50	40,510	838.04	97.96	31.08	262.76	1 229.84
	1,554.8	419.01	48.98	14	131.38	613.37
		68 %	8 %	2 %	21 %	100 %
Ripening 51-60	11,156	363.73	41.4	9.5	83.46	498.09
	678.6	181.86	20.7	4.27	41.73	248.56
		73 %	8 %	2 %	17 %	100 %
Mature 61-80	10,653	418.43	55.95	13.4	90.62	578.4
	788	209.22	27.97	6.03	45.3	288.52
		73 %	10 %	2 %	16 %	100 %
Older than mature 80 and >	617	36.9	4.93	1.19	7.99	51.01
	69.5	18.45	2.46	0.52	4.01	25.44
		73 %	10 %	2 %	16 %	100 %

In total	63,781	1 664.29	201.99	56.22	447.5	2 370
	3,105.4	832.14	100.99	25.29	223.75	1,182.17
Percentage distribution of biomass		70.2	8.5	2.4	18.9	100 %

In determining the biomass supply and carbon stock in forest ecosystems, special importance is given to the morphological-structural distribution of individual forest fractions by fractions [12, 22, 32, 38, 39], modeling their dynamics [37]. Percentage distribution of fractions and the number of their stocks are mainly determined by forest species composition, genetic-morphological characteristics of the species [40, 41], as well as the age structure of the forest, forest density, climatic-soil conditions [12, 37, 42-44].

The percentage of biomass distribution of fractions in the total biomass of the subalpine birch forests of Georgia significantly differs from all other deciduous trees. The biomass share of the stem of crooked birches in total biomass is 70.2%, which is 7-12% higher than in other deciduous trees. And the share of biomass supply of branches in total biomass is about 2 times smaller and amounts to 8.5%, instead of 16% for other deciduous trees on average. The share of leaf biomass is high, which amounts to 2.4% of the total biomass, in other deciduous trees its supply is in the range of 1.5-2.1% [41, 45-47].

The biomass supplies of their fractions in the subalpine birch forests of Georgia are very small. This is due to the harsh living environment of the subalpine birch forests. Their supplies are the smallest compared to other deciduous species. For example, the average supply of total biomass in birch forests is 2,370 Gg (37.2 Mg·ha<sup>-1</sup>). Their biomass supply of stem is 26.1 Mg·ha<sup>-1</sup>, of a branch - 3.2 Mg·ha<sup>-1</sup>, of a leaf - 0.9 Mg·ha<sup>-1</sup>, and of root biomass - 7.0 Mg·ha<sup>-1</sup>. The biomass supplies of other deciduous fractions are significantly higher. For comparison, biomass supplies of beech forests are the highest. Their total biomass is 191.3 Mg·ha<sup>-1</sup>. According to the fractions, their supplies are: stem - 121.0 Mg·ha<sup>-1</sup>; branch - 31.0; leaf - 3.0; root - 36.3 Mg·ha<sup>-1</sup> [47]. The biomass supplies and carbon stocks are also high in the other deciduous species spread in the subalpine zone. The highest is the biomass of highland oaks, their supply is 95.0 Mg·ha<sup>-1</sup>. According to the fractions, their supplies are: stem - 59.4 Mg·ha<sup>-1</sup>; branch - 15.9; leaf - 2.0; root - 17.7 Mg·ha<sup>-1</sup>. The biomass of highland maple fractions is also high: total biomass - 91.4 Mg·ha<sup>-1</sup>, in their number stem - 56.7 Mg·ha<sup>-1</sup>; branch - 14.4;

leaf - 3.3; root - 17.0 Mg·ha<sup>-1</sup> [6, 7, 22, 27, 41].

The total biomass of birch forests growing on 63,781 hectares in Georgia has been determined at 2,370.0 Gg. They store 1,182.17 Gg of carbon, which corresponds to 4.33 Tg of CO<sub>2</sub> absorbed from the atmosphere.

## 2. Estimation of biomass and carbon stock in the subordinate layer of birch forests of Georgia

The vegetative cover of the subordinate layer plays a central role in the formation of the structure and composition of the forest ecosystem. Its participation and abundance provide the basic elements of biodiversity [48, 49], metabolism [50-53] and forms the future timber structure and diversity [54-56].

According to the data available in the forest sources, the rates of biomass and carbon stocks of young growth, undergrowth, and living soil cover in the forest vary greatly [46, 54-59]. Species composition of young growth and undergrowth in the subordinate layer, their quantity and biomass indicators depend not on the timber supply of the stand, but mainly on forest species composition, forest fruiting period, degree of crown closure (light factor), on the conditions of the locality of plant growth - environmental conditions in which the phytocenosis developed. The same can be said about the species composition of the living soil cover and the degree of their coverage in the forest. Therefore, their biomass and carbon stock values are highly variable over time and are related to environmental conditions. The range of change in the number of their biomass supplies per hectare is very wide, from a few kilograms to several tens of tons. Therefore, the rate of biomass supplies and carbon stocks of young growth, as well as of undergrowth and living soil cover is not a constant value.

It should be noted that the generalization of the data of biomass and carbon stocks of the **subordinate layer** - young growth, undergrowth, living soil cover, obtained at the study of birch forests on such a small area of 128 hectares and one location (on the example of Lagodekhi Reserve) on birch forests spread in Georgia (especially for the conditions of Western Georgia) does not give convincing results. Thus, we consider it necessary to continue research in this direction on subalpine forests, including subalpine birch forests, to

correct the data for the subordinate layer obtained by us (as the forest element most sensitive to the changing environmental conditions of the forest), to make them more precise.

In determining the living biomass and sequestered carbon stocks in the subordinated

layer of the forest, we used the weighted average values of the young growth and living soil cover of biomass supplies obtained in the study in the pilot birch forests selected by us in the form of reference values (coefficients); accordingly, their coefficients are 0.1 and 0.395 Mg·ha<sup>-1</sup>.

**Table 4.** The biomass and carbon stock of the subordinate layer of birch forests of Georgia B/C in Gg

Young growth			Undergrowth		Living cover			Subordinate layer		
above-ground	below-ground	Total	above-ground	below-ground	above-ground	below-ground	Total	above-ground	below-ground	Total
4.47	1.92	6.39	-----	-----	14.82	10.37	25.19	19.29	12.29	31.58
2.01	0.87	2.88	-----	-----	6.67	4.67	11.34	8.68	5.53	14.21

The phytocenological structure of the subordinate layer of subalpine birch forests is highly peculiar.

**Biomass and carbon of young growth.** Subalpine birch forests are represented by pure stands. Their renewal is mainly vegetative, with a root collar. Thus, in the subalpine birch forest, the young growth is usually represented by young birch, it is seldom mixed with single young trees of highland maple and highland oak. The levels of biomass and carbon for young growth of the subalpine birch forests spread in Georgia on 63,781 ha are not high. Their stocks are 6.39 and 2.88 Gg, respectively. Their stocks per unit area are small and amount to: biomass - 0.1 Mg·ha<sup>-1</sup>, carbon - 0.045 MgC·ha<sup>-1</sup>.

**Biomass and carbon of undergrowth.** The Caucasian rhododendron (*Rhododendron caucasicum*) is distributed in the undergrowth in the form of small fragments, blueberry (*Vaccinium myrtillus*), as well as rowan (*Sorbus aucuparia* L.) are found. The total biomass supply in the pilot crooked undergrowth of birch forests is so insignificant that it is permissible to ignore them [25].

**Biomass and carbon of living soil cover.** It is noteworthy that there is a strong development of high grassland living cover in the subalpine forests, mainly in the plain areas, in the uncultivated grounds in the forest. High grassland is dominated by Alpine dock (*Rumex alpinus* L.), heartleaf oxeye (*Telekia speciosa*), ragwort (*Senecio rhombifolius*), Sosnowsky's hogweed (*Heracleum*

*sosnowskyi*), also common aconite (*Aconitum orientale*), rough comfrey (*Symphytum asperum*) [60-62]. The biomass supplies in the living soil cover of birch forests of Georgia amounts to 25.19 Gg, with 11.34 Gg of sequestered carbon. Their stocks per unit area are 0.395 Mg·ha<sup>-1</sup> and 0.178 Mg C·ha<sup>-1</sup>, respectively.

The carbon dioxide - 52.11 Gg (0.817 Mg CO<sub>2</sub>·ha<sup>-1</sup>) - is absorbed from the atmosphere by the subordinate layer (young growth, undergrowth, living soil cover) of subalpine birch forests of Georgia, this value is bound in 31.58 Gg·ha<sup>-1</sup> (t / ha) of biomass in the form of 14.21 Gg of carbon (Mg C·ha<sup>-1</sup>).

### 3. Estimation of the biomass of dead organic substance and carbon stock in birch forests of Georgia

**Dead standing and fallen biome.** Biomass supplies in the dead standing and fallen biome of the subalpine birch forests were not recorded in the inventory materials, so for its determination, we used the coefficient for calculation of pristine forests from the total forest biomass, proposed by the working group of climate change experts [26], according to which the mass, as well as carbon stocks in dead standing and fallen deadwood, should be 23.7 Gg and 11.85 Gg of C, respectively (see Table 5).

**Dead soil cover.** In the subalpine birch forest, there is a dead cover 2-4 cm thick, its biomass supply is up to 319.85 Gg (5.02 Mg·ha<sup>-1</sup>), and it contains 127.94 Gg (2.01 Mg C·ha<sup>-1</sup>) of carbon, which corresponds to 469.16 Gg of carbon absorbed from the atmosphere. (see Table 5).

**Table 5.** Biomass of dead organic substance and carbon stock in birch forests of Georgia (B/C in Gg)

Area/stock ha/thous. m <sup>3</sup>	Dead standing and fallen deadwood	MgC·ha <sup>-1</sup>	Dead forest cover	MgC·ha <sup>-1</sup>	In total	MgC·ha <sup>-1</sup>
63,781	23.7	0.37	319.85	5.02	343.55	5.39
3,105.4	11.85	0.19	127.94	2.0	139.79	2.19

The total biomass and carbon stock of dead organic matter in the subalpine birch forests are 343.55 and 139.79 Gg, respectively. Here, the carbon bound in the organic matter of dead standing and fallen mass is very small 11.85 Gg of C, and carbon bound in the dead organic mass is only 8.5% of the total carbon stock. None of the species are cut in the subalpine birch forests, thus, the formation of dead standing and fallen timber here is a result of a natural, self-thinning process.

#### 4. Mass of dead organic substance and carbon

**Table 6.** The total humus and carbon stock in the mountain-forest-meadow soil of subalpine birch forests of Georgia (B/C in Gg)

Area, ha	Humus stock		Carbon stock		Absorbed carbon dioxide	
	Mg·ha <sup>-1</sup>	Tg	Mg·ha <sup>-1</sup>	Tg	Mg·ha <sup>-1</sup>	Tg
63,781	227.18	14.49	131.54	8.39	482.43	30.77

The mountain-forest-meadow soils developed under the subalpine birch forests are strongly humous. In the whole soil profile, according to the genetic horizons of the soil (soil depth is 0-58 cm on average), the average weighted supply of organic matter is high, amounting to 14.19 Tg (227.18 Mg·ha<sup>-1</sup>) on average. 8.39 Tg of carbon (131.54 Mg C·ha<sup>-1</sup>) is bound in it, which corresponds to 30.77 Tg of carbon dioxide (482.43 MgCO<sub>2</sub>·ha<sup>-1</sup>) absorbed from the atmosphere.

#### stock in the soil of birch forests of Georgia

Typical mountain-forest-meadow soils are formed in the crooked subalpine birch and beech forests and under the sparse maple groves of Georgia. Soils are characterized by undifferentiated profile, low and medium depth, acid reaction of the whole profile, low degree of saturation, high humus [63-66].

Average values of soil organic matter (humus) obtained by granulometric and chemical analysis of soil samples in pilot birch forests are given in Table 6.

#### 5. Accumulated carbon stocks in the total biomass of birch forests of Georgia by pools

In sequestration of the atmospheric carbon, forest formations are considered to be the most powerful carbon pool. There are three main pools of carbon in the forest: I – in the form of living biomass, II – dead organic matter, and III – soil. Table 7 shows the amount of carbon sequestered in carbon pools and their percentage distribution by pools.

**Table 7.** Carbon stocks in subalpine birch forests of Georgia by pools

<b>I In living biomass</b>	<b>1,196.38 Gg</b>	<b>18.76 MgC·ha<sup>-1</sup></b>	<b>12.30 %</b>
a) forest stand	1,182.17 Gg	18.54 Mg C·ha <sup>-1</sup>	
b) subordinate layer	14.21 Gg	0.22 Mg C·ha <sup>-1</sup>	
<b>II In dead organic mass</b>	<b>139.79 Gg</b>	<b>2.19 Mg C·ha<sup>-1</sup></b>	<b>1.44 %</b>
a. dead standing and fallen deadwood mass	11.85 Gg	0.19 Mg C·ha <sup>-1</sup>	
b) dead forest cover	127.94 Gg	2.0 Mg C·ha <sup>-1</sup>	
<b>III In organic substances of soil</b>	<b>8,390.0 Gg</b>	<b>131.54 Mg C·ha<sup>-1</sup></b>	<b>86.26 %</b>
Total carbon stock	9,726.17 Gg	152.49 Mg C·ha <sup>-1</sup>	100 %

According to carbon pools in the subalpine birch forests, from total biomass, only 12.3% (18.76 MgC·ha<sup>-1</sup>) accounts for I - living biomass, II - 1.4% (2.19 Mg C·ha<sup>-1</sup>) accounts for dead organic matter, III - soil organic matter 86.3%

(131.54 Mg C·ha<sup>-1</sup>). The percentage distribution of their stocks differs markedly from that of both Georgia [47] and European forests (excluding Russian forests) [67].

#### Carbon stocks by pools

	I – Living biomass	II – dead mass	III – soil
Georgian forests	78.0 MgC·ha <sup>-1</sup> 34.2 %	8.8 MgC·ha <sup>-1</sup> 3.5 %	142.3 MgC·ha <sup>-1</sup> 62.3 %
European forests	63.9 MgC·ha <sup>-1</sup> 35.7 %	18.6 MgC·ha <sup>-1</sup> 10.6 %	96.6 MgC·ha <sup>-1</sup> 53.9 %
Subalpine birch forests of Georgia	18.76 MgC·ha <sup>-1</sup> 12.3 %	2.19 MgC·ha <sup>-1</sup> 1.4 %	131.54 MgC·ha <sup>-1</sup> , 86.3 %

**6. Mean annual increment of biomass and carbon in subalpine birch forests.** When considering the process of gas exchange in the forest, it is important to determine the annual volume of atmospheric carbon absorbed by the living forest biomass.

Below, the rates of mean annual increment in biomass and carbon stocks of the forest stand and subordinate layers of the subalpine birch forest, their above-ground and below-ground layers ( without leaf and living forest grass cover) are given in the form of a Table.

**Table 8.** Mean annual increment of biomass and carbon stock of subalpine birch forests (Mgha<sup>-1</sup>yr<sup>-1</sup>)

Forest forming species, average age, area, ha	Forest layer	Living biomass and carbon stock, Gg	Mean annual increment of living biomass and carbon stock, Mgha <sup>-1</sup> yr <sup>-1</sup>		
			above-ground	below-ground	In total
Subalpine birch forest, average age 46 yr. 63,781 ha	Forest stand	2,313.78	0.636	0.153	0.789
		1,156.88	0.318	0.76	0.394
	Subordinate	6.30	0.003	0.002	0.005
		2.84	0.0014	0.0006	0.002
	In total, phytocenosis	2,320.08	0.639	0.155	0.794
		1,159.72	0.319	0.077	0.396

According to the forest inventory materials, the average annual age of subalpine birch forests in Georgia is 46 years. In the subalpine zone, birch forests developed during the vegetation period of 3-4 months are characterized by low productivity. We have determined the total volume of live biomass and carbon stock in the forest stand and subordinate layer of birch forests at 2,320.08 Gg (36.38 Mgha<sup>-1</sup>) and 1,159.72 Gg (18.18 MgC·ha<sup>-1</sup>). This does not include carbon bound in their leaf biomass, it is considered to be scattered as soon as it falls. The same can be said for the living (herbage) cover of the forest [25].

The mean annual increment of biomass and carbon stock of subalpine birch forests is not high (Tab.8). The values of mean annual increment of biomass and carbon stocks of forest stand and subordinate layer are small, especially, of the subordinate layer. The mean annual increment is: for the biomass of forest stand - 50.3 Gg·yr<sup>-1</sup>; carbon - 25.15 GgC·yr<sup>-1</sup>; and the subordinate layer, respectively - 0.32 GgC·yr<sup>-1</sup> and 0.14 GgC·yr<sup>-1</sup>. Taking into account the unit area, the mean annual increment in carbon stock sequestered in the biomass of the forest stand and subordinate layer of the subalpine birch forests was 0.394 Mg

$\text{C}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$  and  $0.002 \text{ Mg C}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ , respectively.

The forest ecosystem as a result of photosynthesis not only absorbs carbon dioxide from the atmosphere but at the same time, releases free oxygen in the atmosphere.

$95.37 \text{ GgCO}_2 \cdot \text{yr}^{-1}$  ( $1.5 \text{ MgCO}_2\cdot\text{yr}^{-1}$ ) of carbon dioxide is absorbed annually from the atmosphere during the gas exchange process on 63,781 hectares of subalpine birch forests in Georgia, while  $69.4 \text{ GgO}_2$  ( $1.09 \text{ MgO}_2\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ ) of oxygen are released.

## CONCLUSION

The percentage distribution of biomass and carbon in the individual fractions of the birch forests differs significantly from all other forest forming deciduous trees of Georgia. The share of carbon sequestered in the stem biomass of the subalpine birch forests is 70.4% of the total biomass, which is 7-12% greater than in other deciduous trees, and that of a branch is 2 times smaller and amounts to 8.5%, instead of 16% for other deciduous trees on average. The leaf carbon stock is high, with a share of 2.2% of the total stock. In other deciduous trees, its stock is in the range of 1.5-2.0%. However, the total stock of birch forests per unit area is the smallest compared to other deciduous trees. The average biomass supply in birch forests is  $37.2 \text{ Mg}\cdot\text{ha}^{-1}$ . The biomass supplies of a stem are  $26.1 \text{ Mg}\cdot\text{ha}^{-1}$ , of a branch -  $3.2 \text{ Mg}\cdot\text{ha}^{-1}$ , of a leaf -  $0.9 \text{ Mg}\cdot\text{ha}^{-1}$ , and of a root -  $7.0 \text{ Mg}\cdot\text{ha}^{-1}$ , which is due to the harsh living conditions of the subalpine birch forests.

The principle of percentage distribution of fractions of forest woody species was mainly determined by the genetic-morphological structure formed during their evolutionary development. The difference between biomasses of the tree fraction by species is small, not substantial. The differences are mainly due to the forest density, more precisely to the degree of crown closure.

In total in Georgia, the total supply of above-ground and below-ground biomass of the forest stand of subalpine birch forests growing in Georgia on area 63,781 ha with the age 46 years was determined at 2,370 Gg. The carbon stock sequestered there is  $1,182.17 \text{ Gg C}$  ( $18.53 \text{ MgC}\cdot\text{ha}^{-1}$ ), which corresponds to  $4.33 \text{ Tg CO}_2$  ( $68 \text{ Mg CO}_2\cdot\text{ha}^{-1}$ ) absorbed from the atmosphere.

The phytocenological structure of the subordinate layer of subalpine birch forests

is highly peculiar. It can be said that in the subalpine birch forest formations (especially in crooked birch forests) the undergrowth is not developed. Neither biomass nor carbon stocks of young growth are high – the biomass is  $6.39 \text{ Gg}$  ( $0.1 \text{ Mg}\cdot\text{ha}^{-1}$ ) and carbon –  $2.88 \text{ Gg}$  ( $0.045 \text{ MgC}\cdot\text{ha}^{-1}$ ), but the biomass of herbal cover and carbon sequestered in it are high. Accordingly, their stocks are:  $25.19 \text{ Gg}$  ( $0.395 \text{ Mg}\cdot\text{ha}^{-1}$ ) and  $11.34 \text{ Gg C}$  ( $0.178 \text{ MgC}\cdot\text{ha}^{-1}$ ).

The stocks of atmospheric carbon in Georgian birch forests by pools are presented as follows: carbon sequestered in living biomass -  $18.76 \text{ MgC}\cdot\text{ha}^{-1}$ , which is 12.3% of the total carbon stocks; in dead organic matter -  $2.19 \text{ MgC}\cdot\text{ha}^{-1}$ , 1.4%; in the soil -  $131.54 \text{ MgC}\cdot\text{ha}^{-1}$ , 86.3%. Their total stocks in all pools of birch forests are  $9.73 \text{ TgC}$  ( $152.5 \text{ MgC}\cdot\text{ha}^{-1}$ ).

In the harsh climatic conditions of the subalpine zone, mean annual increments of living biomass and carbon stocks of 46-year-old subalpine birch forests developed during the short vegetation period are small. The mean annual increment in carbon stock sequestered in the biomass of the forest stand and subordinate layer of the subalpine birch forests was  $0.394 \text{ MgC}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$  and  $0.002 \text{ MgC}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ , respectively. The average annual increase in carbon deposited in the living biomass of 63,781 hectares in Georgia is  $25.29 \text{ MgC}\cdot\text{yr}^{-1}$  ( $0.396 \text{ MgC}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ ). The mean annual increment of carbon sequestered in the living biomass of the subalpine birch forests growing in Georgia on the area 63,781 ha, amounts to  $25.29 \text{ MgC}\cdot\text{yr}^{-1}$  ( $0.396 \text{ MgC}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ ).

The volume of carbon dioxide absorbed annually from the atmosphere during the gas exchange process in the phytocenoses of subalpine birch forests in Georgia is  $95.37 \text{ GgCO}_2\cdot\text{yr}^{-1}$  ( $1.50 \text{ MgCO}_2\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ ); the volume of the released oxygen is  $69.40 \text{ GgO}_2\cdot\text{yr}^{-1}$  ( $1.09 \text{ MgO}_2\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ ). The mentioned data are the lowest in comparison to the deciduous forests in Georgia.

## Acknowledgments

This work was supported by Shota Rustaveli National Science Foundation of Georgia (SRNSFG) [grant number № FR/217404, Assessment of Carbon Stock Sequestered in Georgian forests, Establishment of Conversion Coefficient]. 2016-2018.

## References

- [1] R.A. Yuknis, Some patterns of tree growth, Text: R.A. Yuknis, Modeling and control of productivity of forest stands, Kaunas: LithAA, (1983) 118–121 (in Russian).
- [2] V. A. Usoltsev, Modeling of the structure and dynamics of the phytomass of forest stands, Krasnoyarsk: Krasnoyarsk University Publishing House, 1985 (in Russian).
- [3] Qi Chen, Modeling above-ground tree woody biomass using national-scale allometric methods and airborne lidar, ISPRS Journal of Photogrammetry and Remote Sensing 106 (2015) 95–106.
- [4] A. Fowler, K. Low, J. Stone. Earth observation for sustainable development: Using space-based technology for monitoring Canada's forests, Information Forestry, Canadian Forest Service, Pacific Forestry Centre, Victoria; British Columbia, (2002) 6-7.
- [5] V.A. Usoltsev, S.V. Zalesov, Methods for determining the biological productivity of plantations, Ekaterinburg: Ural State Forestry University, 2005 (in Russian).
- [6] Z. Tiginashvili, G.Vachnadze, G. Tsereteli, B. Aptsiauri, Separate fractions phytomass of beech of East Georgia and carbon as stocks bio-energy, International Scientific-Practical Conference “Scientific Issues of the Modernity” Proceeding, Gori-Georgia (2014) 188-192 (in Georgian).
- [7] G.Vachnadze, Z. Tiginashvili, G. Tsereteli, Stocks of the biomass of the subalpine forests and the accumulated in them carbon, Bulletin of the Academy of Agricultural Sciences of Georgia, Vol. 33 (2014) 74-78 (in Georgian).
- [8] Raul Ponce-Hernandez, Parviz Koochafkan, and Jacques Antoine. Assessing carbon stocks and modeling win-win scenarios of carbon sequestration through land-use changes, Food and Agriculture Organization of the United Nations, Rome, 2004.
- [9] V.A. Usoltsev, A.A. Malenko, V.A. Azarenok, Biological productivity of birch forests in Eurasia climatic gradients, Bulletin of Altai State Agrarian University, № 9 (143) (2016) 56-63. (in Russian).
- [10] V.A. Usoltsev, Yu.V. Usoltseva, S.V. Zalesov, Geographical Regularities of Birch Phytomass Distribution in Northern Eurasia, Proceedings of higher educational institutions, “Forest Journal”, № 6 (2002) 7-15 (in Russian).
- [11] D.S. Kapralov, S.G. Shiyatov, P.A. Moiseev, et al. Changes in the composition, structure, and altitudinal position of small forests at the upper limit of their growth in the mountains of the Northern Ural, Ecology, no. 6 (2006) 403–409 (in Russian).
- [12] Z. Ya. Nagimov, P.A. Moiseev, A.A. Bartysh, I. V. Rakhmanov, A. A. Grigoriev, Structure and phytomass of birch stands on the upper border of the forest in the northern Ural, Lesnoy Vestnik 3 (2008) 61-68 (in Russian).
- [13] A.G. Dolukhanov, Subalpine crooked forests of the Caucasus, In the book: Academician V.N. Sukachev on the occasion of his 75th birthday. M., (1956) 158-170 (in Russian).
- [14] A. G. Dolukhanov, The upper limits of the forest and the subalpine belt in the Caucasus mountains, In the book: “High Mountain Geocology”. M., (1976) 46-49 (in Russian).
- [15] G. Nakhutsrishvili, Ecology of herbal plants and phytocenoses of high mountains of Central Caucasus, Tbilisi, 1971 (in Georgian).
- [16] N. Zazanashvili, R. Gagnidze, G. Nakhutsrishvili, Main types of vegetation zonation on the mountain of the Caucasus, Proceedings IAYS Symposium, Uppsala, (2000).
- [17] V.Z. Gulisashvili, Features of the growth of woody plants at the upper and lower limits of distribution, Lesovedenie, 3, (1976) 37-43, (in Russian).
- [18] R. Kvachakidze, Some issues of the study of vertical zonation of vegetation of Georgian forest, Bull. of Georgian Botanical Society, vol.8 (1988) (in Georgian).
- [19] R. Kvachakidze, History of the vegetative cover of Georgia, Tbilisi, 2002 (in Georgian).
- [20] R. Kvachakidze, Geobotanical Regions of Georgia, Publishing house “Metsniereba”, Tbilisi, 2010 (in Georgian).
- [21] V.Matikashvili, Georgian Soviet Encyclopedia, Tbilisi, vol.1 (1975), p. 612 (in Georgian).
- [22] D.G. Zamolodchikov, A.I. Utkin, G.N. Korovin, Specification of the carbon stock according to the conservative-volumetric coefficients of age-dependent plantations, J. Forest Science 3 (1998) 84-93 (in Russian).
- [23] A.I. Utkin, D.G. Zamolodchikov, A.A. Pryazhnikov, Methods to define carbon sequestration, phytomass, and net productivity of forests (as exemplified by the Republic of

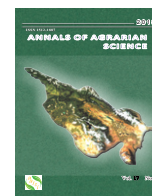
- Belarus). *J. Forest Science*, 1 (2003) 48-57 (in Russian).
- [24] Forest Fund, united registration indicators, The State Department of Forestry of Georgia, Tbilisi, 2005 (in Georgian).
- [25] Guidelines of the IPCC for Efficient Practice of the Land Utilization and Forestry Management sector, IPCC (2003) chapter 3, 3.1-3.199 (in Russian).
- [26] Guidelines of National Inventorying of Greenhouse Gases, IPCC, (2006) Chapter 4, forest areas, 4.1-4.89 (in Russian).
- [27] Z.Tiginashvili, Determination of carbon (C-CO<sub>2</sub>) stocks sequestered in the vegetative cover of Lagodekhi state reserve, SRNSFG grant #31/35 (2013), (in Georgian).
- [28] W. A. Adams, The effect of organic matter on the bulk and true densities of some uncultivated podzolic soils, *Journal of Soil Science*, Vol. 24, No. 1, (1973) 10-17.
- [29] L.K. Mann, Changes in soil carbon after cultivation, *Soil Science* 142 (1986), 279-288.
- [30] M. Bernoux, D. Arrouays, C. Cerri, B. Volkoff, C. Jolivet, Bulk densities of Brazilian Amazon soils related to other soil properties, *Soil Sci. Journal*, Vol. 62 (1998) 743-749.
- [31] M. B. Bernoux, M.D.S. Carvalho, B. Volkoff, C.C. Cerri, Brazil's soil carbon stocks, *Soil Science Society of America Journal* 66 (2002) 888-896.
- [32] V. A. Usoltsev, Growth and structure of phytomass of forest stands, Novosibirsk: Nauka, 1988 (in Russian).
- [33] A. S. Isaev, G. I. Korovin, A.I. Utkin, et al., Estimation of stocks and annual sequestration of carbon in the phytomass of forest ecosystems in Russia, *Lesovedenie*, no. 5 (1993) 3-10 (in Russian).
- [34] A.I. Utkin, D.G. Zamolodchikov, T.A. Gulbe, Ya. I. Gulbe, Allometric equations for phytomass from the data of pine, spruce, birch, and aspen trees in the European part of Russia, *Lesovedenie*, no. 6 (1996) 36-46 (in Russian).
- [35] Andrei Lapenis, Anatoly Shvidenko, Dmitry Shepaschenko, Sten Nilsson, Anantha Aiyyer, Acclimation of Russian forests to recent changes in climate, *Global Change Biology*, 11 (2005) 2090-2102.
- [36] H. Poorter, K.J. Niklas, P.B. Reich, et al., Biomass allocation to leaves, stems, and roots: meta-analyses of interspecific variation and environmental control, *New Phytologist*, Vol. 193 (2012) 30-50.
- [37] Yu. P. Demakov, A. S. Puryaev, V. L. Chernykh, L. V. Chernykh. Using allometric dependencies for assessing the phytomass of various tree fractions and modeling their dynamics, *Bulletin of the Volga Region State Technological University. Ser.: Forest. Ecology. Nature Management*, No. 2 (26), (2015) 19-36, (in Russian).
- [38] G.Gigauri, Diversity of Georgian forest, Tbilisi, 2000 (in Georgian).
- [39] G.S. Vachnadze, Z.T. Tiginashvili, G.V. Tsereteli, B.N. Aptsiauri, Q.G. Nishnianidze, Carbon stock sequestered from the atmosphere by coniferous forests in Svaneti, *J. Annals Agrar. Sci.* 14 (3) (2016) 269-278.
- [40] V.Z. Gulisashvili, Genesis of pine and birch forests of the Caucasus, In collection: Academician V.N. Sukachev on the occasion of his 75th birthday, M.-L. (1956) (in Russian)
- [41] G. Vachnadze, Z. Tiginashvili, G. Tsereteli, B. Aptsiauri, Biomass and Carbon Stock in forest Ecosystems of the Lagodekhi Nature reserve, publishing house L.G.T. „M.G“. Tbilisi, 2013 (in Georgian).
- [42] N.F. Kaplina, Fractional structure of phytomass and annual production of stands and birch trees, Analysis of the production structure of stands, Moscow: Nauka, (1988) 103-117, (in Russian).
- [43] L.N. Rozhkov, Methodological approaches for calculating carbon pools in the forests of Belarus, *Proceedings of BSTU*, no. 1, (2011) 62-70 (in Russian).
- [44] V.A. Usoltsev, A.A. Malenko, V.A. Azarenok, Biological productivity of birch plantations in the climatic gradients of EURASIA, *Bulletin of Altai State Agrarian University* No. 9 (143) (2016) 59-63 (in Russian).
- [45] Z. Tiginashvili, G.Vachnadze, B. Aptsiauri, G. Tsereteli, Stocks of Carbon and Phytomass of Lagodekhi State natural Reserve Beech forest, II International Scientific-Practical Conference, "Bioeconomy and Sustainable Development of Agriculture", *Proceedings* (2013) 444 (in Georgian).
- [46] G.Vachnadze, Z. Tiginashvili, G. Tsereteli, B. Aptsiauri, E. Nakaidze, Q. Nishnianidze, Phytomass and Reserves of Accumulated Carbon of Beech Forests of Eastern Georgia, publishing house "Mtsignobari", Tbilisi, 2015 (in Georgian).

- [47] Z. Tiginashvili, Assessment of Carbon Stock Sequestered in Georgian Forests, Establishment of Conversion Coefficient, SRNSFG, grant № FR/217404, (2016-2018) (in Georgian).
- [48] Charles B. Halpern, Thomas A. Spies, Plant Species Diversity in Natural and Managed Forests of the Pacific Northwest, Ecological Applications, Vol. 5, No. 4, (1995) 913-934.
- [49] <https://www.researchgate.net/publication/258222728>
- [50] N.V. Lukina, L.M. Polyanskaya, M.A. Orlova, Nutrient regime of soils of the northern taiga forests, Moscow: Nauka, 2008 (in Russian).
- [51] K.V. Zvorykina, Composition and above-ground mass of plants of grass-dwarf shrub and moss layers in some types of birch forests on loamy soils, Silvicultural research in the southern taiga subzone. Moscow: Nauka, (1977) 42–50 (in Russian).
- [52] K.S. Bobkova, N.L. Smolentseva, V.V. Tuzhilkina, V.A. Artemov, The cycle of nitrogen and ash elements in a pine-spruce plantation of the middle taiga, Lesovedenie, no. 5 (1985) 3–11 (in Russian).
- [53] Frank S Gilliam, The Ecological Significance of the Herbaceous Layer in Temperate Forest, Ecosystems, BioScience, Vol. 57 No. 10 (2007) 845-858.
- [54] <https://www.researchgate.net/publication/242252719>
- [55] N.A. Bazilevich, A.A. Titlyanova, Biotic circulation on five continents: nitrogen and ash elements in natural ecosystems, Novosibirsk: Nauka 2008 (in Russian).
- [56] Sonia Légaré, Yves Bergeron, David Paré, Influence of Forest Composition on Understory Cover in Boreal Mixedwood Forests of Western Quebec, Silva Fennica, 36(1) (2002) 353-365. <https://www.researchgate.net/publication/235554472>
- [57] V.V. Tuzhilkina, Phytomass structure and carbon stocks in plants of forest ground cover in northeastern European Russia, Plant resources, vol. 48. No. 1 (2012) 44–50 (in Russian).
- [58] M.B. Russell, A.W. D'Amato, B.K. Schulz, C.W. Woodall, G.M. Domke, J.B. Bradford, Quantifying understory vegetation in the US Lake States: a proposed framework to inform regional forest carbon stocks, Forestry: An International Journal of Forest Research, Vol. 87(5) (2014) 629-638.
- [59] D. McKenzie, C.B. Halpern, C.R. Nelson, Overstory influences on herb and shrub communities in mature forests of Western Washington, U.S.A. Can. J. For. Res., 30 (2000) 1655–1666.
- [60] A. Ya. Gulbe, L.S. Ermolova, Dynamics of grass cover as an indicator of changes in environmental conditions during the formation of hanging birch stands on fallow lands, Journal “Actual problems of forest complex”, # 17 (2007) (in Russian).
- [61] A. V. Gryazkin, Influence of environmental factors on the structure and condition of undergrowth, Izvestia of St. Petersburg Forestry Academy. Issue 8 (166). SPb.: SPbFA, (2000) 19-25 (in Russian).
- [62] R.Kvachakidze, Typological study of subalpine forest and bushes in Kakhetian Caucasus. Bull. of Academy of Sciences of Georgian SSR, vol. 8, #3 (1965) (in Georgian).
- [63] R.Kvachakidze, K.Iashagashvili. The vegetation of Caucasian forest of Kakheti, Tbilisi, 1992, (in Georgian).
- [64] L. B. Makhatadze T.F. Urushadze, Subalpine Forests of the Caucasus, Ed. “Forest Industry”, M., 1972 (in Russian).
- [65] G.M. Tarasashvili, Mountain-forest and mountain-meadow soils of Eastern Georgia, ed. “AS GSSR” publishing house, Tbilisi, 1956 (in Russian).
- [66] T.Urushadze, Soils of mountain and forest of Georgia. Publ. house “Sabchota Sakartvelo”, Tbilisi, 1977 (in Georgian).
- [67] T.F. Urushadze, Soils of the Mountain Forests of Georgia. “Metsniereba”, Tb., 1987 (in Russian).
- [68] G.Talakhadze, K.Mindeli, Soils of high mountains of Georgia, Publ. house “Sabchota Sakartvelo”, Tbilisi, 1980 (in Georgian).
- [69] FAO corporate document repository Title: State of World's Forests (2011). [www.fao.org/icatalog/inter-e.htm](http://www.fao.org/icatalog/inter-e.htm)



## Annals of Agrarian Science

Journal homepage: <http://journals.org.ge/index.php>



# Effectiveness of pest management options for managements of brown marmorated stink bug *Halyomorpha Halys* (Hemiptera: Pentatomidae) in hazelnut orchards in the Republic of Georgia

M. Murvanidze<sup>1</sup>, G. Krawczyk<sup>2</sup>, N. Inasaridze<sup>3</sup>, M. Kodua<sup>4</sup>, N. Samsonidze<sup>3</sup>, E. Alimbarashvili<sup>3</sup>, D. Lipartia<sup>4</sup>, R. Shonia<sup>5</sup>, N. Archvadze<sup>3</sup>

<sup>1</sup> Institute of Entomology, Agricultural University of Georgia, 240, D. Aghmashenebeli Alley, Tbilisi, Georgia

<sup>2</sup> Department of Entomology, Fruit Research and Extension Center, Pennsylvania State University, 290 University Drive, Biglerville, PA, USA

<sup>3</sup> Department of Biology, Faculty of Exact and Natural Sciences, I. Javakhishvili Tbilisi State University, 2, I. Chavchavadze Avenue, Tbilisi, Georgia<sup>4</sup> AgriGeorgia. 17, Paliashvili Street, Tbilisi, Georgia<sup>5</sup> Cartlis Agrosystems. 14 Right Bank, Zugdidi, Georgia

Received: 05 May 2021; Accepted: 10 July 2021

## ABSTRACT

Distribution and management of *Halyomorpha halys* (Stål) in commercial hazelnut orchards were investigated during 2019 and 2020. Efficacies of management strategies including attract and kill strategy and border sprays were compared to grower's standard management practices. Seasonality of *H. halys* were similar during both years with the first *H. halys* adults observed near the end of April and all instar nymphs being present from July 20 (820-900 DD<sub>13.9</sub>) to the end of September (1600-1655 DD<sub>13.9</sub>). Although no difference was detected between the treatment types before harvest, after harvest data suggest attract and kill strategy to be the most effective management program during both seasons. Evaluations of hazelnut quality demonstrated higher presence of healthy kernels in nuts collected from trees located in the interior of the block than on trees from the perimeter of the block. Corking damage was higher on nuts collected from trees near the edge of the orchard. Observed increase in corking damage of nuts on trees located inside of the block, was indirectly associated with the mid- and late season *H. halys* feeding in the interior of the orchard. *H. halys* management decisions in hazelnut orchards should be based on the documented presence of *H. halys* populations but growers should also factor in the potential influx of the pest from surrounding areas. In areas surrounded by wild vegetation use of insecticide treated nets as used in the attract & kill plots provides the most effective strategy in protecting nut crop.

**Keywords:** management strategies, attract and kill, monitoring, rescue traps, hazelnut quality.

\* Corresponding author: M. Murvanidze, E-mail address: [m.murvanidze@agr.uni.edu.ge](mailto:m.murvanidze@agr.uni.edu.ge)

## Introduction

Brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), presence in the Republic of Georgia was first documented during the 2015 season [1]. Since the initial

detection, *H. halys* has become and remains a major threat to Georgian agriculture causing intensive damage in hazelnuts (*Corylus avellane* L.). Damage caused by brown marmorated stink bug to the hazelnut sector is widely acknowledged [2, 3]. Georgia is one of the main suppliers of hazelnut

worldwide (5.2% of total world production) [4] and the severity of damage to nuts caused by *H. halys* leads to significant economic losses to Georgian farmers and country budget.

High pressure from *H. halys* populations in pome and stone fruit orchards during the 2010-2011 seasons in the Mid-Atlantic region of USA has resulted in at least a 4-fold increase in the number of insecticide applications caused mainly by additional sprays and by shortening intervals between applications [5]. Frequent applications of broad-spectrum insecticides (e.g., pyrethroids, neonicotinoids, carbamates) negatively influenced populations of beneficial arthropods and often lead to outbreaks of secondary pests [6, 7].

Various pest management tactics within the frame of the Integrated Pest Management (IPM) practices have been developed and tested specifically to limit the number of insecticide applications while at the same time effectively control *H. halys* populations. Some *H. halys* specific management strategies include attract and kill (A&K) approach [6], insecticide treated nets [8], use of window screens [9] and sprays of orchard borders [10, 11, 12, 13]. None of these alternative strategies to manage *H. halys* were evaluated in Georgia prior to this project.

The presence of stink bugs in fields, timing of reproductive activities and migration to overwintering sites is influenced mostly by abiotic factors such as ambient temperature and length of daylight [14, 15, 16]. As degree day (DD) models have not been developed for *H. halys* in our region, we aimed to investigate phenology of this insect pest to find out if there is any edge effect congregation like in other orchard crops such as apple. Precise information about DD accumulation is especially useful for farmers when pesticide applications should target only specific stages during insect life cycle [17].

The main objectives of this project were to compare the effectiveness of grower's standard management, border applications of insecticides, and "attract and kill" (A&K) strategy applied within the integrated pest management program for *H. halys* in the western region of the Republic of Georgia. The following hypotheses were tested: 1) orchard blocks surrounded with insecticide treated nets used as an attract and kill strategy and blocks where orchard border sprays were applied will have lower numbers of *H. halys* compared to blocks with only grower's standard insecticide practices.

This hypothesis is based on the assumption that *H. halys* is a perimeter driven insect pest [10, 18] and protection of the orchard borders by A&K stations and/or border applications of insecticides might effectively control *H. halys* populations inside of the orchard and at the same time reduce the necessity for multiple applications of insecticides on the whole area of orchard; 2) the results of management strategies are influenced by both the numbers of *H. halys* and the location of hazelnut trees within the orchard (edges *versus* inside of the block); and 3) the phenology of *H. halys* is similar from year to year [19].

## Material and methods

### Field site

Our investigations were conducted in Zugdidi Municipality, Samegrelo Region, Republic of Georgia (Sakartvelo). This area represents part of the Kolkheti Lowland and is characterized by high precipitation (annual average precipitation between 1500 – 2000mm, with maximal amount – 4500mm) [20] and rich and diverse wild vegetation [21].

The experimental site was located in Darcheli village (N42.4182282° E41.6508108°). It was composed of a 12-hectare hazelnut orchard planted in 1995-1996. The orchard was surrounded by the Patara Enguri River from western and southern sides, by corn field from the eastern side and by an uncultivated field from the northern side. Hazelnut trees were planted at a spacing of about 4m between the trees and 5m between the rows (Figure 1).

Different management strategies were deployed in three blocks with each block having approximately 2 ha in size: 1) attract and kill (A&K) treatment with block A&K 1 surrounded from one side by the river and from two other sides by hazelnut trees, and block A&K 2 surrounded by the river, corn field and hazelnut trees; 2) border (BRD) treatment with block BRD 1 surrounded by the river, open area and hazelnut trees, and block BRD 2 surrounded by corn field, open area and hazelnut trees; and 3) grower standard (GS) treatment with block GS 1 surrounded by the river and hazelnut trees and block GS 2 surrounded by the corn field and hazelnut trees (Figure 1). As the whole orchard received grower's standard management treatments (details see below) GS blocks were regarded as the control and the

effectiveness of BRD and A&K strategies were compared to them. The GS application scheme was decided by a farmer following standard recommendations for the region developed by Georgia National Food Agency (<https://nfa.gov.ge/Ge/Page/BrownMarmoratedStinkBug>).

In order to determine the direction of pest influx into the orchard, five traps (numbered 13-17) were placed on wild vegetation surrounding the plot (Figure 1)

#### *Monitoring of H. halys population*

Monitoring of *H. halys* was conducted during 2019 and 2020 from late April until mid-October. Stink Bug Rescue traps (Sterling International Inc, Spokane, WA, USA) were used for standard stink bug monitoring. Each trap was baited with a single BMSB monitoring lure, Pherocon BMSB (Trece Inc, Adair, OK, USA) and were placed on the twigs of the hazelnut trees, approximately 1.5m above ground, shaded by the leaves. Two traps were placed inside each block for the total of twelve monitoring traps in six blocks. Five additional Rescue traps were placed around the orchard in unmanaged areas and utilized as a control. Information from traps was utilized to assess the movement of *H. halys* from surrounding areas into the orchard.

All A&K stations were placed outside of the hazelnut orchard; surrounding A&K blocks as indicated on Figure 1. This was done in order to attract bugs away from the orchard towards the sprayed nets. Plastic black nets used as window screens (Scley Insect Nets, KAEM Inc., Baranowo, Poland) were fixed from two sides on the wooden frame about 1.5 -2m each. Individual nets were baited with five Pherocon *H. halys* monitoring lures. White square polyethylene tarp (2m × 2m) was placed on the ground around each trap base to collect dead adults and nymphs. The distance between individual A&K stations was about 50m. A total of fourteen A&K stations were utilized around the A&K blocks (Figure 1).

Traps were monitored for *H. halys* nymphs and adults in every other week. Additionally, during each visit visual observations were conducted to evaluate the presence of alive stink bugs within a 10m diameter from each treatment tree and around A&K stations. Rescue traps were used season long but pheromone lures were replaced every 12 weeks as recommended by manufacturer. *H. halys* adults and nymphs starting from the 2<sup>nd</sup> instar

were counted and recorded. The first instar *H. halys* nymphs were not present inside the traps as they usually aggregate only on or around egg masses they hatched from. During 2019 on-site monitoring of *H. halys* was conducted from 4 May to 12 October while during the 2020 season monitoring was conducted from 9 May to 15 October. Local weather information was obtained from the meteorological station (iMETOS® Pessl Instruments GmbH, Austria) located nearby (N41.7911679°, E42.4726416°). Degree-day accumulations were calculated as  $DD = (t^{\circ}\text{min} + t^{\circ}\text{max})/2 - 13.9$  with the 13.9 °C used as the lower temperature developmental threshold [22].

#### *Treatments*

##### *Standard management practices*

Grower's standard management practices (GS) which included applications of pesticides and fertilizers were applied to whole orchard as presented in Tables 1 and 2. No additional treatment was applied to GS blocks. Full orchard insecticide applications were terminated in the end of June during the 2019 season and in early July during the 2020 season. Decision on termination of treatments was made by the farmer based on the results of *H. halys* captures by monitoring traps and hazelnut quality.

##### *“Attract and Kill” stations*

Plastic nets used for the A&K stations were sprayed weekly from 5 May to 9 September in 2019 and from 9 May to 4 October in 2020 using a pyrethroid insecticide bifenthrin, (Talstar, 10% active ingredient, FMC Chemical S.p.r.l., USA). The spray solution for this treatment was prepared as solution of 60mL bifenthrin in 12L of water (Tables 1 and 2). Applications were performed using backpack sprayer Solo Port 423 (Solo® Kleinmotoren GmbH, Germany) from the distance 1.2m from the nets.

##### *Border sprays*

In order to reveal effectiveness of border sprays for controlling *H. halys* population, blocks used for the border spray treatment were treated by spraying exclusively only border tree rows that were located at the border of the orchard and surrounded area. This was done in addition to the GS applications as shown in Tables 1 and 2. The sprays reached about 3m deep from orchard border to the interior of the orchard. Pesticide applications inside the orchard

and around BRD blocks were applied using 400L of solution per hectare using a Jinma 254 tractor (Jiangsu Yueda Group, Jiangsu, China) equipped by Jarret orchard sprayer (Eko 400L, Poland). Border sprays were maintained through the whole season as indicated in Tables 1 and 2.

#### *Evaluation of hazelnut injuries.*

During 2019 and 2020, injuries to hazelnuts were evaluated three times each year using nuts collected from each block on 7 July, 20 July and 3 August during the 2019 season and 12 July, 24 July and 7 August during the 2020 season. Total crop yield was harvested on 5 August in 2019 and on 10 August in 2020. Hazelnuts in each block were picked from six trees, with four trees located in the outer rows and two – in the interior of each block. From each tree 10 hazelnuts were randomly collected for a total 60 hazelnuts from each block, 360 nuts in total per count. Kernel quality was classified as healthy, corked, blank or with developed fungal disease (mold) as described by Hedstrom et al. [23]

#### *Data analyses*

For results processing, the data were split into two subunits: before harvest (5 August 2019 and 10 August 2020) and after harvest.

The effects of treatments were compared using parametric (one-way and two-way ANOVA) and nonparametric (Independent Samples Kruskal Wallis test) tests. For the comparison of seasons and treatment type statistics the nonparametric test (Independent Samples Kruskal Wallis H *aka* One-Way ANOVA on ranks) was used as assumptions of normality. Equal variances were violated as for before as after harvest data. The analysis of mean ranks of captured bugs were done for each category of variables (season, treatment type). In order to examine the influence of more than one factor (season, treatment strategy, harvest) on *H. halys* population before and after the harvest, Two-way ANOVA with multiple comparison Dunnett's test (for Treatment Type effects) was used, which evaluated interaction between different predictors (years  $\times$  treatment type/harvest etc.). Dunnett test regarded GS blocks as a control and compared all other groups against it. For the assessment of distribution of hazelnut injury categories Independent Samples Kruskal Wallis H test was applied. Comparisons and assessments of statistical significance for full data sets from

different plots, years and management strategy were performed at  $p < 0.05$ . All data analysis was performed using statistical software IBM SPSS Statistics for Windows, v.23.

## **Results**

The first *H. halys* adults in hazelnut orchards were observed in the beginning of May during both seasons when average daily high temperatures exceeded 17°C in 2019 and 15°C in 2020 (89 and 81 DD<sub>13.9</sub>, respectively from January 01). Eggs were first detected by visual observations in the beginning of June, while the second instar nymphs were observed in the traps located outside the orchard, in wild vegetation on 23 June 2019 and 5 July 2020 at 598 and 620 DD<sub>13.9</sub>, respectively (Figure 2).

#### *Comparison of H. halys populations between plots with alternative management strategies*

The overall *H. halys* captures were lower during the 2020 season than during the 2019 ( $H(1)=12.5$ ,  $p < 0.001$ ). The analysis of variations by seasons (2019, 2020) and by treatment type (A&K, BRD, GS) before harvest performed using Kruskal Wallis H test demonstrated difference between the captures by season ( $H(1)=12.5$ ,  $p < 0.001$ ) and no difference between treatment types ( $H(2)=4.158$ ,  $p = 0.125$ ). The numbers of captured bugs were extremely low before the harvest as almost no nymphs were detected and adult population was regulated by GS management practices (Figure 3).

Number of captured stink bugs started to increase after the harvest when the farmer stopped application of insecticides and the orchard was protected only by A&K stations surrounding A&K blocks and border sprays around BRD blocks (Table 3, Figure 3). Two-Way ANOVA analysis of overall *H. halys* captures revealed interaction between the seasons and post-harvest number for adults, nymphs and both of them combined ( $F(1,308)=28.252$ ,  $p < 0.001$ ;  $F(1,308)=6.802$ ,  $p < 0.05$ ;  $F(1,308)=31.512$ ,  $p < 0.001$  correspondingly), as well as for harvest and treatment type for adults and total number of bugs ( $F(1,306)=4.84$ ;  $p < 0.05$ ;  $F(1,306)=3.90$ ;  $p < 0.05$ ). There was no significant interaction between the seasons and treatment type for combined (before and after harvest) data ( $F(2, 306) = 0.003$ ,  $p > 0.05$ )

*A strong difference ( $H(1)=101.299$ ,  $p < .001$ ) exists between H. halys numbers captured before*

and after harvest. The difference between the seasons (2019, 2020) was maintained after the harvest for both, adults ( $H(1)=9.158$ ,  $p=0.002$ ) and nymphs ( $H(1)=4.21$ ,  $p=0.04$ ) and there were differences detected in captures per trap between A&K and BRD blocks during both seasons ( $H(1)=6.297$ ,  $p < 0.04$  for adults and nymphs combined).

The Dunn's pairwise comparison tests revealed that after the harvest, blocks with border application of insecticides demonstrated higher number of *H. halys* captures compared to the A&K blocks ( $p < 0.05$ , adjusted using the Bonferroni correction) (19.636,  $p=0.04$ ) which had lowest number of captured stink bugs, both, adults and nymphs (Figure 3).

#### Evaluation of hazelnut injuries.

The lowest percentage of healthy kernels was observed in nuts collected from trees located in the outer rows (margins) and healthy nuts increased on trees located in the interior of the managed blocks. Corking damage by *H. halys* was higher on nuts collected from trees located on the outside rows of the orchard. Percentage of blank nuts was generally low compared to other types of damage (Figure 4). Overall hazelnut quality was strongly associated with the location of trees (outer vs interior rows) ( $F(4,150)=190.03$ ,  $p < 0.001$  for 2019 and  $F(4,150)=413.474$ ;  $p < 0.001$ , for 2020). The increased levels of injured nuts in outside rows supports the notion of *H. halys* to be a perimeter driven insect pest in hazelnut.

The Kruskal Wallis test revealed differences in distribution of hazelnut injury by the number of kernels within each category for both 2019 ( $H(4)=98.06$ ;  $p < 0.001$ ) and 2020 ( $H(4)=112.89$ ;  $p < 0.001$ ) seasons. Number of healthy kernels were higher ( $p < 0.05$ ) compared to the damaged kernels in both years and in all blocks, but there were no differences between the damaged kernel categories (blank, corked, mold and shrivel) as well as there were no differences in damage between the treatment types for 2019 ( $H(2)=0.804$ ;  $p=0.669$ ) and 2020 ( $H(2)=0.545$ ;  $p=0.761$ ) (Figure 4).

## Discussion

Seasonality of *H. halys* was similar during both seasons. Emergence of the first generation nymphs during the 2019 season started earlier than during the 2017 season [19]. The 2017 year study was

performed in another orchard, located only about 30 km from the 2019 and 2020 site, but both of them share similar regional peculiarities. The early emergence of BMSB nymphs can be potentially associated with higher temperatures in 2019, while in 2020 the DD accumulation curve was similar to that of the 2017 season (Figure 2). *H. halys* adults and all nymphal instars were observed in all blocks from mid-July to mid-September ( $DD_{13.9}$  889 - 1719). In laboratory observations conducted in Minnesota, *H. halys* showed maximum survival and fecundity at 27 and 23°C, respectively, while the mean generation time was the shortest at 30°C [24]. During field-based experiments, where constant temperature cannot be maintained, the average daily temperatures in the range of 20-27°C supports higher *H. halys* populations and presence of overlapping generations.

Finding second instar nymphs near the end of September supports the hypothesis there are at least two adult generations of *H. halys* in subtropical regions of Georgia [19], while in the eastern part of the country presence of this pest in apple and peach orchards was not confirmed [25]. Two generations of *H. halys* are also reported for regions at similar latitudinal range as Georgia. Two generations of *H. halys* are documented for Maryland and West Virginia [26], for Minnesota [25] and for Eastern United States [27]. Similar pattern is observed by our investigation showing, in addition to overwintering *H. halys* adults in the spring, two adult peaks on traps located outside the orchard – in mid-June and mid-September (Figure 5).

Using insecticide treated nets as an alternative management strategy against *H. halys* was first proposed in 2014 at the peak of the infestation of apple orchards in Pennsylvania (G. Krawczyk, personal communication). Multiple field and laboratory experiments were performed to evaluate the efficacy of insecticide treated nets baited with *H. halys* attractants as a management tool to attract and kill the pest and in consequence to reduce numbers of pesticide applications inside plots [8, 28]. Similar version of the attract and kill strategy is relying on utilizing wild trees baited with pheromone lures and located outside or at the border of the orchard which are being frequently treated with insecticides effective against *H. halys*. This approach has been demonstrated to be very effective in killing of high numbers of stink bugs through the growing season [6, 29], namely, two

years trials showed reduction in fruit damage inside of blocks by half to a third compared with the standard blocks treated with insecticides only [29]. Insecticide treated nets were also tested and found very effective in killing pests of storage products. Warehouses deploying nets show 89-100% reduction of storage insects attacking commodities [30]. Our investigation also proved effectiveness of A&K stations surrounding orchard blocks in killing *H. halys* adults and nymphs. Monitoring traps located inside the A&K blocks showed lowest captures of *H. halys* through the study. Although the difference with other management strategies was not clearly evident before harvest when total number of captured bugs within the orchard was low, this difference became significant after the harvest when standard treatment practices were abandoned by the farmer (Table 3, Figure 3).

Surprisingly, additional applications of pyrethroid insecticides around the border of the hazelnut orchard did not reduce the numbers of *H. halys* in the interior of the blocks as effective as attract and kill strategy ( $p > 0.005$ ). The number of captured *H. halys* in the BRD blocks after harvest were even higher than in blocks designed for only grower's standard applications. Our finding contradicts with study which demonstrated that the border application of insecticides in peach orchards significantly reduced the volume of insecticide applications compared to standard blocks, however the border targeting applications still effectively managed *H. halys* and other key pests keeping them at levels close to levels observed in orchards utilizing standard practices of pesticide application [10].

Protection of hazelnuts by alternative management strategies lead to reduction in the number of insecticide applications in the experimental plots, where the last full application of insecticides was performed on 15 June in 2019 and 5 July in 2020. This decision was made by the farmer based on the monitoring results and the hazelnut quality. After these dates, no more whole orchard insecticide applications were made and only the protected parts of the orchard were the blocks with A&K nets and with border spray treatments (Tables 1 and 2). Unfortunately, but consequently an increase in the number of observed stink bugs within the orchard began after mid - August when hazelnuts were already harvested (Figure 5).

The wild vegetation surrounding orchard

area most likely provides shelter for *H. halys* populations and let them survive insecticide applications done in the managed areas. Stink bug adults and nymphs developing outside of the orchard can continuously migrate into the interior of the orchard, making *H. halys* mobile forms a typical border driven pest [31]. In order to observe this occurrence, we used traps (traps number 13-17) located outside the orchard placed on wild vegetation in locations serving as potential source of *H. halys* (Figure 1). Trap number 14 captured high numbers of stink bugs during both study years (Figure 6). This trap was located on the bank of the Patara Enguri River, with dense forest vegetation on the other bank of the river. Width of the river on this location is about 60m which seems an easy obstacle to be crossed knowing the *H. halys* adults are capable to travel 2 to 5 km in 24 hours [32, 33, 34]. However, the trap number 15 that was also located on the bank of the river about 120 m away from the other bank of the river, captured much lower numbers of stink bugs (Figure 6). This difference in the number of captured stink bugs at least partially can be attributed to both, difference in the width of the river and absence of vegetation in immediate surroundings of the trap number 15 (Figure 1). As expected, traps located in areas without surrounding wild vegetation (16 and 17) recorded the lowest captures of stink bugs. As trap number 14 with highest number of captured bugs was placed adjacent to A&K blocks (Figure 1), it serves as additional evidence supporting effectiveness of A&K strategy.

*H. halys* is known to feed on hazelnuts at all stages of kernel development. Early stage feeding can result in blank kernels while feeding on mature hazelnut leads to development of corking damage [23]. In semi-field trials conducted in northwestern Italy, *H. halys* was found to be more harmful species compared to the native bug species causing the highest damage of kernels [2]. Recent investigation provided in California tree crops show also almond to be very effective attractant for *H. halis* [35]. The higher numbers of captured stink bugs at the orchard borders than in the interior of the orchard coupled with higher numbers of corking damage of hazelnut kernels collected from trees at the orchard edges (Figure 4) supports the hypothesis of *H. halys* being a perimeter driven insect pest. This type of behavior was also observed in our earlier studies [19] as well as by other investigations [5, 6, 18, 34]. The level of blank hazelnut was

very similar during both years, while the levels of corking damage in nuts was higher during the 2020 season (Figure 4). The levels of nut damages did not appear to be correlated with different pest management strategies. Although A&K blocks show much less numbers of captured stink bugs, the levels of corking damage were slightly higher on the trees located on the border of A&K blocks, while numbers of blank kernels were higher on border trees of BRD blocks in 2019 season. In GS blocks incidences of different types of damage were similar, however higher prevalence of blank and corked kernels was observed on trees located in the borders of the blocks (Figure 4).

In summary, under environmental conditions existing in Kolkheti Lowland (Western Georgia), the results of this research documented overwintering adults of *H. halys* started to emerge at the end of April and first-generation eggs were observed in early June. Overlapping generations were observed from the mid-July to mid-September. Landscape features influenced *H. halys* pressure as measured by traps, as orchards surrounded by dense wild vegetation were under higher pressure of *H. halys* due to potential influx of pests from surrounding areas. Under such conditions, special attention should be directed to the utilization of alternative management approaches such as attract and kill strategies. Insecticide treated nets should be placed outside of the orchards to be able to attract bugs coming from both, the area surrounding the orchard as well as from the interior of the orchard. Use of attract and kill stations can be utilized to reduce the numbers of insecticide applications. In orchards not surrounded by wild vegetation, these strategies can potentially decrease the necessity of insecticides use and reduce the number of sprays by half, from 5-6 times in 2018 (personal communication) to 3 times (Tables 1 and 2).

### Acknowledgements

The authors express the gratitude to the head of the plant protection service of the National Food Agency Dr. Nikoloz Meskhi for his help in identifying the sites and organizing the field work. We also wish to thank Kote Vekua from “Nergeta ltd” for providing the meteorological data. We want to express our cordial gratitude to the reviewers whose comments and suggestions contributed to improvement of the manuscript. The Investigation was financed by Shota Rustaveli National

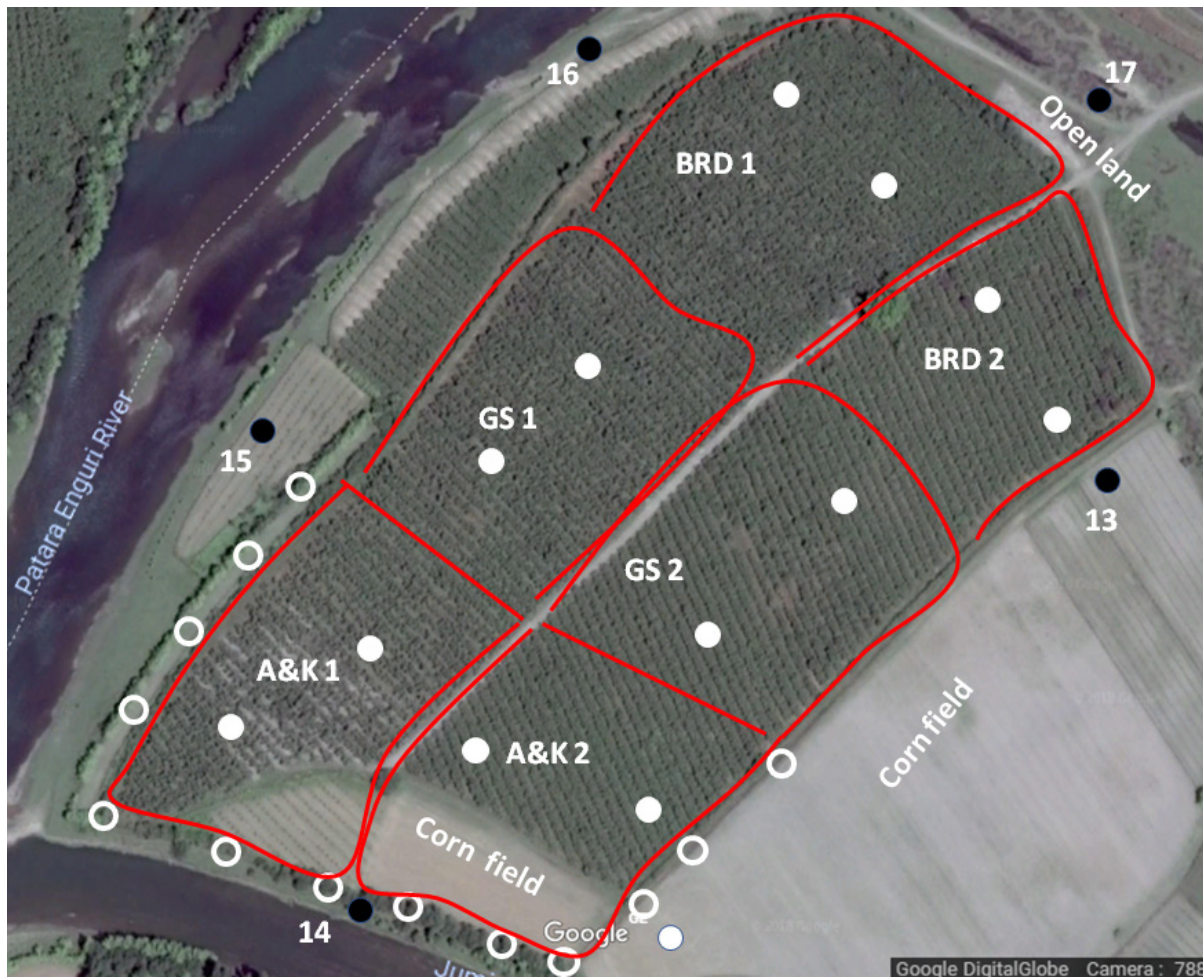
Scientific Foundation project NFA- SRNSFG – 18-115 “Biology and risk assessment of the brown marmorated stink bug, *Halyomorpha halys* in Western Georgia’s crop systems; evaluations of various monitoring tools and alternative management practices”

### References

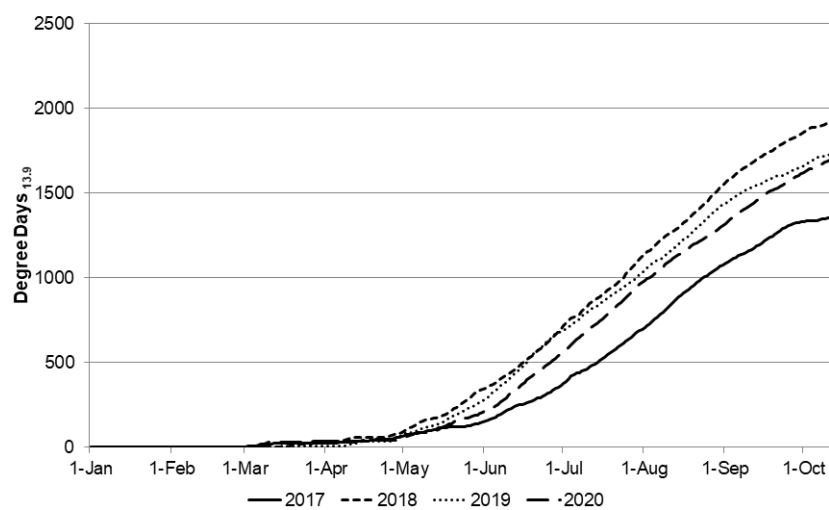
- [1] Gapon DA. First records of the brown marmorated stink bug *Halyomorpha halys* (Stål, 1855) (Heteroptera, Pentatomidae) in Russia, Abkhazia, and Georgia. *Entomological Review*, 96, 2016: 1086-1088
- [2] Bosco L., Moraglio, S. T., Tavella L. *Halyomorpha halys*, a serious threat for hazelnut in newly invaded areas. *Journal of Pest Science*, 91, 2018: 661-670. DOI:10.1007/s10340-017-0937-x
- [3] Güncan A & Gümüş E. Brown Marmorated Stink Bug, *Halyomorpha halys* (Stål, 1855) (Hemiptera: Heteroptera, Pentatomidae), a New and Important Pest in Turkey. *Entomological news* 128, 2019: 168-176.
- [4] FAOSTAT (2016) Food and Agriculture Data. <http://www.fao.org/faostat>
- [5] Leskey TC, Short BD, Butler BR & Wright SE. Impact of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål), in mid-Atlantic tree fruit orchards in the United States: case studies of commercial management. *Psyche: A Journal of Entomology*, 2012: ID 535062. doi:10.1155/2012/535062
- [6] Morrison III WR, Lee DH, Short BD, Khirmian A & Leskey TC. Establishing the behavioral basis for an attract-and-kill strategy to manage the invasive *Halyomorpha halys* in apple orchards. *Journal of Pest Science*, 89, 2016: 81-96. doi:10.1007/s10340-015-0679-6
- [7] Rice KB, Bergh CJ, Bergmann EJ, Biddinger DJ, Dieckhoff C, Dively G, Fraser H, Garipey T, Hamilton G, Haye T, Herbert A, Hoelmer K, Hooks CR, Jones A, Krawczyk G, Kuhar T, Martinson H, Mitchell W, Nielsen AL, Pfeiffer DG, Raupp MJ, Rodriguez-Saona C, Shearer P, Shrewsbury P, Venugopal PD, Whalen J, Wiman NG, Leskey TC & Tooker JF. Biology, ecology, and management of brown marmorated stink bug (Hemiptera: Pentatomidae). *Journal of Integrated Pest*

- Management*, 5, 2014: A1-A13. doi:10.1603/IPM14002
- [8] Kuhar TP, Short BD, Krawczyk G. & Leskey TC. Deltamethrin-Incorporated nets as an integrated pest management tool for the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae). *Journal of Economic Entomology*, 110, 2017: 543-545. doi: 10.1093/jee/tow321
- [9] Mooneyham KL, Aigner Jr JD. & Kuhar TP. Control of brown marmorated stink bug with Insecticide-Treated Window Screens. *Arthropod Management Tests*, 41. 2016: 1-1. doi: 10.1093/amt/tsw021
- [10] Blaauw BR, Polk D. & Nielsen AL. IPM-CPR for peaches: incorporating behaviorally-based methods to manage *Halyomorpha halys* and key pests in peach. *Pest Management Science*, 71, 2015: 1513–1522. doi:10.1002/ps.3955
- [11] Short BD, Khimian A & Leskey TC. Pheromone-based decision support tools for management of *Halyomorpha halys* in apple orchards: development of a trap-based treatment threshold. *Journal Pest Science*, 90, 2017:1191-1204. doi:10.1007/s10340-016-0812-1
- [12] Akotsen-Mensah C, Blaauw B, Short B, Leskey TC, Bergh JC, Polk D & Nielsen AL. Using APM-CPR as management program for apple orchards. *Journal of Economic Entomology*, 113, 2020: 1894-1902. doi: 10.1093/jee/toaa087
- [13] Leskey TC, Short BD & Ludwick D. Comparison and refinement of integrated pest management tactics for *Halyomorpha halys* (Hemiptera: Pentatomidae) management in apple orchards. *Journal of Economic Entomology*, 113, 2020: 1725-1734. doi: 10.1093/jee/toaa067
- [14] Nielsen AN, Scheerer PW & Hamilton GC. Toxicity of insecticides to *Halyomorpha halys* (Hemiptera: Pentatomidae) using glass-vial bioassays. *Journal of Economic Entomology*, 101, 2008: 1439-1442.
- [15] Nielsen AN, Fleischer Sh, Hamilton GC, Hancock T, Krawczyk G, Lee JC, Ogburn E, Pote JM, Raudenbush A, Rucker A, Saunders M, Skillman VP, Sullivan J, Timer J, Walgenbach J, Wiman NG, Leskey TC. Phenology of brown marmorated stink bug described using female reproductive development. *Ecology and Evolution*, 7, 2017: 6680-6690. DOI: 10.1002/ece3.3125
- [16] Berg JC, Morrison WR III, Josef SV. & Leskey TC. Characterizing spring emergence of adult *Halyomorpha halys* using experimental overwintering shelters and commercial pheromone traps. *Entomologia Experimentalis et Applicata*, 162, 2017: 336 - 345. DOI: 10.1111/eea.12539
- [17] Murray, M. Using degree days to time treatments for insect pests. 2008. Retrieved July 7, 2016 from [http://digitalcommons.usu.edu/extension\\_curall/978/](http://digitalcommons.usu.edu/extension_curall/978/)
- [18] Joseph SV, Stallings JW, Leskey TC, Krawczyk G, Polk D, Butler B. & Bergh JC. Spatial distribution of brown marmorated stink bug (Hemiptera: Pentatomidae) injury at harvest in mid-Atlantic apple orchards. *Journal of Economic Entomology*, 107, 2014: 1839–1848. doi:10.1603/EC14154
- [19] Murvanidze M, Krawczyk G, Inasaridze N, Dekanoidze L, Samsonadze N, Macharashvili M, Khutsishvili S & Shengelia S. Preliminary data on the biology of brown marmorated stink bug *Halyomorpha halys* (Hemiptera, Pentatomidae) in Georgia. *Turkish Journal of Zoology*, 42, 2018: 617-624. doi:10.3906/zoo-1802-34
- [20] Williams L, Zazanashvili N, Sanadiradze G & Kandaurov A. An ecoregional conservation plan for Caucasus. WWF. Tbilisi. 2006. 220p.
- [21] Akhalkatsi M & Tarkhnishvili D. Habitats of Georgia. Tbilisi, Georgia. GTZ. 2012. 118p.
- [22] Nielsen AN & Hamilton GC. Life history of the invasive species *Halyomorpha halys* (Hemiptera: Pentatomidae) in Northeastern United States. *Annals Entomological Society of America*, 102, 2009: 308-616.
- [23] Hedstrom CS, Shearer PW, Miller JC. & Walton VM The Effects of Kernel Feeding by *Halyomorpha halys* (Hemiptera: Pentatomidae) on Commercial Hazelnuts. *Horticultural Entomology*, 107, 2014: 1859-1865.
- [24] Govindan BN & Hutchison WD Influence of temperature on age-stage, two-sex life tables for a Minnesota-acclimated populations of the brown marmorated stink bug (*Halyomorpha halys*). *Insects*, 11, 2020: 1-26.
- [25] Japoshvili G, Arabuli T, Salakaia M, Tskaruashvili Z, Kirkitadze G, Talamas EJ. Surveys for *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) and its biocontrol

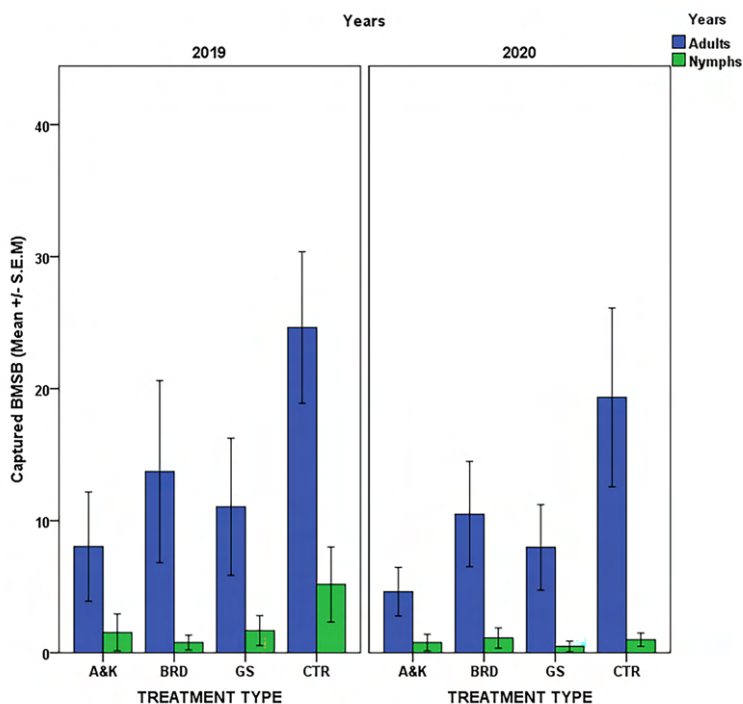
- potential by parasitic wasps in the Republic of Georgia (Sakartvelo). *Phytoparasitica*, 2021, <https://doi.org/10.1007/s12600-021-00949-1>
- [26] Leskey TC, Agnello A, Bergh CJ, Diveli GP, Hamilton GC, Jentsch P, Khrimian A, Krawczyk G, Kuhar TP, Lee DH, Morrison III WR, Polk DF, Rodriguez-Saona C, Shearer PW, Short BD, Shrewsbury PM, Walgenbach JF, Weber D, Welty C, Whalen J, Wiman N, Zaman F. Attraction of the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae) to traps baited with semiochemical stimuli across the United States. *Environmental Entomology*, 44, 2015: 746-756. <https://doi.org/10.1093/ee/nvv049>
- [27] Acebes-Doria AL, Agnello AM, Alston DG, Andrews H, Beers EHJ, Bergh C, Bessin R, Blaauw BR, Buntin GD, Burkness EC, Chen Sh, Cottrell TE, Daane KM, Fann LE, Fleischer SJ, Guédot C, Gut LJ, Hamilton GC, Hilton R, Hoelmer KA, Hutchison WD, Jentsch P, Krawczyk G, Kuhar TP, Lee JC, Milnes JM, Nielsen AL, Patel DK, Short BD, Sial AA, Spears LR, Tatman K, Toews MD, Walgenbach JD, Welty C, Wiman NG, Van Zieren J. & Leskey TC Season-long monitoring of the brown marmorated stink bug (Hemiptera: Pentatomidae) throughout the United States using commercially available traps and lures. *Journal of Economic Entomology*, 113, 2020: 159-171, <https://doi.org/10.1093/jee/toz240>
- [28] Sabbatini Peverieri G, Binazzi F, Marianelli L & Roversi PF. Lethal and sublethal effects of long-lasting insecticide-treated nets on the invasive bug *Halyomorpha halys*. *Journal of Applied Entomology*, 142, 2018: 141-148. <https://doi.org/10.1111/jen.12428>
- [29] Morrison III WR, Blaauw BR, Short BD, Nielsen AL, Berg JC, Krawczyk G, Park YL, Butler B, Khrimian A & Leskey TC Successful management of *Halyomorpha halys* (Hemiptera: Pentatomidae) in commercial apple orchards with an attract-and-kill strategy. *Pest Management Science*, 75, 2019: 104-114. doi:10.1002/ps.5156
- [30] Wilkins RV, Campbell JF, Zhu KY, Starkus LA, McKay T & Morrison III RW Long-lasting insecticide incorporated netting and interception traps at pilot-scale warehouses and commercial facilities prevents infestation by stored product beetles. *Frontiers in Sustainable Food Systems*, 4, 2021: 1-28. doi: 10.3389/fsufs.2020.561820
- [31] Basnet S, Kuhar TP, Laub CA. & Pfeiffer DG Seasonality and distribution pattern of brown marmorated stink bug (Hemiptera: Pentatomidae) in Virginia vineyards. *Journal of Economic Entomology*, 108, 2015: 1902-1909. DOI: 10.1093/jee/tov124
- [32] Lee DH & Leskey TC. Flight behavior of foraging and overwintering brown marmorated stink bug *Halyomorpha halys* (Hemiptera: Pentatomidae). *Bulletin of Entomological Research*, 105, 2015: 566-573. doi:10.1017/S0007485315000462
- [33] Wiman NG, Walton VM, Shearer PM, Rondon SI & Lee JC Factors affecting flight capacity of brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae). *Journal of Pest Science*, 88, 2015: 37-47.
- [34] Blaauw BR, Jones VP, Nielsen AL Utilizing immune marking techniques to track *Halyomorpha halys* (Hemiptera: Pentatomidae) movement and distribution within a peach orchard. *Peer J* 4, 2016: e1997. doi: 10.7717/peerj.1997
- [35] Stahl JM, Scaccini D, Daane KM Field survival of brown marmorated stink bug *Halyomorpha halys* (Hemiptera: Pentatomidae) on California tree crops. *Environmental Entomology*, 2021: nvab055. <https://doi.org/10.1093/ee/nvab055>



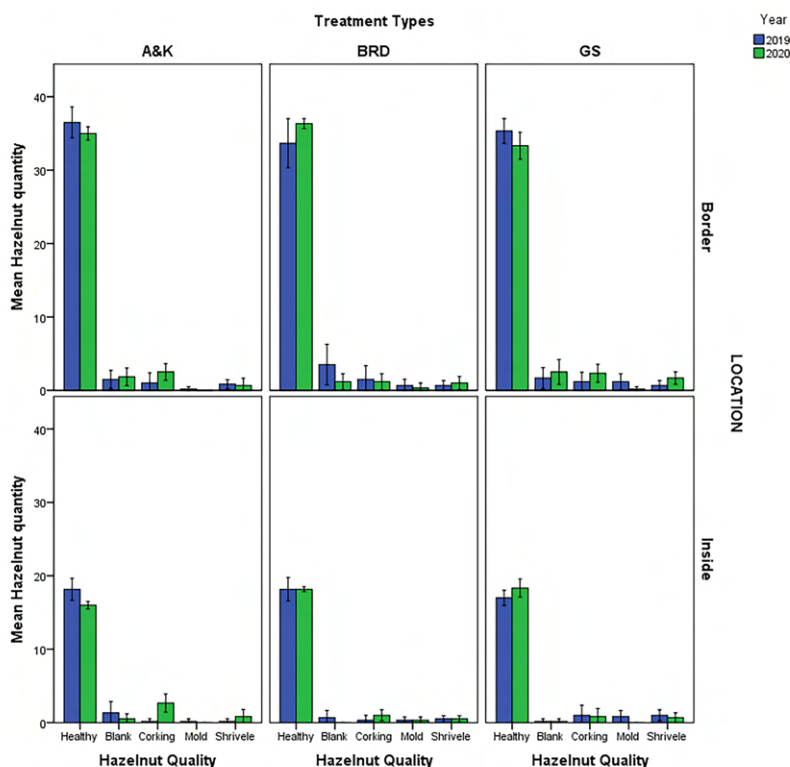
**Figure 1.** Placement of *H. halys* attract and kill stations and monitoring traps within the experimental plots. Solid white circles indicate placement of monitoring Rescue traps inside the orchard; solid black circles indicate placement of Rescue traps outside the orchard; open circles indicate placement of A&K stations (A&K – attract and kill; BRD – border applications; GS – grower’s standard); numbers indicate respective numbers of Rescue traps outside of the orchard



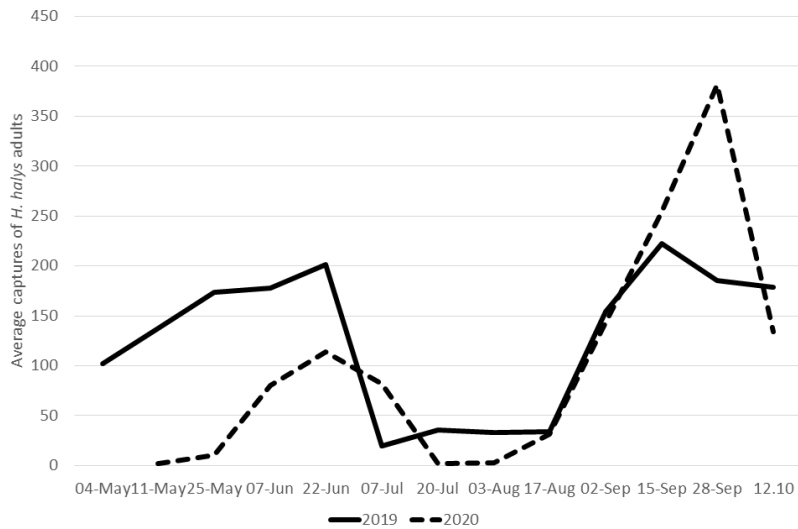
**Figure 2.** Accumulation of developmental degree days (DD)<sub>13.9</sub> for the 2017-2020 seasons in the Zugdidi Municipality, Samegrelo Region, Western Georgia.



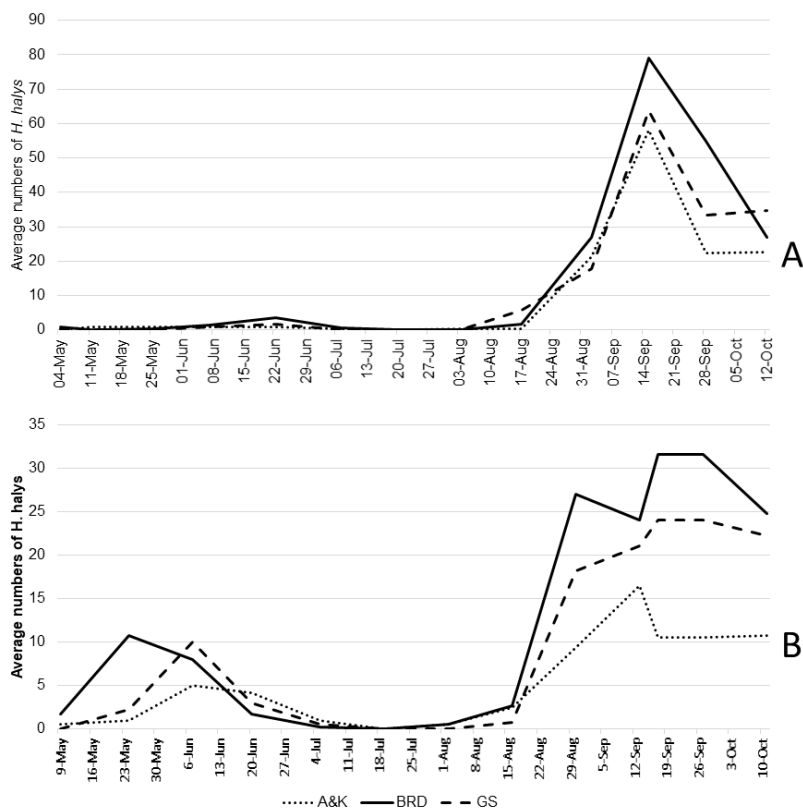
**Figure 3.** Differences in numbers of captured *H. halys* between the 2019 and 2020 seasons before and after the harvest for adults and nymphal instars in plots with different management strategies (A&K – attract and kill; BRD – border applications; GS – grower’s standard; CTR – traps #13-17, located outside the orchard, on wild vegetation).



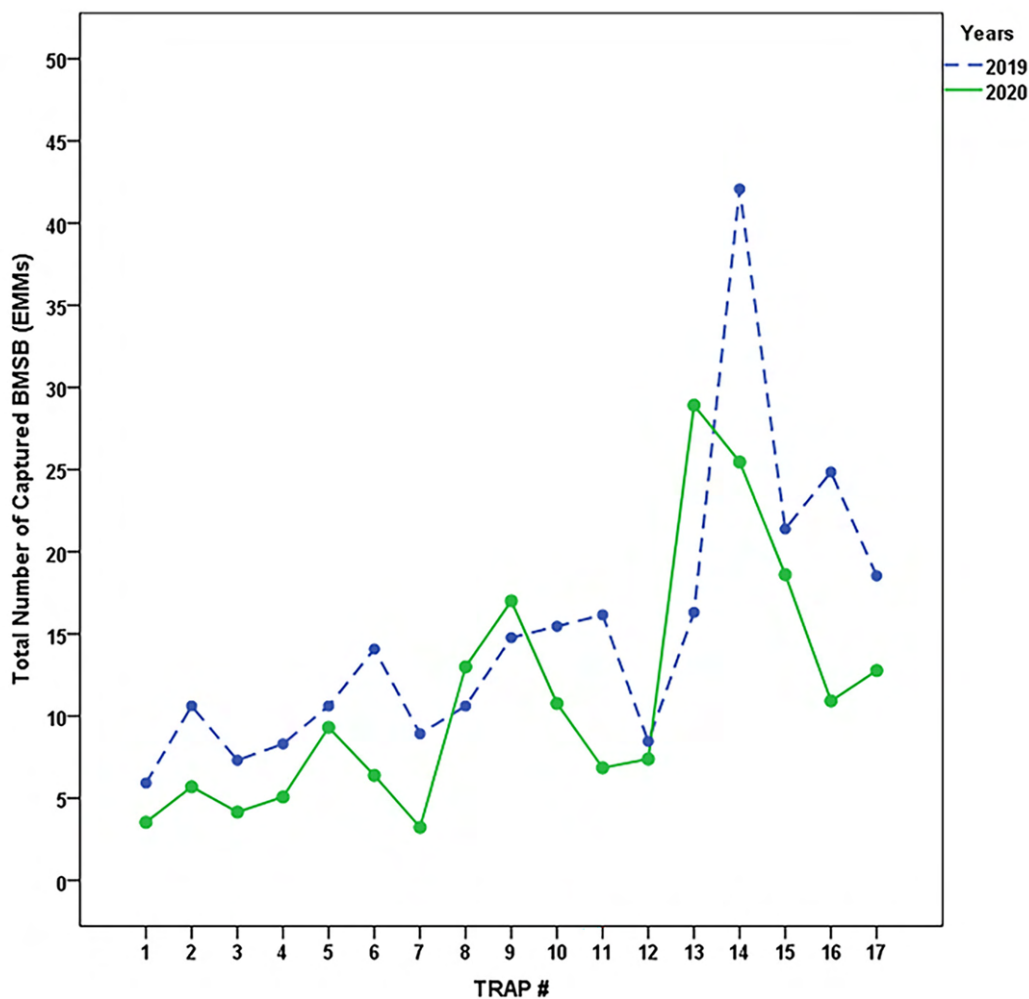
**Figure 4.** Estimation of hazelnut average quality by treatment type (A&K – attract and kill; BRD – border applications; GS – grower’s standard). “Border” – refers to the margin row of trees in each block that serve as “border” between the orchard and surrounding area, “Inside” – refers to the trees located in the interior of the corresponding block



**Figure 5.** Average seasonal captures of *H. halys* adults by traps located outside the orchard during 2019 and 2020 seasons.



**Figure 6.** Seasonality of *H. halys* adults and nymphal instars in plots with different management strategies: a) Season 2019; b) Season 2020 (A&K – attract and kill; BRD – border applications; GS – grower’s standard).



**Figure 7.** Estimated marginal means (EMMs) for the number of *H. halys* adults and nymphal instars captured in the traps during both study seasons. Numbers: 13-17 indicate location numbers of Rescue traps utilized out of the orchard. Location of each trap is shown on Figure 1.

**Table 1.** Pesticide and fertilizer\* applications at the experimental plot for 2019 season. All rates per 400 liter of water per ha.

Date 2019	Location	Volume/ha	Pesticide* and rate of application
8 Apr.	All blocks	400 l	Kocide (2kg)+Superkill Forte (1l)+Trend (200gr)
9 Apr.	All blocks	400 l	Kocide (2kg) + Kaiso (300gr) +Trend (200gr)
24 Apr.	All blocks	400 l	Grogrin Gel 27-27-27 vegetation (3 kg)
10 May	All blocks	400 l	Bayomca (400 ml)
15 May	All blocks	600 l	Bališta (600ml) + Grogrin Gel 27-27-27 vegetation (3kg) +Trend (200gr)
21 May	BRD	400 l	Talstar (450gr) + Trend (250gr)
27 May	All blocks	400 l	Gurador (150gr)
7 Jun.	BRD	400 l	Lannate (400 gr) + Trend (100 gr)
15 Jun.	All blocks	400 l	Talstar (400gr) + Kodafil maximus 8-11-3, (500 gr), Gurador (100gr)

25 Jun.	All blocks	600 l	Grogrin fruit 18-11-59 3kg
20 Jul.	BRD	400 l	Talstar (600gr) +Trend (150gr)
10 Aug.	BRD	400 l	Lannate (400 gr) +Trend (100 gr)
24 Aug.	BRD	400 l	Talstar (450g)
06 Sep.	BRD	400 l	Talstar (450gr) +Trend (250gr)
12 Sep.	All blocks BRD	400 l	Ortus (500g) Talstar (450g)

\*Pesticide descriptions: **Kocide**- copper (II) 8% + hydroxyl 53; **Superkill Forte** - cypermethrin, 50 g/l + chlorpyrifos, 500 g/l; **Trend** -ethoxylated isodecile alcohol, 90% water solution; **Grogrin** – NPK fertilizer, contains **B, Cu, Fe, Mn, Mo, Zn**; **Bayomca** – triadimenol, 250g/l ; **Balista** – alpha cypermethrin, 100g/l; **Talstar** – bifenthrin, 100g/l; **Lannate**– methomyl 200g/l; **Ortus** – fenpyroximate 51, 2g/l.

**Table 2.** Pesticide and fertilizer\* applications at the experimental plot for 2020 season. All rates per 400 liter of water per ha.

Date 2020	Location	Volume/ha	Pesticide* and rate of application
9 Apr.	All blocks	400 l	Kocide (2kg)+Superkill Forte (1l)+Trend (200gr)
26 Apr.	All blocks	400 l	Grogrin Gel 27-27-27 vegetation (3 kg)
12 May	All blocks	400 l	Saga (1l) + Mystik (0.5l) + Grogrin Gel (2kg) + Trend (250mg)
26 May	All blocks	24 l	Kaiso (300gr) + Broder (100mg)
26 May	BRD	24l	Talstar (300mg) – Broder (100mg)
6 Jun.	All blocks	400 l	Talstar (600mg) + Broder (200mg) Kaiso (300g) + Broder (200mg)
9 Jun.	BRD	400l	Talstar 800g
21 Jun.	BRD	400 l	Talstar 800g
05 Jul.	All blocks	400 l	Prius (1l) + Grogrin Gel (2.5kg), Fulpas (0.5l) + Talstar (0.6l)
23 Jul.	BRD	400 l	Talstar 800g
31 Jul.	BRD	400 l	Talstar 800g
28 Aug.	BRD	400 l	Talstar 800g
04 Oct.	BRD	400 l	Talstar 800g

\*Pesticide descriptions: **Kocide**- copper (II) 8% + hydroxyl 53; **Superkill Forte** - cypermethrin, 50 g/l + chlorpyrifos, 500 g/l; **Trend** - ethoxylated isodecile alcohol, 90% water solution; **Grogrin** – NPK fertilizer, contains **B, Cu, Fe, Mn, Mo, Zn**; **Talstar** – bifenthrin, 100g/l; **Mystik** – Tebukonazol 250g/l; **Kaiso** – Lambda-cyhalothrin, 50g/kg; **Broder** – chlorpyrifos 250ml; **Saga** – chlorpyrifos, 480g/l; **Fulpas** – penconazole 100g/l; **Prius** – pyrimethanil, 400g/l.

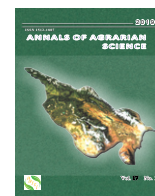
**Table 3.** Average captures of BMSB adults and nymphal instars in stink bug Rescue traps once in two weeks in plots with different management practices. Average numbers of individuals were counted from 2 traps in each managed block and from 5 traps at the control site outside the orchard (A&K – attract and kill; BRD – border applications; GS – grower’s standard; CTR – unsprayed control). Before harvest season envelopes period from the beginning of the monitoring to August 5 for 2019 and to August 10 for 2020 when crop was harvested; after harvest - from the second week of August to the end of the season.

Season 2019

Period	Adults						2 <sup>nd</sup> and 3 <sup>rd</sup> instars						4 <sup>th</sup> and 5 <sup>th</sup> instars					
	GS1	GS2	A&K1	A&K2	BRD1	BRD2	GS1	GS2	A&K1	A&K2	BRD1	BRD2	GS1	GS2	A&K1	A&K2	BRD1	BRD2
Before harvest	1	0.2	1.5	0.4	2	1.5	0	0	0	0	0	0	0	0	0	0	0	0
After harvest	62	50	41	40	63	76	0.4	4	2	0	0	2	5	7	11	2	4	2

Season 2020

Period	Adults						2 <sup>nd</sup> and 3 <sup>rd</sup> instars						4 <sup>th</sup> and 5 <sup>th</sup> instars					
	GS1	GS2	A&K1	A&K2	BRD1	BRD2	GS1	GS2	A&K1	A&K2	BRD1	BRD2	GS1	GS2	A&K1	A&K2	BRD1	BRD2
Before harvest	5	4	2	6	3	10	0	0	0	0	0	0	0	0	0	0	0	0
After harvest	36	22	16	15	27	49	0.3	2	1	5	1.5	6	1.5	0	1	0	1	0.6



## AROMA COMPOUNDS OF GRAPE AND WINE

I. Pipia\*, T. Nozadze

Agricultural University of Georgia, Institute of Molecular Genetics,  
Kakha Bendukidze University Campus, 240 David Aghmashenebeli Alley, 0159, Tbilisi, Georgia

Received: 12 September 2021 Accepted: 29 October 2021

### ABSTRACT

Aroma compounds represent the most important factors that play a crucial role in the formation of wine character. Generally, wine's aroma depends on the grape variety, the winemaking process and environmental factors, such as soil, weather, water, sunlight etc. Here we present a short review of the types of volatile aroma compounds (grape-derived aromas, aromas produced from the process of fermentation, maturation aromas), the chemistry of aromas, and the genetic bases of aroma formation.

**Keywords:** Aroma compounds, Grape, Wine

\*Corresponding Author: Dr. I. Pipia, E-mail address: [i.pipia@agruni.edu.ge](mailto:i.pipia@agruni.edu.ge)

### Introduction

From the ancient times the cultivated grape (*Vitis vinifera* ssp. *vinifera*) represents the most important horticultural crop worldwide. For human society, the importance of grapevines goes into two main usages: grapes as table fruits and as a source of wine or other products of grapes (i.e., juice, vinegar). Among sweetness, acidity, tannin levels, and wine body types (light, medium, full-bodied), aromas are the most important factors contributing to the formation of wine character. The terms "wine aroma" and "wine bouquet" have two independent meanings. The "aroma" corresponds to the smells unique to the grape variety, while "bouquet" represents the new smells formed as a result of winemaking process of fermentation and aging [1].

The main contributors to wine quality are volatile aroma compounds, which are grouped into three classes: grape-derived aromas (primary aromas, varietal aromas), aromas produced from the process of fermentation (secondary aromas, fermentative aromas), and maturation aromas (tertiary aromas, aging aromas) [2, 3, 4]. Besides, it should be

noted that there are several additional factors that are affecting grape aromas. Among them are soil, weather, sunlight, water, nitrogen fertilization, and fungicide treatment [5].

In general, it is very difficult to pinpoint the perception and characterization of wine aromas. This difficulty stems from the above-mentioned different origins of aromas as well as the perception of flavors defined by human sensory systems. The brain interprets the taste of wine through the gustatory system, which is responsible for a taste, and the olfactory system, responsible for a smell. The gustatory system is made up of the so-called taste cells in the mouth. These cells are responding to that chemical compound of food that makes all five known tastes: salty, sweet, bitter, sour, and umami. Olfaction activates when aroma compounds enter the nasal cavity (by inhalation through the nose or mouth). The molecules of the mentioned compounds bind to receptors that signal to the specific olfactory bulb, which initiates a cascade of neural signals responsible for smell recognition, memory, and emotion. According to some authors,

the flavor is not presented in the food - it is created by the brain from food. It is proposed that humans can differentiate more than 1 trillion olfactory stimuli. The human nose can perceive some of the compounds only at very high concentrations, while others are even at low levels [4, 6, 7]. In difference from the human sense of aromas, there are a number of laboratory methods that can accurately detect aromatic compounds at any concentration. Among them, the most usable is Gas-Chromatography coupled with Mass-Spectroscopy (GC-MS) and High-performance liquid chromatography (HPLC analyses) [8, 9].

### Grape derived aromas

Grape-derived aroma compounds are important contributors to the wine bouquet as they offer a unique set of wine aromas. These aromas are called as prime or varietal aromas of wine and are aromas associated with fruit (e.g., peach, blackberry), herbal (e.g., mint, oregano), and flower (e.g., roses, iris) flavors. In the overall aroma composition, grape varieties are mostly similar, differences lie in the rations of the compounds contributing to the aroma profiles [10].

It is determined that in grapes there are at least seven different systems or pools of aroma precursors, but not all of them are contributing to the formation of wine flavor (i.e., the Strecker amino acid system and the fatty acid/peroxygenase system) [11].

The actual aroma of grapes involves compounds in three related categories: 1). Free aromas – aroma compounds presented in the grape skins. This category represents very small amounts of compounds in most grape varieties, and displays weak, rather neutral odors and flavors; 2). Aroma molecules which are formed by enzymatic/catalytical processes triggered during the disruption of fruit tissues. A number of aldehydes, alcohols, and ketones formed as a result of the peroxidation of fatty acids are examples of this category. As the abundance of these molecules has 6 carbons, they are often called C6-compounds; 3). Aroma molecules which are made in the buccal cavity by the activity of salivary or bacterial enzymes. It has been shown that glutathione and cysteinyl precursors (odorless cysteine-S-conjugates) can release the aromatic thiol by the action of buccal microbiota [11]. It should be noted that the certain aromas originated from grapes are modified by the

fermentation process or aging.

Most aroma compounds are presented as non-volatile precursors, including glycosidic precursors, polyolic forms, cysteinylated and glutathionylated precursors, or 32 dimethyl sulfide (DMS) precursors [12]. From those compounds, the main quotative ones are glycosidic precursors. Some most popular aroma chemicals, such as linalool and geraniol (terpenoids, the major aroma compounds in both red and white grapes), eugenol and guaiacol (volatile phenols) are derived from the glycosidic precursors already existing in grapes. In muscat-related grapes, varietal wine aromas originated from the various isoprenoid monoterpenes (i.e., linalool, geraniol, nerol, and citronellol) [10].

### Aromas producing from the process of fermentation

Wine is the product of fermentation, which means the transformation of grape juice into an alcoholic beverage by yeast. During fermentation, yeasts transform the sugars of the juice into ethanol and carbon dioxide. The intensive smell of wine formed under the influence of yeast *Saccharomyces cerevisiae* (often referred to as “wine yeast”). But *S. cerevisiae* is not only responsible for the metabolism of grape sugar to alcohol and CO<sub>2</sub>. Grape must contain a mixture of yeast species and so fermentation is not a ‘single-species’ process [13]. In the early stages of fermentation, numerous yeast species may exist, but as the alcohol concentration increases, *Saccharomyces* species progressively take over [14]. Such non-saccharomyces species include: *Hanseniaspora*, *Hansenula*, *Candida*, *Pichia*, *Lachancea*, *Brettanomyces*, *Kluyveromyces*, *Schizosaccharomyces*, *Torulasporea*, *Zygosaccharomyces* and *Saccharomycodes*.

The major odor-active compounds that arrive from the process of fermentation are esters, higher alcohols, carbonyl, and sulfur compounds. In wines, esters are presented in the form of acetate and ethyl esters, which have different fruity perceptions. Acetate esters are the products of a condensation reaction between acetyl-CoA and higher alcohols produced by yeast from amino acid metabolism. Ethyl esters are the results of the esterification of ethanol, and acyl-CoA intermediates as a result of esterase and transferase enzyme activity. Higher alcohols are formed by yeasts as a result of amino acid metabolism (See below).

## Maturation aromas

During the fermentation process, yeasts transform sugars into alcohol, which is accompanied by the formation of secondary aromas. By this time, wine is still immature, it has to rest to harmonize aromas and balance acidity and tannins. The period between the end of fermentation till bottling represents a period of wine maturation or aging. Maturation partially changes the taste of wine and its bouquet. During wine storage, certain changes in wine aroma composition occur. For instance, many esters, formed by the yeast's metabolic activity, are hydrolyzed and a fresh and fruity aroma is lost. At the same time, a synthesis of new esters occurs (i.e., isoamyl acetate, diethyl succinate). In muscat varieties in which the main odor compounds are terpenes, the concentration of monoterpene alcohols declines, and monoterpene oxides are formed. In wine, the bound terpenes are slowly transformed to free volatile terpenes over time, and when this occurs the fruity aroma

of wine is enhanced during maturation [15]. It was shown that the chemical profiles of wines can be significantly changed by the influence of chips used for aging. In particular, in red wines aged with French oak chips smoky, licorice, and toasty aromas were noticed, while in wines aged with American oak chips vanilla, toasty and cacao aromas were detected [16].

## Chemistry of aromas

There are several types of chemical compounds that are the main contributors to the aroma composition generally for both grapes and wines. Among them are aliphatic alcohols, aliphatic acids, aliphatic esters, monoterpenes, norisoprenoids, volatile phenols and benzoids [4]. Table 1 presents the list of some key aroma compounds of grape and wine with the description of their corresponding flavors [summarized from 10, 16, 17, 18, 19, 20, 21].

**Table 1.** Some major aroma compounds of grape and wine with the description of their characteristic odors.

Compounds		Aroma
Aliphatic alcohols	C6-alcohols	“Green” aroma
Higher alcohols	3-methyl-butan-1-ol	Alcohol notes, nail varnish, malt
	Phenylethanol	Floral, rose, honey notes, peach notes
	Methionol	Crushed potatoes
	Propan-1-ol	Alcohol, ripe fruits
Esters	Ethyl butanoate	Pineapple, strawberries
	Ethyl propanoate	Cherries
	Ethyl hexanoate	Green apples, strawberries, blackberries
	2-phenylethyl acetate	Rose, fruity
	Hexyl acetate	Pears, plums, bananas, currants
Monoterpenes	Linalool	Floral aroma with spicy tones and lemon aroma
	Geraniol	Floral, fruity, Citrus
	$\alpha$ -terpineol	Floral, fruity, Citrus
	Citronellol	Floral, fruity
	Nerol	Sweet, fresh aromas
Volatile phenols	Guaiacol	Smokey, ashy aroma
	Eugenol	Spice, clove, smoke
	Vanillin	Smokey
Volatile thiols	3-sulfanylhexan-1-ol (3SH)	Fruity notes
	4-methyl-4-sulfanylpentan-2-one (4MSP)	Fruity notes
Benzenoids	Phenylethanol	Weak floral aroma
	Phenylacetaldehyde	Weak floral aroma
Sulphur compounds	Volatile thiols	Fruity, “green” aroma
	Methanethiol	Rotten Cabbage, Stagnant Water

Aliphatic alcohols can be originated from both grape and yeast fermentation. C6-alcohols which are formed from the corresponding C6-aldehydes are a group of plant volatiles with a “green” aroma, remindful of leaves and fresh-cut grass. The fusel short-chain alcohols are formed by yeasts during the fermentation process from amino acid catabolism. They can negatively impact wine aroma. Aliphatic alcohols also can act as precursors of esters – other types of aroma compounds. During the fermentation the yeasts produce: a). Long aliphatic acids with 6 and more carbon from fatty acids; b). Short and branched acids from amino acids, and c). Sugar-derived acetic acids. Aliphatic acids can be turned into esters and lactones [10].

As mentioned above, there are two main classes of esters in wines: ethyl esters and acetate esters. While the concentration of ethyl esters of medium-chain fatty acids depends on the concentration of the fatty acid precursor, its concentration does not regulate the concentration of acetate esters [10].

Terpenoids represent the most important organic compounds which are contributing to the formation of aromas of grape varieties and wine's bouquet [22]. They can be found in grapes, musts and wines [23]. Among the different classes of terpenoids (hemiterpenoids, diterpenoids, sesquiterpenoids, etc.) the monoterpenes (substances with 10 carbon atoms built from 2 isoprenoids units) and sesquiterpenes (substances with 15 carbon atoms built from 3 isoprenoids units) appear to be the most important aroma compounds of fruits [23, 24]. The chemical bases of the most floral and citrusy notes in wine are monoterpenes (a C<sub>10</sub> class of terpenes). These compounds are mainly derived from grape varieties and are predominantly glycosylated. In well-known aromatic grape varieties, such as Riesling, Muscat d'Alexandrie etc., the major monoterpenes are linalool, geraniol, nerol, citronellol and  $\alpha$ -terpineol [18]. Monoterpenes are products of terpene synthase enzymes (see below).

Like monoterpenes the floral or fruity notes have group of carotenoid-derived metabolites – norisoprenoids, which are synthesized by enzyme carotenoid cleavage dioxygenases. Wine norisoprenoids with important sensory properties are: 2,2,6-trimethylcyclohexanone (TCH), beta-damascenone, beta-ionone, vitispirane, actinidiol, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), riesling acetal and 4-(2,3,6-trimethylphenyl)buta-

1,3-diene (TPB) [25].

Volatile phenols give to wine spicy aroma notes. They originated from ferulic acid or related metabolites. Most of these compounds are presented as glycosides in grapes, that can be hydrolyzed during the process of winemaking. The most often founded volatile phenols in wine are: 4- ethylguaiacol (4- EG), 4- ethylphenol (4- EP), 4-methylguaiacol, vinylphenols, guaiacol, eugenol, and vanillin. The last one from the list – vanillin can also originate from aging in oak barrels. Some volatile phenols are associated with the non-pleasant aroma. The example of such volatile phenol is guaiacol with smokey and ashy aroma [19].

Volatile thiols, such as 3-sulfanylhexan-1-ol (3SH) and 4-methyl-4-sulfanylpentan-2-one (4MSP), are also important contributions to the wine fruity aromas. Many of thiol compounds are found in Sauvignon blanc wines. The source of thiols is fermentation process [20, 21].

Benzenoid-derived metabolites (i.e., phenylethanol, phenylacetaldehyde) gives to wine floral and fruity flavors [21]. In comparison to grapes in wines the concentration of benzenoids is much higher, that means that the source of these compound are not only grapes, but also yeasts.

More than 800 volatile aroma compounds have been identified in wines [8]. In the standpoint of the source, the core volatile compounds of wines can be classified as follows [5]:

Pyrazines - Originate in grapes.

Terpenes - Usually originate in grapes, but can be made by some yeasts and molds (but not *Saccharomyces*).

Shikimic acid derivatives - Formed by aromatic amino acid metabolism, originate in the plants, microbes, oak barrels.

Lactones – Originated in grapes, microbes, and oak barrels.

Esters - Alcohol: ethanol or alcohol from the degradation of amino acids, purine, and pyrimidine, Acid: acetic acid or acid from the degradation of amino acids or biosynthesis of fatty acids.

Higher alcohols or fusel oils - Formed mostly by microbes, but can be made by plants.

Acids – Made from the plant or microbes

Phenolic compounds – Made by plants

Sulfur-containing compounds – i.e., thiols are made during the fermentation process.

## Genetic bases of aroma compounds

The varietal aromas originated in grape berries by a variety of enzymes (i.e., terpene synthases, carotenoid cleavage dioxygenases, O-methyl transferases, cytochromes P450). It is known that genetic variation in the genes associated with aroma biosynthesis cause differences in aromas between grapevine varieties. For instance, an allelic variant of a terpenoid biosynthetic gene – 1-deoxy-D-xylulose-5-phosphate synthase, is responsible for the accumulation of terpenoids especially in Muscat and Gewürztraminer grapes [4].

Martin et al., reported findings from the analysis of the updated 12-fold sequencing and assembly of the grapevine genome that place the number of predicted terpenoid synthase genes (VvTPS) at 69 putatively functional VvTPS, 20 partial VvTPS, and 63 VvTPS probable pseudogenes. This work included information about gene architecture and chromosomal location [26]. Possible variations in the relative expression of terpene synthase (TPS) genes that depend on the organ were studied by Matarese et al., [22]. Tabidze et. al., reported the resequencing results of four Georgian grape cultivars - Chkhaveri, Saperavi, Meskhuri mtsvane, and Rkatsiteli, belonging to different haplogroups [27]. Annotation of chromosomal DNA of Georgian grape cultivars by MEGANTE annotation system shows the existence totally 66,745 predicted genes in all four cultivars. Among detected coding sequences, 106 predicted genes and 43 pseudogenes of terpene synthase genes were found in chromosomes 12, 18 random (18R), and 19. It should be noted that four novel TPS genes not presented in reference to Pinot noir DNA were detected. Two of them - germacrene A synthase (Chromosome 18R) and (-) germacrene D synthase (Chromosome 19) can be identified as putatively full-length proteins.

Aromatic norisoprenoids (C9, C10, C11, C13 molecules) with the floral/fruity aromas in grapes and wines, are produced from the carotenoids through the cleavage of carotenoid cleavage dioxygenases (CCD). By recombinant expression studies of *VvCCD1* it was shown that the gene encoded a functional CCD [28, 29]. Two cDNA encoding O-methyltransferases were isolated from Cabernet Sauvignon. By recombinant studies it was shown that O-methyltransferases are able to methylate non-volatile hydroxypyrazine to produce methoxypyrazines - volatile, grape-derived aroma compounds with herbaceous flavors [30]. By using

of bioinformatical analyses Iiu et al., identified a sum of 236 *VvCYPs* (the cytochrome P450 (CYP) superfamily) divided into 46 families and clustered into nine clans [31].

## Conclusion

The wine character is made by complex interactions between, on the one hand, varietal aroma profiles, fermentation and maturation processes, and environmental factors (i.e., soil, water, sunlight), on the other. As aroma compounds are one of the most important parameters in determining the quality of grape-derived products, such as wine, grape juice, and vinegar, the understanding of the general profiles of aroma content, wine aroma sources, and molecular bases of varietal aroma formation are the most important for the human society as this knowledge can help to enhance the quality of wines and to derive certain flavors in them.

## References

- [1] Alvaro C. Jimenez, Understanding of Wine, A Brief Guide to Wine Exploration
- [2] Matarese F., Scalabrelli G., D'Onofrio C., Analysis of the expression of terpene synthase genes in relation to aroma content in two aromatic *Vitis vinifera* varieties, *Functional Plant Biology*, (2013) 40:552–565.
- [3] Belda I, Ruiz J, Estéban-Fernández A, et al. Microbial Contribution to Wine Aroma and Its Intended Use for Wine Quality Improvement. *Molecules*, (2017) 22(2):189. Published 2017 Jan 24. doi:10.3390/molecules22020189
- [4] Ilc T., Werck-Reichhart D. and Navrot N., Meta-Analysis of the Core Aroma Components of Grape and Wine Aroma. *Front. Plant Sci.*, (2016) 7:1472. doi: 10.3389/fpls.2016.01472
- [5] González-Barreiro C., Rial-Otero R., Cancho-Grande B., Jesús Simal-Gándara J., Wine Aroma Compounds in Grapes: A Critical Review, *Critical Reviews in Food Science and Nutrition*, (2015) 55:2, 202-218, DOI: 10.1080/10408398.2011.650336
- [6] Neuroenology: The Brain Science Behind Wine testing, By Emily Oleisky, <https://gravitywinehouse.com/blog/neuroenology-science-of-wine-tasting/> 2021
- [7] Bushdid C, Magnasco MO, Vosshall LB, Keller A. Humans can discriminate more

- than 1 trillion olfactory stimuli, *Science*, (2014) 343(6177):1370-2. doi: 10.1126/science.1249168. PMID: 24653035; PMCID: PMC4483192.
- [8] Toci, A. T., Crupi, P., Gambacorta, G., Dipalmo, T., Antonacci, D., and Coletta, A., Free and bound aroma compounds characterization by GC-MS of Negroamaro wine as affected by soil management. *J. Mass Spectrom.*, (2012) 47, 1104–1112. doi: 10.1002/jms.3045
- [9] Moroşanu, A.-M.; Luchian, C.E.; Niculaua, M.; Colibaba, C.L.; Tarţian, A.C.; Cotea, V.V. Assessment of Major Volatile and Phenolic Compounds from ‘Fetească Regală’ Wine Samples after Pre-fermentative Treatments using GC-MS Analysis and HPLC Analysis. *Not Bot Horti Agrobo*, (2018) 46(1):247-259. DOI:10.15835/nbha46110889
- [10] Styger G., Prior P., Bauer F.F., Wine flavor and aroma, *Journal of Industrial Microbiology and Biotechnology*, Vol. 38, Issue 9, (2011) pg. 1145, <https://doi.org/10.1007/s10295-011-1018-4>
- [11] Ferreira V, Lopez R., The Actual and Potential Aroma of Winemaking Grapes, *Biomolecules*, 2019; 9(12):818. Published 2019 Dec 3. doi:10.3390/biom9120818
- [12] Alegre Y, Arias-Pérez I, Hernández-Orte P, Ferreira V., Development of a new strategy for studying the aroma potential of winemaking grapes through the accelerated hydrolysis of phenolic and aromatic fractions (PAFs), *Food Res Int.* (2020) 127:108728. doi: 10.1016/j.foodres.2019.108728. Epub 2019 Oct 9. PMID: 31882095.
- [13] Neil P. Jolly, Cristian Varela, Isak S. Pretorius, Not your ordinary yeast: non-Saccharomyces yeasts in wine production uncovered, *FEMS Yeast Research*, Vol. 14, Issue 2, (2014), pg. 215–237.
- [14] Mas A, Guillamón JM and Beltran G Editorial: Non-conventional Yeast in the Wine Industry. *Front. Microbiol.*, (2016) 7:1494. doi:10.3389/fmicb.2016.01494
- [15] Wine Aging, M. Dharmadhikari, (2016) <http://www.extension.iastate.edu/wine/w-aging>
- [16] Dumitriu Gabur GD, Teodosiu C, Gabur I, Cotea VV, Peinado RA, López de Lerma N. Evaluation of Aroma Compounds in the Process of Wine Ageing with Oak Chips, *Foods*, (2019) 10;8(12):662. doi: 10.3390/foods8120662. PMID: 31835490; PMCID: PMC6963919.
- [17] Baron M., Prusova B., Tomaskova L., Kumsta M., Sochor J., Terpene content of wine from the aromatic grape variety ‘Irsai Oliver’ (*Vitis vinifera* L.) depends on maceration time, *Open Life Sciences*, vol. 12, no. 1 (2017) pp. 42-50. <https://doi.org/10.1515/biol-2017-0005>
- [18] Pardo E., Rico, J., Gil, J.V. et al., De novo production of six key grape aroma monoterpenes by a geraniol synthase-engineered *S. cerevisiae* wine strain, *Microb Cell Fact* 14, 136 (2015). <https://doi.org/10.1186/s12934-015-0306-5>
- [19] Parker M., Osidacz P., Baldock G.A., Hayasaka Y., Black C.A., Pardon K.H., Jeffery D.W., Geue J.P., Herderich M.J., Francis L.I., Contribution of Several Volatile Phenols and Their Glycoconjugates to Smoke-Related Sensory Properties of Red Wine, *J. Agric. Food Chem.* (2012) 60, 10, 2629–2637.
- [20] Helwi, P., Guillaumie, S., Thibon, C. et al. Vine nitrogen status and volatile thiols and their precursors from plot to transcriptome level. *BMC Plant Biol* 16, 173 (2016). <https://doi.org/10.1186/s12870-016-0836-y>
- [21] Renault, P., Coulon, J., Moine, V., Thibon, C., & Bely, M., Enhanced 3-Sulfanyhexan-1-ol Production in Sequential Mixed Fermentation with *Torulaspora delbrueckii*/ *Saccharomyces cerevisiae* Reveals a Situation of Synergistic Interaction between Two Industrial Strains, *Frontiers in microbiology*, (2016) 7, 293. <https://doi.org/10.3389/fmicb.2016.00293>
- [22] Matarese F., Cuzzola A.R., Scalabrelli G., D’Onofrio C., Expression of terpene synthase genes associated with the formation of volatiles in different organs of *Vitis vinifera*. *Phytochemistry* 105 (2014), DOI: 10.1016/j.phytochem.2014.06.007
- [23] Lengyel E., Primary aromatic character of wines, *Acta Universitatis Cibiniensis Series E: Food Technology* Vol. XVI, no.1 (2012).
- [24] Dunlevy J., Kalua C., Keyzers R., Boss P. The Production of Flavour & Aroma Compounds in Grape Berries. In: Roubelakis-Angelakis K.A. (eds) *Grapevine Molecular Physiology & Biotechnology*. Springer, Dordrecht, 2009
- [25] Mendes-Pinto MM., Carotenoid breakdown products the-norisoprenoids-in wine aroma,

- Arch Biochem Biophys. (2009) 483(2):236-45. doi: 10.1016/j.abb.2009.01.008. PMID: 19320050.
- [26] Martin D.M., Aubourg S., Schouwey M.B., Daviet L., Schalk M., Toub O., Lund S.T., Bohlmann J., Functional Annotation, Genome Organization and Phylogeny of the Grapevine (*Vitis vinifera*) Terpene Synthase Gene Family Based on Genome Assembly, FLeDNA Cloning, and Enzyme Assays. BMC Plant Biology (2010) 10(1):226, DOI: 10.1186/1471-2229-10-226
- [27] Tabidze V., Pipia I., Gogniashvili M., Kuneauri N., Ujmajuridze L., Pirtskhalava M., Vishnepolsky B., Hernandez AG., Fields CJ., Beridze T., Whole genome comparative analysis of four Georgian grape cultivars, Mol. Genet. & Genomics, (2017) 292(6):1377-1389. doi: 10.1007/s00438-017-1353-x.
- [28] Mathieu S., Terrier N., Procureur J., Bigey F., Günata Z., A Carotenoid Cleavage Dioxygenase from *Vitis vinifera* L.: functional characterization and expression during grape berry development in relation to C13-norisoprenoid accumulation, Journal of Experimental Botany, Volume 56, Issue 420, (2005) pg. 2721–2731, <https://doi.org/10.1093/jxb/eri265>
- [29] Meng N, Wei Y, Gao Y, Yu K, Cheng J, Li X-Y, Duan C-Q and Pan Q-H (2020) Characterization of Transcriptional Expression and Regulation of Carotenoid Cleavage Dioxygenase 4b in Grapes. Front. Plant Sci. 11:483. doi: 10.3389/fpls.2020.00483
- [30] Dunlevy JD., Soole KL., Perkins MV., Dennis EG., Keyzers RA., Kalua CM., Boss PK., Two O-methyltransferases involved in the biosynthesis of methoxypyrazines: grape-derived aroma compounds important to wine flavour. Plant Mol Biol. 2010 Sep;74(1-2):77-89. doi: 10.1007/s11103-010-9655-y. Epub 2010 Jun 23. Erratum in: Plant Mol Biol. 2013 Mar;81(4-5):523. PMID: 20567880.
- [31] Jiu S., Xu Y., Wang J., Wang L., Liu X., Sun W., Sabir Ia, Ma C., Xu W., Wang S., Abdullah M., Zhang C., The Cytochrome P450 Monooxygenase Inventory of Grapevine (*Vitis vinifera* L.): Genome-Wide Identification, Evolutionary Characterization and Expression Analysis. Front. Genet. (2020) 11:44. doi: 10.3389/fgene.2020.00044

