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Genotype diversity and pathogenicity of Beauveria spp. Isolates from different ecoregions of Georgia

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A B S T R A C T

In this study entomopathogenic fungi *Beauveria* spp. from various ecoregions of Georgia have been studied morphological and on the genome level. After morphological analysis of monocultures, 6 strains were selected for further investigations. Several DNA fragments of isolated strains were amplified and sequenced, ITS region (the rRNA gene cluster), EF1 (the Elongation Factor 1-alpha) and (the intergenic) Bloc region. A BLAST analysis of sequences strains and their morphological studies has shown that the strains belong to two species: (1) *Beuveria bassiana* (strain- Bb001; Bb006) and (2) *Beauveria pseudobassiana* (strain - Bb007; Bb009, Bb010; Bb011). The regional differences between strains were identified through phylogenic tree. Base on this research, it was confirmed that the samples taken from the same region were located at the same species on phylogenic tree. This is a first report where most virulent strains identified and their virulent against the fall webworm, *Hyphantria cunea* have been studied. Two entomopathogenic fungal strains: Bb001 and Bb011 evaluated the pathogenicity on the larvae (L5-L7) *H. cunea.* Both fungal isolate were pathogenic. The mortality 68% - 71 % for *B.bassiana* (Bb001) and 76.9% -87% for *B.pseudobassiana* (Bb010) were observed. Mortality caused by Bb001 was significantly different between the concentration ($p<0,05$). One-way Anova, Single factor, $\alpha=0.01$: significance difference were found between the pairs of treatments: for Bb001 $1x10^8$ and $1x10^7$: $p=0,0025$, $F=18.7$; for Bb010 $1x10^8$ and $x10^7$: $p=0,000156$, $F=44.6$; for Bb001 $1x10^8$ and Bb010 $1x10^8$: $p=0,0001$, $F=46$. *B. pseudobassiana* proved highly pathogenic. Maximum of larvae mortality were observed in 4-9 day after treatment, were as in variance Bb010 mortality indicate later 5 -13 day.

Keywords: *Beauveria bassiana, Beauveria pseudobassiana*, morphological and molecular study, ITS, EF1, BLOC region.

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Introduction

Entomopathogenic fungi (EPF) are distributed in a wide range of habitats, including aquatic, forest agricultural, pasture, desert, and urban habitats [1-3]. Such fungi have the ability to regulate invertebrate pest populations and play an important role in biological control of various harmful insects and mites [4-7]. EPF, which naturally reside in soil, comprise an environmental reservoir of insect pest antagonists and are readily isolated from soils in various ecosystems [8].

Beauveria (Hypocreales: Cordycipitacea) is an anamorphic genus of soil borne facultative necrotrophic arthropod-pathogenic fungi [1, 9-11]. Many species of *Beauveria* grow naturally in all continents, except Antarctica, Arctic and have a cosmopolitan distribution [12]. The fungus has been isolated from more than 700 insect species belonging to nine orders [13-16].

There are several benefits of using *Beauveria* spp. to control invertebrate pest populations: it does not harm non-target organisms or the environment, it is easy to mass-produce and disseminate, it causes rapid host mortality, it has the potential to recycle in the environment, it is compatible with agricultural chemicals, and it is susceptible to genetic selection and DNA transformation [17-22]. Traditional identification of *Beauveria* spp. is principally based on conidial morphology. However, molecular phylogenetic analyses have revealed that the *Beauveria* genus includes cryptic species [23,24].

Beauveria spp. are widely used entomopathogenic fungi in agriculture. These fungi naturally produce biologically active toxins during their life cycle, which cause sustained, significant death of harmful insects of various species without human intervention. Importantly, this fungus is highly effective in controlling vectored human pathogens, specifically adult mosquitoes [25,26]. Also, pathogens that occur as saprotrophs and plant endophytes [27]. Therefore, it is crucial to undertake molecular characterization of *Beauveria* spp. for developing an effective strategy of biocontrol.

The South Caucasus region, particularly Georgia, is distinguished by its diversity of climate zones and biological species. Thus, some level of genetic diversity of entomophatogenic fungi in this region is expected due to the variability and uniqueness of local climates and host organisms. Local strains of such fungi may be adapted to their environment, which renders them of particular interest for use in biological control. Georgia has a large diversity of such fungi, due to its varying altitudes, eco-and cropping systems, which offers a unique opportunity to study local insect pathogens.

The objective of this study was to explore diverse habitats as potential sources of local strains of the entomopathogenic fungus *Beauveria,* their relative distribution in various geographical areas in Georgia, and their potential application as biological control agents against the target insect.

Materials and methods

Study site and collections of samples

Soil samples were obtained in 2007-2009, from four different geographical areas at different altitudes (600-1700 m a.s.l), representing different agro-and forest ecosystems of Georgia.

Isolation of entomopathogenic fungi

Single insects with the symptoms characteristic of entomopathogenic fungus have been detected in the population of Spruce bark beetles - *Ips typographus* L. adults and Colorado Potato beetles – *Leptinotarsa decemlineata* Say.

Fungi isolated from *I.typographus* and *L.decemlineata* were cultivated on artificial media and incubated at 23 ± 2° C for 12-15 days.

Colony growth and morphology

Colony descriptions and measurements were determined from cultures grown on full strength potato dextrose agar (PDA) (DifcoTM) at 23 ± 2° C in darkness at 14 day from inoculation. Terms and notations used to describe colony coloration, hyphae and conidia [28-30]. Microscopic measurements of conidiogenous cells and conidia were taken from PDA cultures at 5–15 days and images were acquired with a light microscope. Terminology for conidial shape follows [31], hyphae and conidia's measurements were taken.

Mycroscopial analyses

Infected insects, at first were examined for under stereomicroscope (MBC-9, USSR, magnification 40x). For the identification of entomopathogenic fungi, symptomatic insects and pathological material were incubated for five days in plastic vials for the moisture atmosphere.

Isolates of fungi cultivated on the artificial media: Potato Dextract Agar (PDA), and *Beauveria* selective media (BSM), for 10-14 days at 23±2^{0}C, until they developed feature permitting their identification as to species or genus [30,32].

The conidia were stained with lactophenol-cottonblue and examined with light and phase- contrast microcopy, to accurately detect morphological peculiarities (Zuzi,S120; magnification 400x, 1300x) for entomopathogenic microorganisms [24,28].

DNA Extraction and PCR amplification

Genomic DNA was extracted using the DNeasy Plant Mini kit (Qiagen, USA) [33]. Extracted DNA was quantified by gel electrophoresis [34]. PCR amplification of *Beauveria* spp. DNA was performed in 25 µl reaction volumes using primer pairs indicated in Table 1. ITS region (ITS4-ITS5)

Table. 1. *PCR Primers used in the study*

Name	Sequence [5´-3`]	PCR Fragment Size (bp)
ITS4 Fwd	5 ' -TCCTCCGCTTATTGATATGC-3 '	600
ITS5 Rev	5 '-GGAAGTAAAAGTCGTAACAAGG-3 '	600
EF2F Fwd	5 '-GGAGGACAAGACTCACATCAACG-3 '	700
1567R Rev	5 '-ACHGTRCCRATACCACCSATCTT-3	700
B22U Fwd	5 ' -GTCGCAGCCAGAGCAACT-3 '	1500
B822L Rev	5 ' -AGATTCGCAACGTCAACTT-3 '	1500

of the rRNA gene cluster [14, 33,35]. Elongation Factor 1-alpha [36,37] and the intergenic Bloc region [7,38].

For the ITS region, the PCR conditions included 3 min initial denaturation at 94 °C, 35 cycles of 1 min at 94 °C,1 min at 50 °C, and 1.5 min at 72 °C followed by final extension of 7 min at 72 °C. In the case of EF1-a and Bloc regions, 3 min initial denaturation at 94 °C, 35 cycles of 1 min at 94 °C,1 min at 55 °C, and 1 min at 72 °C followed by final extension of 10 min at 72 °C.

Bands of expected size were run on a 1% agarose gel containing ethidium bromide in 1X TAE buffer and were visualized by UV illumination using a digital imaging system. Nucleic acid concentration and purity ratios were obtained using NanoDrop.

Purified PCR products were sequenced from both ends using the following primers (Table 1).

Sequencing

Approximately 3-20ngof DNA were used in sequencing reactions with the ABI Prism® BigDye® Terminator Cycle Sequencing Ready Reaction kit v3.1 (Applied Biosystems, Foster City, CA). Cycle sequencing was performed on a GeneAmp® PCR System 9700 or 2720 Thermal Cycler (Applied Biosystems) according to the manufacturer's protocol (BigDye® Terminator v3.1 Cycle Sequencing Kit Protocol, Rev A, Applied Biosystems). Dye terminators were removed from the cycle sequencing reactions using Multiscreen-HV plates (Millipore, Mississauga, ON) loaded with Sephadex G-50 superfine (Sigma, Oakville, ON). Clean reactions were analyzed on an Applied Biosystems 3730 DNA Analyzer (Applied Biosystems). A minimum read length of 700bp was generated for each of the reactions. Chromatograms were analyzed using ABI Prism® DNA Sequencing Analysis Software Version 4 (Applied Biosystems) to generate quality target sequences within the software's clear confidence range. Single nucleotide polymorphism (*SNP*) analysis, Indels (insertion/deletion) and mutation *detection,* and phylogenetic tree construction were performed using the computer programs Mafft and Blast **[39-41**] http://www.ncbi.nlm.nih.gov)

Target Insect – The fall webworm (FWW)

The fall webworm (FWW), Hyphantria cunea Drury (Lepidoptera: Arctiidae) is a one of most dangerous polyphagous pests in Georgia. Different instars of living larvae of H. cunea were collected manually from orchards and forest trees in West Georgia.

Inoculums preparation

Fungal suspensions of the isolates were prepared from 4 week-old cultures grown on PDA at 23±2°C, with good sporulation, using distilled water containing 0.01% (w/v) Twin 80. The obtained suspension was filtered through two layers of sterile muslin into a sterile 50-mL plastic tube, to remove the medium and fungal debris and was then shaken for 5 min using a vortex for homogenization. The concentration of spores in the suspensions from each fungus was determined using a haemocytometer and adjusted to two concentrations of $1x10^7$ and $1x10^8$conidia ml^{-1} for bioassay [30].

Bioassay

The 5th and 7th instars of larvae (L5-L7) target insects - H. cunea performed for the bioassay was treated with fresh culture suspension of Bb 001 and Bps010 (1.0 X 107, 1.0 X 108), placed in a glass jar with mulberry tree leaves and kept at room temperature ~23 ºC (day) /18 ~ ºC (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 was recorded on 3-18 days after treatment [32].

Data Analysis

All mortality data were corrected for control mortality Abbott's formula (1925) [42]. The percentage of larvae mortality for each concentration was analyzed using one way ANOVA; means were separated by Turkey's mean separation test. Mortality was considered statistically significant ($P<0.01$).

Results and analysis

Fungal distribution and isolation

Adult beetles of *Ips typographus*, infected with entomopathogenic fungi were found under tree bark in the maternal gallery of *Picea orientalis* in Borjomi-Bakuriani gorgeous. The fungi were identified using microscopic preparations made directly from mycelia developing on beetles in dead bark. After morphological analysis of monocultures, individual isolates of *Beauveria* spp. were collected. Conidia dimensions were between (1.5) 2.0 – 3.0 (4.0) x (1.5) 2.0 – 2.5 (- 3.0) µm.

Samples were obtained from 2 different geographical sites at different altitudes (600-1700 meters above sea level (m a.s.l.) and climate zones, each representing a unique agricultural and forest ecosystem in Georgia. First investigation site is the Borjomi-Bakuriani region, located on the South Caucasian Mountain, second is the south slope of the Great Caucasus Mountain.

Adults of *Leptinotarsa decemineata* with mycosis symptoms were collected in the potato filed. Six isolates of *Beauveria* spp. were obtained from two regions of Georgia, as described in Table 2.

Morphological study of fungal isolation

Colony growth characteristics and appearance are similar among the majority of *Beauveria* species. All six isolates were cultured on full-strength Potato dextrose agar (Ø 90mm) at 23 ± 2° C at 12-15 days grows average 10–40 mm diam. The culture appears as white to light yellow colonies having a cottony, powdery, velutinous and woolly texture that frequently becomes farinaceous as conidia accumulate on the surface of aging cultures.

Microscopic observation of colony growth and conidia arrangement showed general and typical characteristics of *Beauveria.* After morphological analysis of monocultures, two species of *Beauveria* were identified: (1) *Beuveria bassiana* (Bb001; Bb006) and (2) *Beauveria pseudobassiana* (Bb007; Bb009, Bb010; Bb011). Morphological characterization of two *Beauveria* species are given in Table 3.

Table. 2. *Sites of investigation*

Name of isolates	Host	Region	Geographical location (lat.N,long.E)	Altitude	Habitat		year
					Habitat	Sub-habitate	
Bb001	*Ips typographus*	Borjomi-Bakuriani, LCM*	41⁰ 52' 40'' 43⁰ 22' 35''	950	Natural	Forest	2007
Bb006	*Ips typographus*	Borjomi-Bakuriani, LCM	41⁰ 45' 45'' 43⁰ 30' 58''	1000	Natural	Forest	2008
Bb007	*Leptinotarsa decemlineata*	Shovi, GCM**	42⁰ 35' 36'' 43⁰ 00' 88''	1800	Cultivated	Field crop	2009
Bb009	*Leptinotarsa decemlineata*	Shovi, GCM	42⁰ 23' 76'' 43⁰ 10' 13''	1800	Cultivated	Field crop	2009
Bb010	*Leptinotarsa decemlineata*	Shovi, Glola, GCM	42⁰ 33' 36'' 43⁰ 00' 58''	1730	Cultivated	Field crop	2009
Bb011	*Leptinotarsade cemlineata*	Shovi, Glola, GCM	42⁰ 44' 07'' 43⁰ 77' 01''	1780	Cultivated	Field crop	2009

*LCM- Lesser Caucasian Mountain; GCM**- GrateCaucasian Range

Table. 3. *Morphological characterization of Beauveria spp. isolates from different ecoregion of Georgia*

Species	Colony description	Colony size (Ø mm)	Size of conidia (µm)	Form of conidia
Beauveria bassiana (Bb001; Bb006)	cottony, powdery, velutinous, white or yellow white appressed to agar surface	13–30	(1.5) 2.0 – 3.0 (4.0) x (1.5) 2.0 – 2.5 (- 3.0)	globose, subglobose , broadly ellipsoid forming short chains
Beauveria pseudobassiana (Bb007; Bb009, Bb010; Bb011)	subvelutinous, velutinous to cottony, closely appressed to agar surface white or yellowish white	14–34	2–3 X 1.5–2.5 (1.5) 2.0 – 3.0 (3,5) x (1.2) 1.5 – 2.0 (- 2.5)	subglobose, ellipsoid

Colony of *Beauveria bassiana* on full strength PDAat 12 days (23°C), 13–30 mm diam., cottony, powdery, velutinous, white or yellow white, appressed to agar surface, at first white and becoming creamyyellowish white. Conidia aggregated in ball-shaped, spherical clusters among hyphae and white in mass. Colony reverseun colored to yellowish white. Vegetative hyphae septate, branched. Conidiogenous cells solitary, usually dense clusters, denticulate rachis, produced hyphae and conidia. Conidia (1.5) 2.0 – 3.0 (4.0) x (1.5) 2.0 – 2.5 (- 3.0)µm, globose, subglobose, broadly ellipsoid forming short chains.

Colony of *Beauveria pseudobassiana* growth on full-strength PDA 14–34 mm diam. at 12 day at 23°C. Surface mycelium subvelutinous, velutinous to cottony, closely appressed to agar surface, white or to yellowish white. Conidia aggregated as, 0.1 mm spherical clusters, white in mass, conidia occasionally. Forming a farinaceous surface layer in older cultures. Colony reverse either uncolored or yellowish white. Vegetative hyphae septate, branched, hyaline. Conidia 2–3 X 1.5–2.5 (1.5) 2.0 – 3.0 (3,5) x (1.2) 1.5 – 2.0 (- 2.5) µm, primarily subgloboseor broadly ellipsoid, rarely ellipsoid, occasionally with inconspicuous hilum at base, hyaline, aseptate walls thin and smooth (Fig. 1).

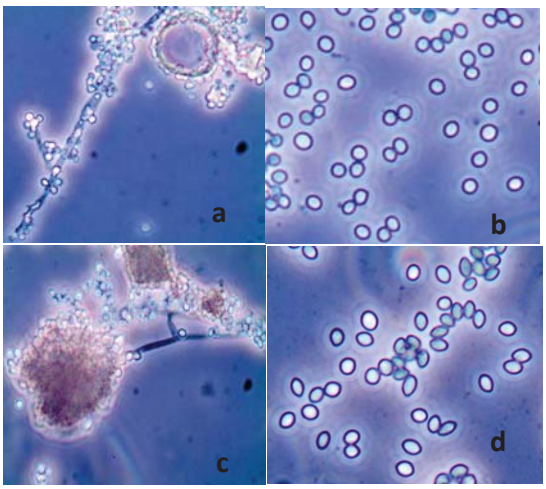

Fig.1. *Conidiogenous cells and conidia of Beauveria species. a-b B.bassiana; c-d B.pseudobassiana Molecular characterization of Beauveria spp.*

In samples that were obtained from two different regions of Georgia, the following sequence differences were identified: two samples from the Borjomi region and three samples from Shovi were identical to one another, respectively (Table 4).

The following genetic markers for characterization of *Beauveria* were used: the ITS region of the rRNA gene cluster, a region of EF1-α and the intergenic Bloc region. Mega6, Mafft, Blast, and DNASTAR were used to analyze nucleic acid sequence data [43].

Table. 4. *Strains isolated from two different regions of Georgia*

Strain designator	Short name of isolates*	Origin, species	Region of Isolates
Bb001	1B	*Beauveria bassiana*	Borjomi-Bakuriani
Bb006	2B	*Beauveria bassiana*	Borjomi-Bakuriani
Bb007	3S	*Beauveria pseudobassiana*	Shovi, CM*
Bb009	4S	*Beauveria pseudobassiana*	Shovi, CM
Bb010	5S	*Beauveria pseudobassiana*	Shovi, Glola, CM
Bb011	6S	*Beauveria pseudobassiana*	Shovi, Glola, CM

*Short name of isolates were given according for region

In this study we characterized genotypes of the entomopathogenic fungi of *Beauveria spp.* from different geographical and ecological regions of Georgia.To the best of our knowledge, this report describes the first time that *Beauveria* spp. have been characterized in Georgia using molecular genetics.

Strains from different geographical regions were compared using phylogenetic analysis of sequences of multiple gene fragments. Samples obtained from the same region were grouped together as clades within the phylogenetic tree (Fig. 2, 3).

Fig.2. *EF1- alpha region of samples 5S, 4S, 3S, 2S (Shovi) and 6b, 1b (Borjomi)*

Fig.3. *ITS region 4S, 3S, 2S (Shovi) and 5B, 1B (Borjomi)*

Sequences of Georgian species were compared to other strains from database. Georgian strains were found to belong to multiple clades, with the greatest homology in samples belonging to the A (*Beauveria bassiana*) or C (*Beauveria pseudobassiana*) clade [12]. Samples collected in this study were identified as *Beauveria bassiana* (99-100% coincidence) through comparison of sequences obtained from the ITS region of the ribosomal gene with those published in GenBank.

ITS5 and ITS4 primers are verified reference sequences for PCR amplification of unknown species. Partial sequences of two nuclear loci, including EF1-α and the intergenic Bloc region, were also used in analysis. EF1-α was selected for analysis because of its widespread use in phylogenetic studies of *Beauveria* and other fungi [23, 44]. Bloc is a nuclear intergenic region initially developed for investigation of cryptic speciation in *B. bassiana* [38].

Georgian DNA samples, which were compared with database samples, have shown that selected strains of *Beauveria* spp. are located in diferent clades. The highest level of homology was detected between samples located in A and C clade (Fig. 4).

The EF1-alpha and the intergenic Bloc region were also compared (in contrast to ITS region - the rRNA gene cluster, EF1-alpha, Bloc region are more variable and contains more information) of Georgian samples with the samples taken from various country, located in the different clades.

Strains from Borjomi belong to clade A (*B.bassiana*), whereas strains from Shovi belong to clade C (*B. pseudobassiana)* (Fig. 4).

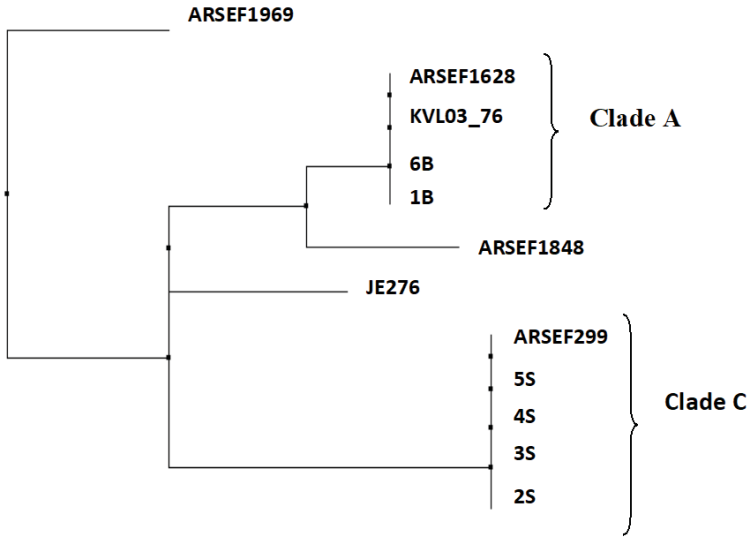

Fig.4. *Elongation factor-1a (EF1-alfa) phylogeny of Beauveria bassiana (B1, B6, S2, S3, S4, S5) Constructed Neighbour Joining tree using PID(Waterhouse et al 2009).The GenBank accession used for the analyses are Beauveria bassiana samples: AY531896(ARSEF 1628), EF193188 (KVL 03-76), AY531904(ARSEF 1848), EF193181(KVL 03-114), DQ376244(JE276), EF193192(KVL 03-91), AY531921, (ARSEF 292)AY531923(ARSEF 299), AY531907(ARSEF1969).*

Only two samples were obtained for the Bloc region (Borjomi region). Both samples are identical and differ by one SNP from GenBank isolates. Comparison of KVL 03_76 [7] with Georgian samples is described in Table 5.

Table. 5. *SNPs in Bloc region of Beauveria Bassiana*

Samples	Nucleotide position	Nucleotide
KVL 03 76	1118	G
B1, B6	549	A

After identification, we selected two entomopathogenic fungal strains: Bb001 and Bb011 and evaluated the pathogenicity on the larvae (L5-L7) *Hyphantrya cunea*. Both fungal strains were pathogenic. The mortality 68% - 71 % for *B.bassiana* (Bb001) and 76.9% -87% for *B.pseudobassiana* (Bb010) were observed (Fig. 5).

Mortality caused by Bb001was significantly different between the concentrations ($p<0,05$). One-way Anova, Single factor, $\alpha=0.01$: significance difference was found between the pairs of treatments: for Bb001 $1x10^8$ and $1x10^7$: $p=0,0025$, $F=18.7$; for Bb010 $1x10^8$ and $x10^7$: $p=0,000156$, $F=44.6$; for Bb001 $1x10^8$ and Bb010 $1x10^8$: $p=0,0001$, $F=46$.

B. pseudobassiana proved highly pathogenic against *H. cunea* causing 76.9 % and 87,6 % mortality in both cases and the highest rates of fungal outgrowth (Fig. 5).

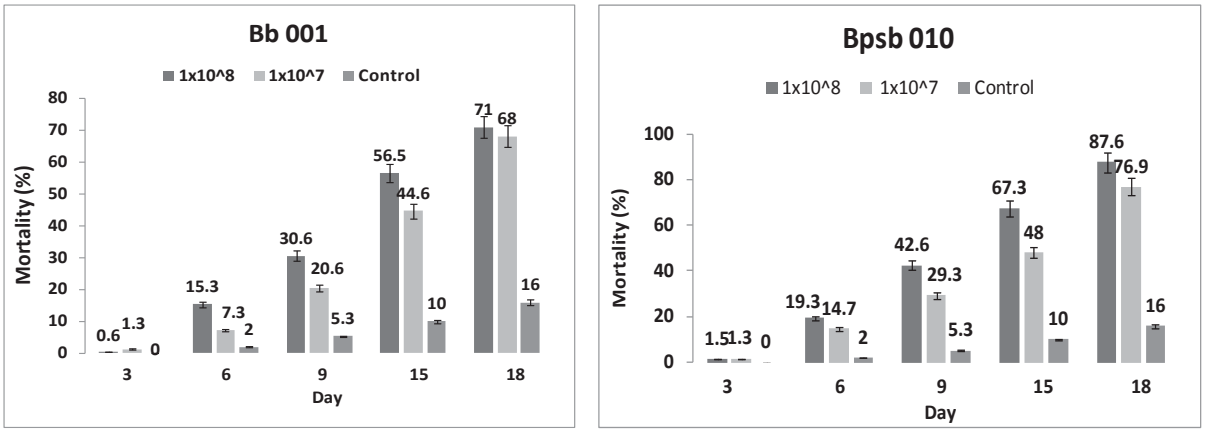

Fig.5. *Mortality (%) of larvae H. cunea after treatment with different concentration of EPF in laboratory per replicate (Mean %±SD), significant level α=0.01*

At high concentration ($1x10^8$) of promising isolates Bb001 showed significantly different results compared to Bb010. Maximum of larvae mortality were observed in 4-9 day after treatment, were as in variance Bb010 mortality indicate later at 5 -13 day (Fig. 6). However, virulence varied considerably. Development of mycosis in variance Bb001 were observed in the L5-L6 larvae and in cocoon, in variance Bb010 mycosis symptoms mostly were observed in L6-L7 instars larvae. A quick development of mycosis was observed in variance of Bb010.

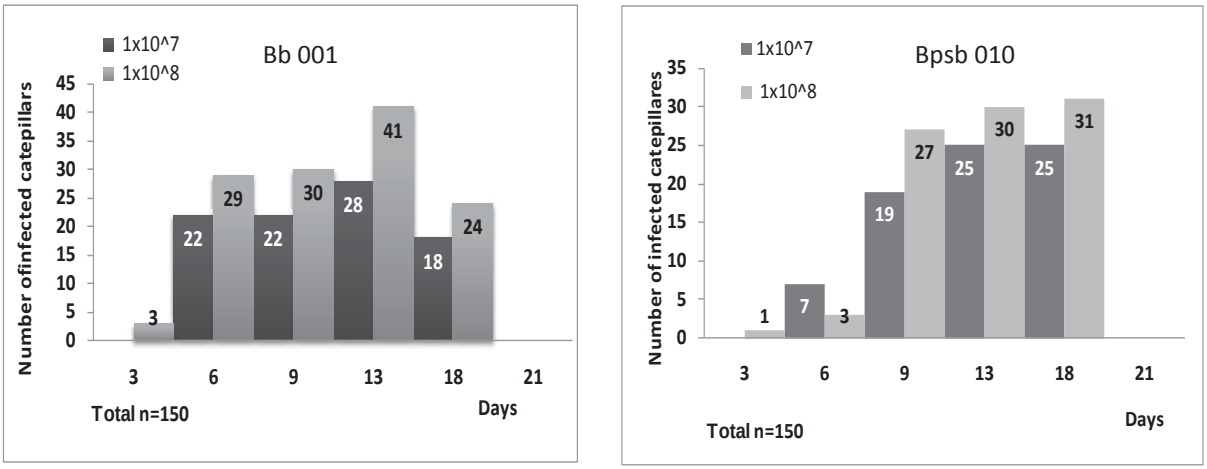

Fig.6. *More susceptible time (in days) for the appearance of mycosis infection in larvae of H. cunea by Bb001 and Bb011.*

Vertical transmission of Beauveria spp.

The surviving larvae hidden under the leaves and cordon, made cocoons and were transformed into pupae. They have been left overwinter until spring at 8±5 ° C.

In the spring adult moths started to appear from the pupae, followed by mass emergence in 4-5 days with subsequent mating. The mass laying eggs lasted for 10-12 days. The numbers of oviposition groups and eggs were significantly larger in variance of Bb010 (2 ovipositions group) then in Bb001 (Fig. 7).

The emerged adults in Bb001was 68.4-15% and inBb010 - 48.4-42.18 60%. The larvae hatched seven to ten days later (Bb001 - 76.3%, Bb010-70%, Control - 89.5%) and began feeding on fresh leaves intensively in-group.

It should be noted, that in the instance of Bb010 a dimorphic male with incompletely formed right wings was observed.

These results suggest that theBb001and Bb010 isolates may be used to control *H. cunea*.

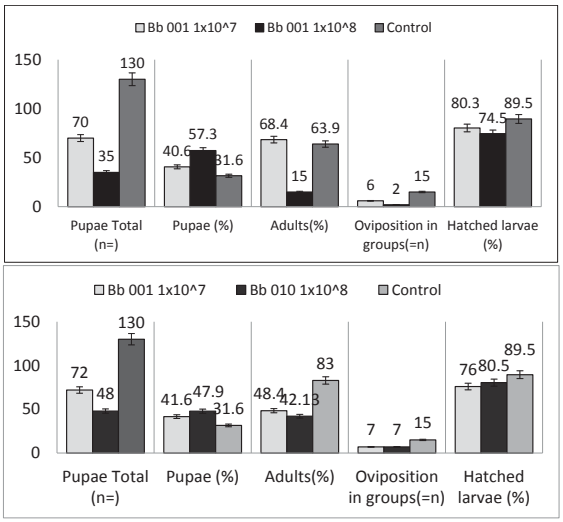

Fig.7. *The number of emergences adult of H. cunea from the overwinter pupae and they reproductive rate (=n - number)*

According to the results, the number of larvae that transformed into pupae was considerably less in the experimental group than in the control group.

Conclusion

Beauveria is a cosmopolitan anamorphic, a facultative soil-borne pathogen that has well-established characterization of the genus [45]. The genus *Beauveria* is characterized morphologically by globose to flask-shaped conidiogenous cells from which one-celled, terminal holoblastic conidia are produced in sympodial succession on an indeterminate, denticulate rachis. *Beauveria* species are distinguished principally according to characteristics of their conidia, which are typically smooth-walled, hyaline, 1.5–5.5 mm and globose to cylindrical or vermiform [46,47].

For the study of the genetic diversity and molecular ecology of *Beauveria, B. bassiana* and *B. brongniartii* are redescribed, and typifications are proposed for these species. Genealogical criteria were used to delimit the six novel clades orphy logenetic terminals that are described here as new species, including: *B. asiatica, B. australis, B. kipukae, B.pseudobassiana, B. sungii B.varroae*. and *B. amorpha*has been published and an epitype specimen has been proposed [10,24], which occur as multispecies assemblages in both natural and agricultural habitats [7,38].

Fall webworm (FWW) - *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) is the most polyphagous insects all over the world's, has invaded in Georgia and well adapted to local climate condition. There are found rare Georgian endemic plant in Caucasus within its host range (some 600 plant species). Population of *H. cunea* increased last two decade, so the Georgian Government has declared as a national problems, because our purpose was to evaluate efficiencies of local isolates of *Beauveria* spp. on the *H. cunea* [48]. Within the framework of the assessment of the biodiversity of *Beauveria* spp. isolated from different ecoregion of Georgia, has been evaluated using both morphological criteria and the molecular taxonomic characterization. Previous classification of isolates to presents *Beauverri* spp.

Morphologically and the molecularly different single-spore-derived cultures here referred: (i) *Beauveria bassiana*, (Bb001; Bb006) isolated from *I.typographus* population in the Lesser Caucasus Mountain and (ii) *Beauveria pseudobassiana* (Bb007; Bb009; Bb010; Bb011) isolated from the population *Lepthinotarsa decemlineata* and from South slope of the great Caucasus Mountain soil.

In this study, we characterized genotypes of the entomopathogenic fungi *Beauveria* from various regions of Georgia. To the best of our knowledge, this report describes the first time that *Beauveria*

spp. The goal of our research was to test the isolated virulent *Beauveria* sp. against a target insect species. The fall webworm – *H. cunea* appeared to bean excellent candidate because it is one of the most dangerous polyphagous insects in Georgia that affects more than 600 species of plants. Two isolates from the mountanious regions of both East and West Georgia were tested. In both cases, that the virulence of these strains towards the *H. cunea* was high. There are preliminary data where used to ascertain the entomopathogenic activity of the isolates.

Sequencing data of the IF1 a, ITS and and the BLOC DNA regions of the *Beauveria* isolates confirmed the presumed species: sample 6, 1 was confirmed as *Bauvaria bassiana*, while samples 2,3,4,5 were confirmed as *Beauveria pseudobassiana*. The initial evaluation of these species was done on the lowest taxonomic level. At the same time, the picturesclearly show the difference between the conidia of *B. bassiana* and those of *B. psedudobassiana*. About distribution of *B. pseudobassiana,* it is found both in Europe and North America, and likely elsewhere [24, 45, 49].

GenBank database material of *B.bassiana* with respect to other *Beauveria* species and the fact that above all older entries are referred to as *B. bassiana* [45]. Compared the sequencing results with the genebank's outdated data, all samples were determined to belong to *B. pseudobassiana*, despite the fact that, according to the modern classification, part of these amples can now be classified as *B. pseudobassiana*. Molecular testing, therefore, turned out to be more effective in species-specific identification of the highly virulent fungi isolates from the distinct ecological areas of Georgia.

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