

Testing local isolates of entomopathogenic microorganisms against Brown Marmorated Stink Bug *Halyomorpha halys* in Georgia

Medea Burjanadze,* Natalia Kharabadze, and Nona Chkhidze

Agricultural University of Georgia, Vasil Gulisashvili Forest Institute, 0159, Tbilisi, Georgia

Abstract. The brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae) (BMSB) is an exotic invasive insect which has spread extensively and established in new area of Black sea regions of Georgia. During 2017-2019, infected adults with fungal disease symptoms were found in population of *H. halys* from different regions of Western Georgia. Three isolates of entomopathogenic fungus *Beauveria bassiana* sensu lato and one of *Isaria cf fumosorosea* were recovered. Mycoinsecticide Bover-Ge, based on a local strain of *B. bassiana*-024 (isolated from soil high mountain of Caucasus Range, provide molecular identification in CABI-UK, gave a unique cultural number - IMI # 501797 and keep in CABI Genetic Recourse Collection) two concentrations (1×10^7 and 1×10^8 conidia/ml) was tested on the adults of *H. halys* in laboratory and its efficiency reached 72.0-90.5%. Under laboratory conditions, local isolates of nematodes *Heterorhabditis bacteriophora* and *Steinernema borjomiensis* were tested on *H. halys* adults. At three increasing concentrations (200, 500 and 1000 IJs/mL), *H. bacteriophora* caused bug mortality at the rates of 33-47 %. As for *Steinernema borjomiensis*, the respective values were 13-33 %.

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål) is native for East Asia. It is nowadays distributed throughout the US, Canada, Europe as a severe invasive agricultural pest [1,2]. BMSB is widely polyphagous, it feeds on over 170 host plants, many of them are of agricultural importance, such as fruit, vegetables, row crops, and ornamentals [3,4]. Following its first detection in 2015, *H. halys* has been spread extensively and established in new areas of Black sea regions becoming a key pest of many crops in Western Georgia [5]. At present BMSB is highly active, forming outbreaks foci in agricultural and urban landscapes and making the phytosanitary situation 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.

The aim of our study was to identify pathogens in populations of *H. halys* in Georgia and evaluate their potential for the pest control. During 2017-2019, adults of *H. halys* were collected from different regions and sites (Samegrelo, Guria, Imereti) of West

* Corresponding author: m.burjanadze@agruni.edu.ge

Georgia. More than 350 insects were studied. In different *H.halys* populations, adults developed mycosis symptoms on the body were observed. Three isolates of entomopathogenic fungus *Beauveria bassiana* sensu lato and one of *Isaria* cf *fumosorosea* were identified (Table 1; Figure 1). Adults infected by *Beauveria bassiana* and by *Isaria* cf *fumosorosea* 0.3 % were observed at 0.9 and 0.3 % prevalence rate, respectively.

Table 1. Sites of collection adults of *Halyomorpha halys*

Isolate names	Region	Geographical location (lat.N,long.E)	Altitude	Habitat		year
				Habitat	Sub-habitat	
<i>Beauveria bassiana</i> MB-101	Samegrelo	42°33'17" 41°51'24"	100	Orchards	Apple	2017
<i>Beauveria bassiana</i> MB-102	Samegrelo	42°01'58" 41°56'28"	73	Cultivated	Hazelnut s	2018
<i>Beauveria bassiana</i> MB-103	Guria	42°33'17" 41°51'24"	50	Cultivated	Hazelnuts	2018
<i>Isaria</i> cf <i>fumosorosea</i> MB-110	Guria	42°05'47" 42°12'04"	30	Cultivated	Hazelnuts	2018

Morphological characters were obtained for cultures grown on solid potato dextrose agar (PDA) at 23 ± 2° C in darkness for 14 days after inoculation [6-8], including measurements of conidiogenous cells and conidia acquired with a light microscope (Zuzi, S120; magnification of 400× and 1300×).

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 bassiana* sensu lato conidia dimensions were between (1.5) 2.0 – 3.0 (4.0) x (1.5) 2.0 – 2.5 (3.0) µm. In case of *Isaria* cf *fumosorosea* conidia dimensions were between (2.5) 3.0 – 4.0 (4.6) x (0.7) 1.0 – 2.0 (2.5) µm [9].

In 2019, a mycoinsecticide Bover-Ge (powder) was registered by National food agency of Georgia as a biopesticide. The bioformulation is based on a local Georgian strain of *B. bassiana*-024 from high mountain soil of Caucasus Range, supported by molecular identification in CABI-UK, gave a unique cultural number - IMI # 501797 and keep in CABI Genetic Recourse Collection. The product, tested at two concentrations (1x10⁷ and 1x10⁸ conidia/ml) against stink bug adults under laboratory conditions showed efficacy of 72.0-90.5% at 12 days post treatment (Figure 2).

Two entomopathogenic nematodes isolated from Georgian ecosystems *Heterorhabditis bacteriophora* (HRB, GEO)[10] and new species of entomopathogenic nematode, *Steinernema borjomiense* n. sp., was isolated from the body of the host insect, *Oryctes nasicornis* (Coleoptera: Scarabaeidae), in Georgia, in the territory of Borjomi-Kharagauli [11] were used to assay their effectiveness against adult *H. halys*. Under laboratory conditions (22°C and 80% RH), the nematodes were used in the following doses: 1:1000, 1:500, 1:200 infective juveniles (IJs)/ml. *H. bacteriophora* and *S. borjomiense* at the concentrations of 1:1000, 1:500 and 1:200 induced mortality of 46,6% -33,3%, 33,3%-32% and 33,3%-13,3%, respectively at 9 days post treatment of adults *H.halis* (Figure 3).

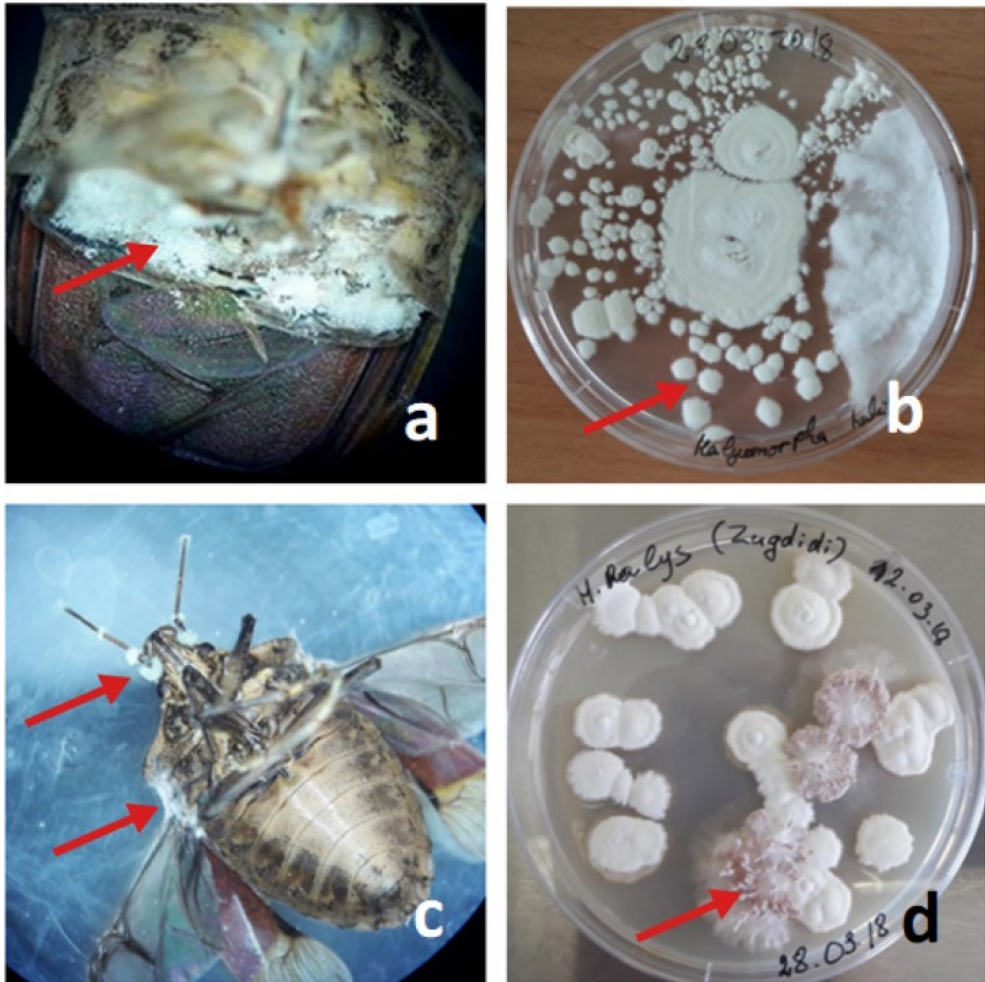


Figure 1. Isolation entomopathogenic fungi from different population of BMSB – *H. halys*: **a**- infected adults by *Beauveria Bassiana*; **b** – colony develop on the PDA media; **c** - infected adults by *Isaria fumosorosea*; **d** - colony develop on the PDA media.

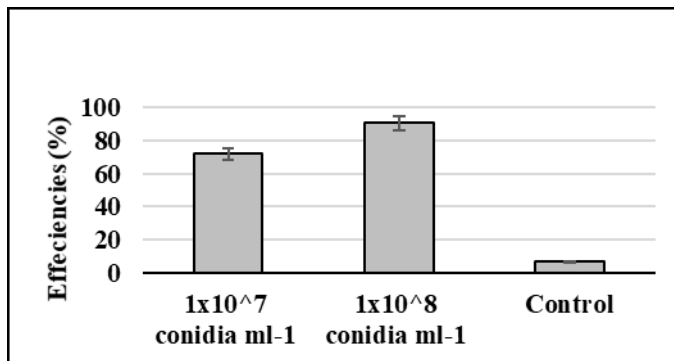


Figure 2. Efficiency of Bover-Ge on the adult *Halyomorpha halys* under laboratory conditions

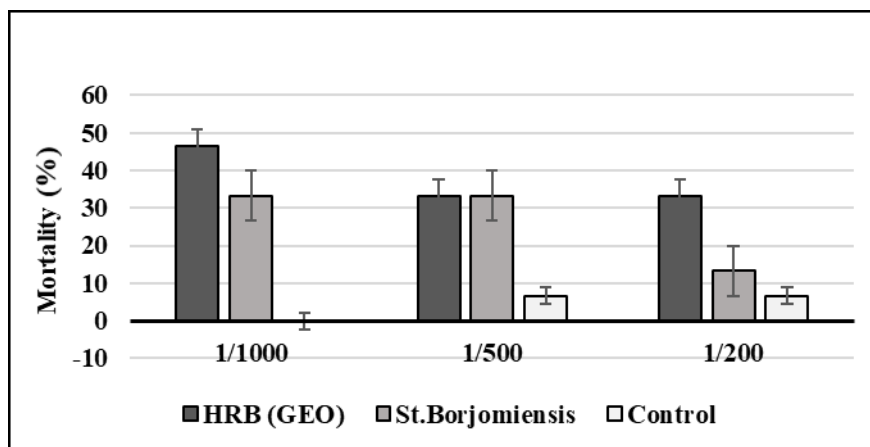


Figure 3. Mortality (%) of adults of *H. halys* due to infection with entomopathogenic nematodes

These results demonstrate high efficiency of entomopathogenic fungi and low potential of the nematodes to control *H. halys*. Further trials are still needed to fully assess the efficacy of these entomopathogens and formulations under different environmental and field conditions. These studies are important both for Georgia and adjacent territories where the pest is also widespread [12].

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