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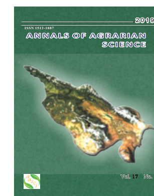

Bover-Ge – mycopesticedes for the control Brown marmorated stink bug - Halyomorpha halys (Stål) (Hemiptera: Pentatomidae)

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A B S T R A C T

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^{TM} based on a local strain of entomopathogenic fungus *Beauveria bassiana*, with tree concentrations ($1x10^6$, $1x10^7$ and $1x10^8$ 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.

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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, mycopesticiede, 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 (2.2×10^{10} conidia g^{-1}). The formulation were diluted to deliver the desired test concentrations 1×10^6; 1×10^7 and 1×10^8 conidia ml^{-1}. 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 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 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_{50}) for the different treatments. The mortality data were transformed to probits, while the concentrations were transformed into Probit log10 (dose). Before analysis LC_{50} values were estimated from the probit lines.

For the determination of lethal time (LT_{50}) 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_{50}), 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 (1×10^8 conidia/ml) reached 93.3% and 53.3%, whereas at concentrations of 1×10^7 and 1×10^6 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_{50} levels. The concentration required to cause 50% mortality of *H. halys* for different are presented in Table 1. All

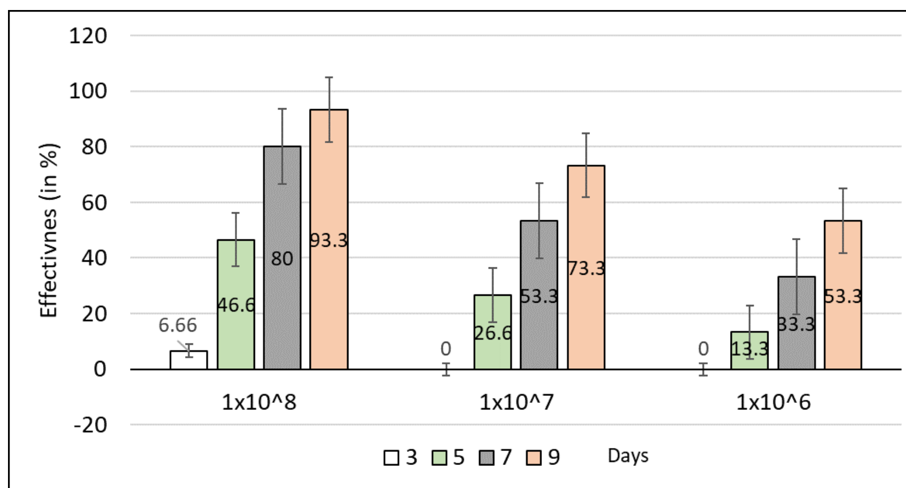

Fig. 1. Efficiencies of Bover-Ge on the adult *H .halys* according to days in laboratory

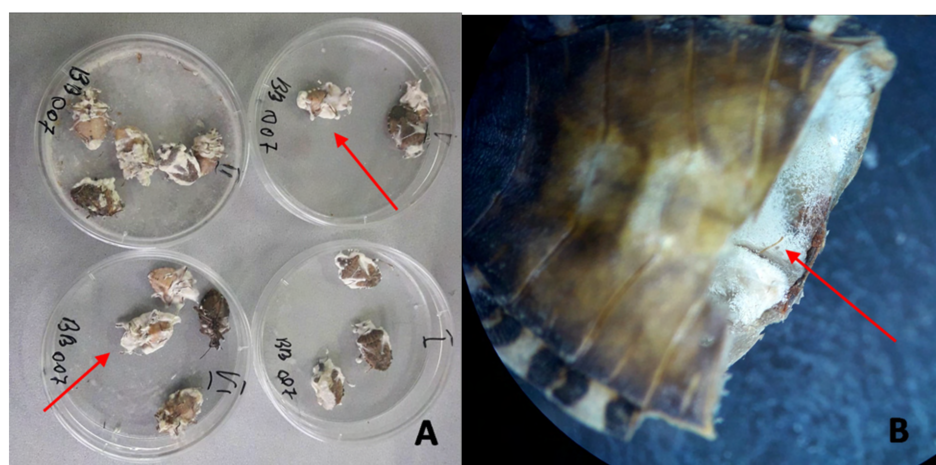

Fig. 2. Effeciencies Bover-Ge on the adult *H. halys* in laboratory

three concentration are pathogenic with a mean mortality ranging from 33.3 to 93.3% (P ≤0.05). The highest percentage of mortality was observed 9 days after treatment. Bover-Ge in high concentration 1×10^{8} conidia/ml significantly more virulent than the other tested concentrations. Probit analysis used to analyse the mortality results revealed that 1×10^{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 ≤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 (1×10^{7} and 1×10^{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. hayls* adults exposed to the control and the selected concentration 1×10^{7} and 1×10^{6} conidia/ml adult indicated no significant difference between the mortality times (Tables 3 and 4). Survival curves for treatments with concentration 1×10^{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 1×10^{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_{50} of *H. halys* adult treated by the Bover-Ge in deferent concentration (ANOVA, $P \leq 0.05$)

Strain	Slope±SE	LC%50 PPM	95% Fiducial CI		Chi-test (χ2) Sig	df	*P-value*
			Lower	Upper			
1×10^8 conidia/ml	0.721±0.322	879.3	205.4	3764.2	0.871	1	0.015
1×10^7 conidia/ml	1.255±0.228	64.2	22.9	179.6	0.997	1	0.034
1×10^6 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_{50} of *H. halys* adult on 9 days after treatment *by Bover-Ge*

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

[a] Percent of dead individuals at the end of experiment corrected for mortality in control using Abbott's formula;

[b] The mean survival time and its standard error were underestimated because the largest observation was censored;

[c] Total number of individuals in bioassay.

Table 3. Mortality (%), mean survival time and LT_{50} of *H. halys adult* 7 days after treatment by *Bover-Ge*

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

[a, b, c] For explanations see Table 2

Table 4. Mortality (%), mean survival time and LT_{50} (days) of *H.halys* adult 5 days after treatment by Bover-Ge

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

[a, b, c] For explanations see Table 2.

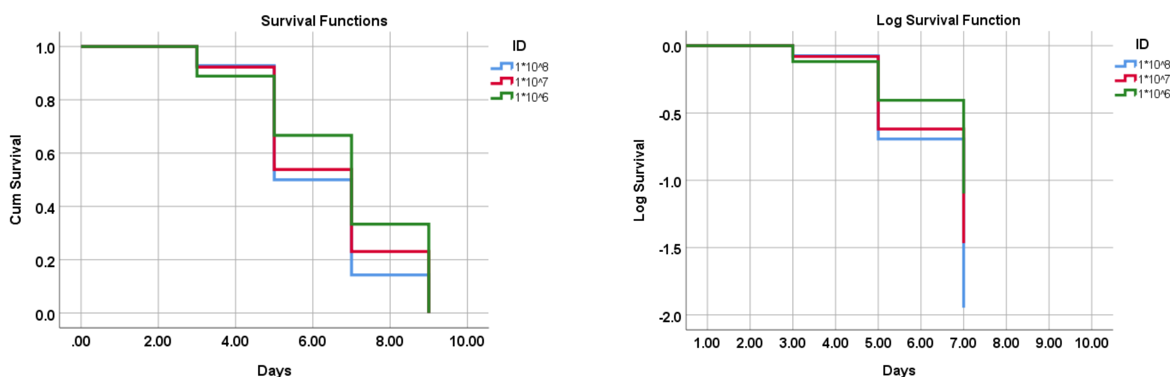

Figure 5. Survival curves (Lethal concentration LC_{50}) of H. *halys* adults treated by Bover-Ge.

Also to establish postmigration efficacy of mycopesticides, Bover-Ge was tested on the migration stage of *H. halyes* 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 (1x10^{8} conidia $^{ml-1}$)	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 1×10^8 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 (1×10^8 conidia/ml) reached 93.3% and 53.3%, whereas at concentrations of 1×10^7 and 1×10^6 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

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