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Use of Biological Agents to Control the Number of *Halyomorpha halys* Stål

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Abstract

Background: *Halyomorpha halys* Stål, commonly known as the brown marmorated stink bug, poses a significant threat to various crops, necessitating repeated seasonal insecticide applications for control. However, these methods carry environmental and human health risks. Considering these concerns, there is a pressing need to explore alternative, eco-friendly approaches to managing H. halys populations. This study sets out to investigate innovative strategies that minimize reliance on harmful chemicals while still effectively controlling the stink bug menace. By delving into the realm of sustainable pest management, the aim is to develop methods that strike a balance between pest control efficacy and environmental stewardship. This study aims to explore eco-friendly approaches to managing *H. halys* populations.

Methods: The research involved monitoring the population dynamics of *H. halys* in the central zone of the Krasnodar Territory, Russia. It also evaluated the efficacy of local parasitic insects *Pediobius cassidae* and *Anastatus bifasciatus* in reducing pest infestations, conducted laboratory tests on the insecticidal properties of essential oils from plants such as wormwood, coriander, and Siberian fir, and performed field trials on biological control agents.

Results: Infestation rates by parasitic insects remained relatively low, ranging from 5-10%. Laboratory experiments demonstrated the potent insecticidal properties of essential oils derived from wormwood, coriander, and Siberian fir. Field trials further confirmed significant mortality rates of *H. halys* specimens when exposed to biological control agents.

Conclusions: The study underscores the potential efficacy of utilizing parasitic insects and plant-derived essential oils in managing *H. halys* populations. These findings offer practical insights for promoting sustainable agricultural practices in the central zone of the Krasnodar Territory.

Introduction

The brown marmorated stink bug *Halyomorpha halys* Stål is a broad polyphage, therefore, the degree of its harmfulness is very high [1]. Feeding on the juices of plants from various families, the pest not only worsens the quality of agricultural products (apples, figs, tangerines, persimmons, plums, cherry plums, sweet peppers, soybeans, etc. [2-4] but can also spread various diseases, including phytoplasmic and viral [5,6] which together causes huge economic damage to agriculture [7,8].

In the places of the original natural habitat, *H. halys* does not cause such harm as in the newly developed territories. This is due to the long-established ecology of the species, as it occupies a certain niche in its original habitat and has its second-order consumers. These include entomophages, including parasitic insects [9,10], and pathogens (*Beauveria bassiana* and *Metarhizium anisopliae*), which restrain their reproduction [11,12]. No natural enemies have been found on the territory of the Krasnodar Territory, Russia, that can effectively restrain *H. halys* population. Among the potential agents regulating the number of *H. halys* specimens, representatives of the families Scelionidae, Eulophidae, and Eupelmidae are noted by several authors [13,14]. These egg-eating insects are non-specific and in natural conditions do not demonstrate a high effect on deterring pest reproduction [15-17]. Thus, the main way to control its population now is the use of biological and chemical preparations [18], mechanical collection from wintering sites [13], and the use of pheromone traps [14,19].

Due to the insufficient effectiveness of natural egg-eating entomophages living in the Krasnodar Territory, the restriction of the use of chemical insecticides in recreational areas, and the negative consequences of the use of chemicals, there is a need to find other methods to limit its reproduction and harmfulness [20].

Such methods may include the use of preparations based on living organisms and their waste products (fungi, bacteria, viruses), as well as substances of natural origin, which include essential oils of plants or their synthetic analogues [21,22].

Insecticides based on bacteria, fungi, and viruses or their biologically active compounds have long been known as an alternative to synthetic preparations to control invertebrate pests. Understanding the risks to the environment and human health from the use of chemical pesticides [23], changing standards for chemical residues in food, and increasing demand for organic agricultural products contribute to a significant increase in the use of biological and biorational preparations [24,25].

Essential oils are important and are used by humans for various purposes: in pharmaceuticals, cosmetics (as flavourings), and agriculture (as suppressants for pathogenic microorganisms, as herbicides, acaricides, and insecticides) [26,27]. Essential oils have low toxicity to mammals, decompose rapidly in the environment [28], and contain complex mixtures of biologically active components with multi-modal activity against target insect populations [29,30]. Research into the use of plant essential oils as insecticidal agents or repellent substances has had positive results and is recommended by several authors for use in integrated plant protection systems [29,31-34].

Methods

Laboratory studies were carried out during 2018-2020 at the Federal Research Center for Biological Plant Protection (FRCBPP) (Krasnodar, Russia). In the experiments, we used *H. halys* on imaginal stage collected manually from trees and shrubs, on soybean crops and plots adjoining the farms, as well as using a pheromone trap (Figure 1). A strictly specified volume of the test substance was applied to the abdomen of the imago using a micro-syringe applicator. The effectiveness of each biologically active substance (BAS) was judged by the survival rate of stink bugs on the first, third, and fifth days after application. *H. halys* imagos served as a control variant without the application of biologically active substances. The experiment was carried out four times (at least 15 specimens in each variant). The reliability of the differences between the variants was checked using Duncan's test.

Figure 1: Pheromone trap for catching *H. halys*, FRCBPP, 2020.

Field tests of low-risk biorational preparations took place at the experimental site of the FRCBPP (eight-field research crop rotation, 2020-2022) in soybean crops of the Arleta variety. The area of each plot was 10 m^2. The experiment was carried out three times. The treatment was carried out using a 5-liter manual sprayer of the 77330 model (manufactured by FIT IT, China). The following preparations were used in the experiment: experimental FRCBPP preparation with a rate of application of 2 l/ha (active ingredient: a complex of essential vegetable oils); Biostop, P (active ingredient: a complex of biological objects *Bacillus thuringiensis*, *B. bassiana*, and *Streptomyces sp.* 3NN strain), with the rate of application equalling 100 g/ha (Invivo LLC, Russia). The control plot was not treated in any way. The records were carried out three times: on the third, fifth, and tenth days after treatment. Statistical processing based on the results of the calculations was carried out using Duncan's test using the STATISTICA software package, version 13 (StatSoft, Inc., USA).

In the natural conditions of the Central Zone of the Krasnodar Territory in 2020, the dynamics of the *H. halys* population were studied.

Simultaneously with the study of the dynamics of the pest population, a search was carried out for *H. halys* egg-laying sites to consider the number of eggs infected with natural parasitic insects from the total number of detected eggs. For this purpose, route surveys of various *H. halys* habitats (trees and shrubs, field plots with soybeans) were carried out, during which *H. halys* eggs were collected (40 egg sets were collected in total) and further observations were carried out in laboratory conditions. The infestation of eggs was judged by the darker color and subsequent emergence of parasites.

Laboratory screening of several vegetable essential oils and their compositions was carried out to search for active substances capable of influencing *H. halys* vital activity. In the field, we tested the following biorational preparations: Biostop, P, and the FRCBPP experimental preparation (based on vegetable oils) [15,16].

Results

The first specimens of *H. halys* were discovered at the end of May with 1-2 specimens per trap (Figure 2). *H. halys* pheromone is aggregative and attracts adult insects (females and males) and larvae (mainly at ages IV-V). By the second decade of June, the number of insects had increased to 26.7 per trap and then dropped sharply. The peak number was observed in the middle of the first decade of July, and the trap mainly contained *H. halys* larvae. Then the number of stink bugs dropped. Further, until mid-September, there was a gradual increase in the number of trapped specimens. From the third decade of September, the number of stink bugs began to fall and in early October amounted to 4 specimens/trap.

In 2019-2020, two species of parasitic insects were recorded in the Krasnodar Territory: *Pediobius cassidae* Erdos (Hymenoptera: Eulophidae) and *Anastatus bifasciatus* Geoffroy (Hymenoptera: Eupelmidae) infesting *H. halys* eggs.

Parasites were bred from parasite-infested eggs collected in nature. Then, *Podisus maculiventris* and *H. halys* eggs were used to maintain the parasite population in the laboratory (Table 1).

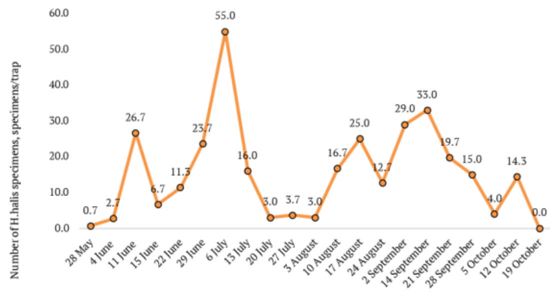

Figure 2: Dynamics of the *H. halys* population, 2020.

As can be seen from the data presented in Table 1, natural parasites in the laboratory effectively infested the eggs of stink bugs. Infestation with the parasite *P. cassidae* was 47.9% for Podisus eggs and 68.4% for *H. halys* eggs while the parasite *A. bifasciatus* infested 66.6 and 75.0% of eggs, respectively. A comparison of the infestation of eggs of the marmorated stink bug and predatory Podisus stink bug with parasites showed that *P. cassidae* and *A. bifasciatus* caught in nature equally effectively infested the eggs of both species of stink bugs, which makes it possible to further develop parasites of the marmorated bug on Podisus eggs in laboratory conditions and release them in the early spring period, when the number of natural parasites is insufficient to regulate the reproduction of the pest. The absence of significant differences in infestation according to Duncan's test shows that the breeding of parasites is possible on Podisus eggs.

Infestation of *H. halys* eggs by natural egg-eating populations in the field in 2020 was observed in the second to third decade of May and the first decade of June. Of the 40 egg sets collected as a result of route surveys, three were infested with parasites (*P. cassidae* emerged out of one egg set, and *A. bifasciatus* out of two). On average, the infestation rate of eggs was 5-10%, which did not lead to a significant decrease in the number of *H. halys*. With the onset of high temperatures and low humidity, which were observed for most of the summer period, infection of the pest's eggs was not noted.

In experiments devoted to the search for BAS capable of having a depressing effect on *H. halys* vital activity, essential oils of plants from the following families were used: Umbelliferae (coriander, dill, fennel oils), Compositae (wormwood oil), and Abies (Siberian fir oil).

Type of parasite	*P. maculiventris*			*H. halys*		
	Number of eggs, pcs.	Contains parasites, %	Infested, %	Number of eggs, pcs.	Contains parasites, %	Infested, %
P. cassidae Erdos	$98 \pm 0.7^{e*}$	47^{a}	47.9^{a}	76 ± 0.6^{c}	42^{a}	68.4^{a}
A. bifasciatus Geoffroy	87 ± 0.5^{d}	58^{a}	66.6^{a}	64 ± 0.6^{b}	48^{a}	75.0^{a}

*Note: There are no statistically significant differences between variants marked with the same letter indexes when compared within the columns according to Duncan's test at a 95% probability level.

Table 1: Infestation of stink bug eggs with parasites, 2020, 2021
Source: compiled by authors

Variant	Dose, mg/specimen	Before treatment, specimens	After treatment, days							
			Number of insects, specimens				Death of insects, %			
			1	3	5	10	1	3	5	10
Wormwood essential oil	1.0	4.5^{cde}	0.25^{a*}	0.25^{a}	0^{a}	0^{a}	95.8^{cd}	95.8^{cd}	100^{d}	100^{d}
Siberian fir essential oil	1.0	3.8^{cd}	2.5^{b}	0.25^{a}	0.25^{a}	0.25^{a}	37.5^{ab}	93.7^{cd}	93.7^{cd}	93.7^{cd}
Coriander essential oil	2.0	4.8^{def}	4.3^{cde}	2.5^{b}	0.75^{a}	0.25^{a}	10.0^{a}	48.7^{b}	85.0^{c}	95.0^{cd}
Dill essential oil	1.0	6.0^{f}	5.5^{ef}	4.8^{def}	4.8^{def}	3.8^{cd}	8.3^{a}	20.8^{ab}	20.8^{ab}	37.5^{ab}
Fennel essential oil	1.0	5.0^{def}	4.8^{de}	4.8^{de}	4.3^{cde}	3.3^{bc}	5.0^{a}	5.0^{a}	15.0^{a}	35.0^{ab}
FRCBPP experimental preparation	2.0	11.3^{g}	1.25^{a}	0.5^{a}	0.5^{a}	0.5^{a}	89.3^{c}	96.7^{cd}	96.7^{cd}	96.7^{cd}
Control	-	4.0^{cd}	4.0^{cd}	4.0^{cd}	4.0^{cd}	4.0^{cd}	-	-	-	-

*There are no statistically significant differences between the variants marked with the same letter indices according to Duncan's test at a 95 % probability level.

Table 2: Laboratory assessment of the biological activity of preparations based on essential oils against *H. Halys* adult specimens, 2020-2022
Source: compiled by authors

Variant	Application rate	Number of *H. halys* larvae and adults, specimens/plant				Decrease in number adjusted for control after treatment by day of counts, %		
		Before treatment	After treatment					
			3	5	10	3	5	10
Biostop, P	100 g /ha	2.8^{abc*}	1.8^{ab}	1.6^{ab}	0.5^{a}	37.4^{a}	54.1^{ab}	70.1^{ab}
Experimental FRCBPP preparation	2.0 l/ha	2.5^{ab}	0.8^{a}	0.6^{a}	0.5^{a}	68.1^{ab}	81.9^{b}	64.2^{ab}
Control	-	3.4^{bc}	3.8^{bc}	5.1^{c}	2.9^{abc}	-	-	-

*There are no statistically significant differences between the variants marked with the same letter indices according to Duncan's test at a 95 % probability level.

Table 3: Testing of bio-rational preparations against *H. halys*, FRCBPP research crop rotation, 2020-2022
Source: compiled by authors

The essential oils of wormwood, Siberian fir, and coriander showed high efficiency (Table 2). On the first day, in the variant using wormwood essential oil, insect mortality reached 95.8%, and on the fifth 100%, Siberian fir essential oil led to the death of 93.7% of *H. halys* specimens, while coriander essential oil on the fifth day caused 85% mortality of stink bugs. The use of the FRCBPP experimental preparation caused the death of 96.7% of the insects. Essential oils of fennel and dill showed a weak toxic effect against *H. halys* imago.

Plants with biologically active compounds have long been successfully used to control various crop pests and human infections [26,35,36]. Wormwood is an example of a plant that has been successfully used as a source of safe insecticides for pest control [34]. In this study, the toxic effect of wormwood essential oil on *H. halys* also showed high effectiveness in laboratory conditions, which makes it a promising agent for research in the field of *H. halys* population control in integrated and biological plant protection technologies.

Since 2017, the FRCBPP has been working on the selection of preparations that are least dangerous to the environment and beneficial organisms that can regulate the number of *N. halys*. In 2020-2022, the effect of Biostop, P and the experimental FRCBPP preparation was studied in the agro-climatic conditions of the Central zone of the Krasnodar Territory (Table 3). The experimental FRCBPP preparation showed the greatest effectiveness on the third day (68.1%), while the effectiveness of the preparation Biostop, P was 37.4%. However, on the 10th day, the death rate of insects in the variants was 64.2% (experimental FRCBPP preparation) and 70.1% (Biostop, P).

In previous studies, the chemical preparation Euphoria, KS (belongs to the group of pyrethroids and neonicotinoids) was tested in combination at a reduced concentration with experimental FRCBPP preparations, which showed 100% effectiveness in the field [15,16]. In the experiments of scientists at the Lazarevsky experimental station, the positive effect of several chemical preparations of various groups was described, including pyrethroids, neonicotinoids, organophosphorus compounds, an inhibitor of chitin synthesis and their combinations [13].

However, despite the high effect obtained when using chemical preparations, the negative consequences for the environment and human health contribute to the abandonment of the use of chemical insecticides. There is a worldwide trend in the consumption of food products made using safe and preferably natural plant protection products [34]. Therefore, the search for effective biological agents should be continued.

Sequence Analysis of Constructs

Proper orientation of specific genes into the vector was finally verified through sequence analysis performed by BGI Tech Solutions Hongkong.

Discussion

Due to the study of the population dynamics of *H. halys* in the central zone of the Krasnodar Territory, data were obtained on the peaks in the pest population, which were noted in early July and mid-September. Our observation aligns with similar studies conducted in other countries, which also report mid-summer and early autumn as critical periods for pest activity. For example, research in Western Slovenia observed peak populations of *H. halys* during late July and early August, slightly earlier than our findings [37]. Additionally, studies in North America, where H. halys is indigenous, show a broader peak period from June to September, suggesting that the pest's native environment offers a more conducive habitat for longer periods of activity [38].In the natural conditions of the Krasnodar Territory, parasitic insects *P. cassidae* and *A. bifasciatus* infected about 5-10% of *H. halys* eggs, which did not lead to a significant decrease in *H. halys* number and did not significantly affect the dynamics of the pest population in 2020. However, both species are promising as biological agents for controlling the abundance of *H. halys* and other stink bugs during their artificial reproduction in the laboratory and subsequent release. For example, a study conducted in the northeastern United States demonstrated that *Trissolcus japonicus*, another parasitic wasp, significantly reduced H. halys populations through targeted releases [39]. These findings suggest that controlled breeding and release of parasitic insects can effectively manage pest populations, especially when integrated into a broader pest management strategy.

In laboratory conditions, the parasite *P. cassidae* infested 68.4% of *H. halys* eggs, while *A. bifasciatus* infested 75%.

Of the six tested BAS based on essential oils, wormwood essential oil (the mortality of *H. halys* imago on the tenth day was 100%), coriander oil (95.0%), and Siberian fir oil (93.7%) showed high effectiveness against *H. halys*.

The treatment of soybean crops in the conditions of the Central zone of the Krasnodar Territory with environmentally safe preparations made it possible to achieve the death of 70.1% of *H. halys* specimens when using Biostop, P and 64.2% in the variant with the experimental FRCBPP preparation.

Our studies showed that parasitic insects such as *P. cassidae* and *A. bifasciatus*, although they infect only a small part of *H. halys* eggs in natural conditions, have the potential as biological agents to control this pest. Laboratory tests have also confirmed their high effectiveness in infesting *H. halys* eggs. Regarding BAS based on essential oils, experiments have shown that some of them, such as wormwood essential oil, coriander oil, and Siberian fir oil, are highly effective in destroying the brown marble bug. An important result is also the identification of environmentally friendly preparations for the treatment of soybean crops, which can reduce the number of *H. halys* by a significant percentage. Thus, the results of the study provide important practical recommendations for the control of *H. halys* in the agro-climatic conditions of the central zone of the Krasnodar Territory and emphasize the importance of using a variety of control methods to ensure sustainable agriculture.

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Author Contributions

Irina Sergeevna Agasyeva conceptualized the research project and designed the methodology in consultation with Vladimir Ismailov. Maria Petrishcheva conducted the data analysis and interpretation under the supervision of Anton Nastasiy. All authors contributed to drafting and revising the manuscript, providing critical intellectual input, and approving the final version for publication.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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